Synthesis and structure-activity relationships of CD39 and CD73 inhibitors

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Contents

1 Introduction

1.1 Purinergic signaling

Purinergic signaling is the signaling mediated by extracellular purine nucleotides/nucleoside (ATP, ADP, adenosine) and pyrimidine nucleotides (UTP, UDP) (**Figure 1.1**). Extracellular nucleotides, phosphorylated nucleosides, participate ubiquitously in cell-to-cell communication in the so-called purinergic signaling pathways.[1] They are also the precursors of DNA and RNA with many physiological functions, and regulate the metabolism of substances *in vivo*.[2] These signaling molecules activate purinergic receptors and regulate the functions of most tissues and cells in health and disease. Short-term or fast purinergic signaling within a few seconds is observed in neurotransmission, neuromodulation, secretion, acute inflammation and chemoattraction.[3-4] Long-term purinergic signaling affects cell proliferation, differentiation and apoptosis, which can be maintained even for weeks due to the long-term trophic events triggered by ATP.[5-6]

In 1929, the key purinergic signaling molecule ATP was isolated by Karl Lohmann, and extracellular effects of purine nucleotides on the heart and on arterial pressure responses were reported by Drury and Szent-Györgyi.[7-8] ATP is the substance for intracellular energy storage and supply, which is produced by glucose in the cellular metabolism in 3 steps by glycolysis (yielding pyruvate, acetyl coenzyme A and low amounts of ATP), the tricarboxylic acid cycle (NADH) and finally by oxidative phosphorylation (large amounts of ATP).[9] In the cells, millimolar concentrations of ATP are present for this purpose, while extracellular signaling requires much lower concentrations. Modulation of purinergic signaling was proposed for a variety of therapeutic applications in the central nervous system, for cardiovascular diseases, airway diseases, disorders of the eye, ear, olfactory organ and tongue, immune system, inflammation, infection, diabetes, obesity, gut disorders, kidney, lower urinary tract, liver, reproductive system, skin, and musculoskeletal diseases among others.[10-12] The

research on purinergic signaling also accelerates the understanding of potential toxic reactions or side-effects of old/novel drugs by the interactive mechanism between purinergic receptors and drugs.

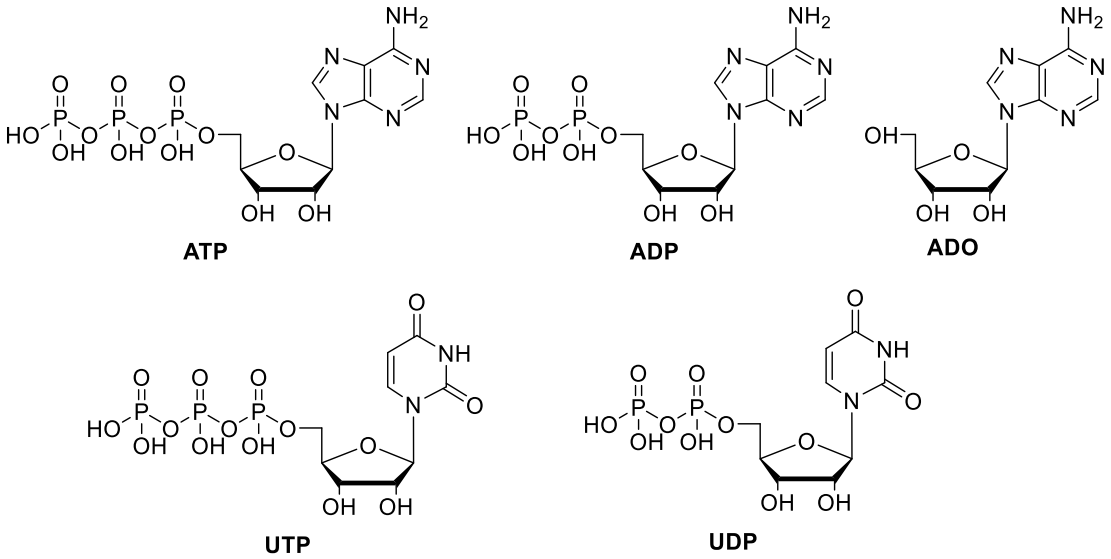


Figure 1.1. Extracellular purinergic signaling molecules including adenine nucleotides/nucleoside (ATP, ADP, ADO) and pyrimidine nucleotides (UTP, UDP). (ATP: adenosine triphosphate; ADP: adenosine diphosphate; ADO: adenosine; UTP: uridine triphosphate; UDP: uridine diphosphate.)

1.2 Purinergic receptors (purinoceptors)

Purinergic receptors (purinoceptors) were described in 1976 by Geoffrey Burnstock to be activated by extracellular ATP or by adenosine.[13] In 1978, Geoffrey Burnstock basically divided the purinergic receptors into 2 types, P1 (purinergic-1) and P2 (purinergic-2), activated by the agonists adenosine and ATP/ADP, respectively.[14] P2 receptors are also divided into 2 types: P2X and P2Y. P1 receptors and P2Y receptors are purinergic G protein-coupled receptors (GPCRs) while P2X receptors are ligand-gated ion channel (LGIC) receptors in human tissues that are common biological targets in pharmaceutical research.[15-17] Later on, a third family, P0 receptors were proposed, activated by adenine (**Figure 1.2**).[18-19] These GPCRs are present in rat, mouse and hamster, but not yet identified in human on a molecular level. They belong to the family

of Mas-related G protein-coupled receptors (MRGPRs).[20]

P1 receptors are divided into 4 subtypes: A_1, A_{2A}, A_{2B} and A_3, all activated by adenosine.[14] Until 2019, twenty agonists and antagonists of P1 receptors had entered clinical trials, but only two agonists are approved by the FDA (U.S. Food and Drug Administration); regadenoson as a diagnostic agent, and adenosine for the treatment of PSVT (paroxysmal supraventricular tachycardia) and Wolff-Parkinson-White syndrome, and also as a diagnostic agent in MPI (myocardial perfusion imaging).[21] The A_{2A}-selective antagonist istradefylline was first approved in Japan for the treatment of Parkinson's disease,[22] and then much later, also in the USA in 2019.[23]

P2X receptors are subdivided into 7 types: P2X1 to P2X7, all are activated by ATP.[18] P2X receptors display high Ca^{2+}, K^+ and Na^+ permeability, which can be triggered by ATP.[24] P2Y receptors are subdivided into 8 types: $P2Y_1$ (mainly activated by ADP), $P2Y_2$ (UTP, ATP), $P2Y_4$ (UTP), $P2Y_6$ (UDP), $P2Y_{11}$ (ATP), $P2Y_{12}$ (ADP), $P2Y_{13}$ (ADP) and $P2Y_{14}$ (UDP, UDP-glucose and UDP-galactose).[18,25-26] $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$ receptors are G_q-coupled receptors, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$ receptors are G_i-coupled receptors.[27-28] The $P2Y_{11}$ receptor can additionally couple to G_s-proteins and activate adenylate cyclase, and $P2Y_{13}$ receptors were reported to couple to G_s-proteins as well.[29-30]

Until 2020, the $P2Y_{12}$ receptor antagonists clopidogrel, prasugrel, cangrelor and ticagrelor had been approved for the treatment of thrombotic diseases,[22] also the first $P2Y_2$ receptor agonist diquafosol was approved in Japan, Korea and China for the treatment of dry eye disease.[31] Research on agonists or antagonists for $P2Y_{12}$ receptors have become the hotspot in drug development for purinergic receptors. Furthermore, there are 23 Chinese herbal compounds that have been reported to target P2 receptors.[5] Among them, the $P2Y_4$ and $P2Y_7$ antagonist rhein is the main active ingredient in LHQW (Lianhuaqingwen, the most famous TCM herbal medicine product for the treatment of COVID-19 in China that has already been approved by dozens of countries)

also showing a good inhibition of angiotensin-converting enzyme 2 with an IC_{50} value of 18.33 µmol/L.[32] Rhein is one of the most promising lead structures from nature for the development of novel drugs for the treatment of COVID-19.

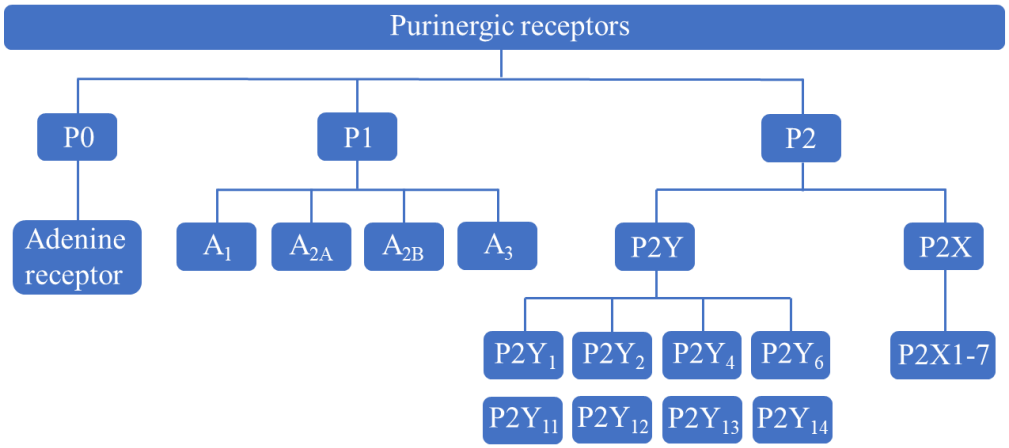


Figure 1.2. Purinergic receptors.

1.3 CD39

Ectonucleotidases are enzymes responsible for hydrolyzing extracellular nucleotides which are expressed on cell surfaces of virtually all mammalian cell types.[33-34] NTPDases (nucleoside triphosphate diphosphohydrolases) are a major class of ectonucleotidases. The family of mammalian NTPDases are membrane-bound nucleotidases that are subdivided into eight distinct subtypes, four of which (NTPDase1, -2, -3 and -8) are located on the cell surface catalyzing the hydrolysis of extracellular nucleotides, while the others (NTPDase4, -5, -6 and -7) are located on intracellular organelles.[35] The structural and functional features of NTPDase1, -2, -3 and -8 (70-80 kDa) are similar; they contain two transmembrane domains (TMDs), a large extracellular loop harboring the catalytic domain, and consist of approximately 500 amino acid residues which share 40% amino acid identity.[36]

NTPDase1 was identified from the surface of B lymphocytes which were infected by the EB (Epstein-Barr) virus as an activation marker by Rowe *et al.* in 1982.[37] It was subsequently officially named CD39 (cluster of differentiation 39, EC 3.6.1.5) during

the 3rd International Workshop and Conference on Human Leucocyte Differentiation Antigens in 1986. CD39 catalyzes the extracellular hydrolysis of ATP and ADP in a Ca^{2+}- and Mg^{2+}-dependent manner yielding AMP.[38-39] AMP is subsequently dephosphorylated by CD73 yielding adenosine (**Figure 1.3**).[40] Further nucleoside 5′-tri- and 5′-di-phosphates are also hydrolyzed by CD39, e.g., UTP and UDP. CD39 is highly expressed in spleen, thymus, lung, and placenta,[33,41-43] where it is primarily found on endothelial cells and immune cell populations, such as B-cells, natural killer (NK) cells, dendritic cells, Langerhans cells, monocytes, macrophages, mesangial cells, neutrophils, and regulatory T cells (Tregs).[44]

CD39 regulates nucleotide and nucleoside signaling via P2 and P1 receptors, respectively. Its inhibitors lead to high extracellular levels of ATP and ADP, which activate immune cells via P2 receptors and are thus promising for cancer immunotherapy.[45] Due to its significant role in purinergic signaling, CD39 is a promising target for the modulation of a number of pathological conditions including infections, acquired immune deficiency syndrome (AIDS), autoimmune diseases, atherosclerosis, ischemia-reperfusion injury, and cancers.[40,46-50] Until 2020, three monoclonal antibodies (TTX-030, SRF617 and IPH5201) as CD39 inhibitors have been in clinical phase I trials as monotherapies or in combination with other treatments for different cancers.[51]

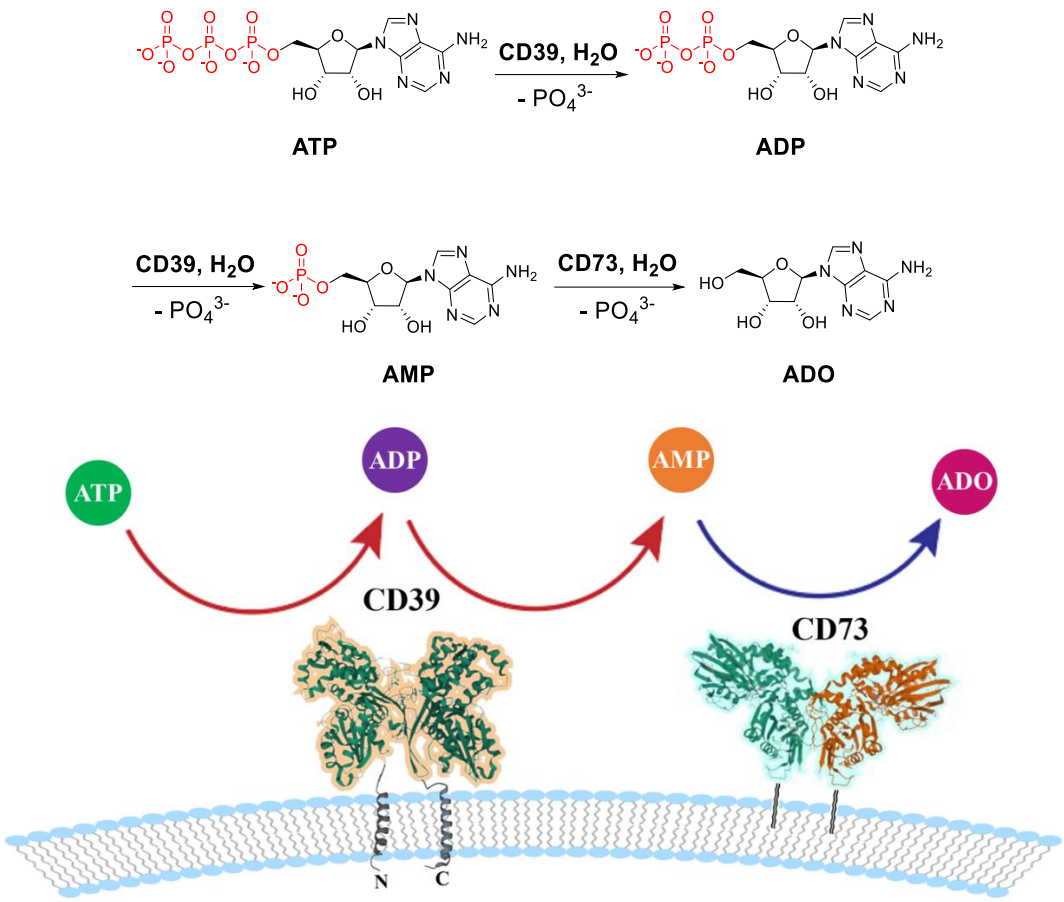


Figure 1.3. Extracellular hydrolysis of nucleotides by CD39 and CD73. (ATP: adenosine triphosphate; ADP: adenosine diphosphate; AMP: adenosine monophosphate; ADO: adenosine; CD39: ecto-nucleoside triphosphate diphosphohydrolase-1; CD73: ecto-5′-nucleotidase.)

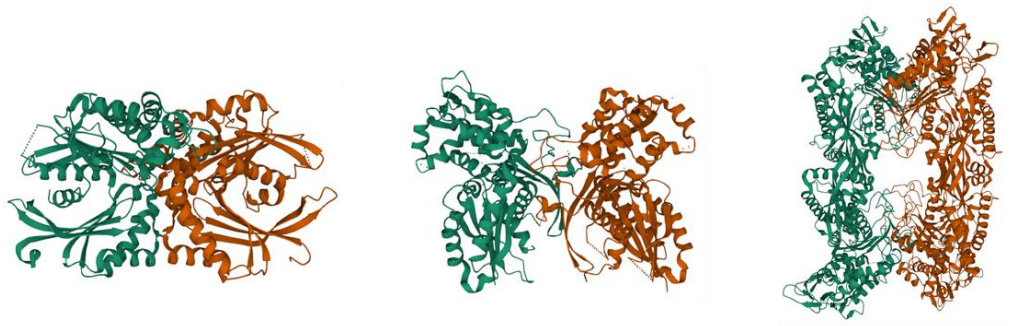
1.3.1 Crystal structure of CD39

CD39 is an extracellular glycoprotein (70-100 kDa), consisting of two TMDs with intracellular short N- and C-termini, and a large extracellular loop with five apyrase-conserved regions (ACR1 to ACR5).[38,52-53] Human CD39 contains 510 amino acids, sharing 75% amino acid sequence identity with murine CD39.[54-55] The TMDs of CD39 appear to have important functions since the truncation of both TMDs can reduce activity by 90%, and its transmembrane helices exhibit a high degree of rotational mobility which is required for activity and is regulated by substrate binding.[55] The ACRs cluster around the interdomain cleft to construct the ligand binding pocket; they

are in charge of the hydrolysis of the substrates; their catalytic residues are D54 (ACR1), T131 and A132 (ACR2), E174 (ACR3), D213, S218 and Q220 (ACR4) and W450 (ACR5).[36,56-57] ACR1, -4 and -5, and the C-terminus of human CD39 have been demonstrated to be necessary to maintain enzymatic activity, structural integrity, and protein expression on cell membranes.[58] ACR1 and ACR4, ACR3 and ACR5, form a pseudo-twofold symmetry axis in the TMDs.[36]

The domain motion of CD39 is performed by four asymmetric and independent subunits on both approximate axes via the intermediate conformation.[59-60] The rotational axis of two pairs of subunits of the rat CD39 crystal structure can lead to opening and closing the active-site cleft, from inactive conformation to active to inactive conformation, performed like a butterfly.[59]

Till now, the crystal structures of *Legionella pneumophila* CD39 (*Lp*CD39), *Rattus norvegicus* CD39 (rat CD39) and *Toxoplasma gondii* CD39 (*Tg*CD39) are the only three types of solved CD39 structures (**Figure 1.4**).[59,61-62] *Lp*CD39 was reported to contain six independent crystal forms and can undergo a domain closure motion of at least 17°.[63] The crystal structure of soluble rat CD39 (PDB: 3ZX3) was determined by molecular replacement of the rat NTPDase2 due to the 40% sequence identity, complexed with decavanadate (3ZX2) and heptamolybdate (3ZX0).[59] The structure of wild-type *Tg*CD39 (PDB: 4JEP) was determined derived from *Toxoplasma gondii* NTPDase3 in its inactive conformation, and is between open and closed states.[60] The C258S/C268S variant of *Tg*CD39 (PDB: 4A5B) is lacking the C258/C268 disulfide bridge that is responsible solely for the activation of the enzyme by thiol compounds, and shows higher activity than wild-type *Tg*CD39.[62] The reduction of the C258/C268 disulfide bridge in the activation loop is responsible for the activation and domain motion of *Tg*CD39.[60,62]



*Lp*CD39 (PDB: 4BRO) rat CD39 (PDB: 3ZX3) Wild-type *Tg*CD39 (PDB: 4JEP)

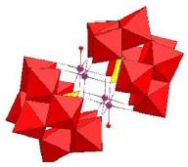
Figure 1.4. Three types of known CD39 crystal structures.[59-60,63] (*Lp*CD39: *Legionella pneumophila* CD39; *Tg*CD39: *Toxoplasma gondii* CD39.)

The crystal structure of human CD39 is still not solved. Nevertheless, homology models of human CD39 were reported based on the crystal structure of rat CD39 (PDB: 3ZX3), and several compounds were docked, e.g., PSB-170621A, 2-((2-(1*H*-indol-3-yl)ethylimino)methyl)-4,6-dichlorophenol, ATP, ARL 67156 and two ARL 67156 analogs.[45,64-65]

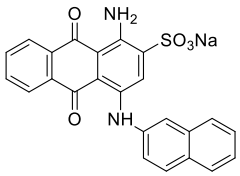
1.3.2 Reported CD39 inhibitors

1.3.2.1 Reported non-nucleotide-derived CD39 inhibitors

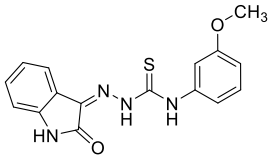
Reported non-nucleotide-derived CD39 inhibitors include PSB-POM142 (**1a**, human CD39, K_i = 0.00388 µM; rat CD39, K_i = 0.480 µM),[66-67] 1-amino-2-sulfo-4-(2-naphthylamino)anthraquinone (**1b**, rat CD39, K_i = 0.328 µM),[68] (*Z*)-*N*-(3-methoxyphenyl)-2-(2-oxoindolin-3-ylidene)hydrazine-1-carbothioamide (**1c**, human CD39, K_i = 0.15 µM; mouse CD39, K_i = 0.006 µM),[69] (*E*)-2-(((2-(1*H*-indol-3-yl)ethyl)imino)methyl)-4,6-dichlorophenol (**1d**, human CD39, K_i = 0.021 µM),[64] ticlopidine (**1e**, human CD39, K_i = 14 µM) and clopidogrel (**1f**, human CD39, K_i = 10 µM),[70] ellagic acid (**1g**, CD39, IC_{50} = 0.50 µM) and 1,2,3,4,6-penta-*O*-galloyl-*β*-*D*-glucopyranose (**1h**, CD39, IC_{50} = 1.56 µM).[71] Their structures are depicted in **Figure 1.5**.



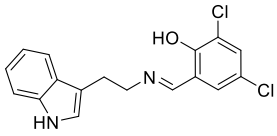
1a PSB-POM142
$K_{10}[Co_4(H_2O)_2(PW_9O_{34})_2]\cdot 22H_2O$
rat CD39, K_i = 0.480 μM[66]
human CD39, K_i = 0.00388 μM[67]



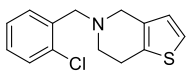
1b 1-Amino-2-sulfo-4-(2-naphthylamino)-anthraquinone[68]
rat CD39, K_i = 0.328 μM



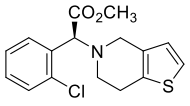
1c (*Z*)-*N*-(3-Methoxyphenyl)-2-(2-oxoindolin-3-ylidene)hydrazine-1-carbothioamide[69]
human CD39, K_i = 0.15 μM
mouse CD39, K_i = 0.006 μM



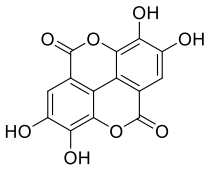
1d (*E*)-2-(((2-(1*H*-Indol-3-yl)ethyl)imino)methyl)-4,6-dichlorophenol[64]
human CD39, K_i = 0.021 μM



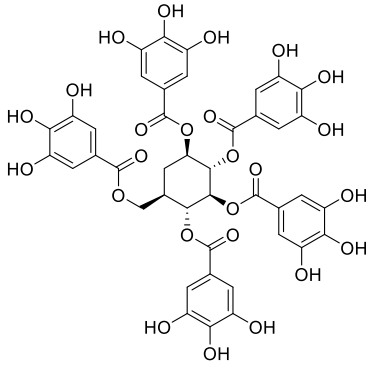
1e Ticlodipine[70]
human CD39, K_i = 14 μM



1f Clopidogrel[70]
human CD39, K_i = 10 μM



1g Ellagic acid[71]
CD39, IC_{50} = 0.50 μM



1h 1,2,3,4,6-Penta-*O*-galloyl-*β*-*D*-glucopyranose[71]
CD39, IC_{50} = 1.56 μM

Figure 1.5. Reported non-nucleotide-derived CD39 inhibitors (all data are taken from literatures). In our laboratory, different results were obtained, e.g., the potency for **1d**, **1e** and **1f** was much lower than the published values.

PSB-POM142 is an inorganic metal cluster discovered as CD39 inhibitor by the Müller group, it is the most potent human CD39 inhibitor described so far.[67] It could also notably inhibit NTPDase2 (human NTPDase2, K_i = 0.0184 μM; rat NTPDase2, K_i =1.53 μM), NTPDase3 (human NTPDase3, K_i = 0.0596 μM; rat NTPDase3, K_i =2.61 μM) and human NPP1 (K_i = 0.0690 μM).[66-67] 1-Amino-2-sulfo-4-(2-naphthylamino)anthraquinone showed potent CD39 inhibition, and being also active at rat NTPDase3 (K_i = 2.22 μM) and less potent at rat NTPDase2 (K_i = 19.1 μM).[68] Compound **1c** was described as a non-competitive human CD39 inhibitor, also

inhibiting mouse NTPDase3 and NTPDase8 with K_i values of 0.07 and 0.44 μM, respectively.[69] Compound **1d**, a Schiff base, was reported to be a competitive and noncytotoxic potent human CD39 inhibitor; it also inhibits human NTPDase3 (K_i = 0.112 μM) and NTPDase8 (K_i = 0.220 μM).[64]

The thienotetrahydropyridine drugs, such as ticlopidine, clopidogrel and prasugrel, are used to inhibit platelet aggregation, e.g., to prevent acute coronary syndrome (ACS), stroke and heart infarction.[72-73] The three compounds are prodrugs of irreversible $P2Y_{12}$ receptor antagonists. The thienotetrahydropyridines ticlopidine and clopidogrel are oxidized by the enzymes CYP3A4, CYP3A5 and CYP2C19, leading to their activation and therapeutic effects after opening of the thiophene ring and producing a reactive thiol.[74] At 100 μM, unmetabolized ticlopidine was reported to strongly inhibit the hydrolysis of ADP by human CD39 in intact COS-7 or HUVEC cells (human umbilical vein endothelial cells, by 99% and 75%) and also that of ATP although less efficiently (by 25% in both cell types).[75] Ticlopidine was reported to be selective for CD39 since it did not inhibit human NTPDase2, NTPDase3, NTPDase8, NPP1 and NPP3 at a concentration of 100 μM in that study.[75] Clopidogrel behaves very similarly to ticlopidine in most experiments due to similar structures. Both compounds facilitated platelet aggregation via the inhibition of vascular CD39, inhibiting ADP hydrolysis more efficiently than that of ATP.[70] Ticlopidine was identified as a non-competitive allosteric inhibitor of CD39 by our ongoing research.[76] In the present PhD project, ticlopidine was selected as a lead compound to develop new small molecule CD39 inhibitors.

The recently reported two CD39 inhibitors, ellagic acid and 1,2,3,4,6-penta-*O*-galloyl-*β*-*D*-glucopyranose were identified by screening. They are natural products with low cytotoxicity, which inhibit CD73 as well, the K_i values being 1.85 μM (human CD73) and 0.04 μM (murine CD73) for ellagic acid, and 10.54 μM (human CD73) for 1,2,3,4,6-penta-*O*-galloyl-*β*-*D*-glucopyranose.[71]

1.3.2.2 Reported nucleotide-derived CD39 inhibitors

The majority of reported CD39 inhibitors are nucleotides, such as 8-BuS-AMP (**1i**, human CD39, K_i = 0.8 μM),[77] 8-BuS-ADP (**1j**, human CD39, K_i = 0.9 μM),[77] 8-BuS-ATP (**1k**, human CD39, K_i = 0.8 μM),[77] ARL 67156 (**1l**, human CD39, K_i = 11 μM),[78] and 8-butylthio-β,γ-bromomethylene-ATP (**1m**, human CD39, K_i = 1.13 μM).[65] Their structures are depicted in **Figure 1.6**.

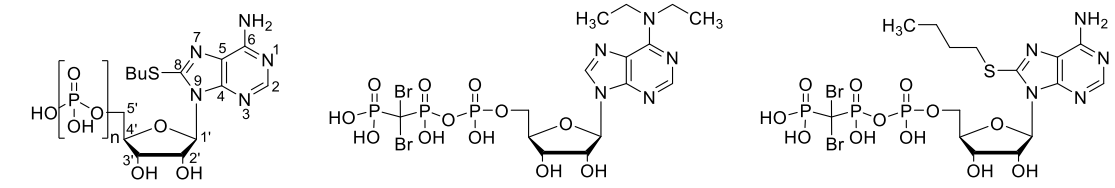


Figure 1.6. Reported nucleotide-derived CD39 inhibitors.

The K_i values showed no difference for 8-BuS-AMP, 8-BuS-ADP and 8-BuS-ATP; this demonstrates that the length of the phosphate chain has no major impact on these 8-substituted nucleotide-derived CD39 inhibitors. At 100 μM, 8-BuS-AMP, 8-BuS-ADP and 8-BuS-ATP significantly inhibited the hydrolysis of ATP by human CD39 in intact COS-7 cells (by 61, 73 and 70%, respectively), showing similar inhibition of the hydrolysis of ADP.[77] ARL 67156 also efficiently inhibited the hydrolysis of ATP (by 48%) and ADP (by 70%) under the same conditions.[78] 8-Butylthio-β,γ-bromomethylene-ATP derived from 8-BuS-ATP and ARL 67156 showed almost the same K_i value as 8-BuS-ATP. It was synthesized and found by our group to be metabolically unstable in human and mouse liver microsomes, and it could notably inhibit other human ectonucleotidases as well, e.g., NTPDase2 (K_i = 22.2 μM), NTPDase3 (K_i = 1.54 μM), CD73 (K_i = 0.831 μM) and NPP1 (K_i = 5.17 μM).[65]

In this study, 8-BuS-AMP was selected as a lead compound to develop new nucleotide-derived CD39 inhibitors due to its relatively high reported potency, its quite high stability, and its synthetic accessibility.

1.3.3 Structure-activity relationships regarding the phosphate chain of adenine nucleotides as CD39 inhibitors

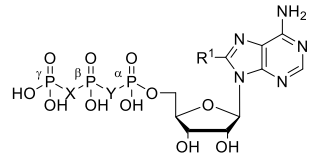
Based on reported articles and our group′s previous research, the presence of a phosphate group or chain in adenine nucleotides plays a vital role for the inhibition of NTPDases and other ectonucleotidases. Without these phosphate chains, the respective adenine nucleosides show no inhibitory activity at CD39 and other NTPDases. The structure-activity relationships (SARs) regarding the phosphate chain of known adenine nucleotides at different human hydrolytic enzymes are summarized in **Tables 1.1** and **1.2**, and **Figure 1.7**.

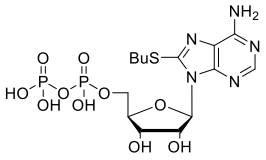
To investigate the inhibitory potency of human CD39, both oxygen atoms of ATP between the P_α and P_β, and between the P_β and P_γ positions have been replaced by different groups. For example, CF_2 (**1o**, K_i = 10.6 μM), CCl_2 (**1p**, K_i = 9.53 μM), and CBr_2 (**1q**, K_i = 5.26 μM) between the P_β and P_γ positions; the inhibitory potency was determined versus the substrate FL-ATP (N^6-(6-fluoresceincarbamoyl)hexyl-ATP).[65] But the inhibition was decreased a lot for CH_2 (**1n**, K_i > 10 μM).[65] Conversely, when CH_2 was introduced between the P_α and P_β positions of ATP (**1r**, K_i = 0.632 μM), the inhibitory potency was significantly higher.[65] According to Lecka *et al.*, the inhibitory potency did not change much between 8-BuS-ATP (**1k**, K_i = 0.8 μM), 8-BuS-ADP (**1j**, K_i = 0.9 μM), and 8-BuS-AMP (**1i**, K_i = 0.8 μM) determined versus ATP as a substrate.[77] This implies that the length of the phosphate chain (mono-, di- and tri-phosphates) is not an important parameter at least in 8-butylthio-substituted adenine nucleotides. However, the appropriate modifications of different atoms within the phosphate chain can offer possibilities to increase the inhibitory activity at CD39. According to Lecka *et al.* again, 8-BuS-ATP (**1k**), 8-BuS-ADP (**1j**) and 8-BuS-AMP (**1i**) are almost inactive at NTPDase2, -3 and -8.[77] But the testing in our group found that 8-BuS-AMP (**1i**) showed moderate inhibition of NTPDase2 and -3 as well with K_i values of 84.6 μM and 99.5 μM, respectively.[79] 8-BuS-AMP is much more potent at CD39 (K_i = 1.1 μM) and inactive at NTPDase8.[79]

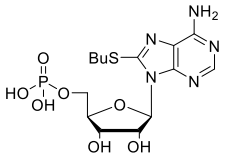
AMPCP (**1s**, 12%) only weakly inhibits CD39 but is selective for NPP1 (K_i = 1.28-16.5 μM) and especially CD73 (K_i = 0.197 μM).[80] Its inhibitory activity at CD39 disappeared when the β-phosphate was replaced by a sulfonate (**1t**), or if both phosphate groups were replaced by sulfonates (**1u**) in AMPCP using ADP (100 μM) as a substrate.[80] The replacement of the β-phosphate (**1s**, K_i = 0.197 μM) by a sulfonate (**1t**, K_i = 49.5 μM) also led to a 251-fold reduction in inhibitory potency at CD73 using [2,8-^{3}H]AMP (5 μM) as a substrate.[80] Furthermore, compounds **1t** and **1u** only showed moderate inhibition at CD73.[80] This indicates that sulfonate replacement of phosphate has negative effects on the inhibitory potency at different phosphohydrolases.

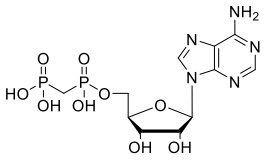
Between two groups of isomeric compounds (**1v** and **1w**, **1x** and **1y**), their stereoisomerism has no effects to the inhibitory activity at different ectonucleotidases.[81] For example, the inhibition of **1v** and **1w** (at a concentration of 100 μM) at NTPDase1 was 19% and 22%, at NTPDase2 was 15% and 13%, at NTPDase3 was 27% and 24%, at NTPDase8 was 1% and 2%, at CD73 was 45% and 23%, at NPP1, K_i values were 4.5 and 1.3 μM, respectively.[81] These 5 sulfonate-substituted ATP derivatives (**1v**, **1w**, **1x**, **1y** and **1z**) appear to show the best potency and selectivity for NPP1.[81] Modifications between the P_α and P_β positions led to more potent inhibitors than modifications between the P_β and P_γ positions.[65,81] It can also been predicted that modifications between the P_α and P_β positions on the triphosphate chain of **1r** or AMPCP (**1s**), namely CBr_2, CCl_2 or CF_2 instead of CH_2, increase inhibitory potency at different ectonucleotidases.

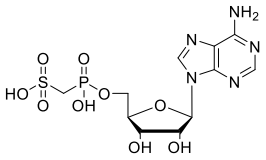
Table 1.1. Inhibitory potency of adenine nucleotide derivatives and analogs at human NTPDase1, -2, -3 and -8

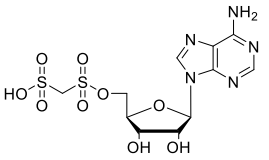


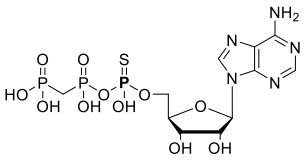


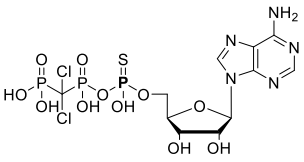


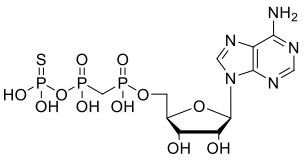












Compd.	X	Y	R^1	K_i ± SEM/SD (µM) (or % inhibition at 10 µM)			
				NTPDase1	NTPDase2[b]	NTPDase3[b]	NTPDase8[b]
1n[65]	CH_2	O	H	>10 (23 ± 6%)[a]	-	-	-
1o[65]	CF_2	O	H	10.6 ± 0.4[a]	-	-	-
1p[65]	CCl_2	O	H	9.53 ± 1.46[a]	-	-	-
1q[65]	CBr_2	O	H	5.26 ± 0.22[a]	-	-	-
1r[65]	O	CH_2	H	0.632 ± 0.024[a]	-	-	-
1k[77]	O	O	$S(CH_2)_3CH_3$	0.8 ± 0.2[b]	Inactive	>100 (26%)	Inactive
1j[77]	*for structure see above*			0.9 ± 0.2[b]	>100 (18%)	Inactive	>100 (14%)
1i[79]	*for structure see above*			1.1 ± 0.62[a]	84.6 ± 42.2[e]	99.5 ± 45.0[e]	Inactive[e]
1s[80]	*for structure see above*			>10 (12 ± 5%)[c]	-	-	-
1t[80]	*for structure see above*			Inactive[d]	-	-	-
1u[80]	*for structure see above*			Inactive[d]	-	-	-
1v[81]	*for structure see above*			>100 (19%)[b]	>100 (15%)	>100 (27%)	>100 (1%)
1w[81]	*for structure see above*			>100 (22%)[b]	>100 (13%)	>100 (24%)	>100 (2%)
1x[81]	*for structure see above*			>100 (1%)[b]	>100 (11%)	>100 (22%)	>100 (5%)
1y[81]	*for structure see above*			>100 (1%)[b]	>100 (10%)	>100 (19%)	>100 (1%)
1z[81]	*for structure see above*			>100 (58%)[b]	>100 (16%)	>100 (40%)	>100 (7%)

[a]Evaluation of enzyme inhibition using 0.5 µM FL-ATP as a substrate.

[b]100 μM Derivative evaluation of enzyme inhibition using 100 μM ATP as a substrate.

[c]Evaluation of enzyme inhibition using 100 μM ADP as a substrate.

[d]No inhibition at 10 μM.

[e]Evaluation at 50 and 100 μM test concentration.

Table 1.2. Inhibitory potency of adenine nucleotide derivatives and analogs at human NPP1, -3 and CD73

Compd.	X	Y	R^1	K_i ± SD/SEM (μM) (or % inhibition at 100 μM)		
				NPP1[a]	NPP3[a]	CD73[a]
1k[77]	O	O	$S(CH_2)_3CH_3$	>100 (58%)	>100 (15%)	>100 (13%)
1j[77]	*for structure see above*			>100 (46%)	>100 (21%)	Inactive
1i[79]	*for structure see above*			51.4 ± 2.1[b]	95.5 ± 34.3[d]	6.2 ± 0.6[e]
1s[80]	*for structure see above*			1.28-16.5[82, c]	Inactive[c]	0.197[83, f]
1t[80]	*for structure see above*			Inactive[c]	Inactive[c]	49.5 ± 0.7[g]
1u[80]	*for structure see above*			>10 (12%)[c]	Inactive[c]	>10 (23%)[g]
1v[81]	*for structure see above*			4.5 ± 0.03	>100 (45%)	-
1w[81]	*for structure see above*			1.3 ± 0.01	>100 (23%)	-
1x[81]	*for structure see above*			0.685 ± 0.005	>100 (40%)	-
1y[81]	*for structure see above*			15.2 ± 0.1	>100 (37%)	-
1z[81]	*for structure see above*			0.02 ± 0.0001	>100 (32%)	-

[a]Evaluation of enzyme inhibition using 100 μM *p*NP-TMP as a substrate.

[b]Evaluation at 20 μM test concentration.

[c]No inhibition at 10 μM, evaluation of enzyme inhibition using *p*NP-TMP as a substrate.

[d]Evaluation at 10 and 100 μM test concentration.

[e]Evaluation at 50 μM test concentration.

[f]No inhibition at 10 μM, evaluation of rat CD73 inhibition using 5 μM [2,8-^{3}H]AMP as a substrate.

[g]No inhibition at 10 μM, evaluation of enzyme inhibition using 5 μM [2,8-^{3}H]AMP as a substrate.

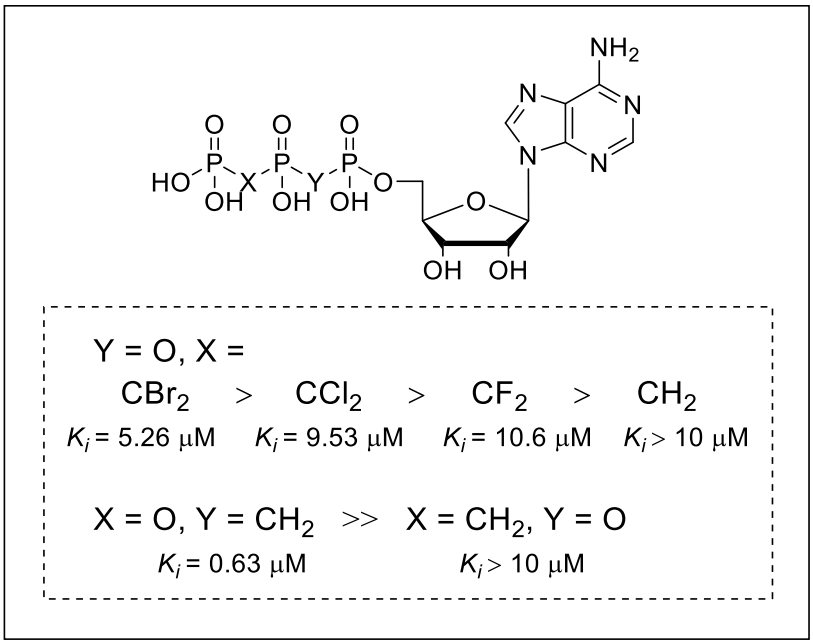


Figure 1.7. SARs of the phosphate chain of the ATP scaffold at human CD39.[65]

1.4 CD73

In 1934, 5′-nucleotidases were identified in heart and skeletal muscle.[84-85] Until now, seven subtypes of human 5′-nucleotidases were found. Among them, six 5′-nucleotidases are located intracellularly in the cytoplasm, mitochondria and erythrocytes, only one 5′-nucleotidase is located extracellularly anchored in the cell membrane, known as ecto-5′-nucleotidase.[36,86] Ecto-5′-nucleotidase was officially named CD73 (EC 3.1.3.5) during the 4th International Workshop and Conference on Human Leucocyte Differentiation Antigens in 1989.

CD39 and CD73 are often co-expressed in various cell membranes to hydrolyze extracellular ATP yielding adenosine.[87-88] CD39 and CD73 are overexpressed to produce adenosine on the surface of a variety of tumor and virally infected cells. Excess adenosine subsequently engages with P1 receptors to halt immune cell differentiation and maturation, and induces the expression of checkpoint proteins (e.g., PD-1 and CTLA-4) to accelerate immune escape of cancers.[89] Inhibiting the ATP→ADO pathway, namely CD39 and CD73, producing extracellular adenosine can efficiently curb tumor cell proliferation and neoangiogenesis.[90] Mittal *et al.* reported that

combination immunotherapy of an A_{2A} receptor antagonist (SCH58261) and checkpoint blockade (anti-CTLA-4, anti-PD-1, or anti-Tim-3 monoclonal antibody) can more potently inhibit high expression of CD73 on tumor cells, compared to any monotherapy.[91] CD73 is now used or considered as an effective clinical tumor biomarker to evaluate the survival, tumor metastasis and prognostic implication in various cancer immunotherapies.[92-93]

Until 2020, six monoclonal antibodies (oleclumab, BMS-986179, CPI-006, NZV930, GS-1423 and TJ004309) and one small molecule (AB680) as CD73 inhibitors are in clinical phase I/II trials as monotherapies or in combination with other treatment approaches for different cancers.[51] The development of CD39 and CD73 as novel checkpoint inhibitor targets is a promising strategy to restore the antitumor immune response and assist cancer therapy.[49,94-95]

1.4.1 Crystal structure of CD73

CD73 is an extracellular surface protein (≈ 140 kDa), which is anchored to the membrane by glycosylphosphatidylinositol (GPI).[96-97] The GPI is a protein linker anchored to the cell membrane surface by serine-523 in the hydrophobic C-terminal domain of CD73 in rat, human and bovine.[36] It consists of the conserved core glycan with glycan side chains, and phosphatidylinositol with its fatty chains are inserted into the outer leaflet of the lipid bilayer.[98] Apart from the membrane-bound CD73, the soluble CD73 (≈ 60 kDa) is also present *in vivo* generated by phospholipase-mediated cleavage of the GPI anchor.[51,97,99] The soluble CD73 consists of amino acid residues 27-549, after its N-terminal residues 1-26 and C-terminal residues 550-574 were cleaved. The cDNA (complementary DNA) sequences of CD73 have been identified for a considerable variety of mammalian species, and the mouse is 86% and 92% identical to human and rat, respectively.[36]

Various crystal structures reveal CD73 to be a noncovalent dimer consisting of two structural domains: the N-terminal domain (residues 27-317) and the C-terminal domain (residues 337-549), which are connected by a hinge region (α helix, residues 318-336) to enable the switch and domain movements between the open and closed conformations.[100] The active site is between the N- and C-terminal domains, and the substrate AMP is buried and hydrolyzed in a Zn^{2+}-dependent manner yielding adenosine in the closed, active conformation.[100-101] Open and closed conformations are the two main reported human CD73 crystal structures which reveal an extensive, 114° conformational switch, and the closed conformation offers a larger and superior binding pocket for ligands for further interactions.[100] The open and closed forms of human CD73 crystals are depicted in **Figure 1.8**.

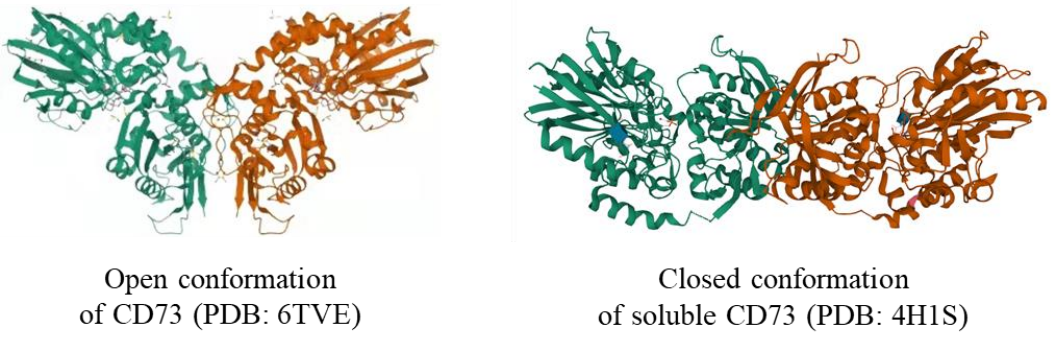


Figure 1.8. Open and closed conformations of CD73 crystal structures.[101-102]

Till now, there are more than 6 kinds of different open, closed or open/closed hybrid human CD73 crystal forms that have been solved (**Table 1.3**).[97,100-110] Two open or closed human CD73 crystals without ligand, and 35 human CD73 complexes with different ligands have been reported and widely studied in the recent decade.

Table 1.3. Summary of published X-ray crystal structures of human CD73 (till January 2022)

PDB code	Resolution (Å)	Ligand	Crystal form	Time
4H1S[102]	2.20	none	(closed)	2012
4H2F[100]	1.85	adenosine	I (open)	2012
4H2G[100]	1.55	adenosine	II (open)	2012
4H1Y[100]	1.58	PSB-11552	II (open)	2012
4H2B[100]	1.70	baicalin	II (open)	2012
4H2I[100]	2.00	AMPCP	III (closed)	2012
6HXW[103]	2.78	IPH53	(closed)	2019
6S7F[97]	2.05	PSB-12379	III (closed)	2019
6S7H[97]	1.85	PSB-12489	III (closed)	2019
6TVE[101]	1.05	none	II (open)	2020
6TVG[101]	1.48	AMPCP	II (open)	2020
6TVX[101]	2.60	PSB-12676	III (closed)	2020
6TW0[101]	2.50	PSB-12690	III (closed)	2020
6TWA[101]	2.00	PSB-12646	III (closed)	2020
6TWF[101]	2.50	PSB-12604	III (closed)	2020
6VC9[104]	2.25	TB19	-	2020
6VCA[104]	3.73	TB38	-	2020
6Z9B[105]	2.17	A830	III (closed)	2020
6Z9D[105]	1.90	AB680	III (closed)	2020
6XUE[106]	1.94	A2396	IV (closed)	2020
6XUG[106]	2.09	A2410	IV (closed)	2020
6XUQ[106]	1.97	A1618	III (closed)	2020
7JV8[107]	2.46	OP-5244	-	2020
7JV9[107]	2.70	CAS: 2319622-58-5	-	2020
6YE1[108]	2.66	A894	IV (closed)	2021
6YE2[108]	2.44	A1202	IV (closed)	2021
7P9N[109]	1.55	AMP	II (open)	2021
7P9R[109]	1.41	GMP	II (open)	2021
7P9T[109]	1.79	dCMP	II (open)	2021
7PA4[109]	1.45	CMP	II (open)	2021
7PB5[109]	1.28	UMP	II (open)	2021
7PD9[109]	1.39	riboflavin	II (open)	2021
7PBA[109]	1.42	IMP	II (open)	2021
7PBB[109]	1.56	caffeine	II (open)	2021
7PBY[109]	1.13	4-nitrocatechol	II (open)	2021
7PCP[109]	1.38	5-iodouracil	II (open)	2021
7BBJ[110]	2.73	mAb19	II (open)	2021

1.4.2 Reported nucleotide-derived CD73 inhibitors

The majority of reported nucleotide-derived CD73 inhibitors are nucleoside methylenediphosphonates (AMPCP derivatives and analogs), such as AMPCP (**1s**, human CD73, K_i = 88.4 nM; rat CD73, K_i = 197 nM),[83] PSB-12379 (**2a**, human CD73, K_i = 2.21 nM; rat CD73, K_i = 9.03 nM),[83] PSB-12489 (**2b**, human CD73, K_i = 0.318 nM; rat CD73, K_i = 0.746 nM),[97] AB680 (**2c**, human CD73, K_i = 0.005 nM)[105] and (((((2*R*,3*S*,4*R*,5*R*)-5-((*Z*)-4-((benzyloxy)imino)-3-methyl-2-oxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)methyl)phosphonic acid (**2d**, human CD73, K_i = 7.96 nM; rat CD73, K_i = 3.67 nM).[111] ((((2*R*,3*S*,4*R*,5*R*)-5-(6-Chloro-4-(cyclopentylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)methyl)phosphonic acid (**2e**, human CD73, IC_{50} = 2.6 nM)[108] and OP-5244 (**2f**, human CD73, IC_{50} = 0.25 nM) were reported as new nucleotide-derived CD73 inhibitors.[107] Their structures are depicted in **Figure 1.9**.

Among these reported CD73 inhibitors, the highly potent, reversible and selective AB680 (**2c**) is the most potent one showing very low clearance and a long half-life; it is currently being evaluated in a phase I clinical trial.[105] AB680 (**2c**) is based on the extensive work by the Müller group.[83,97,101] In this project, we further developed AMPCP derivatives to obtain new nucleotide-derived CD73 inhibitors. In particular, the 7-position was modified by introducing different substituents.

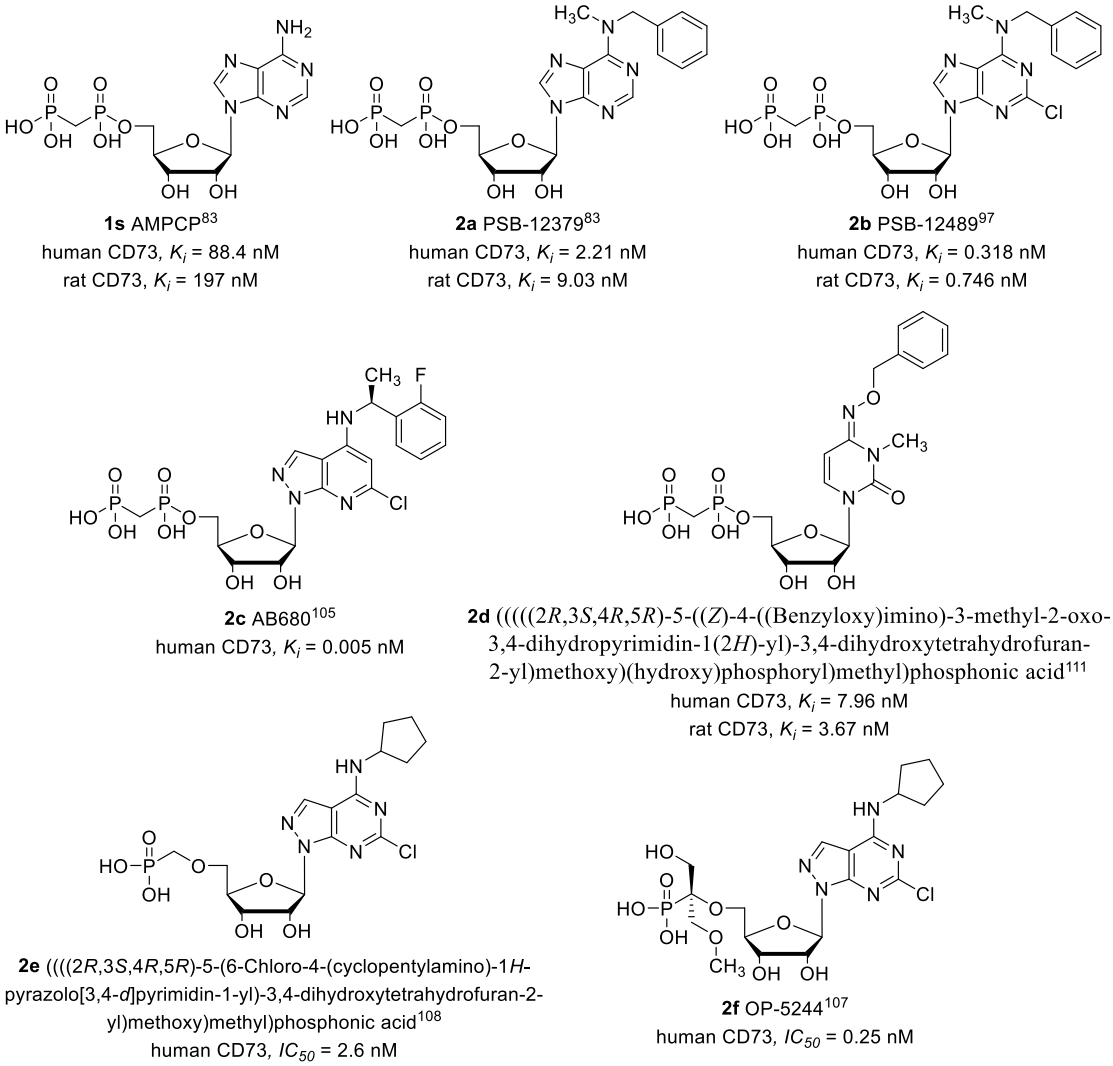


Figure 1.9. Select-reported nucleotide-derived CD73 inhibitors.

Previous studies of the Müller group had identified the potent, selective, and metabolically stable inhibitors PSB-12379 (**2a**) and PSB-12489 (**2b**).[83,97] The pyrimidine nucleoside methylenediphosphonate derivative, compound **2d**, was later reported as a potent CD73 inhibitor as well.[111] The latest reported CD73 inhibitor, compound **2e** is the methylenephosphonic acid analog reported by the Powers group, also showed high potency, selectivity, low clearance and a long half-life *in vivo*.[105,108] Another analog, OP-5244 (**2f**), reported by Du *et al*. introduced hydroxymethylene and methoxymethylene groups at the α-position of the phosphonic acid that increased oral bioavailability and inhibitory potency.[107] It inhibited the production of adenosine completely in both human cancer cells and $CD8^+$ T cells in preclinical studies, and

modulated the AMP→ADO pathway to reverse immunosuppression *in vivo*.[107] To replace the methylenedisphosphonic acid moiety by both unmodified and modified methylenephosphonic acid moieties yielded compound **2e** and OP-5244 (**2f**) as new nucleotide-derived CD73 inhibitors, which expanded the area of small-molecule CD73 inhibitors as promising candidates for novel antitumor drugs.[107-108]

1.5 Further ectonucleotidases

In addition to CD39, NTPDase2, -3, -8 and CD73, cyclic ADP ribose hydrolase (CD38), alkaline phosphatases (APs), and ecto-nucleotide pyrophosphatases/phosphodiesterases (NPPs) are further known families of ectonucleotidases.[80,112-113]

CD38 (EC 3.2.2.5, 46 kDa) is a transmembrane glycoprotein of type II, consists of a short 20-aa (amino acid) N-terminal cytoplasmic tail as the transmembrane domain and a long 256-aa extracellular domain with multiple asparagine-linked glycosylation sites at its C-terminal end.[114-116] It is widely expressed in immune cells and multiple tissues on the cell surface or in intracellular compartments.[113,117] CD38 regulates the Ca^{2+} signaling pathway, NAD^+ (nicotinamide adenine dinucleotide) metabolism, and sirtuin activity by adjusting the balance of cyclic adenosine diphosphate ribose (cADPR), nicotinic acid adenine dinucleotide phosphate (NAADP), and adenosine diphosphate ribose (ADPR) between neutral and acidic pH.[118-119] The current research is mainly focused on targeted therapy of CD38 in cardiovascular diseases, inflammation, autoimmune diseases, hematological malignancy, solid cancers and neurodegenerative diseases.[113-114,118,120-121]

APs (EC 3.1.3.1) are nonspecific homodimeric metalloproteases, containing two Zn^{2+}, one Mg^{2+}, one Ca^{2+} and five cysteine residues (Cys101, Cys121, Cys183, Cys467, and Cys474 in PLAP).[122-123] Zn^{2+} and Mg^{2+} ions are crucial for the catalytic activity of APs which are located at the active site of each monomer.[122] APs can stepwise hydrolyze

ATP to ADP, ADP to AMP, and AMP to ADO, most effectively in an alkaline environment.[96,112] APs are divided into two groups, the tissue-nonspecific alkaline phosphatase (TNAP) and the tissue-specific alkaline phosphatases; the latter are further subdivided into 3 groups, placental alkaline phosphatase (PLAP), intestinal alkaline phosphatase (IAP) and germ cell alkaline phosphatase (GCAP).[124] The molecular weight is 70-90 kDa for IAP, 90-120 kDa for PLAP and GCAP, and 120-150 kDa for TNAP.[125] The amino acid structure of TNAP shares approximately 50% identity with placental alkaline phosphatases (PLAP, IAP and GCAP).[123] TNAP is mainly expressed in liver, bone and kidney; PLAP is mainly formed in the placenta, IAP in the gastrointestinal tract, especially in the duodenum.[122] GCAP is located at primordial germ cells, testes, cervix, thymus, placenta and some neoplastic tissues.[122,125] APs were identified as biomarkers for monitoring disease activity, since they are overexpressed in some diseases, e.g., cancers, bone diseases and chronic kidney diseases.[123,126-127]

The family of NPPs (EC 3.1.4.1) is subdivided into seven distinct subtypes (NPP1-7). Four of them (NPP1, -3, -4 and -5) degrade nucleotides, and the other three (NPP2, -6 and -7) show high affinity for phospholipid-based substrates.[128-129] NPP1-7 are divided into two subgroups based on their primary structures, type I membrane proteins (NPP4-7) and type II membrane proteins (NPP1-3).[130] The ecto-domain of NPP1-3 is composed of two N-terminal short somatomedin B-like domains, a central catalytic domain, and a C-terminal nuclease-like domain.[36,131] The sole catalytic ecto-domain of NPP4-7 is composed of a putative N-terminal signal peptide and a C-terminal TMD.[36] The catalytic domains of all NPPs are conserved zinc-binding sequences sharing 24 to 60% amino acid identity between the human isoforms.[36,132] The structural identity of the catalytic domains of NPP3 with NPP1 (PDB: 4GTW) is 51%, that with NPP2 (3NKM) is 49%, for NPP4 (4LR2) it is 36%, for NPP5 (5VEM) it is 35%, for NPP6 (5EGE) it is 30%, and for NPP7 (5TCD) it is 30%.[132]

NPP1 mainly hydrolyzes nucleoside triphosphates (e.g., ATP to AMP) and diphosphates, but also cGAMP (cyclic guanosine monophosphate-adenosine

monophosphate) to generate AMP and GMP (guanosine monophosphate), which leads to metastatic progression of chromosomally unstable tumors and prevention of protective STING (stimulator of interferon genes) activation in host cells.[133] NPP1 is highly expressed on the surface of osteoblasts and chondrocytes, and its inhibitors have been suggested as novel drugs for the treatment of calcium pyrophosphate (diphosphate) dihydrate deposition diseases and diabetes mellitus type 2.[134-135] NPP2, also called autotaxin, is highly selective to hydrolyze lysophosphatidylcholine yielding lysophosphatidic acid which is implicated in cancer, asthma, fibrosis of the lung and kidney, neuropathic pain, inflammation and cardiovascular events.[136] NPP3, also called CD203c, mainly hydrolyzes ATP to AMP.[128] It is expressed on the cell surface of basophils and mast cells, and it has been investigated as a target for the treatment of allergic diseases and cancers.[132] NPP4 is abundantly present on the surface of human brain vascular endothelium, it hydrolyzes diadenosine triphosphate yielding ADP which induces irreversible platelet aggregation.[137] NPP5 is expressed in the brain, respiratory epithelium, epididymis, kidney and white adipose tissue; it is predicted to play an important role in neuronal functions.[129,138] NPP6 is dominantly expressed in the brain and kidney, and was suggested to be important for the reuptake of physiologically essential choline.[130] NPP7 is located on the surface of microvillus membranes in enterocytes; it can promote cholesterol absorption by affecting sphingomyelin levels in the gut and decrease the risk of colon cancer.[139-140]

2 Aims of the project

2.1 Design and synthesis of ticlopidine derivatives and analogs as novel CD39 inhibitors

Ticlopidine and clopidogrel were reported as CD39 inhibitors with K_i values of 14 and 10 μM at CD39,[70] but no other derivatives or analogs have been studied so far. Investigation of derivatives and analogs of ticlopidine and clopidogrel appears to be very promising with the aim to improve the inhibitory activity at CD39 and enhance selectivity and metabolic stability. In this study, we selected ticlopidine as a lead structure to explore the SARs of this class of CD39 inhibitors.

For the synthesis of ticlopidine derivatives and analogs, the following modifications were targeted (**Figure 2.1**): (A) Introducing halogens, nitro, alkyl and aryl groups on the benzene ring, or replace it by cyclohexane. (B) Elongation of the linker by one methylene unit. (C) Introducing methyl to the linker or to the N-atom. (D) Introducing methyl or phenyl to the piperidine ring. (E) Replacement of the thiophene ring by aryl or H, or introducing Br to it. (F) Replacement of the thienotetrahydropyridine by tetrahydroquinoline, indoline and naphthalene (or their derivatives). (G) Combining the above-mentioned modifications.

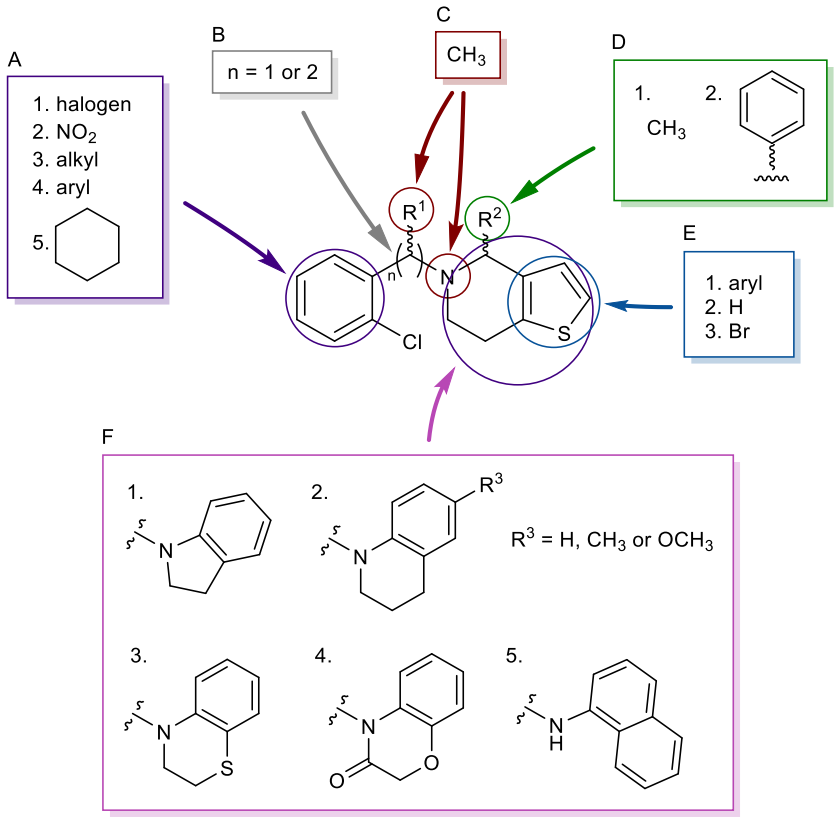


Figure 2.1. Planned structural modifications of the ticlopidine scaffold.

2.2 Design and synthesis of 8-BuS-AMP derivatives and analogs as novel CD39 inhibitors

Most of the reported nucleotide-derived CD39 inhibitors are analogs of the substrate ATP focusing on the modification of the phosphates or substitutions of the N^6- or 8-position.[65,77-78] Till now, no highly potent nucleotides have been reported, and most of the reported nucleotides are metabolically unstable. 8-BuS-AMP is one of the most potent nucleotide-derived CD39 inhibitors so far with a K_i value of 0.8 µM at human CD39,[77] and a very long half-life (human liver microsomes) of 462 min found by our group (unpublished data). The synthesis of derivatives and analogs of 8-BuS-AMP is very promising to improve its inhibitory activity at CD39, retain its metabolic stability and enhance its selectivity versus other ectonucleotidases.

For some collaboration projects, 8-BuS-AMP was synthesized on a large scale (>200 mg) to allow *in vivo* studies.

For the synthesis of 8-BuS-AMP derivatives and analogs, the following modifications were targeted (**Figure 2.2**): (A) Replacement of the 8-butylthio by other alkylthio, arylthio, alkyl, phenyl, alkylamino, alkoxy and OH groups. (B) Replacement of the N at the 7-position by CH, and introduction of Br and aryl residues at the 7-position. (C) Introduction of alkylamino, arylamino and alkylthio groups at the N^6-position. (D) Bridging 1- and N^6-positions with ethylene. (E) Introduction of Cl and NH_2 at the 2-position. (F) Replacing monophosphate by methylenediphosphonate and methylthiophosphate. (G) Combining the above-mentioned modifications.

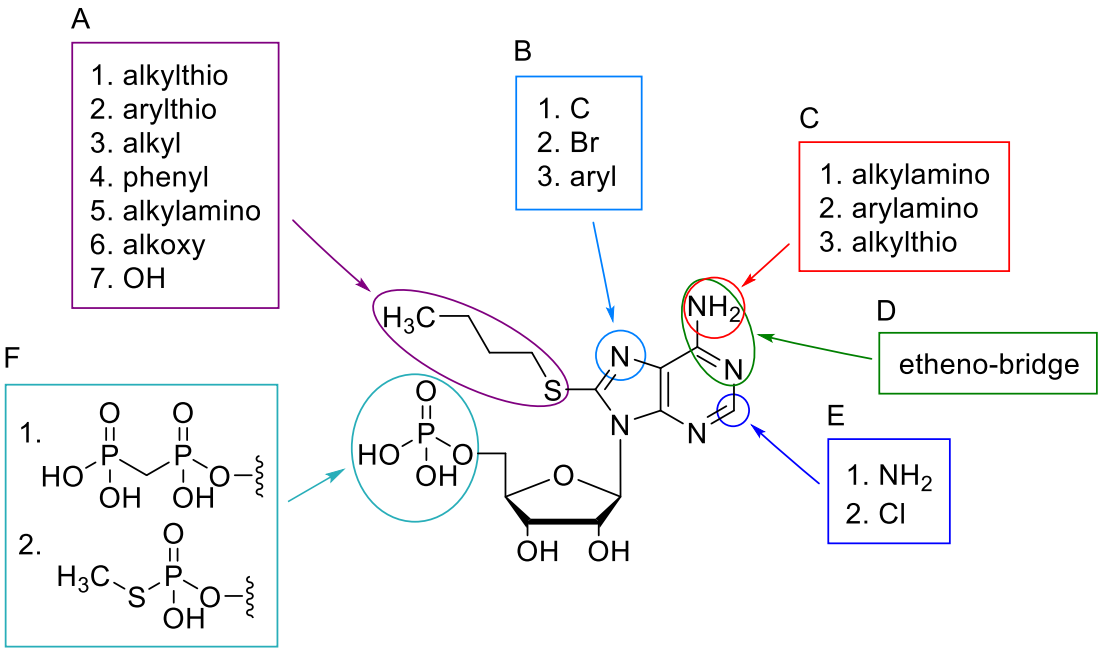


Figure 2.2. Planned structural modifications of the 8-BuS-AMP scaffold.

2.3 Design and synthesis of AMPCP derivatives and analogs as novel CD73 inhibitors

In this study, we selected AMPCP, and its derivatives and analogs as lead structures to further explore their SARs on CD73. Most reported potent AMPCP derivatives and analogs developed as CD73 inhibitors focused on modifications at the N^6- and/or 2-position(s).[83,97,101,105,107-108] In the present study, we mainly modified the 7- or 8-position resulting in novel compounds not previously investigated.

For AMPCP derivatives and analogs, the following modifications were targeted (**Figure 2.3**): (A) Replacement of the CH in the 8-position by N, or introducing a butylthio residue at the 8-position. (B) Replacement of the N in the 7-position by CH, and introducing Br, ethynyl and aryl residues at the 7-position. (C) Introducing 4-phenylbutyl and dibutylamino at the N^6-position. (D) Bridging the 1- and N^6-positions with ethylene. (E) Replacing the methylenediphosphonate by monophosphate. (F) Combining the above-mentioned modifications.

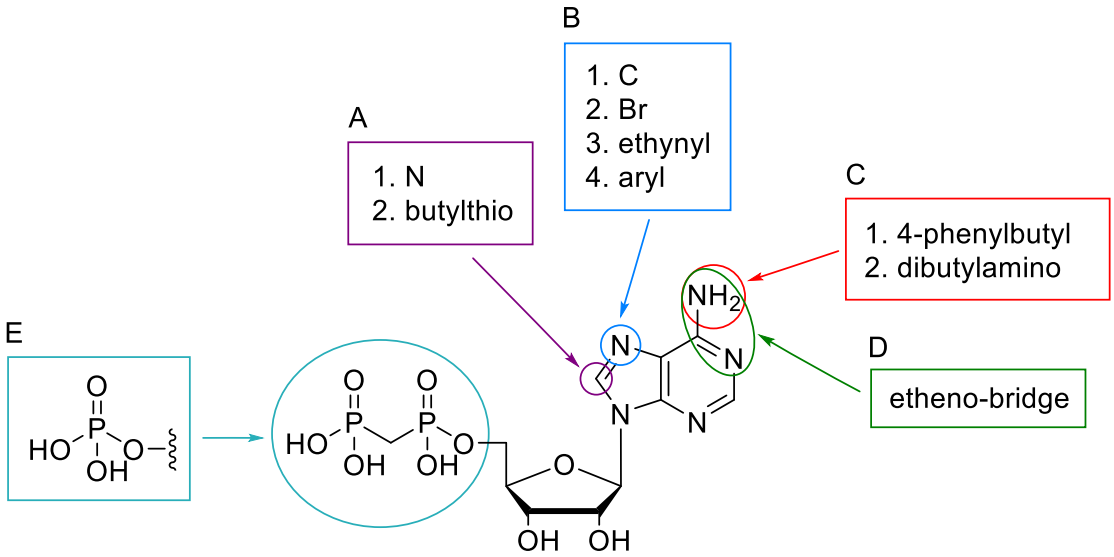


Figure 2.3. Planned structural modifications of the AMPCP scaffold.

2.4 Development of dual CD39/CD73 inhibitors

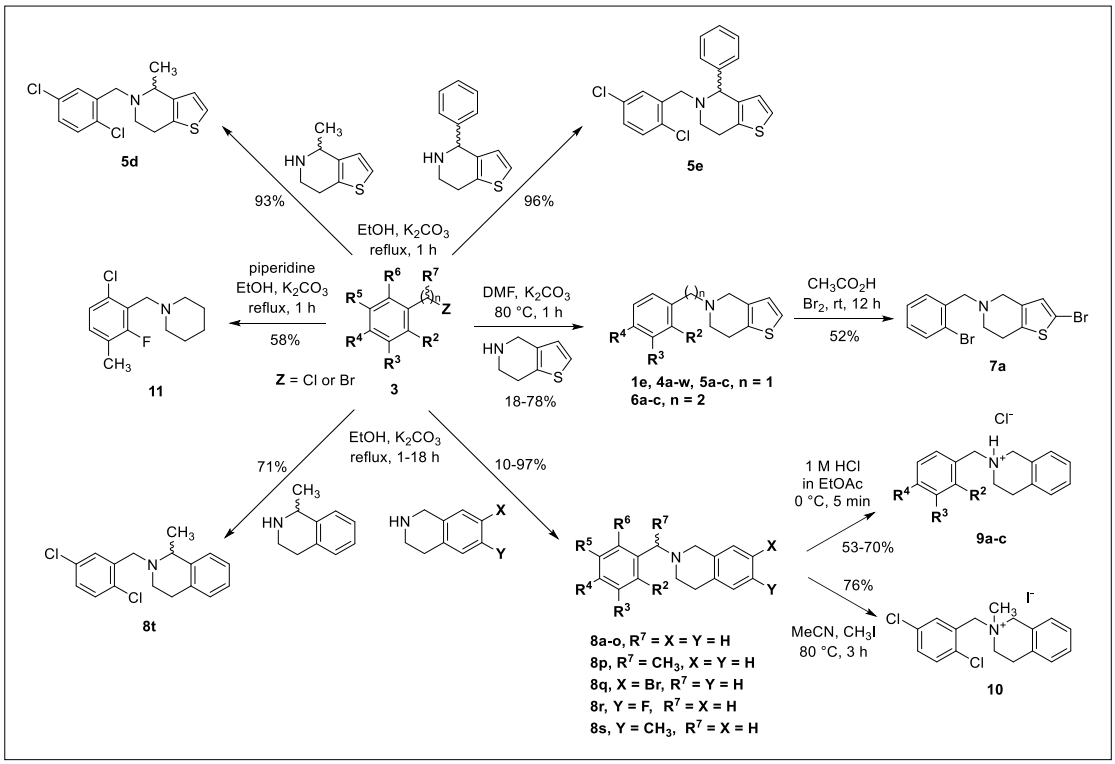
In the past, dual CD39/CD73 inhibitors were seldom reported. And no potent dual CD39/CD73 inhibitors have been described. During this study, selectivity studies of some potent CD39 or CD73 inhibitors on different ectonucleotidases were also performed. Some of them blocked both CD39 and CD73. These inhibitors may act synergistically since they block both pathways of ATP→AMP and AMP→ADO conversion. Based on the initial observation, dual inhibition will be optimized by structural modifications. During this study, it is planned to develop potent dual CD39/CD73 inhibitors.

3 Results and discussion – Part I: Development of novel ticlopidine derivatives and analogs as inhibitors of CD39

3.1 Synthesis of ticlopidine derivatives and analogs

3.1.1 Synthesis of ticlopidine derivatives and analogs (1e, 4a-w, 5a-e, 6a-c, 7a, 8a-t, 9a-c, 10 and 11)

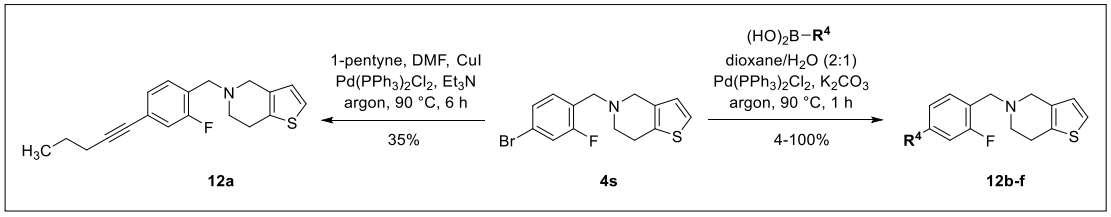
The synthesis of ticlopidine derivatives and analogs **1e**, **4a-w**, **5a-e**, **6a-c**, **7a**, **8a-t**, **9a-c**, **10** and **11** is depicted in **Scheme 3.1**. Compounds **1e**, **4a-w**, **5a-e**, **6a-c** were synthesized by alkylation of 4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine or its derivatives with a variety of benzyl halides in the presence of K_2CO_3 in dimethylformamide (DMF) or EtOH. The resulting product **4c** was subjected to bromination yielding compound **7a**. Products **8a-t** were obtained by alkylation of 1,2,3,4-tetrahydroisoquinoline or its derivatives with a variety of benzyl halides in the presence of K_2CO_3 in EtOH. Three of the oily products were converted to their hydrochlorides by adding 1 mol/L HCl in ethyl acetate yielding **9a-c**. Compound **10** was synthesized by methylation of **8k** with CH_3I in MeCN in analogy to a reported method.[141] Compound **11** was synthesized by alkylation of piperidine with 6-chloro-2-fluoro-3-methylbenzyl bromide in the presence of K_2CO_3 in EtOH.



Scheme 3.1. Synthesis of ticlopidine derivatives and analogs (**1e**, **4a-w**, **5a-e**, **6a-c**, **7a**, **8a-t**, **9a-c**, **10** and **11**). For $\mathbf{R^2}$, $\mathbf{R^3}$, $\mathbf{R^4}$, $\mathbf{R^5}$, $\mathbf{R^6}$ see **Table 3.1**.

3.1.2 Synthesis of 5-(2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4s) derivatives (12a-f)

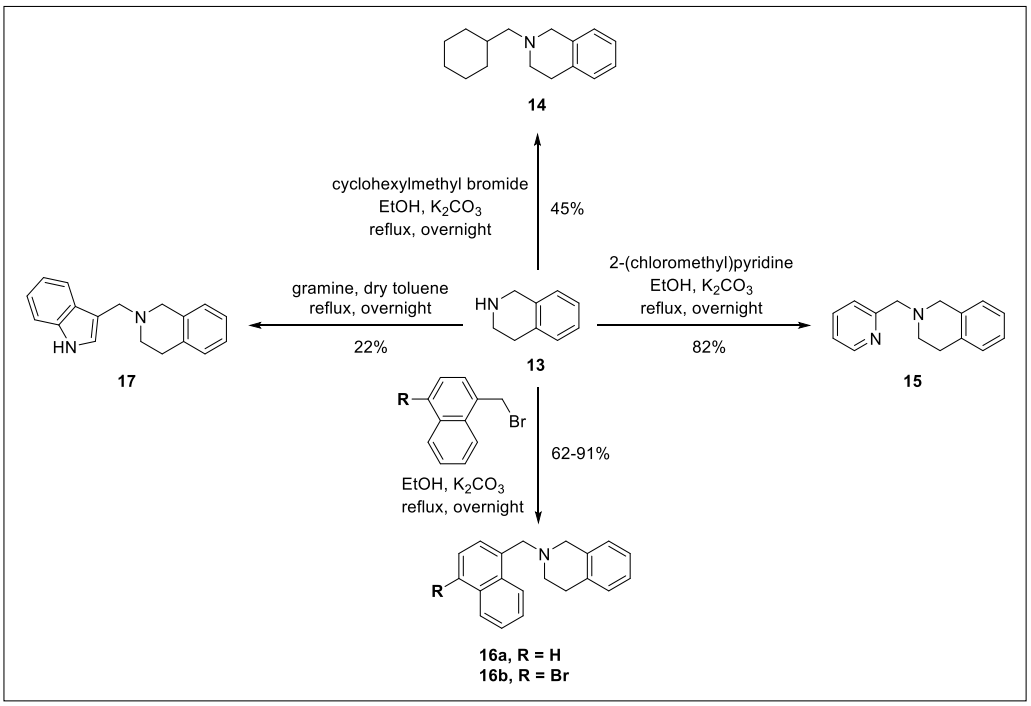
Compound **4s** was further modified as depicted in **Scheme 3.2**. Compound **12a** was synthesized by Sonogashira coupling reaction of **4s** with 1-pentyne according to a reported procedure.[142] Aromatic substituents were introduced by Suzuki reaction in analogy to a reported procedure,[143] employing the appropriate phenyl or heterocyclic boronic acid derivatives yielding **12b-f**.



Scheme 3.2. Synthesis of 5-(2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (**4s**) derivatives (**12a-f**). For $\mathbf{R^4}$ see **Table 3.1**.

3.1.3 Synthesis of tetrahydroisoquinoline analogs (14, 15, 16a-b and 17)

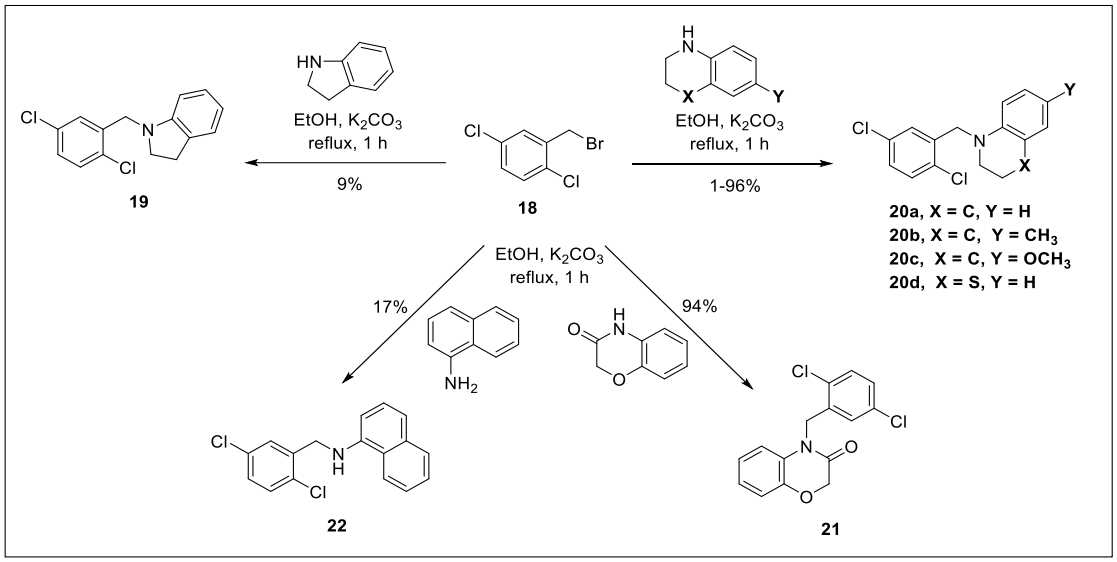
The synthesis of tetrahydroisoquinoline analogs **14**, **15**, **16a-b** and **17** is depicted in **Scheme 3.3**. Compounds **14**, **15** and **16a-b** were obtained by alkylation of 1,2,3,4-tetrahydroisoquinoline with cyclohexylmethyl bromide or a naphtylmethyl bromide derivative in the presence of K_2CO_3 in EtOH. Compound **17** was synthesized according to a reported procedure,[144] by treatment of to 1,2,3,4-tetrahydroisoquinoline dissolved in anhydrous toluene with gramine (*N*,*N*-dimethyl-1*H*-indole-3-methylamine), stirring the mixture under reflux overnight.



Scheme 3.3. Synthesis of tetrahydroisoquinoline analogs (**14**, **15**, **16a-b** and **17**).

3.1.4 Synthesis of ticlopidine analogs (19, 20a-d, 21 and 22)

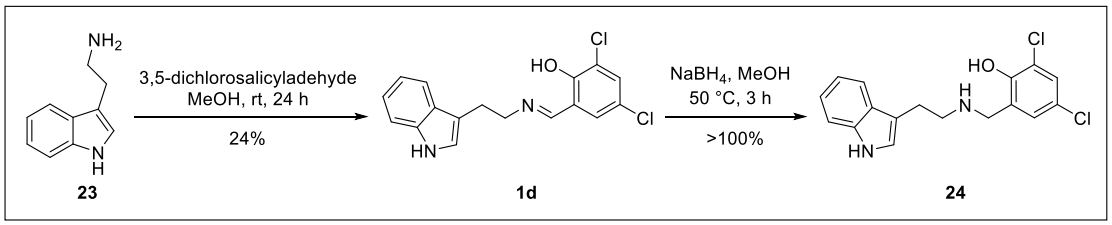
The synthesis of ticlopidine analogs **19**, **20a-d**, **21** and **22** is depicted in **Scheme 3.4**. Compounds **19**, **20a-d**, **21** and **22** were synthesized by alkylation of 2-(bromomethyl)-1,4-dichlorobenzene (**18**) with a variety of 1,2,3,4-tetrahydroquinoline derivatives and analogs or 1-naphthylamine in the presence of K_2CO_3 in EtOH.



Scheme 3.4. Synthesis of ticlopidine analogs (**19**, **20a-d**, **21** and **22**).

3.1.5 Synthesis of (*E*)-2-(((2-(1*H*-indol-3-yl)ethyl)imino)methyl)-4,6-dichlorophenol (1d) and 2-(((2-(1*H*-indol-3-yl)ethyl)amino)methyl)-4,6-dichlorophenol (24)

The Schiff base, **1d** (K_i = 0.021 μM), was previously reported as a potent inhibitor of CD39.[64] Compound **1d** and its reduced product, **24**, were synthesized and investigated in this study for comparison. Firstly, tryptamine was reacted with 3,5-dichlorosalicyladehyde to generate **1d**. Subsequently, **1d** was reduced by $NaBH_4$ to generate **24** according to a reported procedure with small modifications (**Scheme 3.5**).[145]



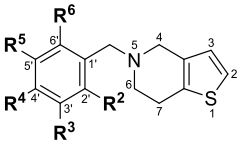
Scheme 3.5. Synthesis of (*E*)-2-(((2-(1*H*-indol-3-yl)ethyl)imino)methyl)-4,6-dichlorophenol (**1d**) and 2-(((2-(1*H*-indol-3-yl)ethyl)amino)methyl)-4,6-dichlorophenol (**24**).

3.2 Pharmacological evaluation of ticlopidine derivatives and analogs at human CD39

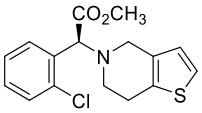
3.2.1 Inhibitory potency of ticlopidine derivatives and analogs at human CD39

All synthesized ticlopidine derivatives and analogs were tested for their inhibitory potency at human membrane-bound CD39 expressed in umbilical cord membrane using 50 µM ATP as a substrate and 100 µM inhibitor in a malachite green assay (n = 3), which is described in **8.5.2**. Results are summarized in **Tables 3.1-3.3**. Subsequently, concentration-inhibition curves for the most potent derivatives and analogs were determined (**Figure 3.1**). The biological testing was performed by Laura Schäkel.

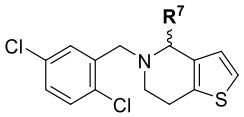
Table 3.1. Inhibitory potency of thienotetrahydropyridines at human CD39



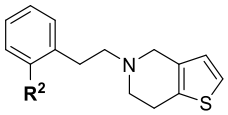
1e, 4a-w, 5a-c, 12a-f



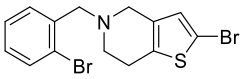
Clopidogrel (1f)



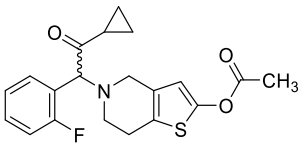
5d, $R^7 = CH_3$
5e, R^7 = Ph



6a, R^2 = H
6b, R^2 = Cl
6c, R^2 = Br



7a



Prasugrel (7b)

Compd.	R^2	R^3	R^4	R^5	R^6	IC_{50} ± SEM (µM) (or % inhibition at 100 µM)
Ticlopidine (1e)	Cl	H	H	H	H	81.7 ± 5.0
Clopidogrel (1f)	*for structure see above*					113 ± 25
Prasugrel (7b)	*for structure see above*					(29%)
4a	H	H	H	H	H	(13%)
4b	F	H	H	H	H	(7%)
4c	Br	H	H	H	H	(44%)
4d	I	H	H	H	H	130 ± 46

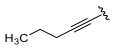
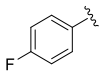
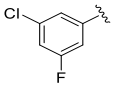
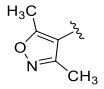
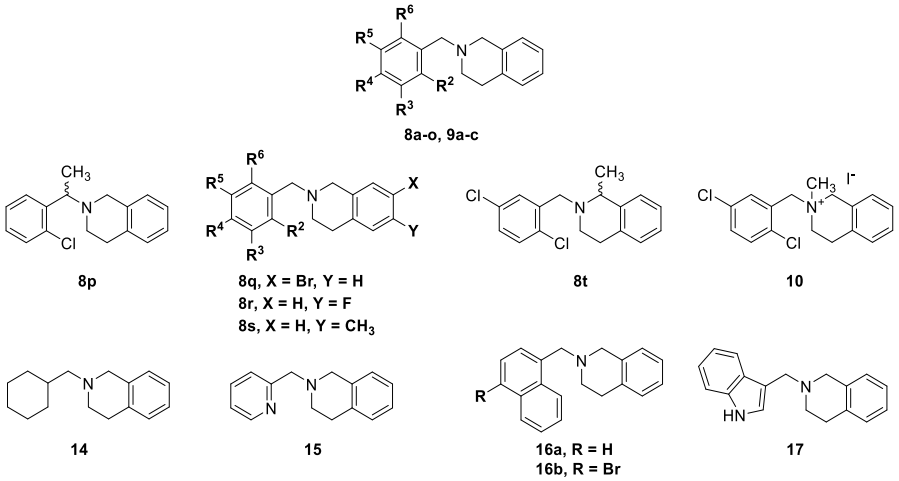
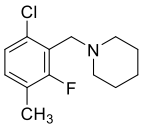
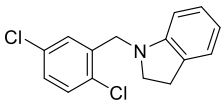
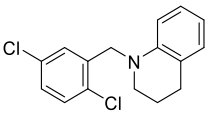
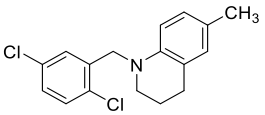
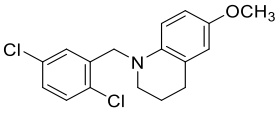
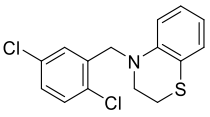
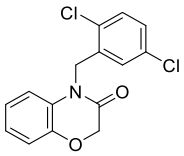
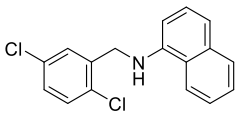
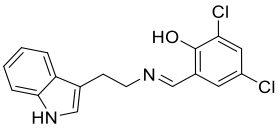
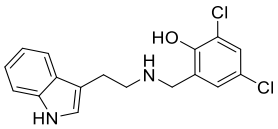
4e	OCH_3	H	H	H	H	(12%)
4f	NO_2	H	H	H	H	(36%)
4g	H	F	H	H	H	(21%)
4h	H	Cl	H	H	H	(46%)
4i	H	Br	H	H	H	(47%)
4j	H	I	H	H	H	52.7 ± 7.6
4k	H	H	F	H	H	(10%)
4l	H	H	Cl	H	H	174 ± 1
4m	H	H	Br	H	H	72.7 ± 14.6
4n	H	H	I	H	H	78.6 ± 16.8
4o	H	H	NO_2	H	H	(23%)
4p	F	Cl	H	H	H	142 ± 6
4q	Cl	Cl	H	H	H	60.9 ± 11.6
4r	F	H	Cl	H	H	91.6 ± 15.0
4s	F	H	Br	H	H	58.7 ± 4.9
4t	Cl	H	Cl	H	H	82.9 ± 26.8
4u	F	H	H	Cl	H	156 ± 16
4v	Cl	H	H	Cl	H	(41%)
4w	F	H	H	H	F	(35%)
5a	Cl	H	H	Cl	F	(46%)
5b	Cl	H	H	CH_3	F	42.7 ± 17.7
5c	F	H	Br	H	F	155 ± 37
5d	*for structure see above*					67.7 ± 16.7
5e	*for structure see above*					(41%)
6a	*for structure see above*					(13%)
6b	*for structure see above*					(27%)
6c	*for structure see above*					(18%)
7a	*for structure see above*					191 ± 28
12a	F	H		H	H	77.0 ± 12.1
12b	F	H		H	H	82.1 ± 6.7
12c	F	H		H	H	(44%)
12d	F	H		H	H	183 ± 57
12e	F	H		H	H	(35%)
12f	F	H		H	H	(33%)

Table 3.2. Inhibitory potency of tetrahydroisoquinolines at human CD39



Compd.	R^2	R^3	R^4	R^5	R^6	IC_{50} ± SEM (μM) (or % inhibition at 100 μM)
8a	F	H	H	H	H	(13%)
8b	Cl	H	H	H	H	128 ± 18
8c	CH_3	H	H	H	H	(24%)
8d	H	I	H	H	H	49.4 ± 6.8
8e	H	CH_3	H	H	H	(16%)
8f	H	H	Br	H	H	(45%)
8g	F	CH_3	H	H	H	(30%)
8h	F	CF_3	H	H	H	(41%)
8i	F	H	Cl	H	H	111 ± 20
8j	F	H	Br	H	H	70.2 ± 16.7
8k	Cl	H	H	Cl	H	39.0 ± 4.5
8l	H	CH_3	Br	H	H	62.6 ± 4.3
8m	Cl	H	H	Cl	F	43.6 ± 5.8
8n	Cl	H	H	CH_3	F	48.1 ± 2.0
8o	F	H	H	CH_3	Cl	70.4 ± 11.5
8p	*for structure see above*					84.2 ± 4.3
8q	Cl	H	H	Cl	H	(40%)
8r	Cl	H	H	Cl	H	133 ± 33
8s	F	H	H	H	H	63.2 ± 15.5
8t	*for structure see above*					90.7 ± 20.5
9a	H	H	CH_3	H	H	(15%)
9b	F	Cl	H	H	H	(45%)
9c	CH_3	CH_3	H	H	H	(41%)
10	*for structure see above*					(19%)
14	*for structure see above*					(10%)
15	*for structure see above*					(16%)
16a	*for structure see above*					(46%)
16b	*for structure see above*					(37%)
17	*for structure see above*					(11%)

Table 3.3. Inhibitory potency of various compounds at human CD39

Compd.	Structure	IC_{50} ± SEM (µM) (or % inhibition at 100 µM)
11		(12%)
19		(40%)
20a		(49%)
20b		(42%)
20c		116 ± 10
20d		(8%)
21		(24%)
22		(28%)
1d		22.3 ± 1.1
24		(17%)

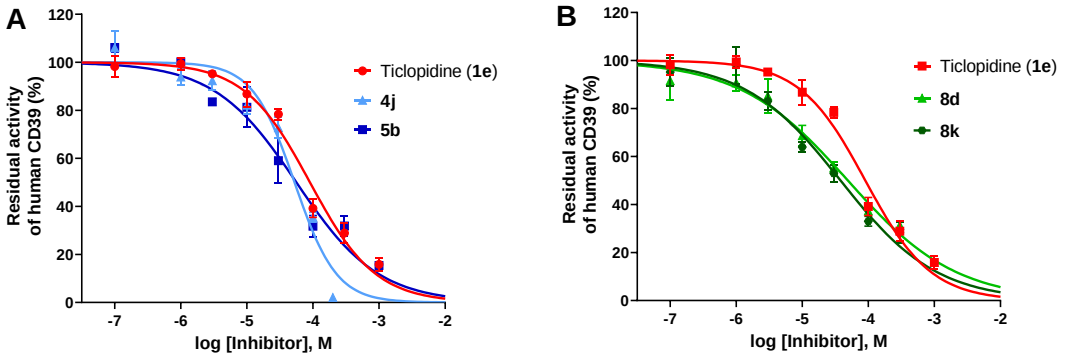


Figure 3.1. Inhibition curves of ticlopidine (**1e**) and selected derivatives **A. 4j** and **5b**, and **B. 8d** and **8k**.

3.2.2 Structure-activity relationships of ticlopidine derivatives and analogs

Based on the test results of CD39 inhibition, insights into the SARs of the ticlopidine (**1e**) scaffold were obtained. **Figures 3.2** and **3.3** give selected overview of SARs for thienotetrahydropyridines and tetrahydroisoquinolines, respectively.

Substitution at the linker methylene chain with a carboxylate ester in clopidogrel (**1f**) proved similarly potent compared to ticlopidine (**1e**), while the structurally related prasugrel (**7b**) was not a potent inhibitor of CD39. A single methyl group at the linker seems to improve the potency of the tetrahydroisoquinoline derivative **8p** (IC_{50} = 84.2 µM) compared to its analog **8b** (IC_{50} = 128 µM). A methylene linker was superior to an ethylene linker (**6a-c**). Benzyl (**4a**, 13% inhibition at 100 µM), cyclohexyl (**14**, 10%), 2-pyridyl (**15**, 16%) and 3-indolyl (**17**, 11%) groups showed the same low inhibitory activity when there was no further substituent on them. Halogen-substitution in *ortho*-, *meta*-, or *para*-position was tolerated except F-substitution. For example, the rank order of potency in *ortho*-position on the ticlopidine scaffold was Cl ≥ I ≥ Br > NO_2 > OCH_3, H, F. The most addition of multiple halogens proved advantageous for increasing overall inhibitory activity. For example, the combination of *o*- and *m*-chloro derivatives of ticlopidine (**1e**) and **4h** generated a more potent inhibitor **4q** showing an IC_{50} of 60.9 µM. 2-Bromothiophene (**7a**) and tetrahydroisoquinoline derivatives **8a-t** and **9a-c**

showed no or a minor decrease in inhibition compared to their corresponding thienotetrahydropyridine analogs. This exchange results in compounds that can no longer be metabolically activated to irreversible $P2Y_{12}$ receptor antagonists.[146] Thus, $P2Y_{12}$ inhibition can be abrogated, while maintaining the activity at CD39. The lack of an aromatic ring at that position led to a loss of inhibitory activity (**11**, 12% inhibition at 100 µM). Indoline (**19**, 40%) and tetrahydroquinoline analogs (**20a-c**, 49%, 42%, 44%) slightly decreased the activity compared to the tetrahydroisoquinoline analog (**8k**, 59%). However, inhibitory activity of **8k** was highly decreased or abrogated in 2*H*-1,4-benzoxazin-3(4*H*)-one (**21**, 24%), 1-naphthylamine (**22**, 28%) and 3,4-dihydro-2*H*-1,4-benzothiazine (**20d**, 8%) analogs. Methylation of **8k** yielded **10** (19%) also resulting in a really inactive compound.

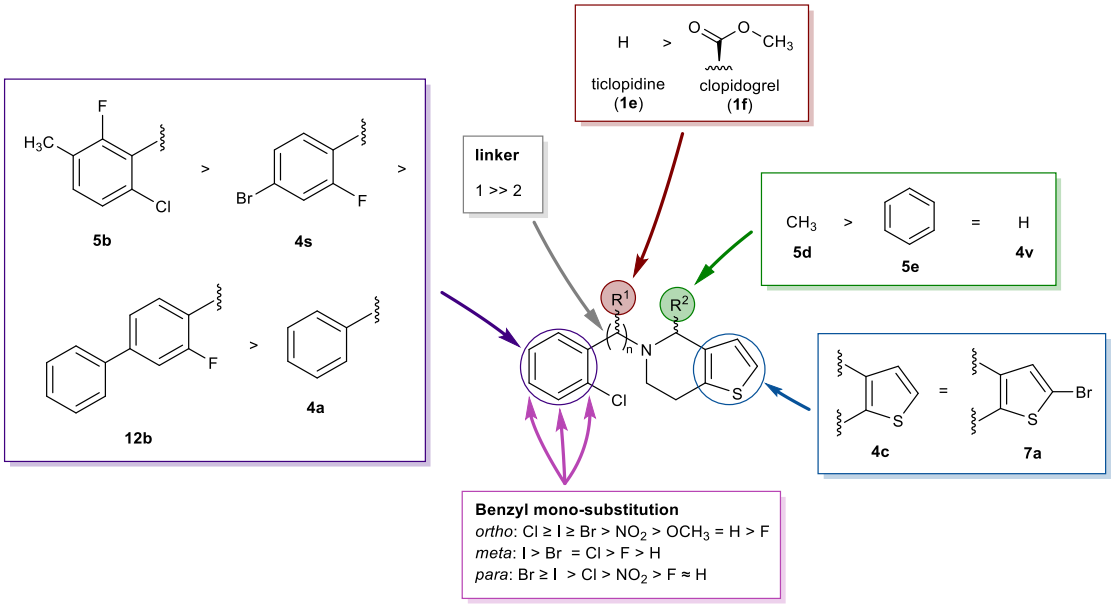


Figure 3.2. Selected SARs of thienotetrahydropyridine derivatives.

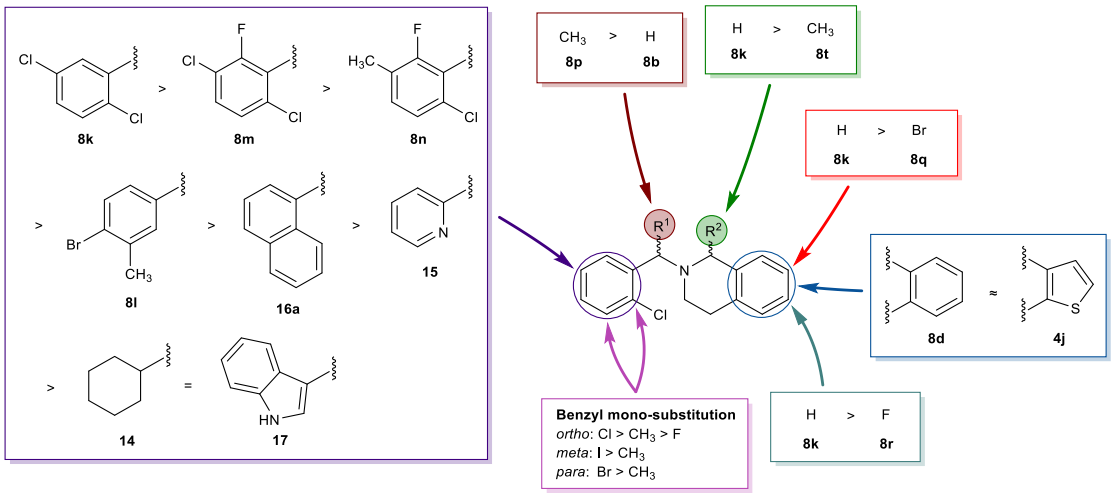
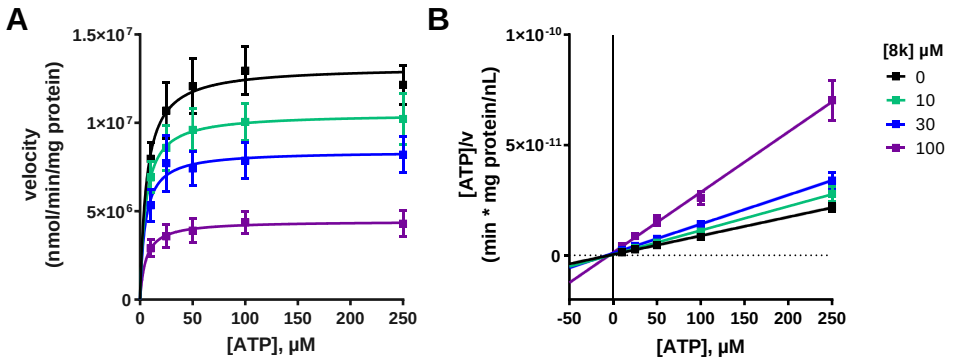


Figure 3.3. Selected SARs of tetrahydroisoquinoline derivatives.

3.2.3 Inhibition type determination for 8k

The inhibition type of the most potent CD39 inhibitor of this series, **8k** was determined analogously to ticlopidine by determination of Michaelis-Menten kinetics as depicted in **Figure 3.4**. Compound **8k** was found to be a non-competitive inhibitor of CD39 like ticlopidine. A K_i value of 51.4 ± 7.4 µM was calculated, which is in agreement with the determined IC_{50} value (see **Table 3.2**). The experiments and their analysis were performed by Laura Schäkel.



C

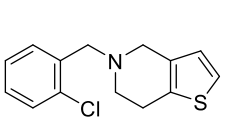
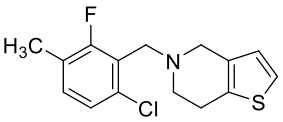
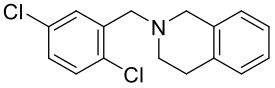
	[8k]			
Best-fit values	**0 µM**	**10 µM**	**30 µM**	**100 µM**
V_{max} [nmol/min/mg protein]	13,200,000	10,500,000	8,390,000	4,440,000
K_m [µM]	6.06	5.26	4.84	5.53

Figure 3.4. Inhibition type determination for **8k**. Inhibition of ATP hydrolysis by human CD39 expressed in COS-7 cells was determined using the malachite green assay with 0, 10, 30 and 100 µM inhibitor and 10-250 µM ATP as a substrate (n = 3). **A.** Michaelis-Menten plot. **B.** Hanes-Woolf plot where the intersection of lines at the X-axis indicates the non-competitive inhibition type. The K_i value was calculated with GraphPad Prism 8 software by non-linear regression of the Michaelis-Menten plot data with the equation $v_{maxinh} = v_{max} / (1+ [I] / K_i)$[147] to be 51.4 ± 7.4 µM. **C.** V_{max} and K_m values of CD39 in the absence and presence of various concentrations of inhibitor **8k**.

3.2.4 Selectivity studies versus other ectonucleotidases

Potent compounds ticlopidine (**le**), **5b** and **8k** were further investigated at NTPDase2, -3, -8, NPP1, -3, -5, CD38 and CD73. Results are summarized in **Table 3.4**. The biological testing was performed by Laura Schäkel, Salahuddin Mirza, and Katharina Sylvester, respectively.

Table 3.4. Selectivity studies of ticlopidine (1e), 5b and 8k at human ectonucleotidases

	 ticlopidine (**1e**)	 **5b**	 **8k**
Enzyme	***IC*$_{50}$ ± SEM (μM) (or % inhibition at indicated concentration)**		
CD39	81.7 ± 5.0	42.7 ± 17.7	39.0 ± 4.5
NTPDase2	170 ± 24	181 ± 28	145 ± 30
NTPDase3	149 ± 11	72.5 ± 22.9	15.6 ± 1.7
NTPDase8	>300 (28%)	>300 (13%)	>300 (19%)
NPP1	>50 (18%)	>50 (10%)	>50 (11%)
NPP3	>50 (10%)	>50 (1%)	>50 (4%)
NPP5	>50 (-2%)	>50 (-3%)	>50 (-5%)
CD38	>50 (-14%)	>50 (-6%)	>50 (-14%)
CD73	192 ± 37	113 ± 40	102 ± 28

Ticlopidine (**1e**) was reported as a selective CD39 inhibitor among several ectonucleotidases (CD39, NTPDase2, -3, -8, NPP1, -3 and CD73).[70,75] However, when selectivity studies were performed in our group with the respective recombinant enzyme preparations of CD39, NTPDase2, -3, -8, NPP1, -3, -5, CD38 and CD73, its selectivity could not be confirmed. Ticlopidine (**1e**) and two of its potent analogs (**5b** and **8k**) additionally inhibited NTPDase2, -3 and CD73. And they were selective towards NTPDase8, NPP1, -3, -5 and CD38 in our study.

4 Results and discussion – Part II: Development of novel 8-BuS-AMP derivatives and analogs as inhibitors of CD39

4.1 Synthesis of AMP derivatives and analogs

4.1.1 Standard conditions of monophosphorylation

Monophosphorylation was performed according to the Yoshikawa procedure with small adjustments.[148-150] Nucleosides were dissolved in $PO(OCH_3)_3$ and reacted with $POCl_3$ in the presence of proton sponge at 0 °C under argon to yield the reactive 5′-dichlorophosphate intermediates. Hydrolysis by triethylammonium hydrogencarbonate (TEAC) buffer, or H_2O, or saturated aqueous NH_4HCO_3 solution yielded the desired nucleoside monophosphates. The crude mixture was extracted with *tert*-butylmethylether to remove $PO(OCH_3)_3$ and proton sponge, and finally purified by preparative HPLC.

4.1.2 Synthesis and upscaling of 8-BuS-AMP (1i)

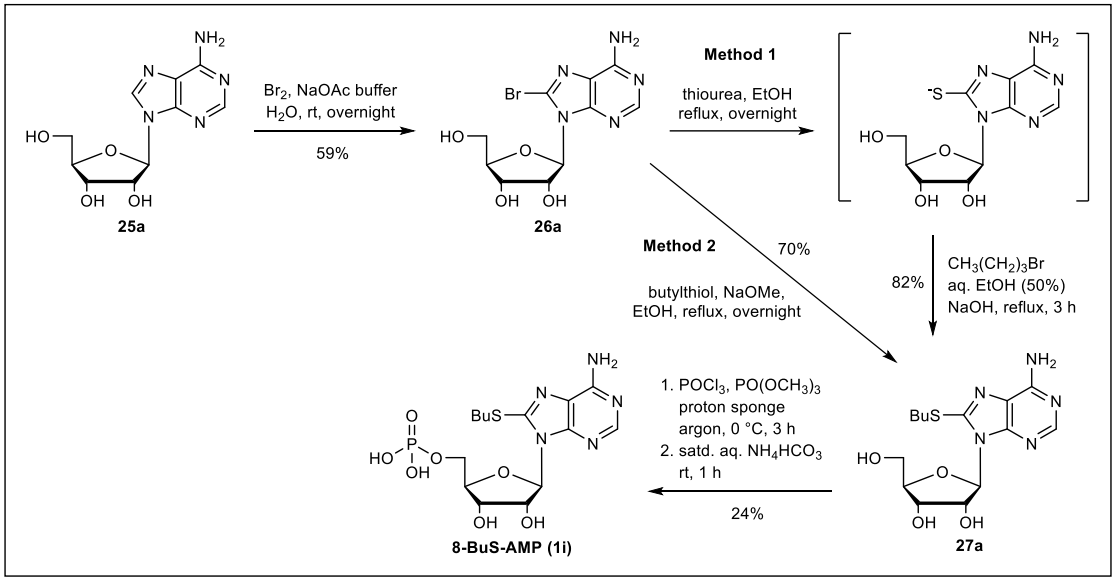
8-BuS-AMP is the most potent reported nucleotide-derived CD39 inhibitor with a K_i value of 0.8 μM,[77] but it has not been completely characterized and is not commercially available. Further chemical and biological research on 8-BuS-AMP is necessary and promising. Therefore, I upscaled the synthesis of 8-BuS-AMP to obtain up to 1 g for extended biological studies.

First, adenosine was brominated by the reported procedures with some modifications.[83,151] To adenosine under acidic conditions was added aqueous Br_2 in the presence of 1 M sodium acetate buffer (pH 4.0). The excess Br_2 was removed by 1 M $NaHSO_3$ buffer at the end of the reaction. The mixture was neutralized with 2 M aqueous NaOH followed by recrystallization and filtration to generate **26a**.

The intermediate **27a** was synthesized from compound **26a** by two different methods. In method 1, it needs longer time, and has more steps, but the yield is higher than in the second method. In method 2, it only needs one step to generate **27a** and its purity

normally is higher than in the first method and more environmentally friendly, but its yield is a little lower.

In method 1, **27a** was synthesized in two steps according to a reported procedure with small changes.[83] Treatment of **26a** with thiourea in EtOH generated the intermediate 8-thioadenosine without purification, which was subsequently basified slightly with 2 M aqueous NaOH, and then alkylated using 1-bromobutane to generate **27a**. In method 2, **27a** was synthesized according to another reported procedure in only one step.[152] Compound **26a** in EtOH was reacted with butylthiol in the presence of NaOMe to generate **27a**. Finally, **27a**, the intermediate in both methods, was monophosphorylated under standard conditions to generate **1i** (**Scheme 4.1**).



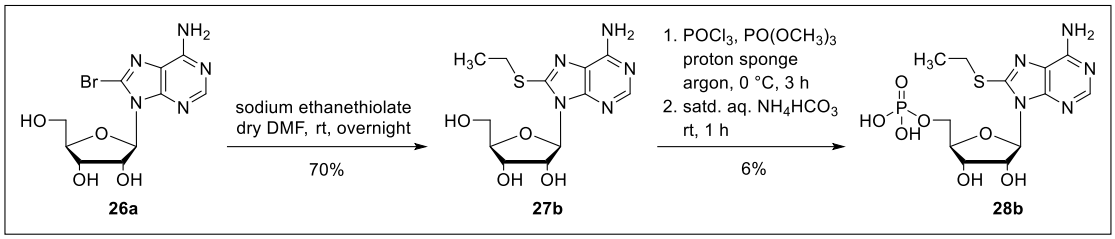
Scheme 4.1. Synthesis and upscaling of 8-BuS-AMP (**1i**).

4.1.3 Synthesis of 8-ethylthio-AMP (28b)

From the previous research of our group and based on reported papers, synthesizing AMP derivatives with the substituents at the 8-position converted via a sulfide bridge is a good strategy because most 8-thio-substituted AMP derivatives have potent inhibitory activities at CD39.[65,77,79]

Compound **27b** was synthesized from **26a** according to a reported procedure.[153] To **26a** in anhydrous DMF was added sodium ethanethiolate. The mixture was stirred at rt

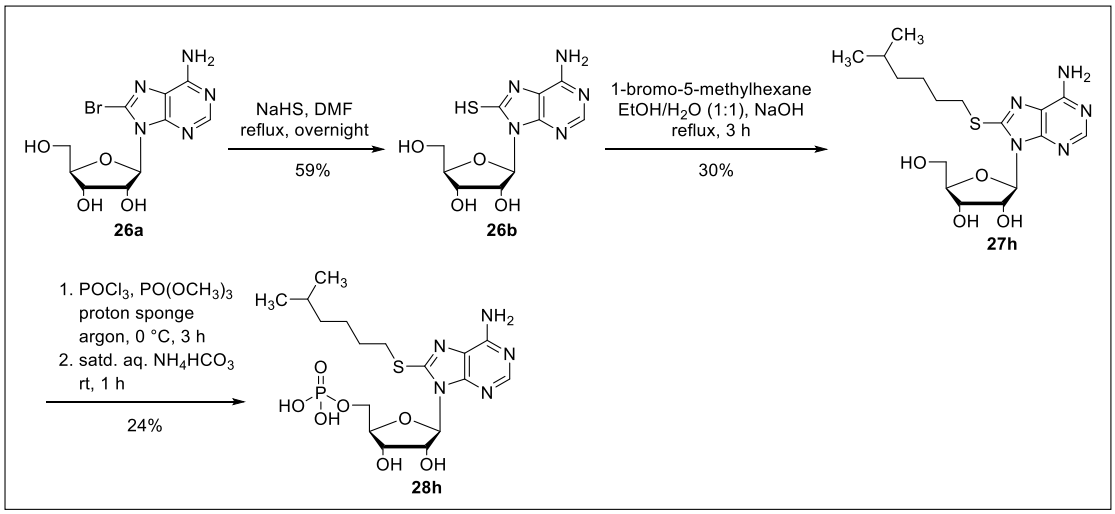
overnight to generate **27b**. Finally, the intermediate **27b** was monophosphorylated under standard conditions to generate **28b** (**Scheme 4.2**).



Scheme 4.2. Synthesis of 8-ethylthio-AMP (**28b**).

4.1.4 Synthesis of 8-(5-methylhexyl)thio-AMP (28h)

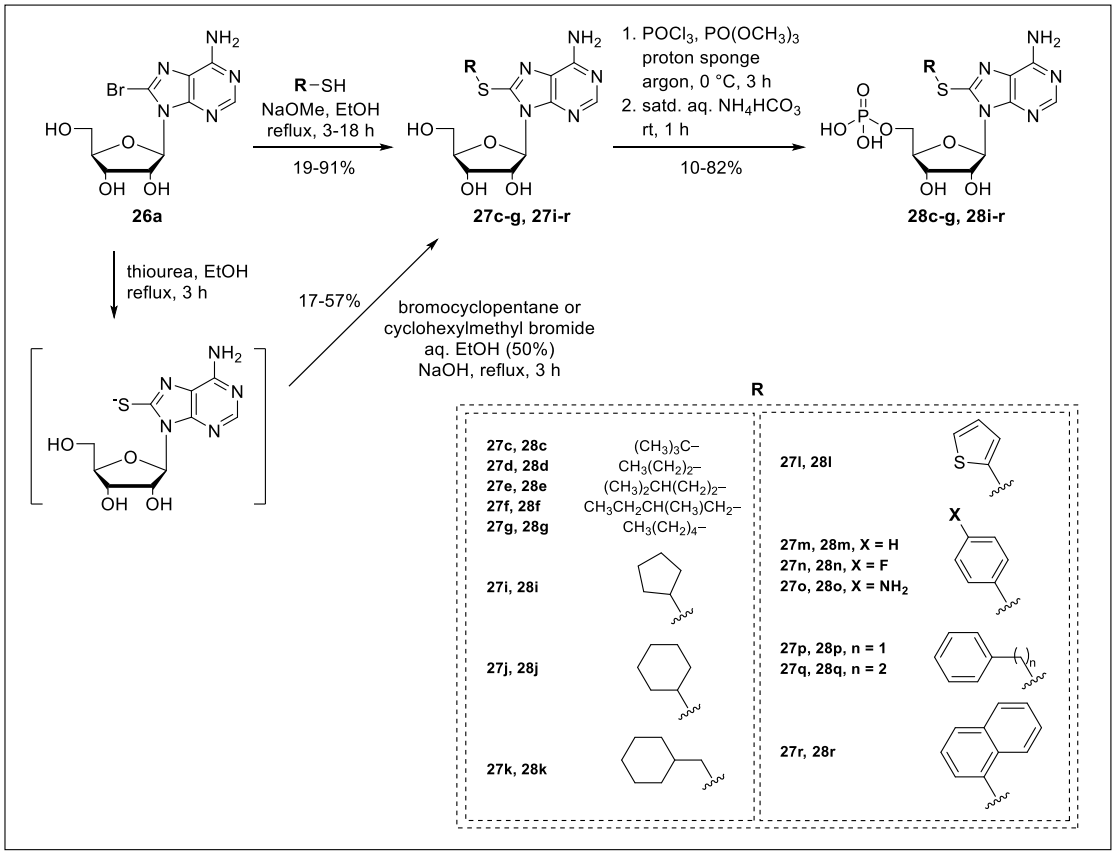
Compound **27h** was synthesized by another strategy: the precursor 8-thioadenosine (**26b**) needed to be synthesized firstly according to a reported procedure with some modifications.[154] To **26a** in DMF was added NaHS, and the mixture was refluxed overnight to generate **26b**. To **26b** in EtOH/H_2O (1:1) was added 1-bromo-5-methylhexane and basified sightly with 2 M NaOH. The mixture was refluxed for 3 h to generate **27h**. Finally, the intermediate **27h** was monophosphorylated under standard conditions to generate **28h** (**Scheme 4.3**).



Scheme 4.3. Synthesis of 8-(5-methylhexyl)thio-AMP (**28h**).

4.1.5 Synthesis of 8-thio-substituted AMP derivatives (28c-g and 28i-r)

In this study, many 8-thio-substituted AMP derivatives were synthesized using the same methods as for **1i**. Most intermediates were synthesized using method 2 of **1i** synthesis in one step by reaction of the appropriate commercially available thiol with 8-bromoadenosine (**26a**). Intermediates **27i** and **27k** were synthesized using method 1 of **li** synthesis in two steps by reactions of thiourea with 8-bromoadenosine, and subsequent reaction with bromocyclopentane or cyclohexylmethyl bromide. Finally, the corresponding 8-thio-substituted adenosine derivatives **27c-g** and **27i-r** were monophosphorylated under standard conditions to generate 8-thio-substituted AMP derivatives **28c-g** and **28i-r** (**Scheme 4.4**).

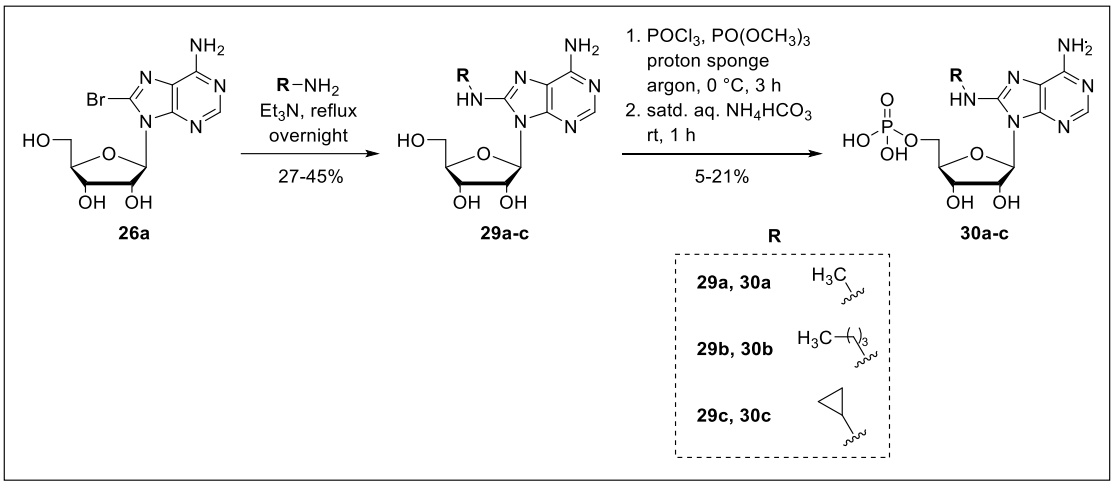


Scheme 4.4. Synthesis of 8-thio-substituted AMP derivatives (**28c-g** and **28i-r**).

4.1.6 Synthesis of 8-amino-substituted AMP derivatives (30a-c)

For the synthesis of 8-amino-substituted adenosine intermediates, 8-bromoadenosine (**26a**) was reacted with the appropriate amine in the presence of Et_3N.[83] The mixture was refluxed to yield the 8-amino-substituted adenosine intermediates **29a-c**. Finally,

these three intermediates **29a-c** were monophosphorylated under standard conditions to generate **30a-c** (**Scheme 4.5**).

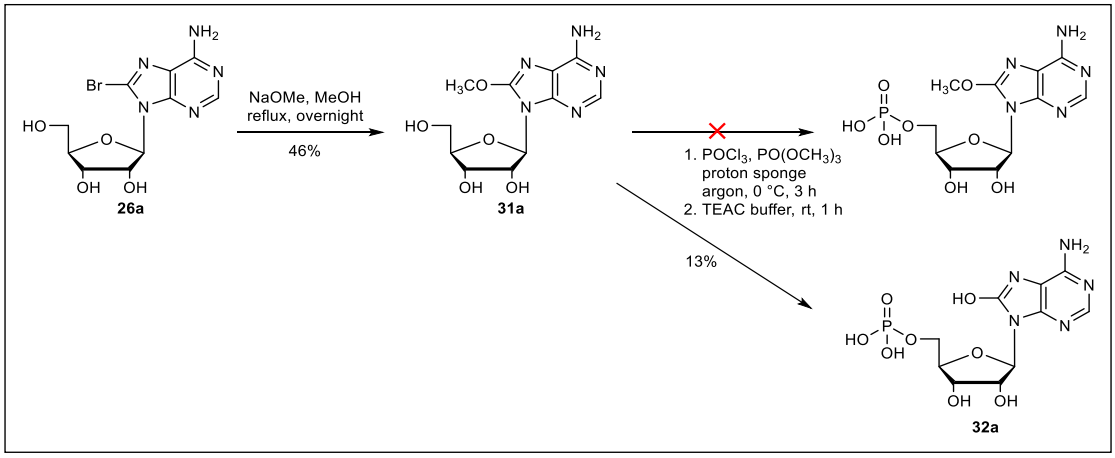


Scheme 4.5. Synthesis of 8-amino-substituted AMP derivatives (**30a-c**).

4.1.7 Synthesis of 8-hydroxy-AMP (32a)

8-Methylamino-AMP and 8-methylthio-AMP were found to be potent inhibitors of CD39 with K_i values of 0.660 µM and 2.22 µM determined in our group.[79] To change the 8-amino or 8-thio linker by an ether linker might also be a good strategy to develop a new CD39 inhibitor. The intermediate **31a** was synthesized from **26a** according to a reported procedure.[155] Compound **26a** was suspended in MeOH in the presence of NaOMe, and the mixture was refluxed overnight to generate **31a**. Finally, the intermediate **31a** was monophosphorylated under standard conditions to generate **32a** (**Scheme 4.6**).

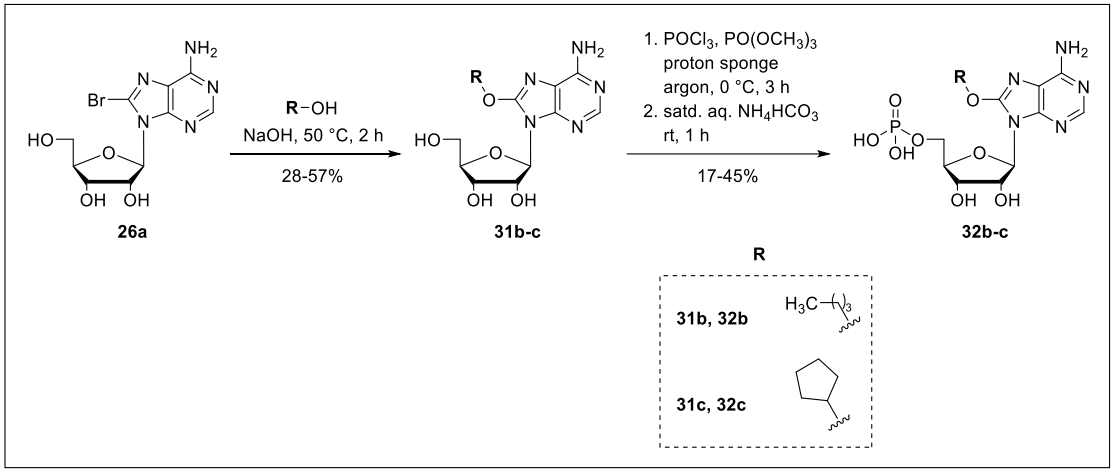
Unfortunately, 8-methoxy-AMP was not obtained since during the hydrolysis procedure by the TEAC buffer under acidic conditions, the 8-methoxy moiety was also hydrolyzed to generate the 8-hydroxy derivative. Saturated aqueous NH_4HCO_3 solution turned out to be the best method for hydrolysis compared to H_2O or TEAC buffer.



Scheme 4.6. Synthesis of 8-hydroxy-AMP (**32a**).

4.1.8 Synthesis of 8-alkoxy-AMP derivatives (32b-c)

For the synthesis of 8-alkoxy-adenosine intermediates, 8-bromoadenosine (**26a**) was suspended in butanol or cyclopentanol in the presence of NaOH, and the mixture was stirred at 50 °C to yield the desired intermediates **31b-c**. Finally, the intermediates **31b-c** were monophosphorylated under standard conditions to generate **32b-c** (**Scheme 4.7**).

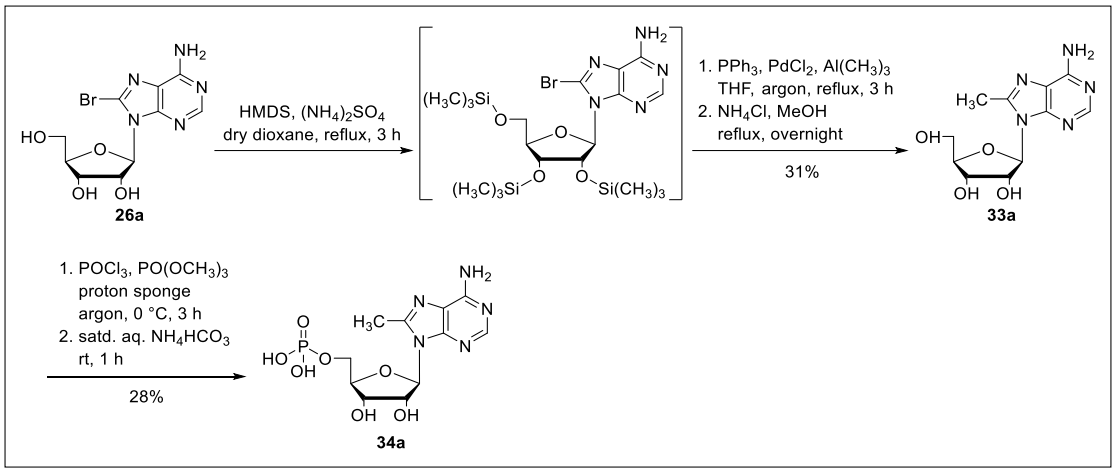


Scheme 4.7. Synthesis of 8-alkoxy-AMP derivatives (**32b-c**).

4.1.9 Synthesis of 8-methyl-AMP (34a)

Compound **33a** was synthesized from **26a** according to a reported procedure.[156] The 2′-, 3′- and 5′-hydroxyl groups needed to be protected by hexamethyldisilazane (HMDS) in the presence of the catalyst $(NH_4)_2SO_4$ in anhydrous dioxane. Trimethylaluminum was

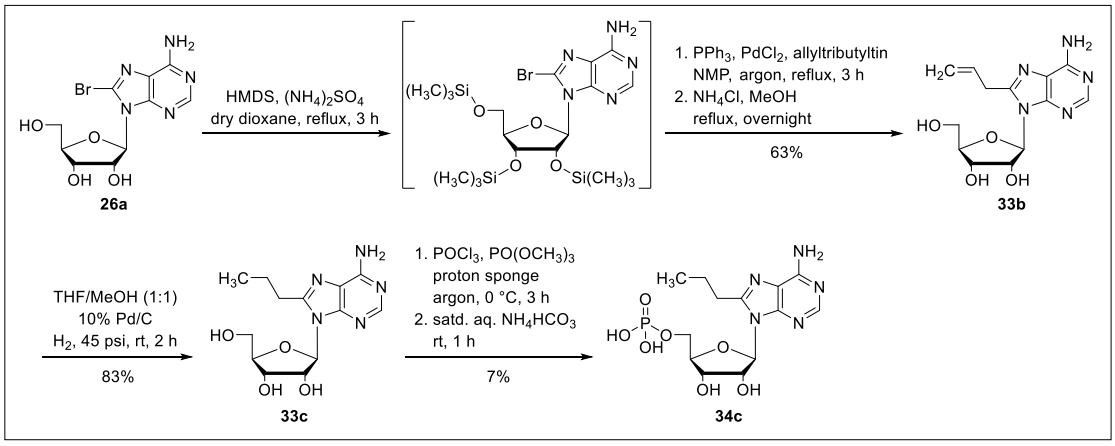
subsequently added together with triphenylphosphine and $PdCl_2$ in THF (tetrahydrofuran) as a catalyst under argon. The mixture was refluxed to introduce a methyl moiety at the 8-position. The 2′-, 3′- and 5′-trimethylsilyl protecting groups were then conveniently removed by NH_4Cl in MeOH to generate **33a**. Finally, the intermediate **33a** was monophosphorylated under standard conditions to generate **34a** (**Scheme 4.8**).



Scheme 4.8. Synthesis of 8-methyl-AMP (**34a**).

4.1.10 Synthesis of 8-propyl-AMP (34c)

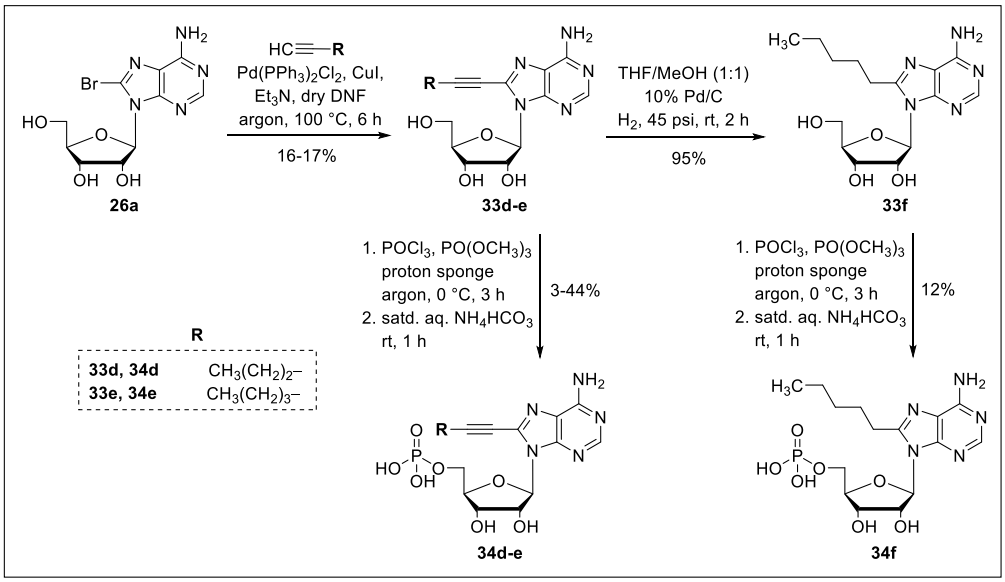
For the synthesis of **33c**, **33b** needed to be synthesized from **26a** according to a reported procedure.[157] The 2′-, 3′- and 5′-hydroxyl groups of **26a** need to be protected by HMDS in the presence of $(NH_4)_2SO_4$ in anhydrous dioxane as they are all susceptible to the subsequent reaction with allyltributyltin, PPh_3 and $PdCl_2$ in *N*-methyl-2-pyrrolidone (NMP) under argon. The trimethylsilyl protecting groups were then conveniently removed by NH_4Cl in MeOH to generate **33b**. Compound **33b** in THF/MeOH (1:1) was subsequently hydrogenated with 10% Pd/C under H_2 (45 psi) to generate the intermediate **33c**. Finally, the intermediate **33c** was monophosphorylated under standard conditions to generate **34c** (**Scheme 4.9**).



Scheme 4.9. Synthesis of 8-propyl-AMP (**34c**).

4.1.11 Synthesis of various 8-substituted AMP derivatives (34d-f) by Sonogashira coupling

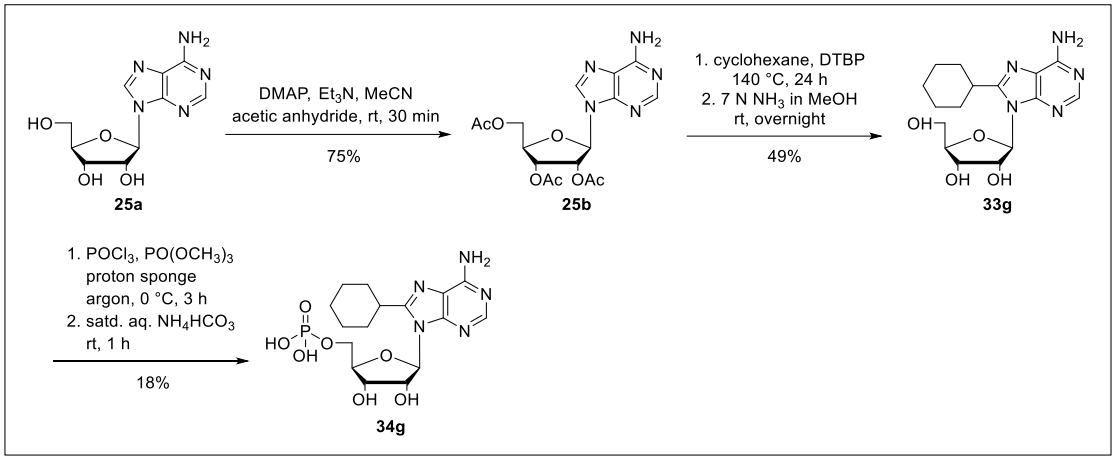
Three more 8-substituted AMP derivatives **33d-f** were synthesized from **26a** according to the reported Sonogashira coupling procedure.[142] To **26a** in anhydrous DMF, Pd $(PPh_3)_2Cl_2$, CuI, Et_3N and 1-pentyne or 1-hexyne were added. The mixture was stirred at 90 °C under argon to generate **33d** or **33e**. Compound **33d** was subsequently hydrogenated with 10% Pd/C under H_2 (45 psi) to generate **33f**. Finally, the intermediates **33d-f** were monophosphorylated under standard conditions to generate **34d-f** (**Scheme 4.10**).



Scheme 4.10. Synthesis of various 8-substituted AMP derivatives (**34d-f**).

4.1.12 Synthesis of 8-cyclohexyl-AMP (34g)

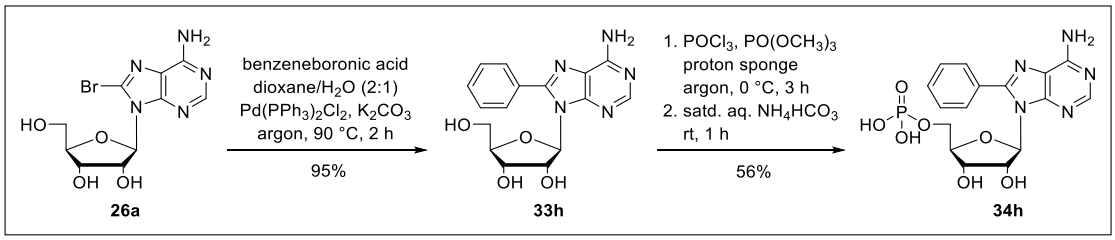
The intermediate **33g** was synthesized from adenosine by a reported procedure with minor modifications.[158] At the first, the hydroxyl groups in the 2'-, 3'- and 5'-positions of adenosine should be protected. To adenosine in MeCN was added DMAP (4-dimethylaminopyridine), Et_3N and acetic anhydride, and the mixture was stirred at rt to yield **25b**. To **25b** in cyclohexane was added DTBP (di-*tert*-butyl peroxide), and the mixture was stirred at 140 °C to introduce a cyclohexyl moiety at the 8-position. Then the 2'-, 3'- and 5'-*O*-acetyl groups were removed by stirring the compound in a solution of 7 N NH_3 in MeOH at rt to yield **33g**. Finally, the intermediate **33g** was monophosphorylated under standard conditions to generate **34g** (**Scheme 4.11**).



Scheme 4.11. Synthesis of 8-cyclohexyl-AMP (**34g**).

4.1.13 Synthesis of 8-phenyl-AMP (34h) by Suzuki reaction

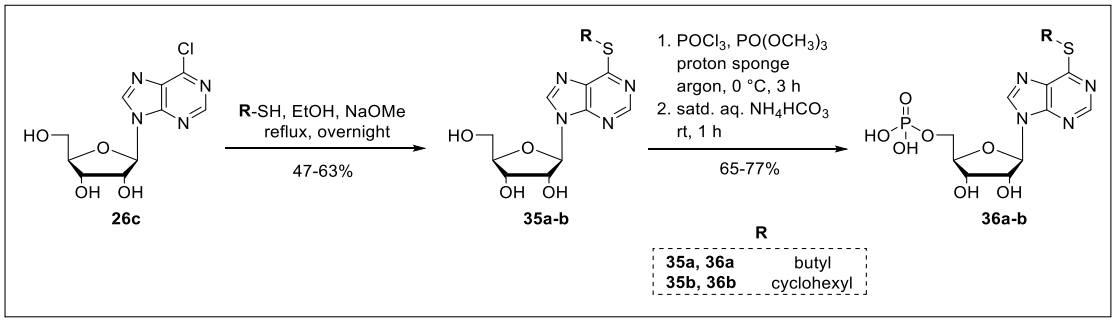
The intermediate **33h** was synthesized from **26a** according to a reported procedure by Suzuki reaction.[143] To **26a** in dioxane/H_2O (2:1) was added benzeneboronic acid, $Pd(PPh_3)_2Cl_2$ and K_2CO_3. The mixture was stirred at 90 °C under argon to generate **33h**. Finally, the intermediate **33h** was monophosphorylated under standard conditions to generate **34h** (**Scheme 4.12**).



Scheme 4.12. Synthesis of 8-phenyl-AMP (**34h**).

4.1.14 Synthesis of 6-alkylthiopurine-*β*-*D*-ribofuranosyl-5′-monophosphates (36a-b)

The N^6-amino group in AMP was replaced by a butylthio residue, a substituent that had led to the potent CD39 inhibitor 8-BuS-AMP (**1i**).[77] Moreover, a cyclohexylthio residue was introduced at the 6-position as well. To 6-chloro-9-(*β*-*D*-ribofuranosyl)purine in EtOH was added NaOMe and 1-butanethiol or cyclohexylthiol, respectively. The mixture was refluxed to generate **35a** or **35b**. Finally, the intermediates **35a-b** were monophosphorylated under standard conditions to generate **36a-b** (**Scheme 4.13**).

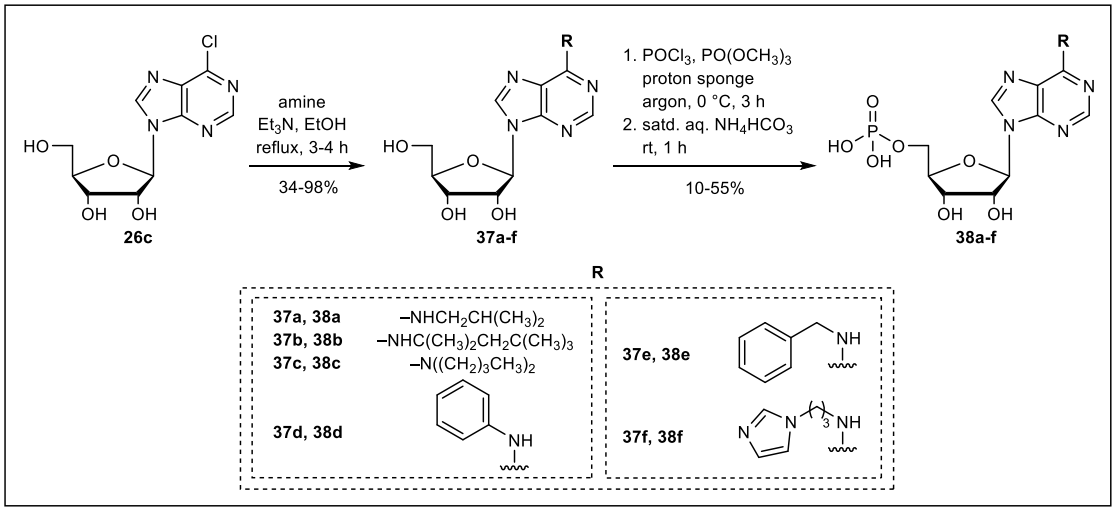


Scheme 4.13. Synthesis of 6-alkylthiopurine-*β*-*D*-ribofuranosyl-5′-monophosphates (**36a-b**).

4.1.15 Synthesis of N^6-substituted AMP derivatives (38a-f)

N^6-(4-Phenylbutyl)-AMP was found to be a potent CD39 inhibitor by our group with a K_i value of 1.40 μM.[79] Thus, modification of the N^6-position of adenosine nucleotides maybe a good strategy to obtain more potent CD39 inhibitors. A series of adenosine intermediates (**37a-f**) with modifications at the N^6-position was synthesized from 6-chloro-9-(*β*-*D*-ribofuranosyl)purine by reaction with appropriate amines in the presence

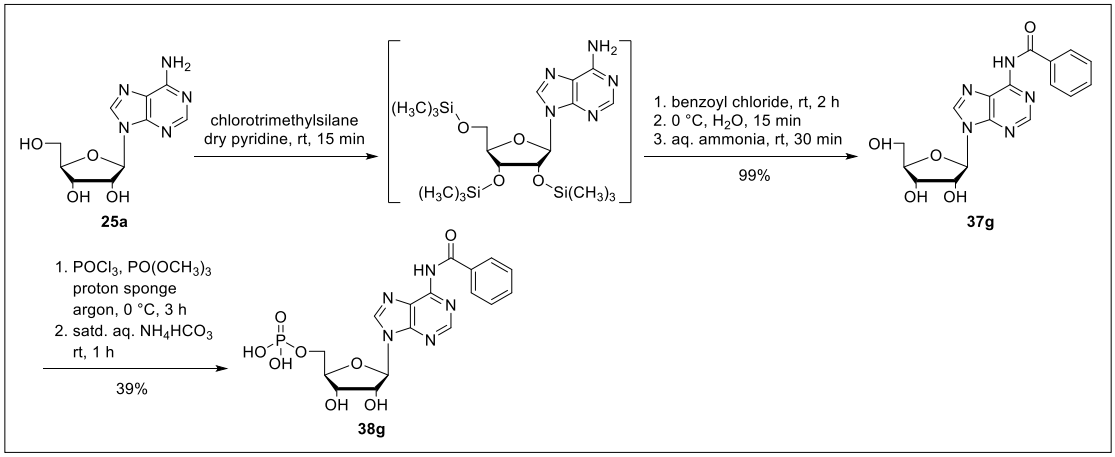
of triethylamine in one step. Finally, the intermediates **37a-f** were monophosphorylated under standard conditions to generate **38a-f** (**Scheme 4.14**).



Scheme 4.14. Synthesis of N^6-substituted AMP derivatives (**38a-f**).

4.1.16 Synthesis of N^6-benzoyl-AMP (38g)

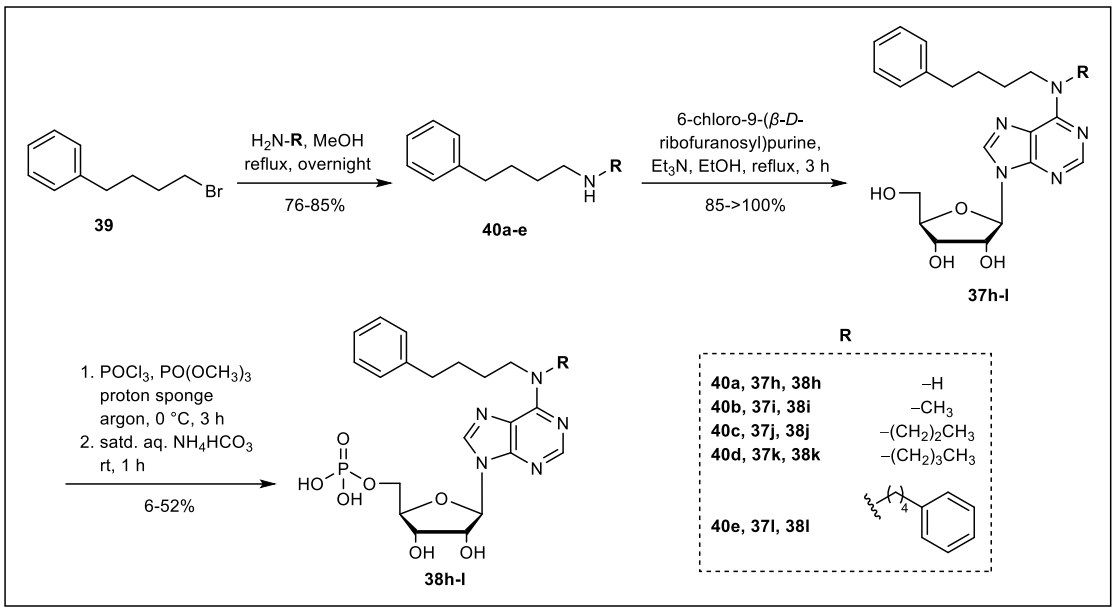
Compound **37g** was synthesized from adenosine according to a reported procedure.[159] For synthesizing **37g**, the 2′-, 3′- and 5′-hydroxyl groups of adenosine needed to be protected with chlorotrimethylsilane in anhydrous pyridine as they are all susceptible to the following reaction with benzoyl chloride. The excess chlorotrimethylsilane was quenched after completion of the reaction by adding H_2O at 0 °C. Trimethylsilyl as a protecting group was then conveniently removed in aqueous ammonia to generate **37g**. Finally, the intermediate **37g** was monophosphorylated under standard conditions to generate **38g** (**Scheme 4.15**).



Scheme 4.15. Synthesis of N^6-benzoyl-AMP (**38g**).

4.1.17 Synthesis of N^6-(4-phenylbutyl)-AMP derivatives (38h-l)

N^6-(4-Phenylbutyl)-AMP and N^6-ethyl-N^6-(4-phenylbutyl)-AMP were identified by our group to be potent CD39 inhibitors with K_i values of 1.40 µM and 7.25 µM, respectively.[79] This shows their modification at the 6-position of AMP by introducing an N^6-(4-phenylbutyl) residue is beneficial. A series of N^6-(4-phenylbutyl)-AMP derivatives was therefore synthesized, accompanied by more modifications at the N^6-position. For the synthesis of **38h-l**, intermediates **37h-l** needed to be synthesized as precursors. For the synthesis of **37h-i**, 6-chloro-9-(*β-D*-ribofuranosyl)purine was directly reacted with commercially available 4-phenylbutylamine or methyl(4-phenylbutyl)amine in the presence of Et_3N in EtOH under reflux. For the synthesis of **37j-l**, there were two reaction steps. First, 4-phenylbutyl bromide and the appropriate amine (propylamine, butylamine, or 4-phenylbutylamine) were reacted in EtOH under reflux to generate the corresponding disubstituted amine derivative (**40c-e**). Then these amines (**40c-e**) were reacted with 6-chloro-9-(*β-D*-ribofuranosyl)purine in the presence of Et_3N in EtOH under reflux to generate **37j-l**. Finally, the intermediates **37h-l** were monophosphorylated under standard conditions to generate **38h-l** (**Scheme 4.16**).

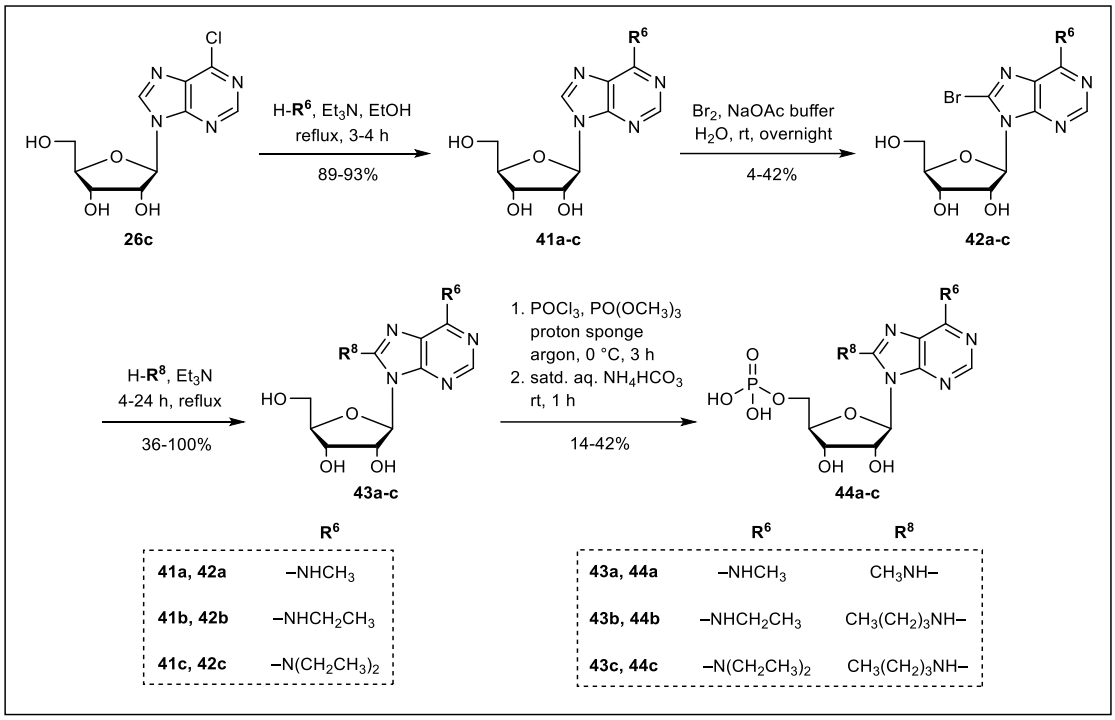


Scheme 4.16. Synthesis of N^6-(4-phenylbutyl)-AMP derivatives (**38h-l**).

4.1.18 Synthesis of 8-, N^6-disubstituted AMP derivatives (44a-c)

Since the known inhibitors 8-BuS-AMP and ARL 67156 have potent substitutions at the 8- or N^6-position, respectively.[77-78] A combination of modifications at both positions might be interesting to investigate. Therefore, a small library of 8- and N^6-disubstituted derivatives was generated by different strategies.

First, methylamine, ethylamine and diethylamine were reacted with 6-chloro-9-(*β-D*-ribofuranosyl)purine to generate **41a-c**. Compound **41a** was generously offered by Dr. Constanze Cerine Schmies.[79] Second, their 8-positions were brominated under acidic conditions by the procedure applied to **26a** to generate **42a-c**. Third, **42a-c** were refluxed in the appropriate amine solution (40% CH_3NH_2/MeOH or butylamine) in the presence of Et_3N to generate **43a-c**. Finally, the intermediates **43a-c** were monophosphorylated under standard conditions to generate **44a-c** (**Scheme 4.17**).



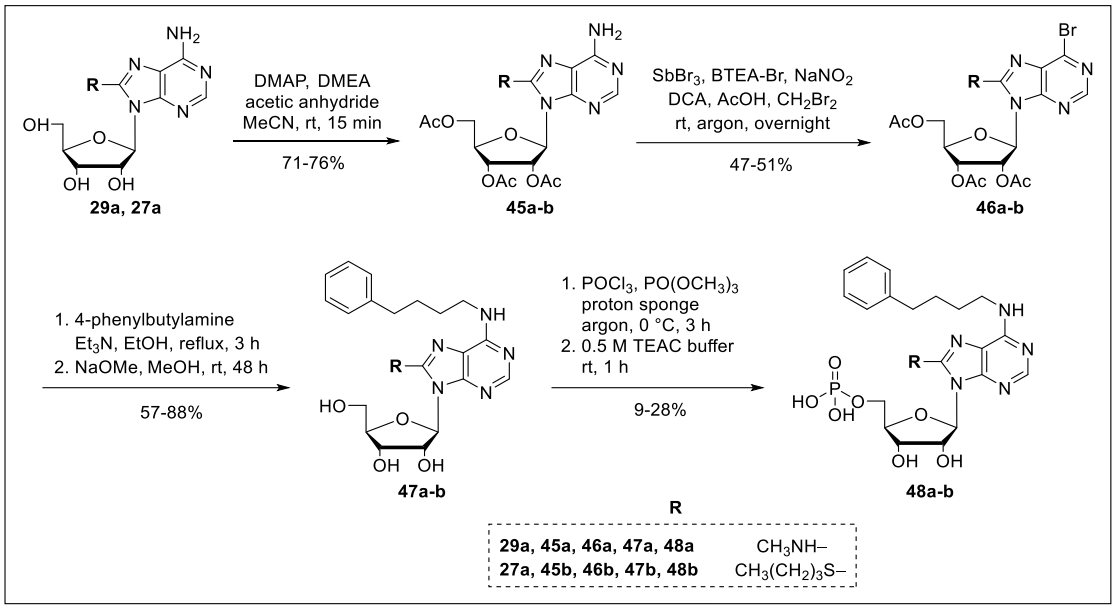
Scheme 4.17. Synthesis of 8-, N^6-disubstituted AMP derivatives (**44a-c**).

4.1.19 Synthesis of 8-methylamino-N^6-(4-phenylbutyl)-AMP (48a) and 8-butylthio-N^6-(4-phenylbutyl)-AMP (48b)

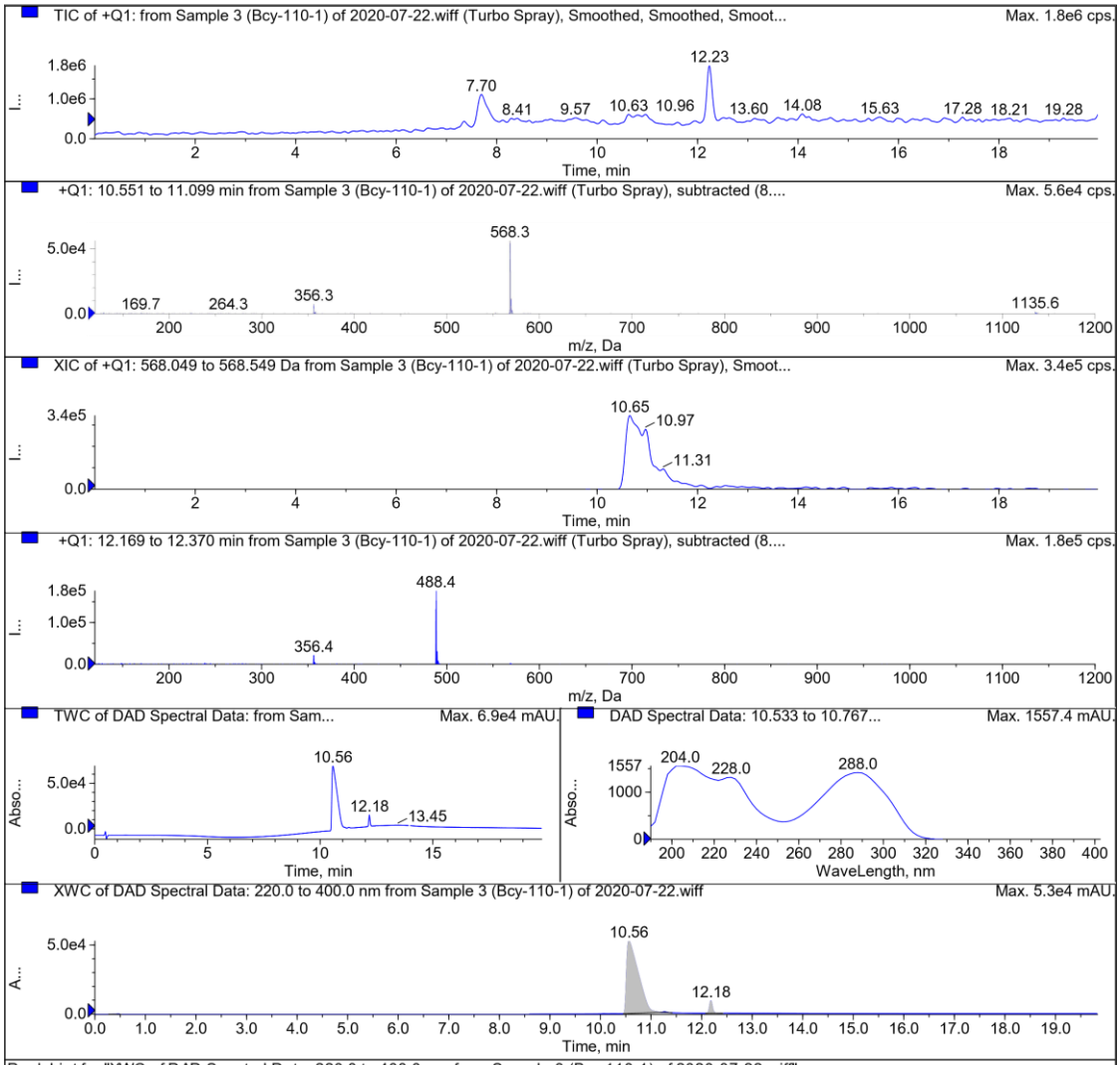
8-BuS-AMP (K_i = 0.8 μM) is the best CD39 inhibitor reported so far.[77] 8-(Methylamino)-AMP (K_i = 0.660 μM) and N^6-(4-phenylbutyl)-AMP (K_i = 1.40 μM) are two similarly potent CD39 inhibitors.[79] The combination of the substituents of 8-BuS-AMP (or 8-(methylamino)-AMP) and N^6-(4-phenylbutyl)-AMP might increase the inhibitory potency and metabolic stability.

For the synthesis of **47a-b**, **29b** and **27a** were used as starting materials. Their N^6-positions were brominated after protecting the 2′-,3′-,5′-OH groups by reported procedures.[160] The 2′-,3′-,5′-OH groups of **29b**/**27a** were protected by reaction with acetic anhydride in MeCN in the presence of DMAP and DMEA (*N*,*N*-dimethylethylamine) to generate **45a**/**45b**. Compound **45a**/**45b** dissolved in CH_2Br_2 was subsequently treated with $SbBr_3$, BTEA-Br (benzyltriethylammonium bromide), $NaNO_2$, DCA (dichloroacetic acid) and AcOH, and the mixture was stirred at rt under argon to generate **46a**/**46b**. Then **46a**/**46b** was aromatized with 4-phenylbutylamine

followed by 2′-,3′-,5′-acetyl groups deprotection by 20% NaOMe in MeOH at rt to generate **47a**/**b**. Finally, the intermediates **47a-b** were monophosphorylated under standard conditions to generate **48a-b** (**Scheme 4.18**). LC-MS spectrum, and ^{1}H and ^{13}C NMR spectra of compound **48b** are depicted in **Figures 4.1** and **4.2**, respectively.



Scheme 4.18. Synthesis of 8-methylamino-N^6-(4-phenylbutyl)-AMP (**48a**) and 8-butylthio-N^6-(4-phenylbutyl)-AMP (**48b**).



Peak List for "XWC of DAD Spectral Data: 220.0 to 400.0 nm from Sample 3 (Bcy-110-1) of 2020-07-22.wiff"

	Time (min)	Area (mAU x min)	% Area	Height (mAU)	% Height	Width (min)	Baseline Type
1	0.4679	2225.5119	0.2532	424.6619	0.6789	0.2467	Base to Base
2	10.5631	8.3862e5	95.3954	5.2606e4	84.1065	0.7467	Base to Base
3	11.2670	2605.7742	0.2964	510.8218	0.8167	0.2200	Base to Base
4	12.1839	3.5648e4	4.0551	9005.4376	14.3979	0.3467	Base to Base

Polarity/Scan Type: Positive Q1 MS Analyst Version: 1.7.2

Figure 4.1. LC-MS spectrum of compound **48b** (retention time: 10.56 min).

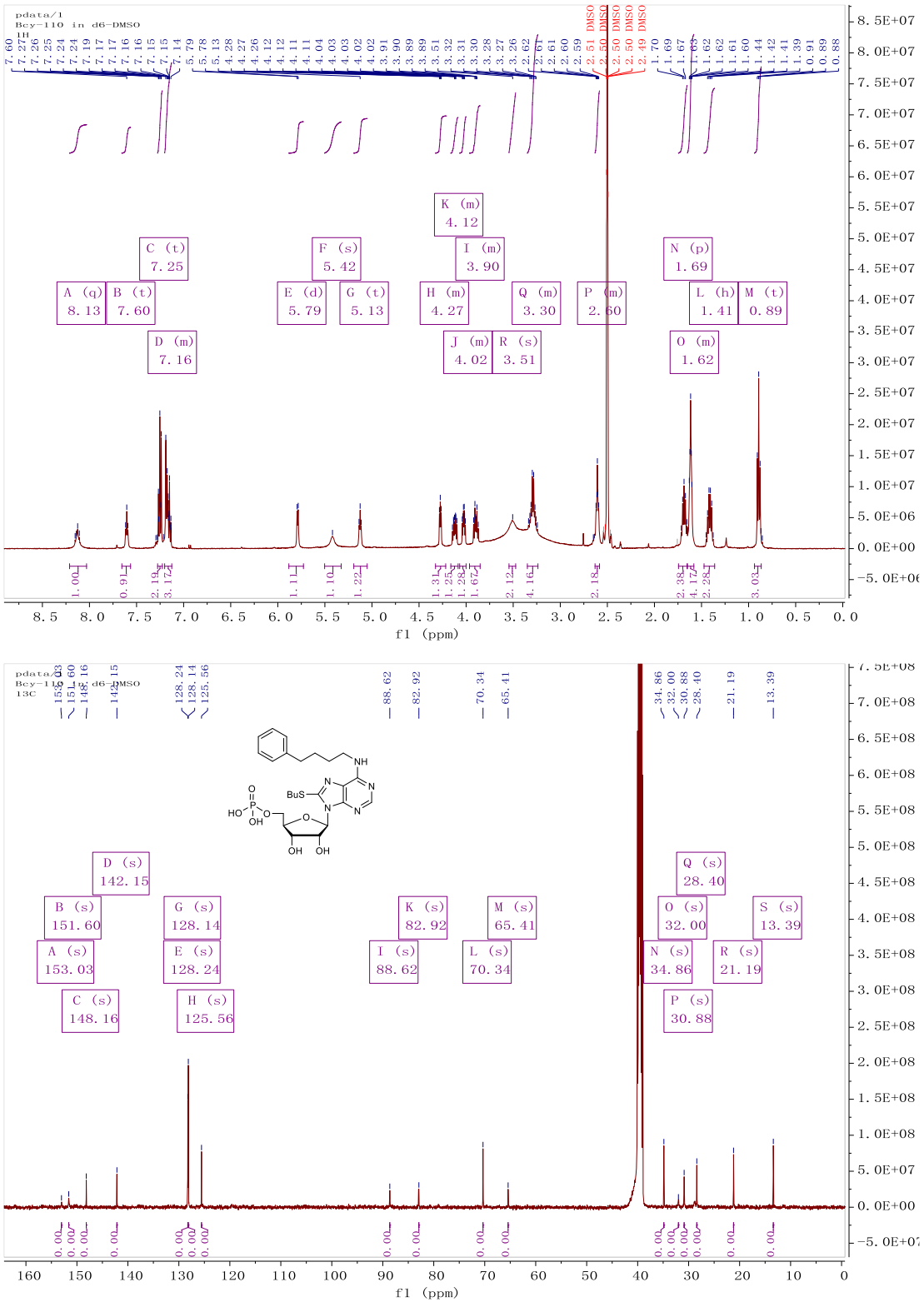
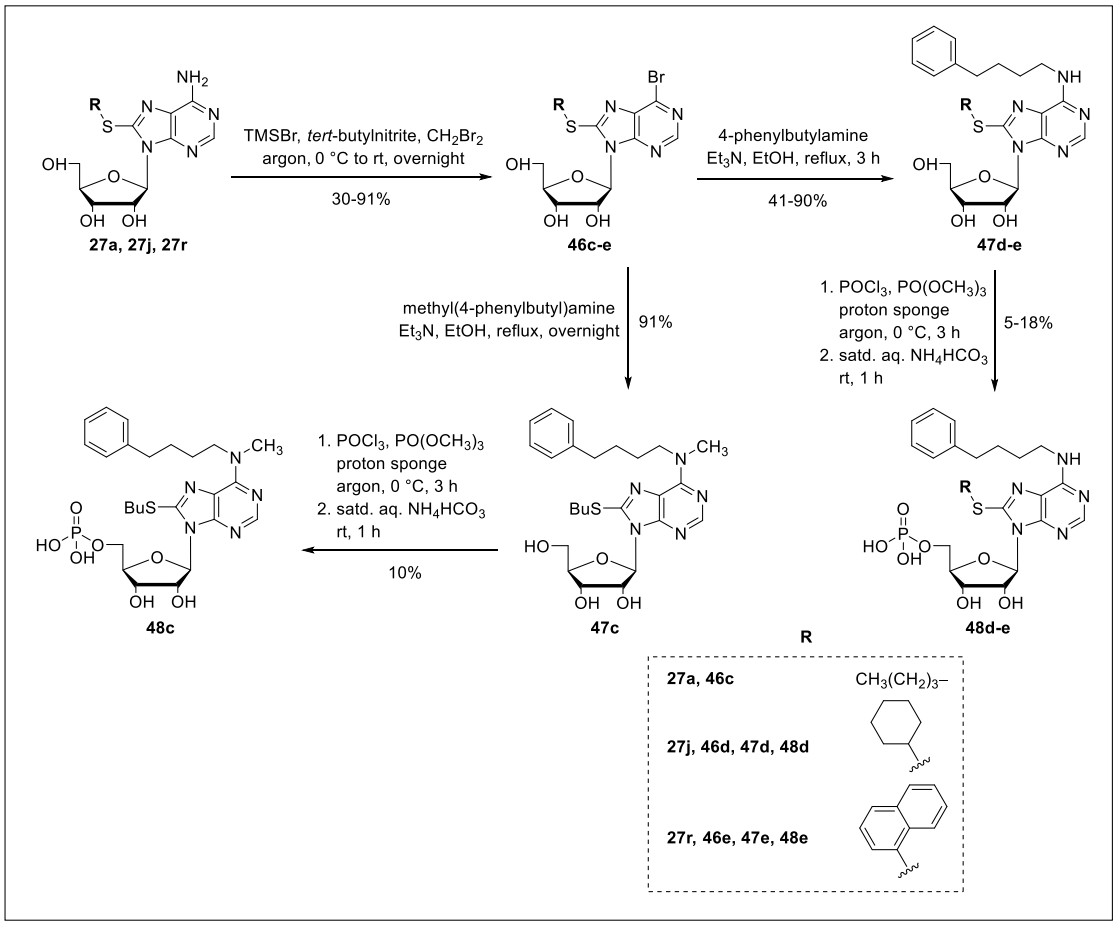


Figure 4.2. ^{1}H and ^{13}C NMR spectra of compound **48b**.

4.1.20 Synthesis of 8-, N^6-disubstituted AMP derivatives (48c-e)

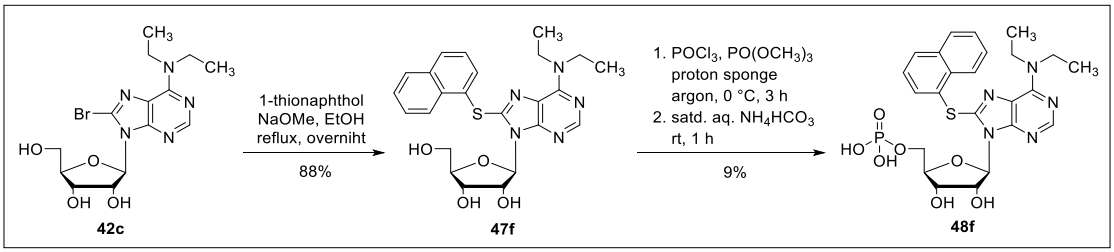
Apart from **48a-b**, more combinations of beneficial substituents were synthesized by an easier route. For synthesizing the intermediate 8-, N^6-disubstituted adenosine derivatives **47c-e**, the adenosine derivatives **27a**, **27j** and **27r** were converted to their 6-bromo analogs at first by a reported procedure with small modifications.[161] To **27a** (or **27j**, or **27r**) in CH_2Br_2 was added TMSBr (bromotrimethylsilane) and *tert*-butylnitrile, and the mixture was stirred overnight increasing the temperature from 0 °C to rt to generate **46c** (or **46d**, or **46e**). To **46c** in EtOH was added methyl(4-phenylbutyl)amine and Et_3N, and the mixture was refluxed to generate **47c**. To **46d**/**46e** in EtOH was added 4-phenylbutylamine and Et_3N, and the mixture was refluxed to generate **47d**/**47e**. Finally, the intermediates **47c-e** were monophosphorylated under standard conditions to generate **48c-e** (**Scheme 4.19**).



Scheme 4.19. Synthesis of 8-, N^6-disubstituted AMP derivatives (**48c-e**).

4.1.21 Synthesis of 8-(1-naphthylthio)-N^6,N^6-diethyl-AMP (48f)

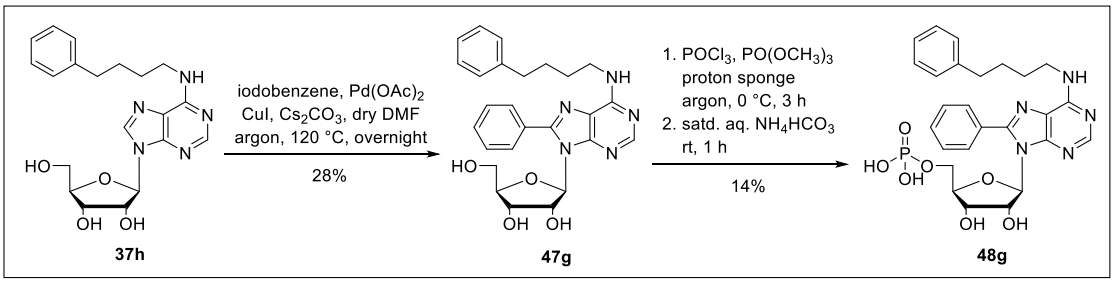
The 1-naphthylthio substituent was introduced at the 8-position of **42c** to generate **47f** by the identical procedure as described for **27r**. Finally, the intermediate **47f** was monophosphorylated under standard conditions to generate **48f** (**Scheme 4.20**).



Scheme 4.20. Synthesis of 8-(1-naphthylthio)-N^6,N^6-diethyl-AMP (**48f**).

4.1.22 Synthesis of 8-phenyl-N^6-(4-phenylbutyl)-AMP (48g)

N^6-(4-Phenylbutyl)-AMP (**38h**) had shown a potent K_i value of 1.40 µM.[79] To introduce some substituents at its 8-position might increase the inhibitory potency of **38h**. Compound **47g** was synthesized by a reported procedure with small modifications.[162] To **37h** in anhydrous DMF was added iodobenzene, $Pd(OAc)_2$, CuI and Cs_2CO_3, and the mixture was stirred in an autoclave at 120 °C under argon to generate **47g**. Finally, the intermediate **47g** was monophosphorylated under standard conditions to generate **48g** (**Scheme 4.21**).

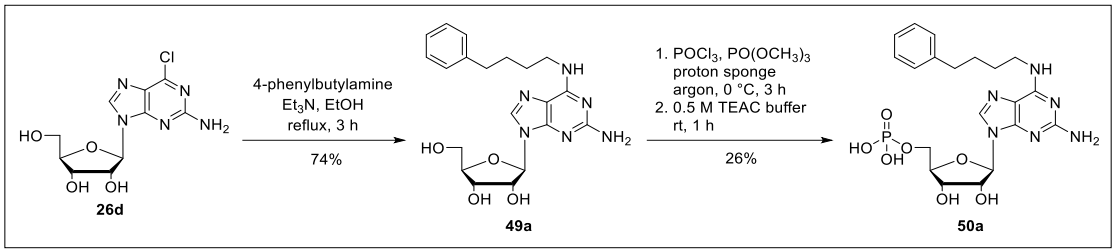


Scheme 4.21. Synthesis of 8-phenyl-N^6-(4-phenylbutyl)-AMP (**48g**).

4.1.23 Synthesis of N^6-(4-phenylbutyl)-2-amino-AMP (50a)

Substitutions at both the 2- and the N^6-position were investigated as well. The beneficial N^6-(4-phenylbutyl) substituent was combined with modifications at the 2-position of AMP. Thus, an amino group was introduced at the 2-position of N^6-(4-phenylbutyl)-AMP (**38h**). The commercially available 2-amino-6-chloro-9-(*β-D-*

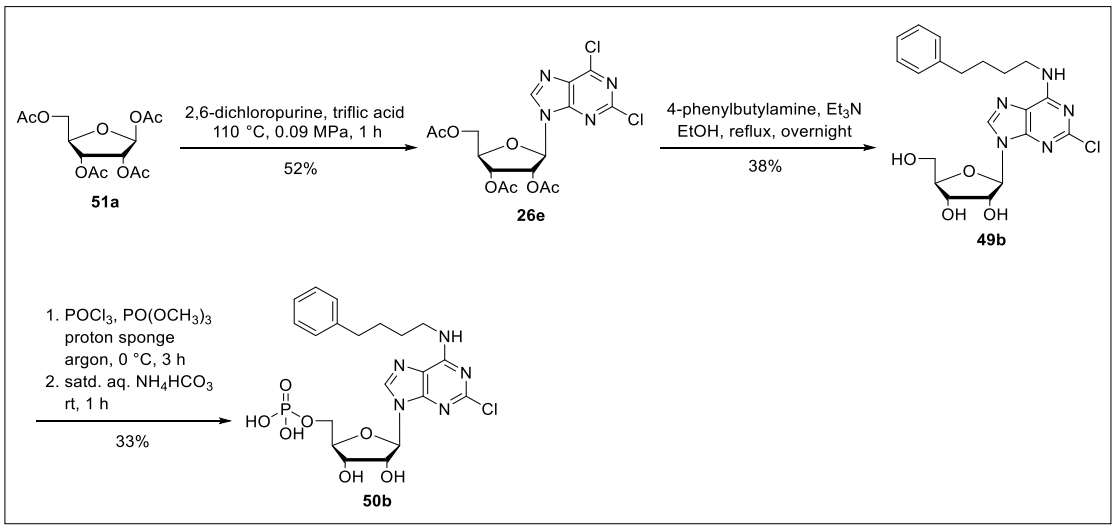
ribofuranosyl)purine was reacted with 4-phenylbutylamine in EtOH in the presence of Et_3N to generate **49a**. Finally, the intermediate **49a** was subsequently monophosphorylated under standard conditions to generate **50a** (**Scheme 4.22**).



Scheme 4.22. Synthesis of N^6-(4-phenylbutyl)-2-amino-AMP (**50a**).

4.1.24 Synthesis of N^6-(4-phenylbutyl)-2-chloro-AMP (50b)

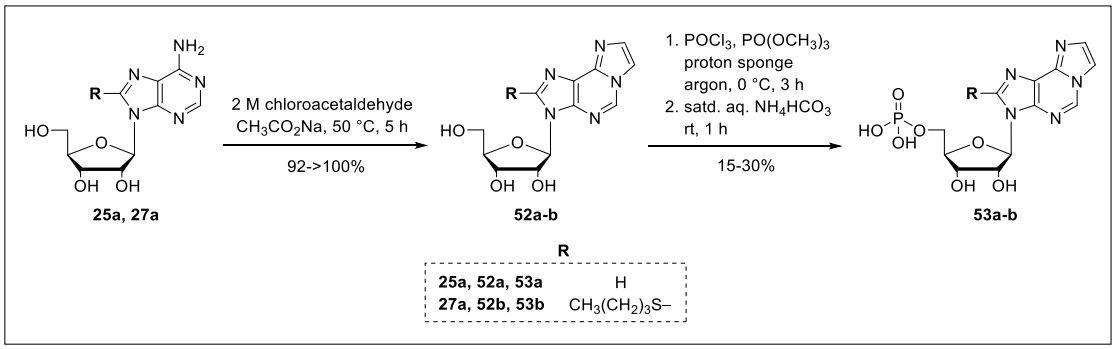
In another synthesis, chloro was introduced at the 2-position of N^6-(4-phenylbutyl)-AMP (**38h**) as well. 2,6-Dichloro-9-(*β-D*-ribofuranosyl)purine is not commercially available, so its 2′-,3′-,5′-tri-*O*-acetyl-protected precursor **26e** was synthesized by a reported procedure with some modifications.[163] Tetraacetylribose was melted at 110 °C and then 2,6-dichloropurine was added in the presence of triflic acid. The mixture was stirred at 110 °C and 0.09 MPa for removing AcOH which was produced during the reaction to generate **26e**. Subsequently, **26e** was reacted with 4-phenylbutylamine in EtOH in the presence of Et_3N to generate intermediate **49b**. Finally, the intermediate **49b** was monophosphorylated under standard conditions to generate **50b** (**Scheme 4.23**).



Scheme 4.23. Synthesis of N^6-(4-phenylbutyl)-2-chloro-AMP (**50b**).

4.1.25 Synthesis of 1,N^6-etheno-AMP (53a) and 8-butylthio-1,N^6-etheno-AMP (53b)

8-BuS-AMP (**1i**) is a potent CD39 inhibitor.[77] To introduce an etheno-bridge between 1- and N^6-position may increase inhibitory potency and lead to a fluorescent ligand. Adenosine was also bridged using the same method. To adenosine/8-BuS-adenosine (**25a**/**27a**) in 2 M chloroacetaldehyde (aq.) was added CH_3CO_2Na, and the mixture was stirred at 50 °C to generate **52a**/**52b**. The intermediates **52a-b** were subsequently monophosphorylated under standard conditions to generate **53a-b** (**Scheme 4.24**).

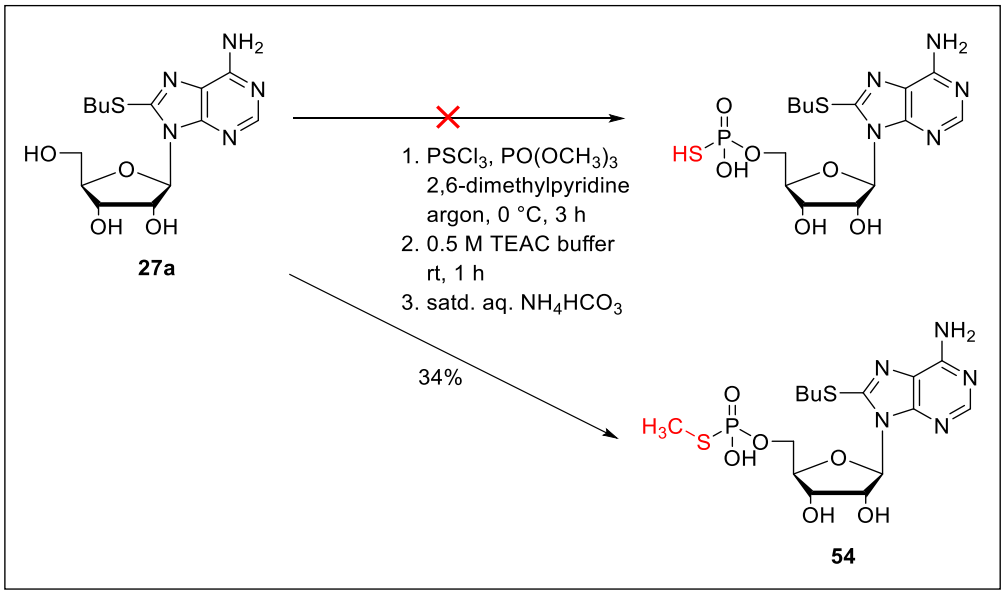


Scheme 4.24. Synthesis of 1,N^6-etheno-AMP (**53a**) and 8-butylthio-1,N^6-etheno-AMP (**53b**).

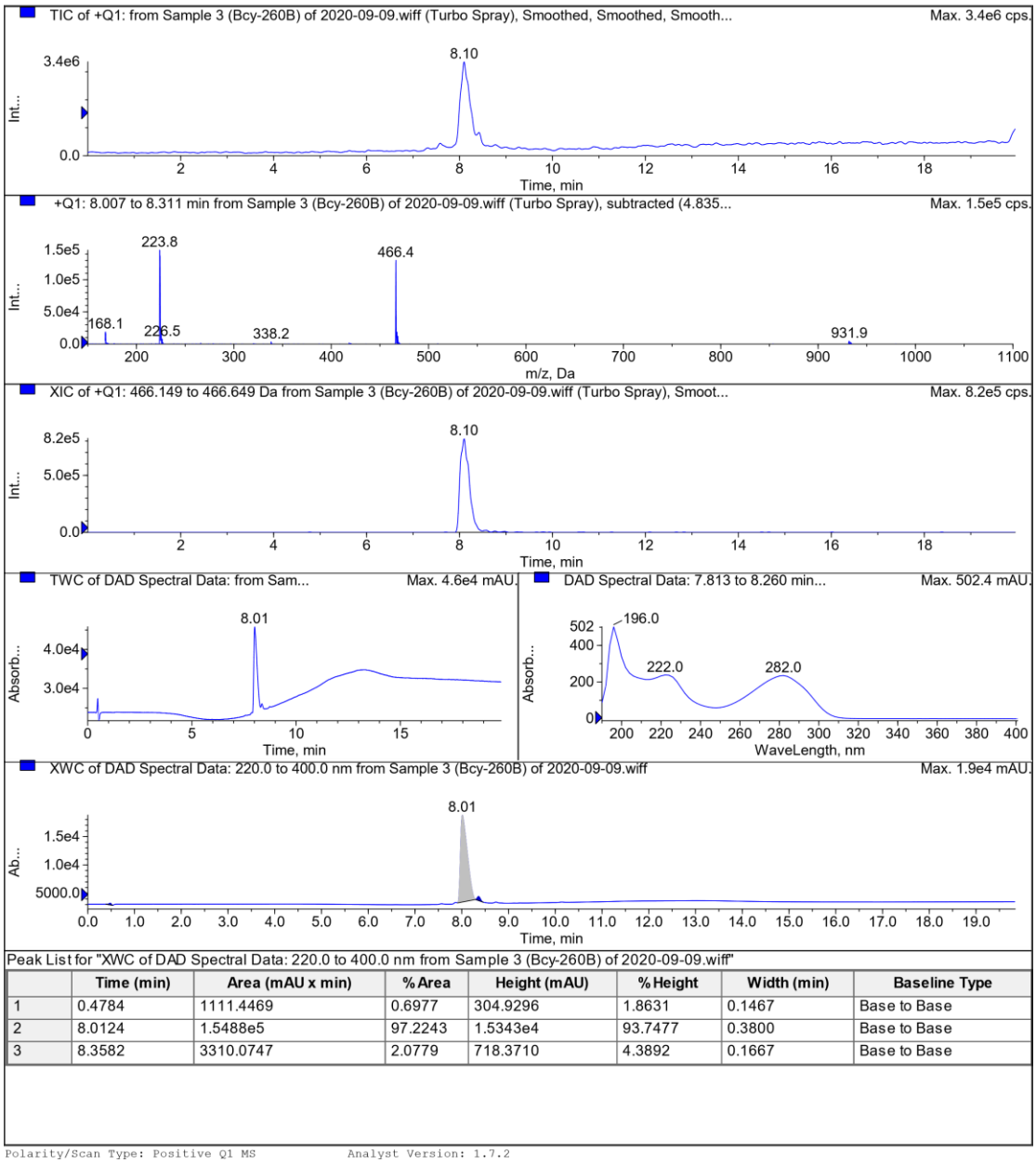
4.1.26 Synthesis of 8-(butylthio)adenosine-5′-*S*-methylthiophosphate (54)

Compound **54** was synthesized by a reported procedure[164] with some modifications by accident. At the beginning, I wanted to replace the O-atom by a S-atom in the phosphate group of 8-BuS-AMP (**1i**). $PSCl_3$ was used for thionation.

Compound **27a** was dissolved in $PO(OCH_3)_3$ and reacted with $PSCl_3$ in the presence of 2,6-dimethylpyridine at 0 °C under argon to yield the reactive 5′-dichlorothiophosphate intermediate. Hydrolysis by TEAC buffer followed by saturated NH_4HCO_3 solution yielded **54** (**Scheme 4.25**). The crude mixture was extracted by *tert*-butylmethylether to remove $PO(OCH_3)_3$ and 2,6-dimethylpyridine, and the product was finally purified by preparative HPLC. LC-MS spectrum, and ^1H and ^{13}C NMR spectra of compound **54** are depicted in **Figures 4.3** and **4.4**, respectively.



Scheme 4.25. Synthesis of 8-(butylthio)adenosine-5′-*S*-methylthiophosphate (**54**).



Peak List for "XWC of DAD Spectral Data: 220.0 to 400.0 nm from Sample 3 (Bcy-260B) of 2020-09-09.wiff"

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1	0.4784	1111.4469	0.6977	304.9296	1.8631	0.1467	Base to Base
2	8.0124	1.5488e5	97.2243	1.5343e4	93.7477	0.3800	Base to Base
3	8.3582	3310.0747	2.0779	718.3710	4.3892	0.1667	Base to Base

Polarity/Scan Type: Positive Q1 MS Analyst Version: 1.7.2

Figure 4.3. LC-MS spectrum of compound **54** (retention time: 8.01 min).

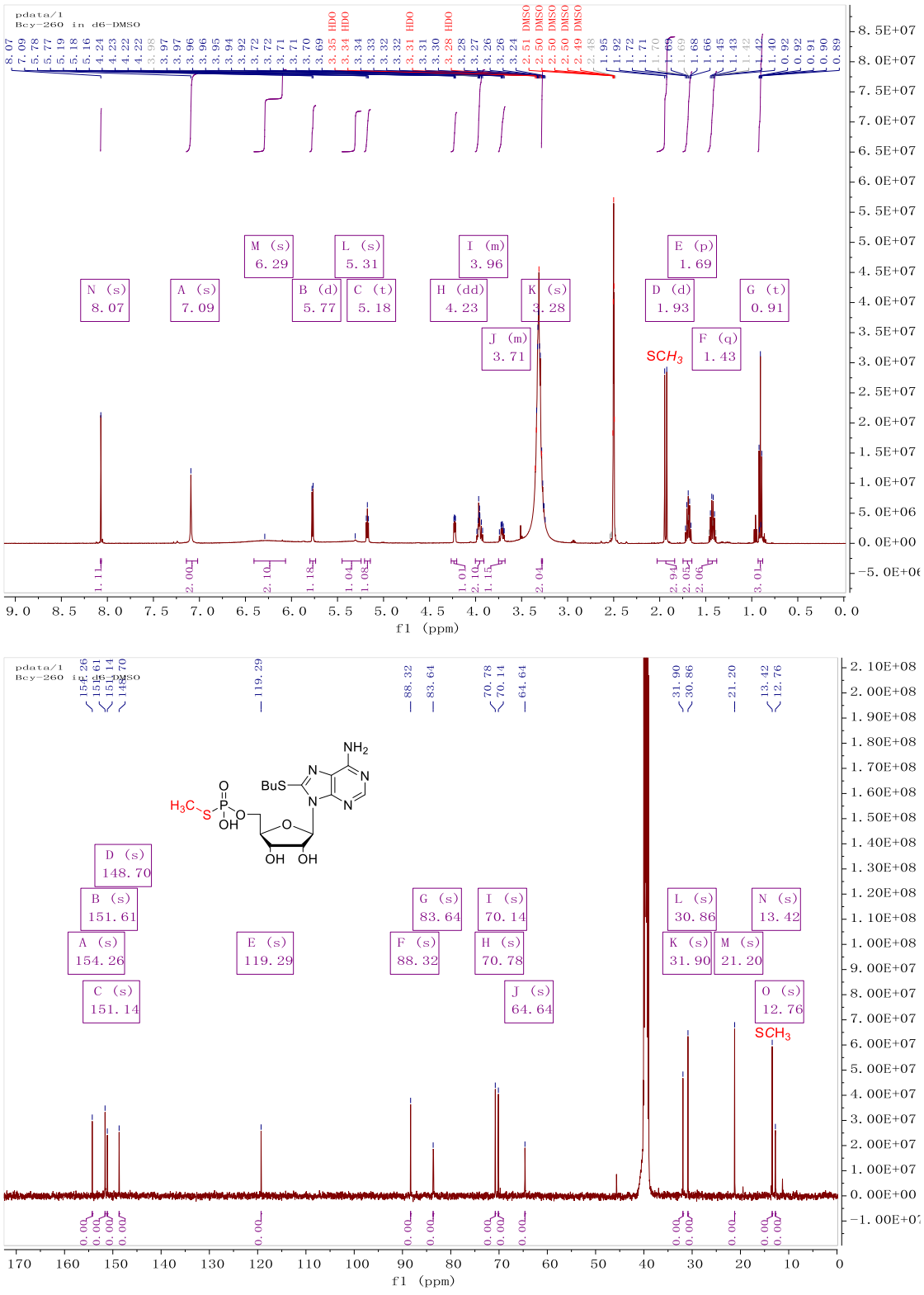


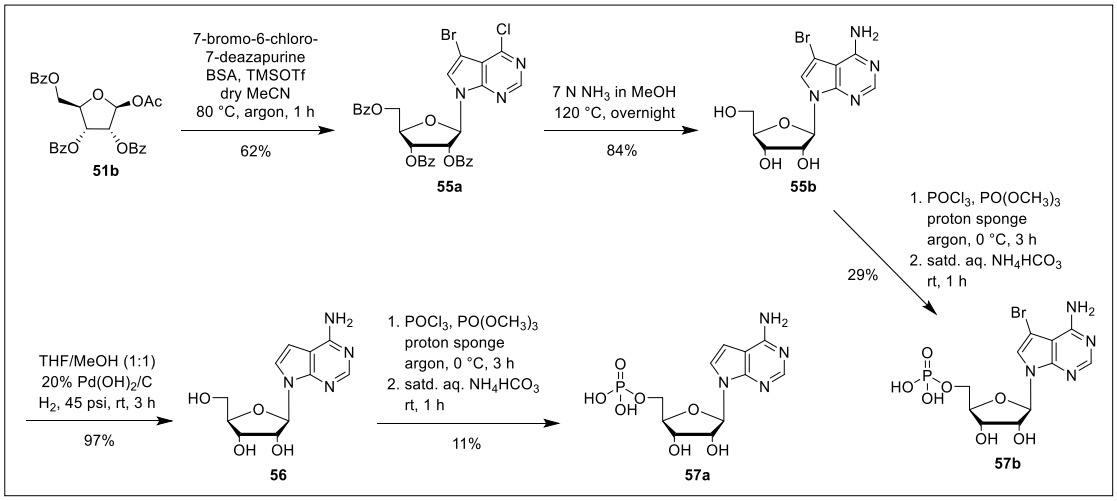
Figure 4.4. ^{1}H and ^{13}C NMR spectra of compound **54**.

4.2 Synthesis of 7-deaza-AMP derivatives and analogs

4.2.1 Synthesis of 7-deaza-AMP (57a) and 7-bromo-7-deaza-AMP (57b)

The Silyl-Hilbert-Johnson (or Vorbrüggen) reaction is the most common method for forming nucleosides, mainly using silylated heterocyclic bases and electrophilic sugar derivatives in the presence of a Lewis acid.[165]

The synthetic procedures to obtain tubercidin (**56**) and its derivatives were reported by Huang *et al*.[166] For the synthesis of **55a**, 7-bromo-6-chloro-7-deazapurine was dissolved in anhydrous MeCN in the presence of BSA (*N*,*O*-bis(trimethylsilyl)acetamide), 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-*D*-ribofuranose and TMSOTf (trimethylsilyl trifluoromethanesulfonate). The mixture was stirred at 80 °C under argon. For the synthesis of **55b**, **55a** was dissolved in ammonia solution (7 N in MeOH) and the mixture was stirred in a sealed flask at 120 °C. For the synthesis of **56**, **55b** was dissolved in THF/MeOH (1:1) in the presence of 20% $Pd(OH)_2/C$. The mixture was shaken with H_2 (45 psi) at rt in a Parr apparatus. Finally, the intermediates **56** and **55b** were monophosphorylated under standard conditions to generate **57a-b** (**Scheme 4.26**).

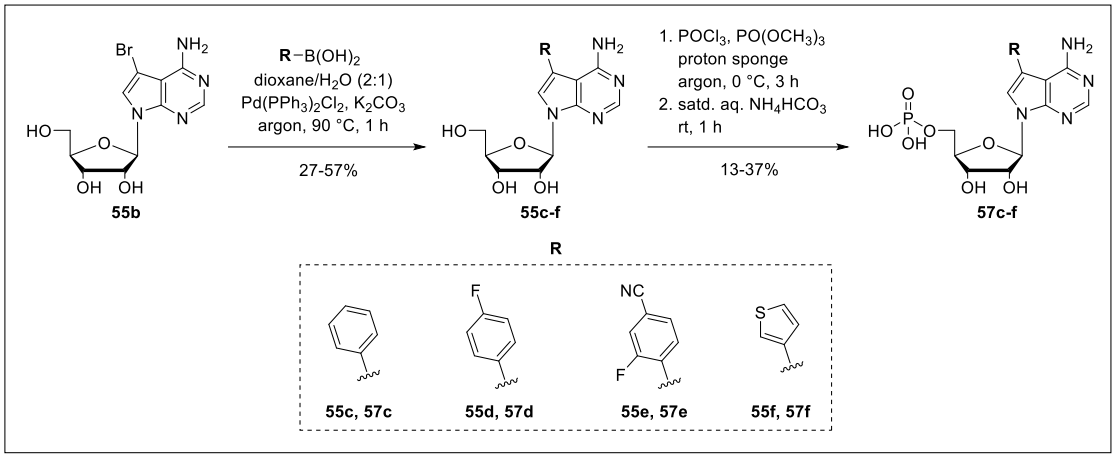


Scheme 4.26. Synthesis of 7-deaza-AMP (**57a**) and 7-bromo-7-deaza-AMP (**57b**).

4.2.2 Synthesis of 7-substituted 7-deaza-AMP derivatives (57c-f) by Suzuki reaction

Compounds **55c-f** were synthesized by the same method as **33h** by Suzuki reaction. Compound **55b** was used as the starting material to react with different aryl-boronic acids (benzeneboronic acid, 4-fluorobenzeneboronic acid, (4-cyano-2-fluorophenyl)boronic acid and 3-thienylboronic acid) to generate **55c-f**. Finally, the

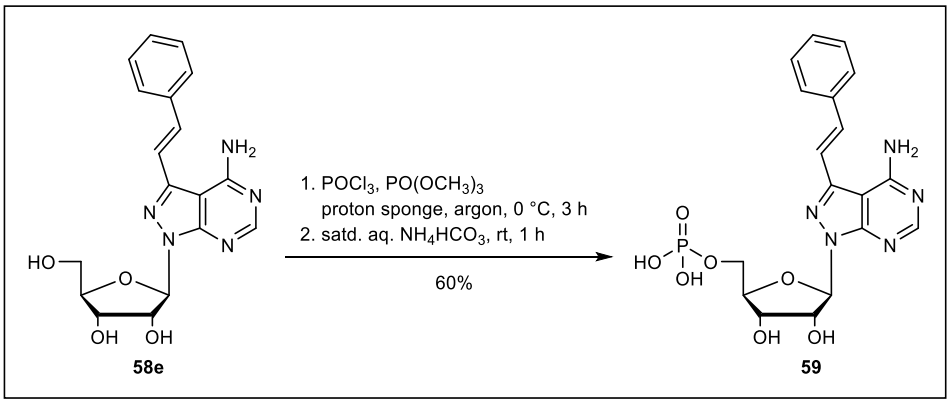
intermediates **55c-f** were monophosphorylated under standard conditions to generate **57c-f** (**Scheme 4.27**).



Scheme 4.27. Synthesis of 7-substituted 7-deaza-AMP derivatives (**57c-f**).

4.2.3 Synthesis of ((2*R*,3*S*,4*R*,5*R*)-5-(4-amino-3-((*E*)-styryl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl phosphoric acid (59)

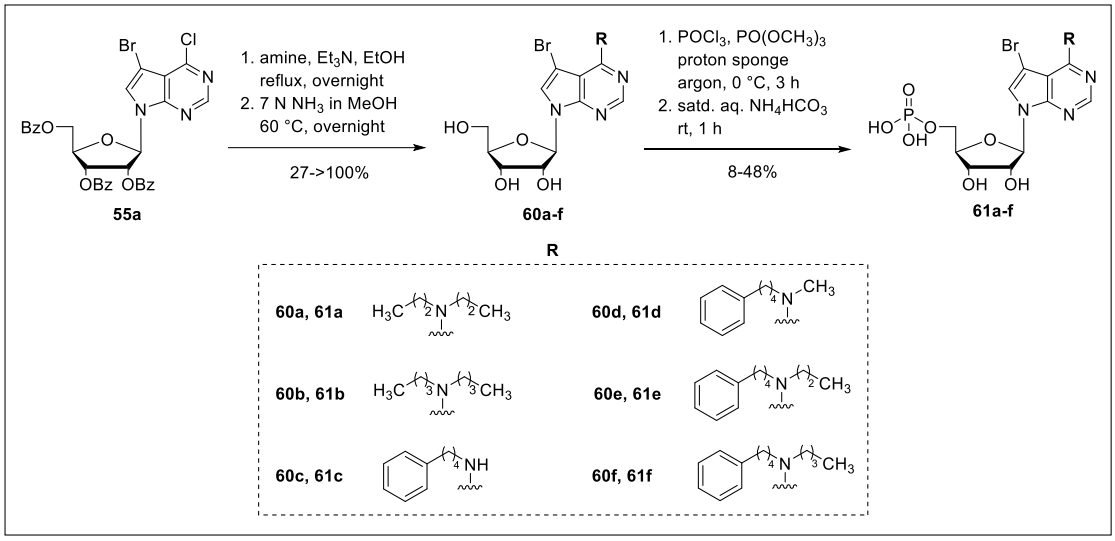
Compound **58e** was generously offered by Prof. Dr. Serge Van Calenbergh and directly monophosphorylated under standard conditions to generate **59** (**Scheme 4.28**).



Scheme 4.28. Synthesis of ((2*R*,3*S*,4*R*,5*R*)-5-(4-amino-3-((*E*)-styryl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl phosphoric acid (**59**).

4.2.4 Synthesis of 7-bromo-7-deaza-N^6-substituted AMP derivatives (61a-f)

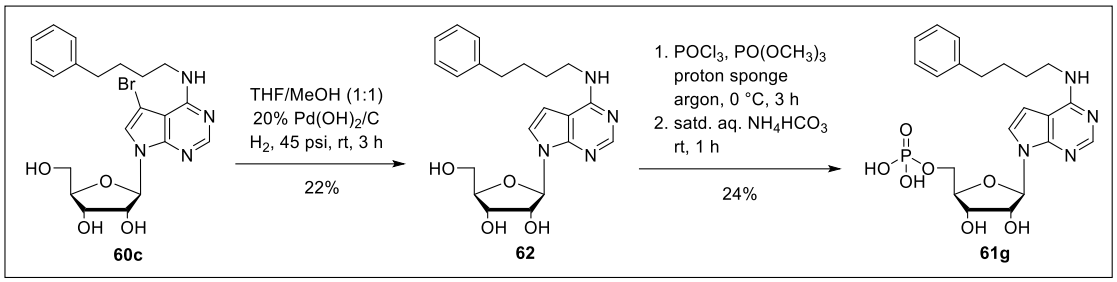
4-Phenylbutyl, the so far best substituent at the N^6-position of AMP, was introduced into 7-bromo-7-deaza-AMP and some derivatives. For this series of compounds, **55a** was chosen as the starting material. To **55a** in EtOH was added the appropriate amine (dipropylamine, dibutylamine, 4-phenylbutylamine, methyl(4-phenylbutyl)amine, 4-phenyl-*N*-propylbutan-1-amine or *N*-butyl-4-phenylbutan-1-amine) and Et_3N, and the mixture was refluxed overnight. Then the solution was dried and stirred in 7 N NH_3 in MeOH at 60 °C overnight to remove the protecting 2′-,3′-,5′-tri-*O*-benzoyl groups generating **60a-f**. Finally, the intermediates **60a-f** were monophosphorylated under standard conditions to generate **61a-f** (**Scheme 4.29**).



Scheme 4.29. Synthesis of 7-bromo-7-deaza-N^6-substituted AMP derivatives (**61a-f**).

4.2.5 Synthesis of 7-deaza-N^6-(4-phenylbutyl)-AMP (61g)

Based on molecular modeling studies of N^6-(4-phenylbutyl)-AMP in our group, we hypothesized that the nitrogen atom in the 7-position was not required or even unfavorable for binding of the nucleotide to CD39, and that compounds lacking this nitrogen atom may be potent CD39 inhibitors. Thus, 7-deaza-N^6-(4-phenylbutyl)adenosine (**62**) was synthesized from **60c** by the same method as described for **56**. The intermediate **62** was subsequently monophosphorylated under standard conditions to generate **61g** (**Scheme 4.30**).



Scheme 4.30. Synthesis of 7-deaza-N^6-(4-phenylbutyl)-AMP (**61g**).

4.3 Pharmacological evaluation of 8-BuS-AMP derivatives and analogs at human CD39

All synthesized 8-BuS-AMP derivatives and analogs were tested for their inhibitory potency at human CD39 expressed in COS-7 cells using 50 µM ATP as a substrate and 50 µM inhibitor employing the malachite green assay (n = 3), which was described in **8.5.2**. The K_i values were calculated for competitive inhibitors from the obtained IC_{50} values using the Cheng-Prusoff equation. Results are summarized in **Tables 4.1**-**4.4**. The biological testing was performed by Laura Schäkel and Areso Ahmadsay.

4.3.1 Structure-activity relationships of 8-substituted AMP derivatives

The K_i value of the lead compound 8-BuS-AMP (**1i**) was 0.847 µM determined in our malachite green assay which was identical to the reported value of 0.8 µM.[77] It was nearly identical (K_i = 1.10 µM) when determined by fluorescence capillary electrophoresis assay. The inhibitory potency in both assays was found to be comparable as K_i values were in the same range for all investigated competitive CD39 inhibitors.

8-Alkylthio or arylthio derivatives and analogs, like the lead structure 8-BuS-AMP (**1i**), were mostly potent inhibitors with K_i values in a low micromolar range. For example, the shorter 8-ethylthio (**28b**, K_i = 2.78 µM) and 8-propylthio (**28d**, K_i = 1.46 µM), as well as the longer 8-pentylthio (**28g**, K_i = 2.99 µM) and 8-(5-methylhexyl)thio (**28h**, K_i = 2.72 µM) substitutions maintained but did not increase potency. 8-Cyclohexylthio

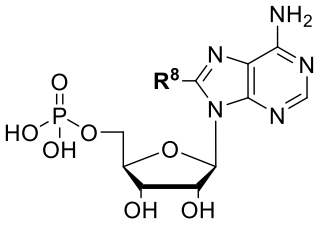
substitution led to a high potency (**28j**, K_i = 0.768 µM), which was 7-fold decreased by the addition of a methylene linker (**28k**, K_i = 5.34 µM).

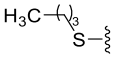
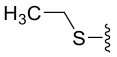
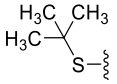
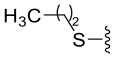
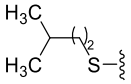
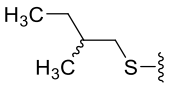
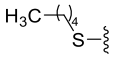
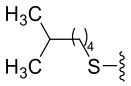
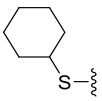
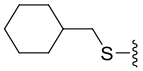
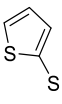
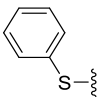
Aromatic thio-substitutions resulted in slightly superior or decreased potency in the 8-position, e.g., 1-naphthylthio (**28r**, K_i = 0.735 µM), 2-thienylthio (**28l**, K_i = 1.51 µM), 8-phenylthio (**28m**, K_i = 3.42 µM), 4-fluorophenylthio (**28n**, K_i = 2.05 µM) and 4-aminophenylthio (**28o**, K_i = 4.02 µM). However, the inhibitory potency was significantly reduced by the deletion of the S atom at the 8-position (compare **28m** with **34h**).

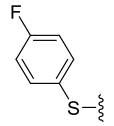
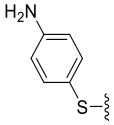
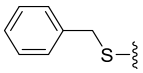
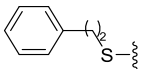
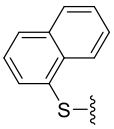
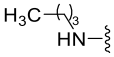
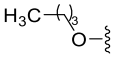
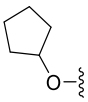
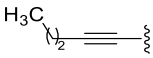
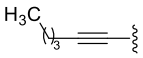
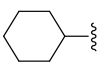
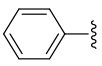
8-Amino substituents are usually not comparable to 8-thio substituents. When replacing the S in 8-BuS-AMP by NH, **30b** showed lower inhibitory potency (K_i = 13.7 µM). However, the 8-methylamino-substituted analog **30a** has a comparable potency to the lead compound 8-BuS-AMP with a K_i value of 0.660 µM (with somewhat lower potency determined in the malachite green assay (K_i = 4.89 µM)). The 8-butyloxy and 8-cyclopentyloxy substituents (**32b**, K_i = 2.56 µM, and **32c**, K_i = 2.17 µM) showed similarly high inhibitory potency as the 8-butylthio- and 8-cyclopentylthio-substituted analogs (**1i**, K_i = 0.847 µM and **28i**, K_i = 1.10 µM). Some saturated and unsaturated aliphatic substituents were also investigated at the 8-position, most of them showing similarly medium inhibitory potency (**34c**, K_i = 8.00 µM; **34d**, K_i = 10.9 µM; **34f**, K_i = 10.8 µM; **34e**, K_i = 7.78 µM and **34g**, K_i = 4.82 µM) except for the 8-methyl derivative (**34a**, 40% inhibition at 50 µM). Data are collected in **Table 4.1**.

The rank order of inhibitory potency of different substitutions at the 8-position is S ≈ O > C > N with regard to the linker.

Table 4.1. Potency of 8-substituted AMP derivatives as inhibitors of human CD39



Compd.	R^8	K_i ± SEM (μM) (or % inhibition at 50 μM)
8-BuS-AMP (1i)		0.847 ± 0.194 (1.10 ± 0.62[a], 0.8 ± 0.1[77])
28b		2.78 ± 0.58
28c		(47%)
28d		1.46 ± 0.17
28e		2.36 ± 0.36
28f		2.18 ± 0.21
28g		2.99 ± 0.14
28h[b]		2.72 ± 0.32 (5.19 ± 1.32)[a]
28i		1.10 ± 0.12
28j		**0.768 ± 0.052**
28k		5.34 ± 3.12
28l		1.51 ± 0.19
28m		3.42 ± 0.62

28n		2.05 ± 0.42
28o		4.02 ± 0.46
28p		2.60 ± 0.69
28q		5.05 ± 0.47
28r		**0.735 ± 0.056**
30a		4.89 ± 1.23 (0.660 ± 0.072)[a]
30b[b]		13.7 ± 1.6
30c		14.9 ± 2.1
32a	HO	(41%)
32b		2.56 ± 0.25
32c		2.17 ± 0.46
34a	CH_3	(40%)
34c		8.00 ± 0.65
34d		10.9 ± 2.5
34e		7.78 ± 0.85
34f		10.8 ± 1.4
34g		4.82 ± 0.70
34h		(27%)
AMP (28a)[79]	H	(10%)[a]

[a]Fluorescence capillary electrophoresis assay: screening at 10 µM, was performed using 0.5 µM fluorescent substrate PSB-170621A and human CD39 expressed in umbilical cord membranes.

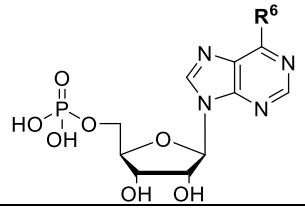
[b]Compounds were synthesized together with Dr. Constanze Cerine Schmies.

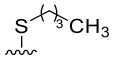
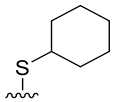
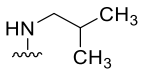
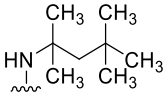
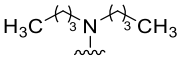
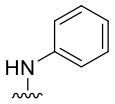
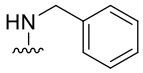
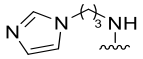
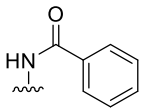
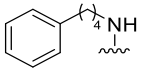
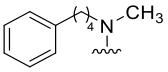
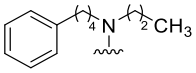
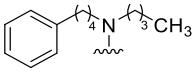
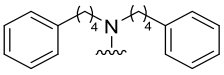
4.3.2 Structure-activity relationships of 6-substituted AMP derivatives

Three alkyl substituents were investigated at the N^6-position. N^6-Isobutyl (**38a**, K_i = 20.5 µM) and N^6,N^6-dibutyl (**38c**, K_i = 11.5 µM) showed medium inhibitory potency, but N^6-(1,1,3,3-tetramethyl)butyl substitution (**38b**) showed almost no inhibition at 10 µM. Introduction of an aromatic ring at the N^6-position led to a potent CD39 inhibitor only if attached via a butyl linker (**38h**, K_i = 7.08 µM), but an N^6-benzoyl-AMP derivative (**38g**), a benzyl derivative (**38e**), or a phenyl derivative (**38d**) showed low potency. Disubstitution of N^6-position combing methyl, propyl, or butyl with a 4-phenylbutyl residue maintained K_i values of around 5 µM (e.g., **38j**, K_i = 5.10 µM). Nevertheless, N^6,N^6-di-(4-phenylbutyl) substitution (**38l**) abolished activity at CD39. When replacing the 6 amino group in **38h** by oxygen, the compound **38n** ($K_i \approx 10$ µM) showed decreased inhibitory potency as previously described (see **Figure 4.5**).[79] The replacement of the whole amino group of **38h** by different thio-substituents, butylthio (**36a**, 15% inhibition at 50 µM) and cyclohexylthio (**36b**, 24%), yielded derivatives with low potency. Data are collected in **Table 4.2**.

The know incomplete rank order of inhibitory potency of different substitutions at the 6-position is N > O with regard to the linker.

Table 4.2. Potency of 6-substituted AMP derivatives as inhibitors of human CD39



Compd.	R^6	K_i ± SEM (µM) (or % inhibition at 50 µM)
36a		(15%)
36b		(24%)
38a		20.5 ± 3.2
38b[a]		(6%)[b]
38c		11.5 ± 0.4
38d		(17%)
38e		(31%)
38f[a]		(11%)[b]
38g		(17%)
38h		7.08 ± 0.68 (1.40 ± 0.12)[b]
38i		9.13 ± 1.85
38j		5.10 ± 0.59
38k		**3.13 ± 0.66**
38l		(0%)

[a]Compounds were synthesized together with Dr. Constanze Cerine Schmies.

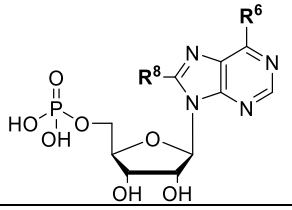
[b]Fluorescence capillary electrophoresis assay: screening at 10 µM, was performed using 0.5 µM fluorescent substrate PSB-170621A and human CD39 expressed in umbilical cord membranes.

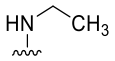
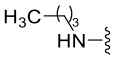
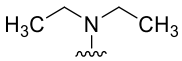
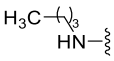
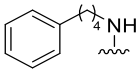
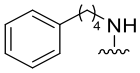
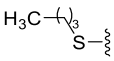
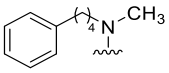
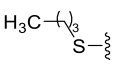
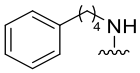
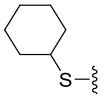
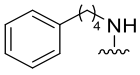
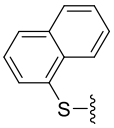
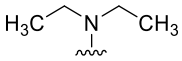
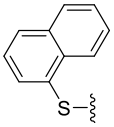
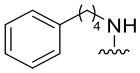
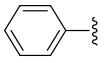
4.3.3 Structure-activity relationships of 8-, N^6-disubstituted AMP derivatives

We combined 8- and N^6-substitution to investigate potential synergistic effects. Methylation of 8-methylamino-AMP (**30a**), and (di)-ethylation of 8-butylamino-AMP (**30b**) at the N^6-position led to decreases in potency. Compound **48f** is a combination of 8-(1-naphthyl)thio-AMP (**28r**) and ARL 67156. However, the substitutions showed no additive effect in potency. In contrast, the addition of the N^6-(4-phenylbutyl) substituent to 8-methylamino-AMP (yield **48a**), 8-BuS-AMP (yield **48b**), 8-cyclohexylthio-AMP (yield **48d**), 8-(1-naphthyl)thio-AMP (yield **48e**) or 8-phenyl-AMP (yield **48g**) led to inhibitors with increased potency. Data are collected in **Table 4.3**.

Overall, the most potent 8-substituents, namely 8-butylthio, 8-cyclohexylthio and 8-(1-naphthyl)thio, are beneficial if combined with different N^6-substituents. The N^6-(4-phenylbutyl)- and N^6-methyl-N^6-(4-phenylbutyl)-substituted derivatives also showed enhanced potency if combined with different 8-substituents on the AMP scaffold. These combinations have led to the most potent CD39 inhibitors of this series, and among all reported nucleotide derivatives and analogs.

Table 4.3. Potency of 8-, N^6-disubstituted AMP derivatives and analogs as inhibitors of human CD39



Compd.	R^6	R^8	K_i ± SEM (µM) (or % inhibition at 50 µM)
44a			(19%)
44b[a]			(17%)[b]
44c[a]			(16%)[b]
48a			1.54 ± 0.36
48b			**0.444 ± 0.061**
48c			1.77 ± 0.03
48d			**0.428 ± 0.132**
48e			**0.329 ± 0.067**
48f			2.20 ± 0.56
48g			6.70 ± 0.11

[a]Compounds were synthesized together with Dr. Constanze Cerine Schmies.

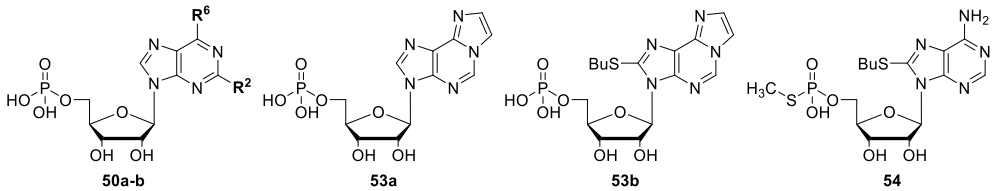
[b]Fluorescence capillary electrophoresis assay: screening at 10 µM, was performed using 0.5 µM fluorescent substrate PSB-170621A and human CD39 expressed in umbilical cord membranes.

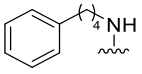
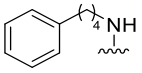
4.3.4 Structure-activity relationships of further 8-BuS-AMP derivatives and analogs

The addition of an amino (**50a**, 17% inhibition at 50 µM) or a chloro substituent (**50b**, K_i = 65.4 µM) at the 2-position of N^6-(4-phenylbutyl)-AMP (**38h**, K_i = 7.08 µM) led to a large decrease in CD39 inhibition. The etheno-AMP derivatives **53a-b** also showed a significant decrease in CD39 inhibition compared to analogous adenine nucleotides. When the phosphate group of 8-BuS-AMP was thionated and methylated (in **54**), its potency decreased. All data are collected in **Table 4.4**.

Overall, the addition of different substituents at the 2-position, an etheno-bridge between N^6 and N^1, and substitution of the phosphate all led to highly decreased inhibitory activity at CD39.

Table 4.4. Potency of further AMP derivatives and analogs as inhibitors of human CD39



Compd.	R^2	R^6	K_i ± SEM (µM) (or % inhibition at 50 µM)
50a	NH_2		(17%)
50b	Cl		65.4 ± 11.8
53a	*for structure see above*		(0%)
53b	*for structure see above*		(48%)
54	*for structure see above*		(20%)

4.3.5 Summary of structure-activity relationships of AMP derivatives and analogs

Apart from the AMP derivatives and analogs described in this chapter, there are some more which were synthesized by Dr. Constanze Cerine Schmies.[79] With all of these AMP derivatives and analogs, some selected and interesting SARs are summarized in the following **Figure 4.5**.

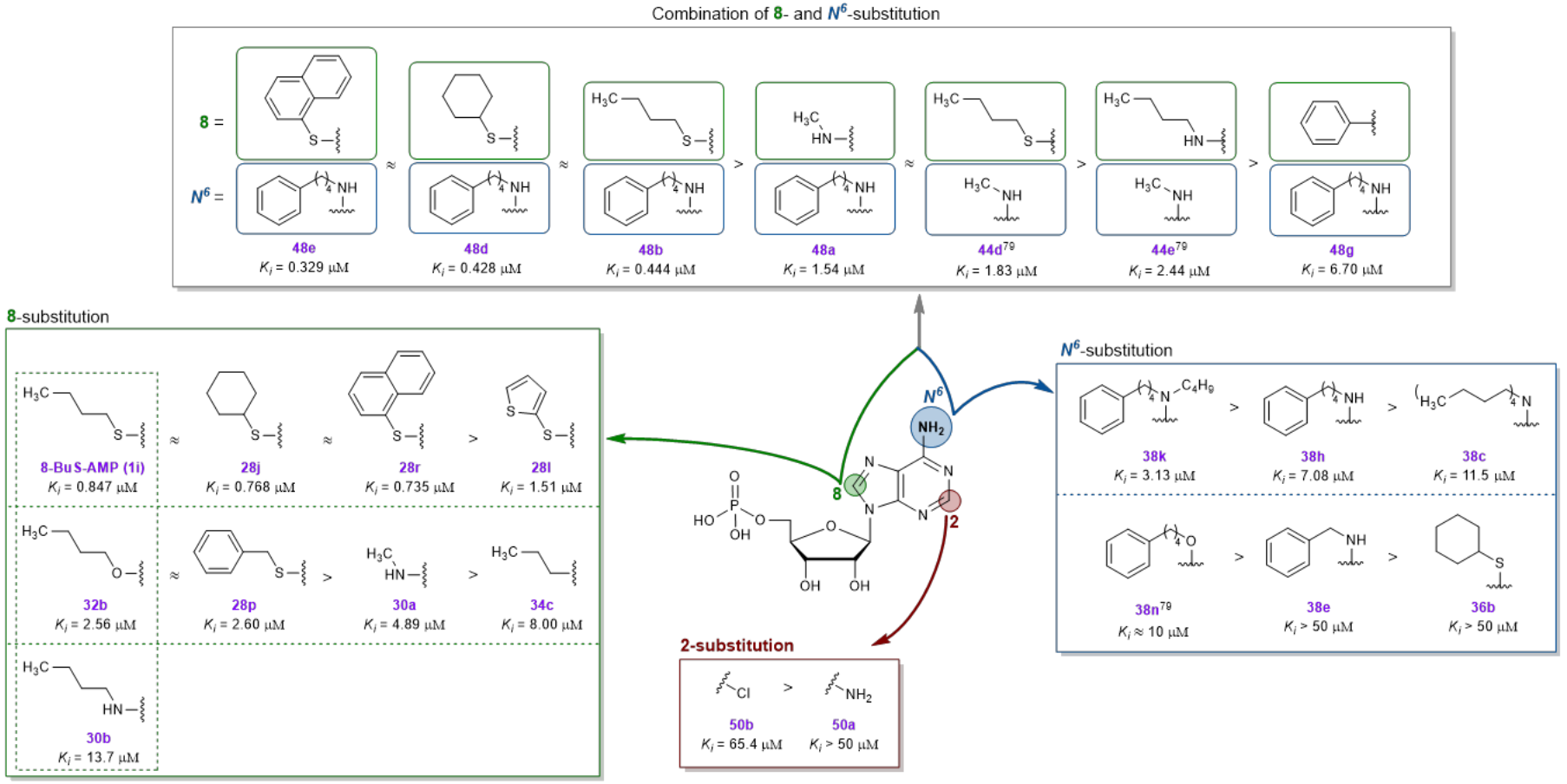


Figure 4.5. Selected SARs of AMP derivatives and analogs as CD39 inhibitors.

4.3.6 Inhibition type determination for 8-BuS-AMP

The inhibition type of 8-BuS-AMP determined in our group was competitive (**Figure 4.6**). The K_i value determined by the crossing of lines in the Dixon plot was determined to be 0.218 µM in this experiment, which is somewhat lower but still in the same range as the K_i values derived from concentration-inhibition curves. Hence the same inhibition type can be assumed for 8-BuS-AMP derivatives and analogs, and K_i values were therefore calculated using the Cheng-Prusoff equation[167] in order to compare inhibition data from different assays. 8-BuS-AMP had previously been described as a predominantly competitive inhibitor of CD39 in a published paper.[77] The present experiments and their analysis were performed by Laura Schäkel.

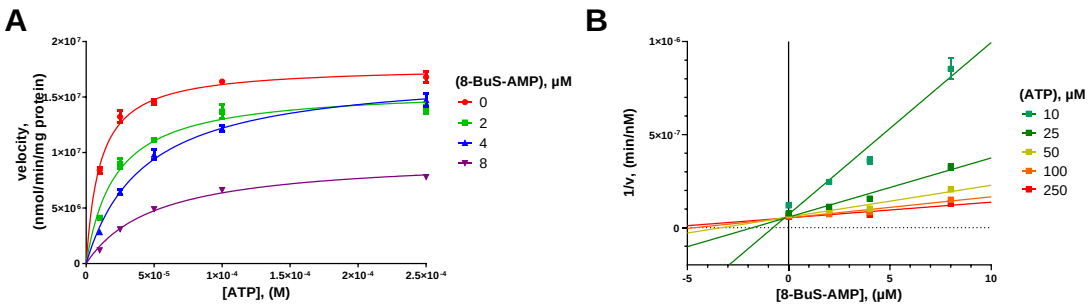


Figure 4.6. Inhibition type determination for 8-BuS-AMP (**1i**). **A.** Michaelis Menten plot of CD39 inhibition by 2, 4 and 8 µM 8-BuS-AMP and 10, 25, 50, 100 and 250 µM ATP as substrate obtained with the malachite green assay, **B.** Dixon plot used for K_i determination. The experiment was performed three times each in duplicates (n = 3).

4.4 Pharmacological evaluation of 7-deaza-AMP derivatives and analogs at human CD39

All synthesized 7-deaza-AMP derivatives and analogs were tested for their inhibitory potency at human CD39 as described in **4.3**. Results are summarized in **Tables 4.5** and **4.6**. The biological testing was performed by Laura Schäkel and Areso Ahmadsay.

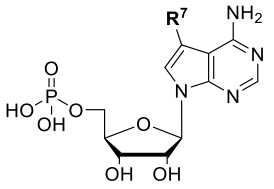
4.4.1 Structure-activity relationships of 7-substituted 7-deaza-AMP derivatives and analogs

Based on our molecular modeling studies, we initially hypothesized that the nitrogen atom in the 7-position of the adenine nucleobase is not required or even unfavorable for binding to CD39, and compounds lacking this nitrogen atom maybe potent CD39 inhibitors. Their inhibitory potency is presented in **Table 4.5** and SARs are depicted in **Figure 4.7**.

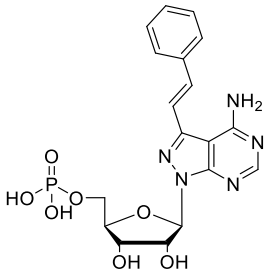
The replacement of the 7-nitrogen atom of AMP by CH resulted in an inactive compound **57a** similar to AMP (**28a**). The addition of Br to the 7-position of 7-deaza-AMP (**57a**), slightly increased the inhibition to 27% at 50 µM. A series of aromatic substituents was introduced at the 7-position of the 7-deaza-AMP scaffold, and the resulting compounds showed K_i values ranging from 4.28-9.57 µM. Another compound **59**, an analog of 7-deaza-AMP in which an 8-N was introduced, and which also contained a styryl residue in the 7-position, showed potent inhibition (K_i = 1.89 µM).

Overall, the replacement of 7-N by CH is not favorable, but the introduction of aromatic substituents at the 7-position increases the inhibitory potency at CD39.

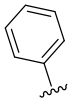
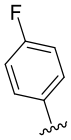
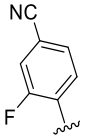
Table 4.5. Potency of 7-substituted 7-deaza-AMP derivatives and analogs as inhibitors of human CD39



57a-f



59

Compd.	R^7	K_i ± SEM (µM) (or % inhibition at 50 µM)
57a	H	(-7%)
57b	Br	(27%)
57c		4.60 ± 1.69
57d		4.28 ± 0.36
57e		9.57 ± 1.15
57f		5.20 ± 0.31
59	*for structure see above*	**1.89 ± 0.07**

4.4.2 Structure-activity relationships of N^6-substituted 7-deaza-AMP derivatives

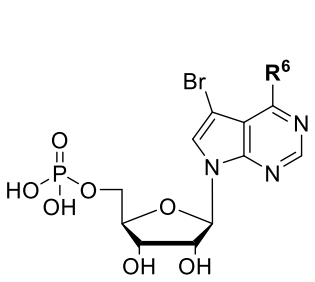
The N^6-position of 7-deaza-AMP was investigated. The inhibitory potencies of these compounds are collected in **Table 4.6** and the SARs are depicted in **Figure 4.7**.

The activity of 7-bromo-7-deaza-N^6,N^6-dipropyl-AMP (**61a**, 37% inhibition at 50 µM) was decreased compared to N^6,N^6-dipropyl-AMP (K_i = 15.5 µM).[79] However, the activity of 7-bromo-7-deaza-N^6,N^6-dibutyl-AMP (**61b**, K_i = 12.8 µM) was similar to that of N^6,N^6-dibutyl-AMP (**38c**, K_i = 11.5 µM). An N^6-(4-phenylbutyl) substituent in addition to an N^6-alkyl residue (methyl, propyl or butyl) was introduced at the N^6-

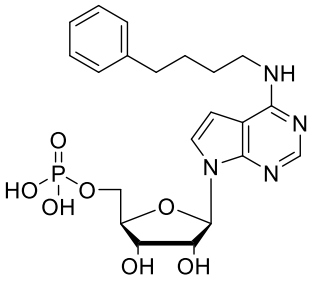
position of 7-Br-7-deaza-AMP. The addition of different alkyl substituents to the N^6-position of 7-bromo-7-deaza-N^6-(4-phenylbutyl)-AMP (**61c**, K_i = 2.32 µM) decreased its potency yielding K_i values of 6.06-10.3 µM. Furthermore, the inhibition of **61c** was significantly decreased to 38% at 50 µM when its 7-Br was removed. Compared to N^6-(4-phenylbutyl)-AMP (**38h**, K_i = 7.08 µM), the potency of **61c** was increased by 3-fold.

Overall, 7-Br-7-deaza-AMP derivatives and analogs have a high potential to maintain or increase potency as CD39 inhibitors compared to AMP derivatives. But removal of the 7-Br substitution decreases their potency.

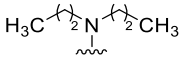
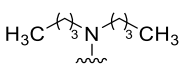
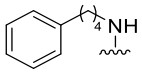
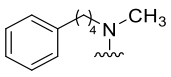
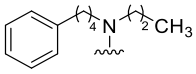
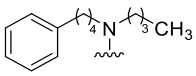
Table 4.6. Potency of (7-bromo)-7-deaza-N^6-substituted AMP derivatives as inhibitors of human CD39



61a-f



62g

Compd.	R^6	K_i ± SEM (µM) (or % inhibition at 50 µM)
61a		(37%)
61b		12.8 ± 0.9
61c		**2.32 ± 0.54**
61d		10.3 ± 0.8
61e		7.19 ± 1.13
61f		6.06 ± 1.62
61g	*for structure see above*	(38%)

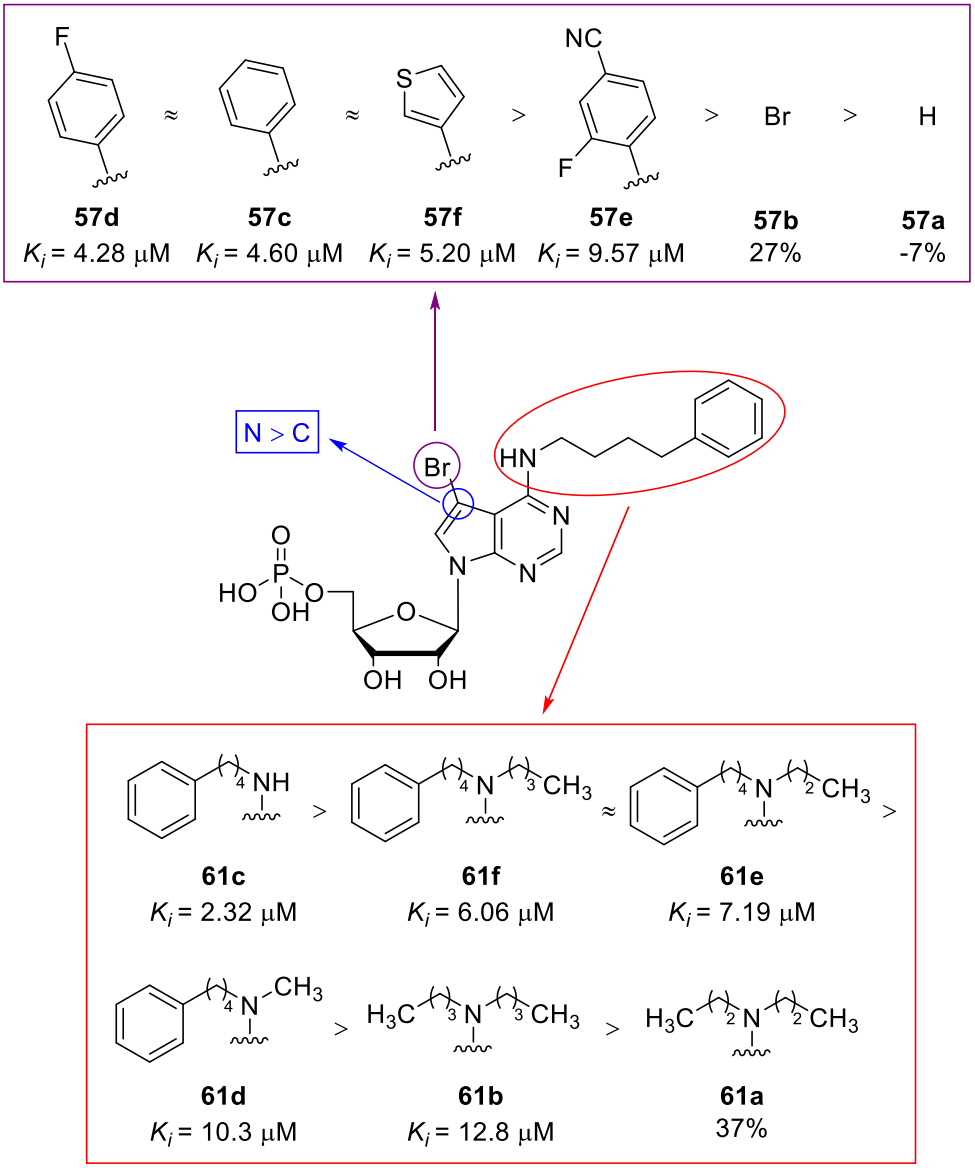


Figure 4.7. SARs of 7-deaza-AMP derivatives and analogs.

4.5 Pharmacological evaluation of potent 8-BuS-AMP derivatives and analogs at soluble human CD39

Seven selected nucleoside monophosphates which are potent at human CD39 were also tested for their inhibitory potency at soluble human CD39 using 150 μM ATP and 50 μM inhibitor by capillary electrophoresis assay (n = 3), which was described in **8.5.3**. The biological testing was performed by Salahuddin Mirza.

8-Butylthio-N^6-(4-phenylbutyl)-AMP (**48b**) is one of the most potent CD39 inhibitors described so far (CD39, K_i = 0.444 μM; soluble CD39, 0.0589 μM). 7-Bromo-7-deaza-

N^6-(4-phenylbutyl)-AMP (**61c**) is the most potent 7-deaza-AMP analog as CD39 inhibitor (CD39, K_i = 2.32 µM; soluble CD39, 1.95 µM,). Except **48a**, all compounds showed higher inhibitory activity at soluble CD39 than at membrane-bound CD39 with an up to 7-fold difference. The predicted reason is that the more flexible structure of soluble CD39 is easier to capture and interact with molecules in mammals than membrane-bound CD39. Concentration-inhibition curves of **48b** and **61c** are depicted in **Figure 4.8**, and all results are summarized in **Table 4.7**.

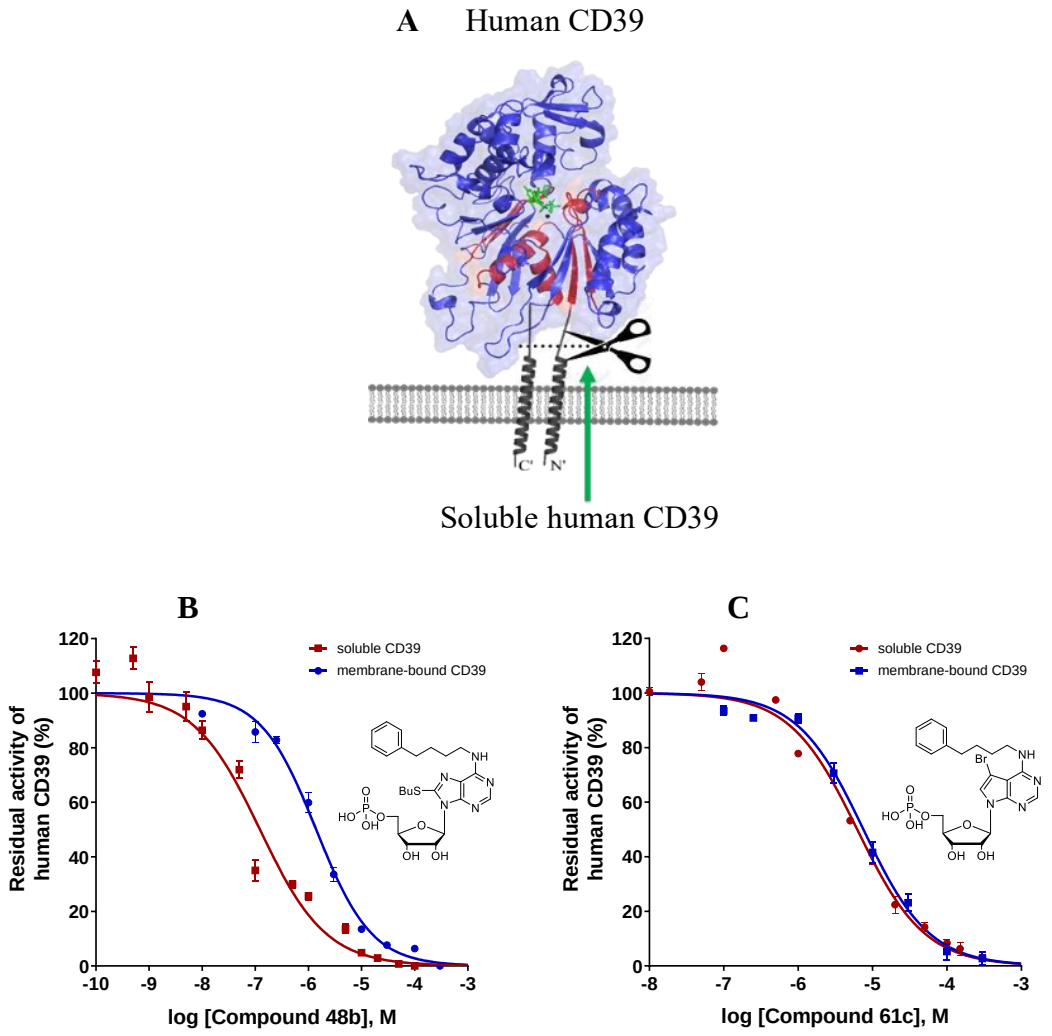
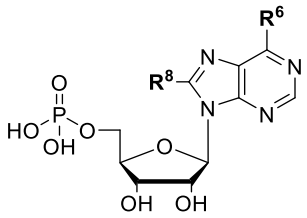
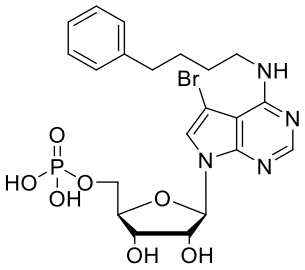


Figure 4.8. Concentration-inhibition curves of **48b** and **61c** at membrane-bound and soluble human CD39. **A.** Structure of membrane-bound and soluble human CD39. **B** and **C.** Concentration-inhibition curves.

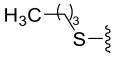
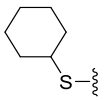
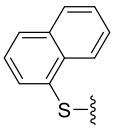
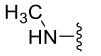
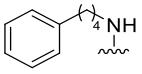
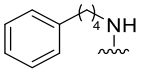
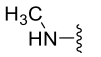
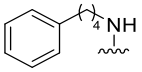
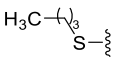
Table 4.7. Inhibitory potency of selected nucleoside monophosphates at soluble and membrane-bound human CD39



1i, 28j, 28r, 30a, 38h, 48a-b



61c

Compd.	R^6	R^8	K_i ± SEM (µM)	
			soluble CD39	membrane-bound CD39
8-BuS-AMP (1i)	NH_2		0.733 ± 0.386	0.847 ± 0.194
28j	NH_2		0.232 ± 0.011	0.768 ± 0.052
28r	NH_2		0.469 ± 0.031	0.735 ± 0.056
30a	NH_2		2.29 ± 0.36	4.89 ± 1.23
38h		H	5.93 ± 3.31	7.08 ± 0.68
48a			8.60 ± 1.19	1.54 ± 0.36
48b			**0.0589 ± 0.0031**	**0.444 ± 0.061**
61c	*for structure see above*		1.95 ± 0.29	2.32 ± 0.54

4.6 Selectivity studies of selected potent 8-BuS-AMP derivatives and analogs versus other ectonucleotidases

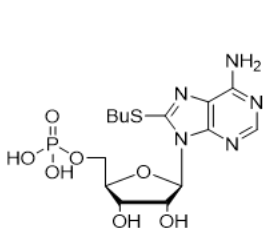
Potent CD39 inhibitors **1i**, **28j**, **28r**, **30a**, **38h** and **48b** were further investigated at NTPDase2, -3, -8, NPP1, -3, -4, -5, CD38 and CD73 at a concentration of 50 µM (n = 3). Results are summarized in **Table 4.8**. The biological testing was performed by Laura Schäkel, Salahuddin Mirza, Vittoria Lopez, Katharina Sylvester and Riham Idris, respectively.

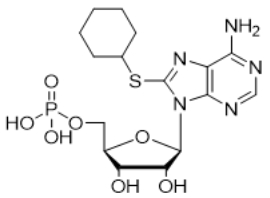
8-BuS-AMP (**1i)** was selective towards NTPDase2, -3, -8, NPP1, -3, -5 and CD38 but moderately inhibited NPP4. It was also potent at CD73. However, in a previous paper **1i** was reported to be inactive at NTPDase2, -3 and -8, and slightly active at NPP1, -3 and CD73.[77] The results are different from our data, especially **1i** was very active at CD73 according to our results.

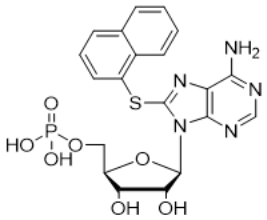
Compound **28j** was selective towards NTPDase2, -3, -8, NPP1, -3, -4, -5 and CD38 but strongly inhibited CD73. Compound **28r** was selective towards NTPDase8, NPP1, -3, -4, -5 and CD38 but moderately inhibited NTPDase2, and it was also potent at NTPDase3 and CD73. Compound **30a** was selective towards NTPDase2, -3, -8, NPP1, -3, -4, -5, CD38 and CD73. It is the first selective CD39 inhibitor in our current study and will be useful as a pharmacological tool compound to study CD39 inhibition specifically.

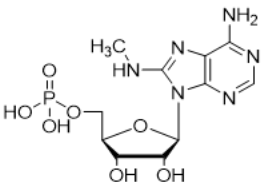
Compound **38h** was selective towards NTPDase3, -8, NPP1, -3, -4, -5 and CD38 but strongly inhibited NTPDase2 and CD73. Compound **48b** was selective towards NTPDase8, NPP1, -3, -4, -5 and CD38 but moderately inhibited NTPDase3. It was also potent at NTPDase2 and CD73.

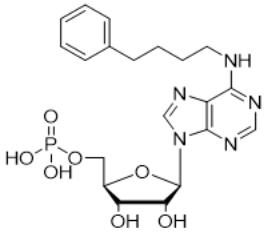
Table 4.8. Selectivity studies of potent 8-BuS-AMP and its derivatives at human ectonucleotidases

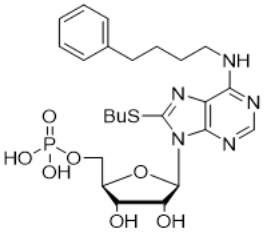

8-BuS-AMP (1i)


28j


28r


30a


38h


48b

Enzyme	K_i ± SEM (μM) (or % inhibition at 50 μM)					
	8-BuS-AMP (1i)	28j	28r	30a	38h	48b
CD39	0.847 ± 0.194	0.768 ± 0.052	0.735 ± 0.056	4.89 ± 1.23	7.08 ± 0.68	0.444 ± 0.061
soluble CD39	0.733 ± 0.386	0.232 ± 0.011	0.469 ± 0.031	2.29 ± 0.36	5.93 ± 3.31	0.0589 ± 0.0031
NTPDase2	>50 (15%)	82.9 ± 17.7	24.3 ± 5.9	>50 (-2%)	1.97 ± 0.62	2.62 ± 0.28
NTPDase3	>50 (22%)	54.8 ± 9.2	4.74 ± 0.45	>50 (33%)	>50 (35%)	29.1 ± 2.3
NTPDase8	>50 (-11%)	>50 (0%)	>50 (8%)	>50 (-26%)	>50 (-11%)	>50 (38%)
NPP1	>50 (25%)	>50 (9%)	>50 (31%)	>50 (15%)	>50 (27%)	>50 (15%)
NPP3	>50 (3%)	>50 (19%)	>50 (14%)	>50 (3%)	>50 (1%)	>50 (23%)
NPP4	27.8 ± 15.0	>50 (4%)	>50 (19%)	>50 (12%)	>50 (32%)	50 (14%)
NPP5	>50 (15%)	>50 (18%)	>50 (10%)	>50 (12%)	>50 (25%)	>50 (2%)
CD38	>50 (15%)	>50 (23%)	>50 (44%)	>50 (12%)	>50 (20%)	>50 (42%)
CD73	2.26 ± 0.130	1.89 ± 0.10	0.739 ± 0.090	>50 (28%)	0.337 ± 0.111	0.817 ± 0.032

4.7 Metabolic stability

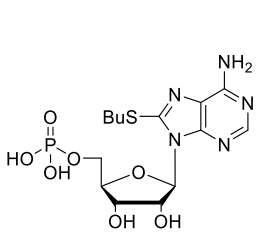
Compounds 8-BuS-AMP (**1i**), **30a**, **38h** and **48b** were further investigated for the metabolic stability which is mainly responsible for drug metabolism *in vivo*. The experiment was performed by Pharmacelsus, Saarbrücken, Germany (https://www.pharmacelsus.com/services/in-vitroadme/) using human and mouse liver microsomes (0.5 mg/mL, mixed gender, pooled) at a concentration of 1 µM. Data points represent means of two separate experiments performed in duplicates.

To ensure that degradation was caused by microsomal enzymes and not due to chemical instability, the stock solution was analyzed by LC/ESI-MS analysis and compounds were conformed to be chemically stable.

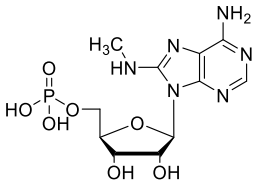
8-BuS-AMP (**1i**) was metabolically stable with half-lives of 462 min and 182 min in human and mouse liver microsomes, respectively. Compound **30a** appeared to be metabolically highly unstable with a half-life of less than 1 min. Compound **38h** was more slowly degraded by human and mouse liver microsomes. Its half-lives were 6 min (human) and 15 min (mouse). Furthermore, compound **48b** was proved to be stable with a half-life of 84 min in human liver microsomes. These bulky residues at the 8- or N^6-position could abolish or delay the degradation by metabolic enzymes. Data are collected in **Table 4.9**.

Therefore, **48b** and 8-BuS-AMP (**1i**) can be recommended for the use *in vivo* and *vitro* studies. Compounds **30a** and **38h** are more suitable as tool compounds for *in vitro* studies.

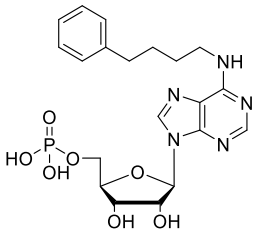
Table 4.9. Metabolic stability evaluation of selected potent CD39 inhibitors in human and mouse liver microsomes ($t_{1/2}$: half-life; CL_{int}: internal clearance.)



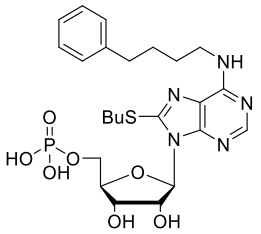
8-BuS-AMP (1i)



30a



38h



48b

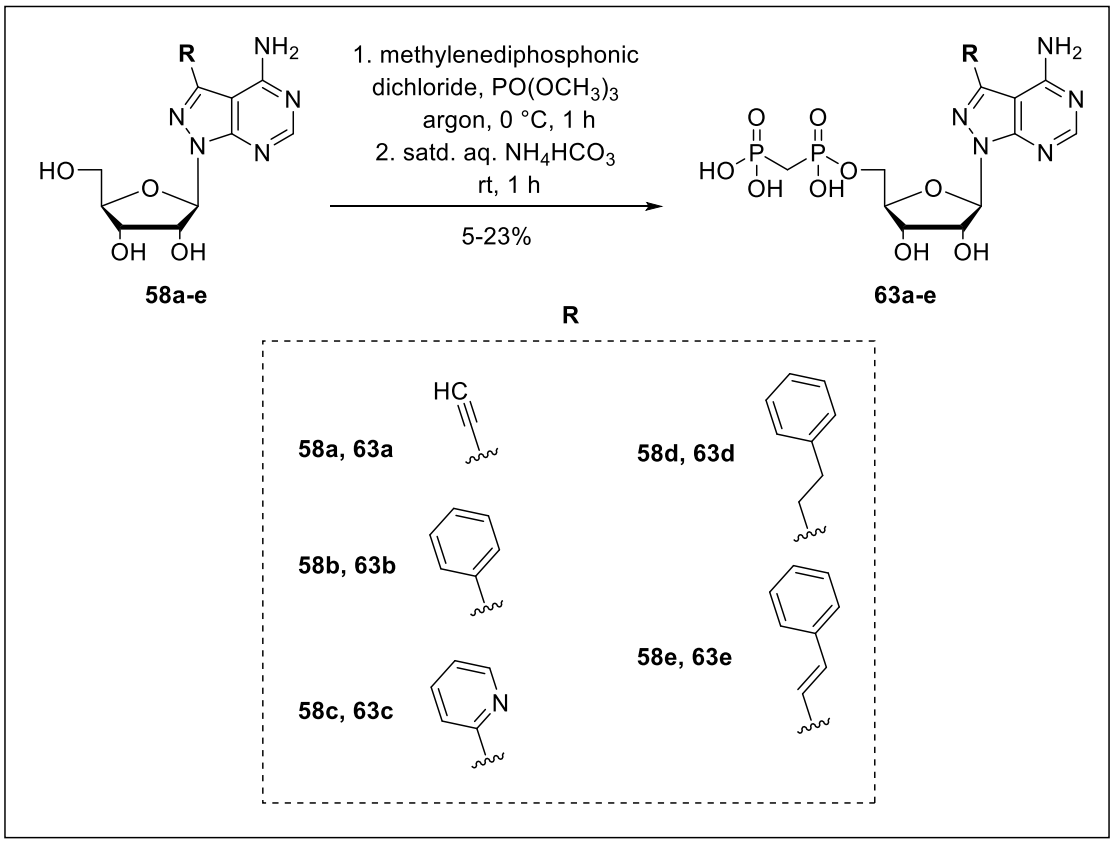
Compd.	Human		Mouse	
	$t_{1/2}$ (min)	CL_{int} (µL/min/mg protein)	$t_{1/2}$ (min)	CL_{int} (µL/min/mg protein)
8-BuS-AMP (1i)	462	3.0	182	7.6
30a[79]	n.a.[a]	n.a.	n.a.	n.a.
38h[79]	6	248.0	15	92.2
48b	84	16.4	-	-

[a]n.a.: no analyte detected.

5 Results and discussion – Part III: Development of novel AMPCP derivatives and analogs as inhibitors of CD73

5.1 Synthesis of pyrazolopyrimidine nucleotides (63a-e)

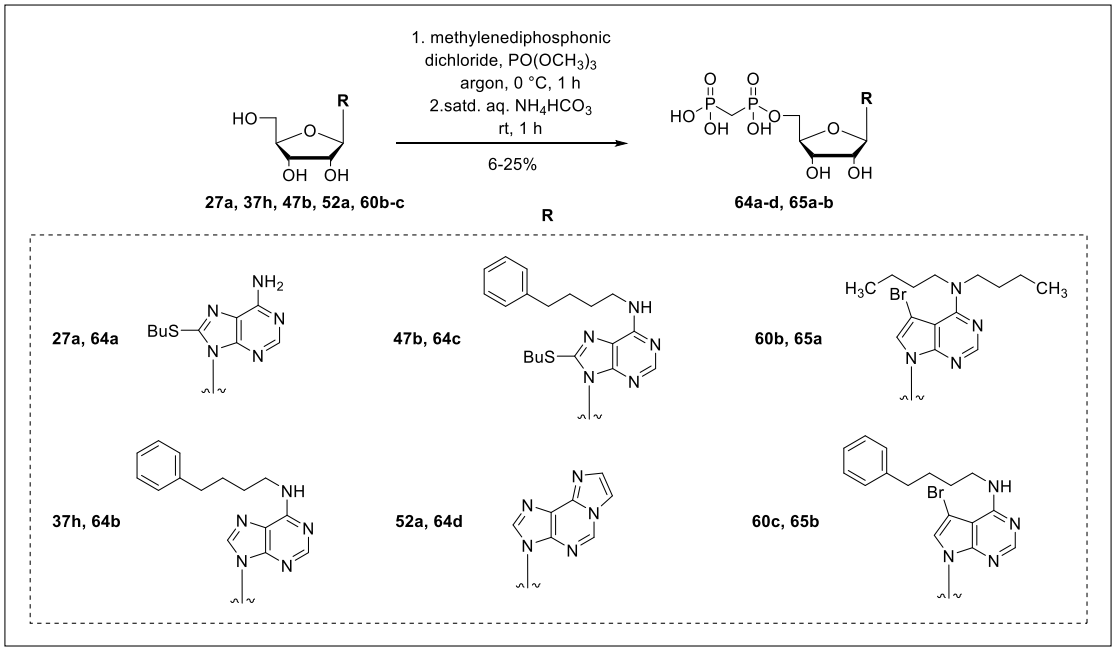
The nucleosides (**58a-e**) for diphosphorylation were generously offered by Prof. Dr. Serge Van Calenbergh. The series of compounds was synthesized by a reported diphosphorylation procedure with small modifications.[83] The synthetic route is depicted in **Scheme 5.1**. The appropriate nucleoside was dissolved in $PO(OCH_3)_3$ and reacted with methylenediphosphonic dichloride at 0 °C under argon. After the reaction was completed, the product was hydrolyzed by saturated NH_4HCO_3 (aq.). $PO(OCH_3)_3$ was finally removed by extracting with *tert*-butylmethylether. The crude aqueous mixture was lyophilized and then purified by preparative HPLC.



Scheme 5.1. Synthesis of pyrazolopyrimidine nucleotides (**63a-e**).

5.2 Synthesis of AMPCP derivatives and analogs (64a-d, 65a-b)

Some intermediates **27a**, **37h**, **47b**, **52a** and **60b-c** were synthesized during my previous work developing CD39 inhibitors and they were also diphosphorylated for developing new CD73 inhibitors. The final products **64a-d**, **65a-b** were generated (**Scheme 5.2**) using the same procedure as for **63a**.



Scheme 5.2. Synthesis of AMPCP derivatives and analogs (**64a-d**, **65a-b**).

5.3 Pharmacological evaluation of pyrazolopyrimidine nucleotides at soluble human CD73

All synthesized pyrazolopyrimidine nucleotides were tested for their inhibitory potency at soluble human CD73 using 5 µM AMP as a substrate and 50 µM inhibitor by radiometric assay (n = 3), which was described in **8.5.4**. The K_i values were calculated for potent inhibitors from the obtained IC_{50} values using the Cheng-Prusoff equation. The biological testing was performed by Katharina Sylvester.

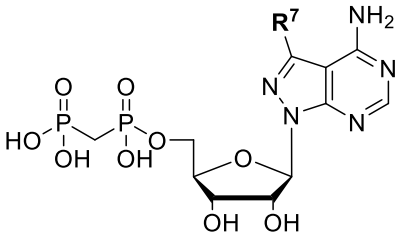
5.3.1 Structure-activity relationships of pyrazolopyrimidine nucleotides

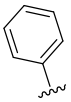
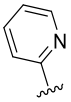
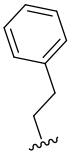
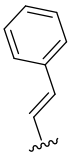
In the present study, 7-substituted pyrazolopyrimidine nucleotides as CD73 inhibitors were investigated. As shown in **Table 5.1**, all the new synthesized pyrazolopyrimidine nucleotides displayed K_i values between 0.00886 μM and 0.160 μM at soluble human CD73. Their SARs are depicted in **Figure 5.1**.

Among these CD73 inhibitors, the ethynyl substituent (**63a** K_i = 0.00886 μM) is superior to aryl and aralkyl substituents. The replacement of phenyl by 2-pyridyl was well tolerated comparing **63b** (K_i = 0.143 μM) with **63c** (K_i = 0.160 μM). On the other hand, a flexible 2-phenylethyl substituent (**63d**, K_i = 0.0301 μM) was almost 4-fold better than the unsaturated 2-styryl substituent (**63e**, K_i = 0.111 μM).

This study filled the gap studying modifications at the 7-position of AMPCP derivatives and analogs, resulting in an inhibitor **63a** with low nanomolar potency. It will be interesting to study its residence time as well as potential species, differences (mouse, rat and human) of these compounds.

Table 5.1. Inhibitory potency of pyrazolopyrimidine nucleotides at soluble human CD73



Compd.	R^7	K_i ± SEM (μM) (or % inhibition at 50 μM)
63a		**0.00886 ± 0.000832**
63b		0.143 ± 0.00993
63c		0.160 ± 0.0176
63d		0.0301 ± 0.00259
63e		0.111 ± 0.00674

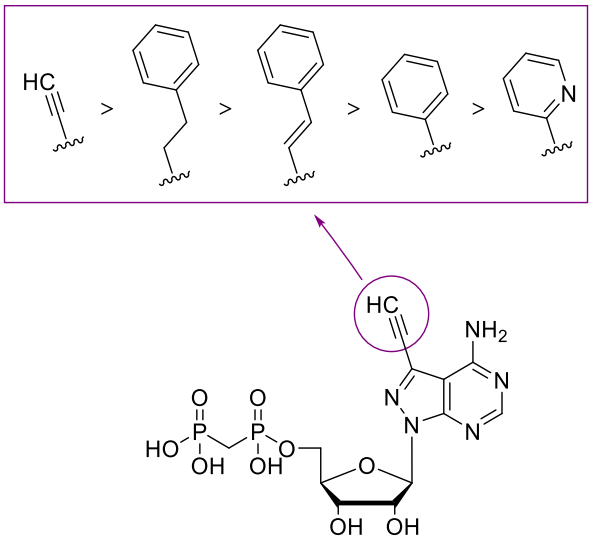


Figure 5.1. SARs of pyrazolopyrimidine nucleotides.

5.4 Pharmacological evaluation of further AMPCP derivatives and analogs at soluble human CD73

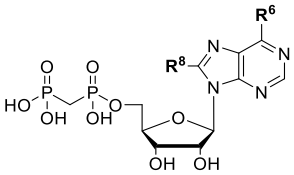
The biological testing was performed by Patrick Riziki using the same method as described in **5.3**.

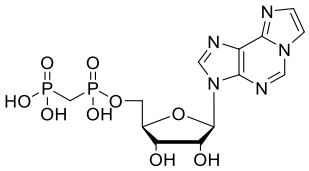
5.4.1 Structure-activity relationships of further AMPCP derivatives and analogs

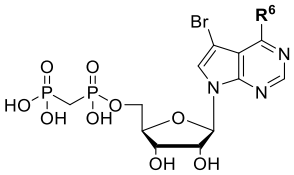
As shown in **Table 5.2**, the synthesized AMPCP derivatives and analogs displayed K_i values between 0.00291 µM and 15 µM at soluble human CD73. Based on the AMPCP scaffold, some substituents were investigated at the 8-, N^6- and 1-positions.

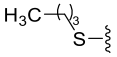
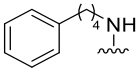
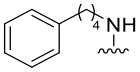
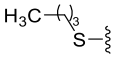
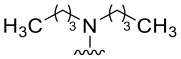
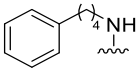
Among these CD73 inhibitors, **64b** (K_i = 0.00291 µM) exhibited the best K_i value compared to other inhibitors due to its lipophilic, bulky 4-phenylbutyl substituent at its N^6-position. The addition of an 8-butylthio substituent to **64b** (in **64c**) highly decreased the activity by 794-fold. The compound with a single 8-butylthio substituent (**64a**, K_i = 0.0882 µM) displayed a medium activity similar to that of AMPCP. These results show that the 8-position is not suitable for modification of AMPCP derivatives and analogs to increase their potency as CD73 inhibitors. Bridging the N^6- and 1-positions by etheno (**64d**, K_i = 15 µM) led to a low activity. An N^6-4-phenylbutyl substituent (**65b**, K_i = 0.0879 µM) was superior to N^6,N^6-dibutyl substituent (**65a**, K_i = 0.329 µM) when adding them to 7-bromo-7-deaza-AMPCP. Furthermore, the 7-bromo-7-deaza-AMPCP scaffold decreased the activity of AMPCP scaffold by 30-fold when the N^6-4-phenylbutyl substituent was present.

Table 5.2. Inhibitory potency of AMPCP derivatives and analogs at soluble human CD73


64a-c


64d


65a-b

Compd.	R^6	R^8	K_i ± SEM (µM)
64a	NH_2		0.0882 ± 0.0242
64b		H	**0.00291 ± 0.000644**
64c			2.31 ± 0.61
64d	*for structure see above*		15 ± 14
65a		-	0.329 ± 0.107
65b		-	0.0879 ± 0.0168

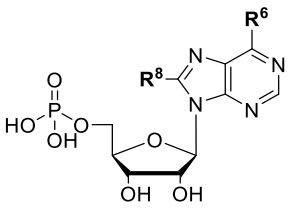
6 Development of dual CD39/CD73 inhibitors

Some selected potent CD39/CD73 inhibitors were tested at both human CD39 and soluble human CD73 for developing dual CD39/CD73 inhibitors. For soluble human CD73, the corresponding biological testing was performed as previously described in **5.3** (by Katharina Sylvester, Patrick Riziki and Riham Idris). For human CD39, the biological testing was performed as previously described in **4.3** (by Laura Schäkel and Areso Ahmadsay).

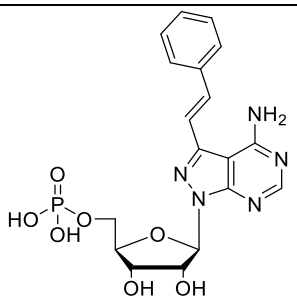
6.1 Selected potent CD39 inhibitors as dual CD39/CD73 inhibitors

Almost all of the selected potent CD39 inhibitors displayed very similar K_i values in the range of 0.2-10 µM for both CD39 and CD73 except **30a** and **48a**. Compounds **30a** and **48a** are potent CD39 inhibitors, but their activity is much lower at CD73. Most of them are very promising to develop novel dual CD39/CD73 inhibitors. Their inhibitory potency at CD39 and CD73 is collected in **Table 6.1**.

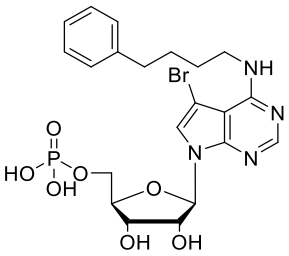
Table 6.1. Inhibitory potency of nucleoside monophosphates at human CD39 and CD73



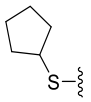
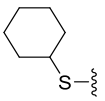
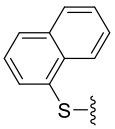
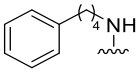
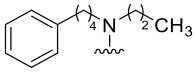
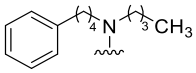
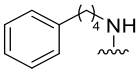
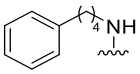
1i, 28d, 28i, 28j, 28r, 30a, 38h, 38j-k, 48a-b, 44d, 44f



59



61c

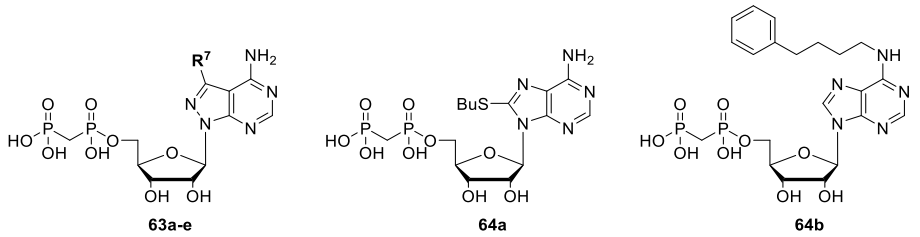
Compd.	R^6	R^8	K_i ± SEM (μM) (or % inhibition at 50 μM)	
			CD39	soluble CD73
8-BuS-AMP (1i)	NH_2	$S(CH_2)_3CH_3$	0.847 ± 0.194	2.26 ± 0.130
28d	NH_2	$S(CH_2)_2CH_3$	1.46 ± 0.17	1.77 ± 0.218
28i	NH_2		1.10 ± 0.12	2.33 ± 0.242
28j	NH_2		0.768 ± 0.052	1.86 ± 0.104
28r	NH_2		0.735 ± 0.056	0.739 ± 0.090
30a	NH_2	$NHCH_3$	4.89 ± 1.23	(28%)
38h		H	7.08 ± 0.68	0.337 ± 0.111
38j		H	5.10 ± 0.59	0.857 ± 0.102
38k		H	3.13 ± 0.66	10.3 ± 2.72
48a		$NHCH_3$	1.54 ± 0.36	20.2 ± 5.56
48b		$S(CH_2)_3CH_3$	0.444 ± 0.061	0.817 ± 0.032
44d[a]	$NHCH_3$	$S(CH_2)_3CH_3$	1.83 ± 0.33[79]	0.239 ± 0.020
44f[a]	$N(CH_2CH_3)_2$	$S(CH_2)_3CH_3$	1.20 ± 0.09	7.06 ± 1.02
59	*for structure see above*		1.89 ± 0.07	3.60 ± 1.80
61c	*for structure see above*		2.32 ± 0.54	1.33 ± 0.23

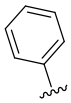
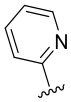
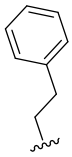
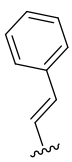
[a]Compounds were synthesized by Dr. Constanze Cerine Schmies.

6.2 Selected potent CD73 inhibitors as dual CD39/CD73 inhibitors

Three of these selected potent CD73 inhibitors also displayed activity at CD39. Compounds **63a-e** are potent CD73 inhibitors, but their activity significantly decreased at CD39. However, **63e** and **64a-b** still displayed K_i values in the range of 3.8-12.2 µM for CD39. Then only three CD73 inhibitors **63e** and **64a-b** are very promising to develop novel dual CD39/CD73 inhibitors. Their inhibitory potency at CD39 and CD73 is collected in **Table 6.2**.

Table 6.2. Inhibitory potency of AMPCP derivatives and analogs at human CD39 and CD73



Compd.	R^7	K_i ± SEM (µM) (or % inhibition at 50 µM)	
		CD39	soluble CD73
63a		(55%)	0.00886 ± 0.000832
63b		(61%)	0.143 ± 0.00993
63c		(62%)	0.160 ± 0.0176
63d		(57%)	0.0301 ± 0.00259
63e		3.82 ± 0.31	0.111 ± 0.00674
64a	*for structure see above*	12.2 ± 1.2	0.0882 ± 0.0242
64b	*for structure see above*	4.03 ± 0.31	0.00291 ± 0.000644

6.3 Comparison of nucleoside monophosphates and methylenediphosphonates as dual CD39/CD73 inhibitors

At CD39, the monophosphate **38h** (K_i = 7.08 μM) showed slightly decreased inhibitory activity compared to the methylenediphosphonate **64b** (K_i = 4.03 μM) of the N^6-(4-phenylbutyl)adenosine nucleotide. However, the monophosphate **1i** (K_i = 0.847 μM) obviously increased the inhibitory activity over the methylenediphosphonate **64a** (K_i = 12.2 μM) of the 8-(butylthio)adenosine nucleotide. It is not sure whether nucleoside monophosphates or methylenediphosphonates are better as CD39 inhibitors based on the current data. Nucleoside monophosphate and methylenediphosphonate do not display large differences. This indicates that we should screen more nucleoside methylenediphosphonates in the future to develop novel CD39 or dual CD39/CD73 inhibitors.

At CD73, the nucleoside methylenediphosphonates are superior to corresponding nucleoside monophosphates absolutely, e.g., **64b** (K_i = 0.00291 μM) and **38h** (K_i = 0.337 μM).

CD39 inhibitors block the pathway of ATP→AMP while CD73 inhibitors block the pathway of AMP→ADO. They may block the hydrolysis pathway of ATP→ADO synergistically and efficiently to reverse cancer immunosuppression. The dual CD39/CD73 inhibitor is extremely promising to provide a better inhibition for cancer immunotherapy than the corresponding single CD39/CD73 inhibitor.

7 Summary and outlook

In conclusion, 78 ticlopidine, and 74 8-BuS-AMP derivatives and analogs including 14 7-deaza-AMP derivatives were successfully synthesized and obtained in high purity. They were characterized at human CD39 (membrane-bound and soluble CD39, respectively). Moreover, 11 AMPCP derivatives and analogs were prepared in high purity and characterized at soluble human CD73 (**Figure 7.1**). More than 100 nucleosides were successfully synthesized and obtained in high purity as precursors for the preparation of various nucleotides.

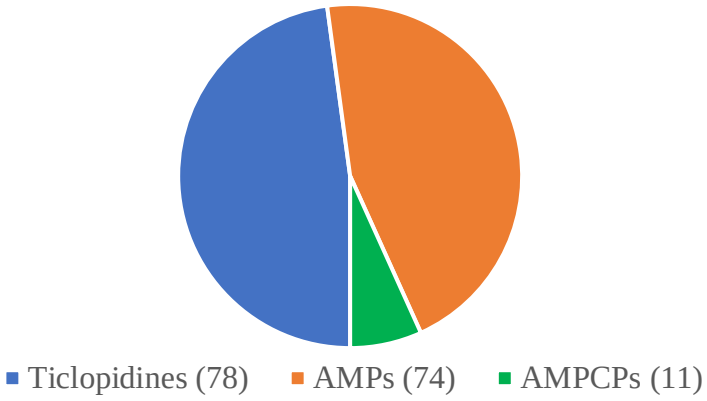


Figure 7.1. Overview of synthesized final products.

7.1 Development of novel ticlopidine-derived CD39 inhibitors

Ticlopidine (**1e**, IC_{50} = 81.7 µM) is a non-competitive, allosteric inhibitor of CD39. The SARs of the synthesized ticlopidine derivatives and analogs are depicted in **Figure 7.2**.

In this study, many modifications were performed based on the scaffold of ticlopidine, i.e., thienotetrahydropyridines, and the analogous tetrahydroisoquinolines. Especially, substitution of the phenyl ring of the benzyl residue was broadly explored. In particular, most halogen substitution was found to be well tolerated. The extension of the methylene unit of ticlopidine decreased CD39-inhibitory potency. Methylation of the N-atom of the tetrahydropyridine ring was not tolerated, while methylation at both neighboring positions was permitted.

Thienotetrahydropyridines such as ticlopidine are prodrugs of irreversible $P2Y_{12}$ receptor antagonists; they are metabolized by cytochrome P450 enzymes in the liver yielding reactive thiols (or sulfenic acids) that react irreversibly with a cysteine residue in the extracellular domain of the ADP-activated $P2Y_{12}$ receptor resulting in antithrombotic activity.[74] In most cases, the inhibitory potency of thienotetrahydropyridines was found to be equal to that of corresponding tetrahydroisoquinolines which cannot be metabolized to yield thiols. Therefore, we replaced the thiophene ring by a phenyl ring. Compounds **8d** (IC_{50} = 49.4 µM), **8k** (IC_{50} = 39.0 µM), **8m** (IC_{50} = 43.6 µM) and **8n** (IC_{50} = 48.1 µM) are potent tetrahydroisoquinolines which will not be converted to thiol-reactive $P2Y_{12}$ receptor antagonists.

Compound **8k** (PSB-21139, 2-(2,5-dichlorobenzyl)-1,2,3,4-tetrahydroisoquinoline) is currently one of the best lead structures for further optimization displaying a K_i value of 51.4 µM. Further research on ticlopidine derivatives and analogs as inhibitors of CD39 may facilitate the development of immune-checkpoint inhibitors for the immunotherapy of cancers and infections.

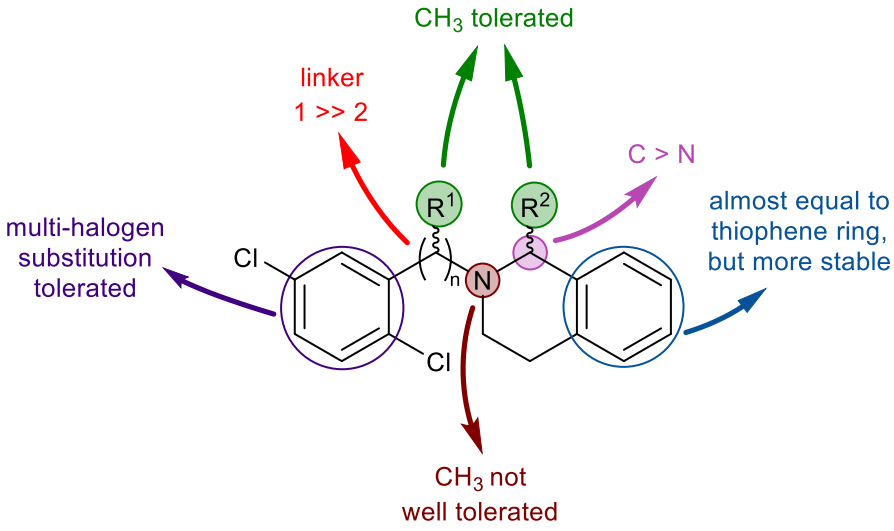


Figure 7.2. SARs of ticlopidine-derived CD39 inhibitors.

7.2 Development of novel 8-BuS-AMP-derived CD39 inhibitors

The metabolically stable 8-BuS-AMP (**1i**) is a competitive inhibitor of CD39. The SARs of the synthesized 8-BuS-AMP derivatives and analogs are depicted in **Figure 7.3**.

A comparison of all synthesized 8-BuS-AMP derivatives and analogs shows that S-linked substituents at the 8-position of the AMP scaffold normally lead to more potent CD39 inhibitors than other substituents at different positions. The combination of some substituents at the 8- and N^6-positions increased the inhibitory potency. On the other hand, substituents at the 2-position, an etheno-bridge between the 1- and N^6-positions, and most substituents at the 6-position on the AMP scaffold were not well tolerated. N^6-(4-Phenylbutyl)-AMP (**38h**) and the corresponding N^6-disubstituted derivatives bearing an additional N^6-methyl- (**38i**), N^6-ethyl-,[79] N^6-propyl- (**38j**) and N^6-butyl- (**38k**) residue were the only N^6-substituted AMP derivatives of the present series that potently inhibited CD39. More combinations of beneficial 8- and N^6-substituents on the AMP scaffold should be investigated in the future.

Compounds **59** and **61c** are novel 7-deaza-AMP analogs identified as potent CD39 inhibitors. Replacing the 7-N by 7-CH in AMP derivatives resulted in inactive compounds but adding aromatic substituents to the 7-position recovered the inhibitory potency. Compound **59** displayed potent inhibition having an N-atom at the 8-position. Based on the results obtained for 8-BuS-AMP derivatives and analogs, the 8-position of 7-deaza-AMP is also very promising to do further modifications for increasing the inhibitory potency. Furthermore, the combination of beneficial modifications at the 8- and/or 7- and/or N^6-positions on the 7-deaza-AMP scaffold may be promising.

Nucleoside monophosphates, nucleoside methylenediphosphonates, and one nucleoside methylthiophosphate were compared with regard to their potency as CD39 inhibitors. The nucleoside methylthiophosphate **54** showed decreased inhibitory potency but the corresponding monophosphate and methylenediphosphonate (**1i** and **64a**) displayed similarly potent inhibition (see **Tables 4.1**, **4.4** and **6.2**). More nucleoside methylenediphosphonates with beneficial substituents are promising to be investigated in the future.

Compound **30a** (PSB-20148, CD39, K_i = 4.89 µM; soluble CD39, K_i = 2.29 µM) is the first selective CD39 inhibitor described so far. It can be recommended for the use as a pharmacological tool compound for *in vitro* studies on CD39, but not for *in vivo* studies due to its metabolic instability. Compounds **48e** (CD39, K_i = 0.329 µM) and **48b** (PSB-20110, CD39, K_i = 0.444 µM; soluble CD39, K_i = 0.0589 µM; half-life, 84 min in human liver microsomes) are the most potent CD39 inhibitors of the present series; they may represent suitable lead structures for further drug development efforts.

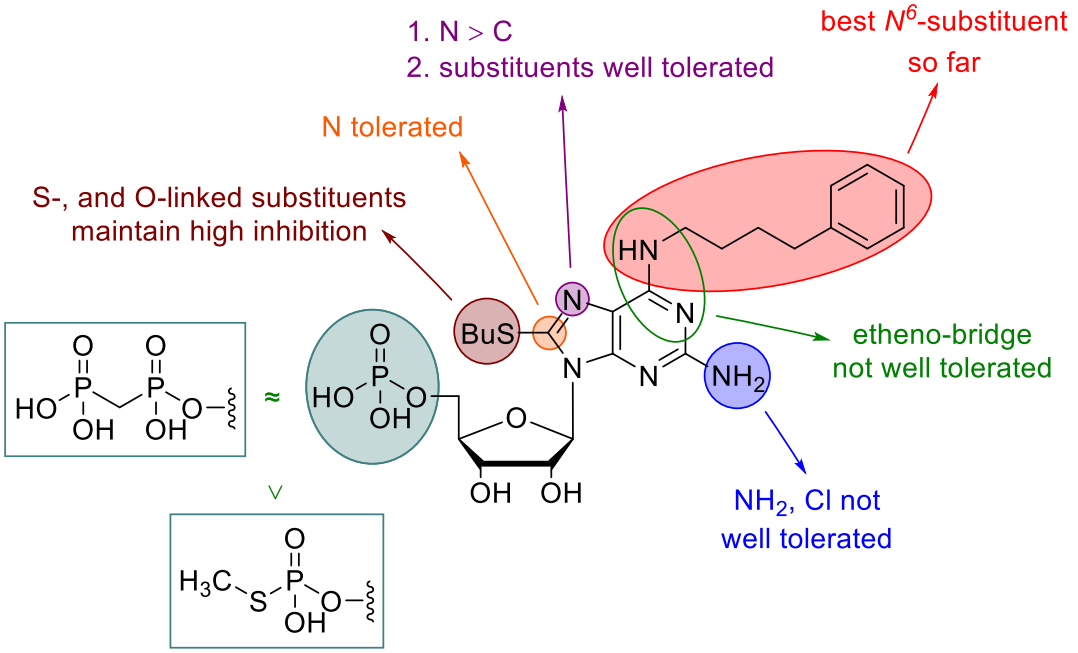


Figure 7.3. SARs of 8-BuS-AMP-derived CD39 inhibitors.

7.3 Development of novel AMPCP-derived CD73 inhibitors

AMPCP (**1s**) has been previously used as a lead structure to obtain potent CD73 inhibitors.[83,97,105,107-108] In the present study, we extended the published SARs. The SARs of the synthesized AMPCP derivatives and analogs are depicted in **Figure 7.4**.

A series of pyrazolopyrimidine nucleotides as novel CD73 inhibitors modified at the 7-position has resulted in K_i values in the low nanomolar range. The introduction of different substituents at the 7-position of pyrazolopyrimidine nucleotides was well tolerated.

Overall, bulky and lipophilic substituents at the N^6-position, combined beneficial substituents at the 2-position of the AMPCP scaffold lead to potent CD73 inhibitors.[83,97,105,107-108] Substituents at the 8-position, or an etheno-bridge between the 1- and N^6-positions of the AMPCP scaffold were not well tolerated. Nucleoside methylenediphosphonates were found to be superior to corresponding nucleoside monophosphates as CD73 inhibitors (see **Tables 6.1** and **6.2**).

Compounds **63a** (PSB-21310, K_i = 0.00886 µM), **63d** (K_i = 0.0301 µM) and **64b** (PSB-21282, K_i = 0.00291 µM) were identified as the most potent CD73-inhibiting nucleotides in this study. Based on the current results, a combination of the most beneficial substituents at the 7- and/or N^6- and/or 2-positions on the AMPCP scaffold could be promising to develop even more potent CD73 inhibitors.

CD73 is currently a major target in anti-cancer research; it bears promise to become one of next immuno-oncological targets for cancer immunotherapy.

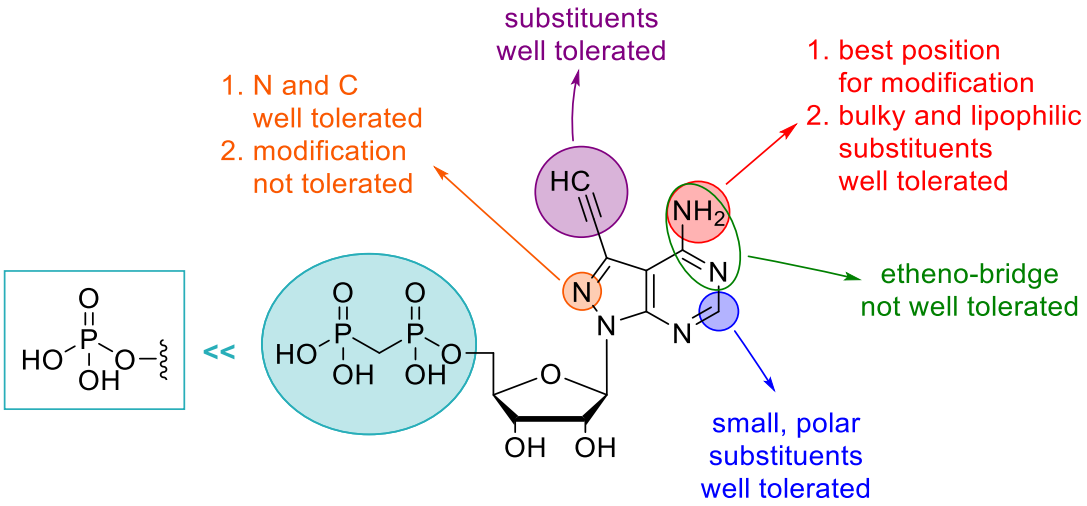


Figure 7.4. SARs of AMPCP-derived CD73 inhibitors.

7.4 Development of dual CD39/CD73 inhibitors

In this study, nucleoside monophosphates and methylenediphosphonates are for the first time described as dual CD39/CD73 inhibitors. Altogether, 15 potent CD39 inhibitors, and 7 potent CD73 inhibitors were tested at both human CD39 and CD73.

Among them, 13 CD39 inhibitors (**1i**, **28d**, **28i-j**, **28r**, **38h**, **38j-k**, **48b**, **44d, 44f**, **59**, **61c**) and 3 CD73 inhibitors (**63e**, **64a-b**) were discovered to be active as dual CD39/CD73 inhibitors (see **Tables 6.1** and **6.2**).

The hydrolysis pathways ATP→AMP and AMP→ADO are blocked by CD39 and CD73 inhibitors, respectively. Dual CD39/CD73 inhibitors may act synergistically inhibiting the hydrolysis of ATP→ADO. These 16 dual CD39/CD73 inhibitors are very promising to be further developed in the future for cancer immunotherapy. Dual CD39/CD73 inhibitors are predicted to be superior to compounds inhibiting only a single target, CD39 or CD73, for the immunotherapy of cancers and infections.

8 Experimental section

8.1 General

Unless stated otherwise, all reagents used in the synthesis were obtained commercially and used without further purification. Thin layer chromatography (TLC) was performed using TLC silica gel 60 F_{254} aluminum 0.255 mm plates, and spots were visualized by UV at 254 nm. Column chromatography was performed using Merck 60 silica gel (0.063-0.200 mm). Flash chromatography was performed on a Büchi system using 24 g HP silica column (RediSep® Rf). Semi-preparative HPLC was performed on a Knauer Smartline 1050 HPLC system equipped with a Eurospher-100 C18 column (250 mm × 20 mm, particle size 10 µm). Organic solutions were concentrated at reduced pressure using a sliding vane rotary vacuum pump (Vacuubrand GmbH). Inorganic solutions were concentrated at reduced pressure using a CHRIST ALPHA 1-4 LSC freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH). Melting points were determined with a BÜCHI Melting Point B-545 apparatus.

The mass of isolated products was determined by electrospray ionization (ESI) mass spectra obtained on a LC-MS instrument (Applied Biosystems API 2000 LC-DAD-UV-MS/MS, HPLC Agilent 1100). The LC-MS samples were prepared by dissolving 0.2-1.5 mg of compounds in 0.5-1.5 mL H_2O/MeOH (1:1) containing 2 mM ammonium acetate. A sample of 10 µL was injected into the HPLC instrument and elution was performed with a gradient of H_2O/MeOH or H_2O/MeCN (containing 2 mM ammonium acetate) from 90/10 to 0/100 for 20 min at a flow rate of 250 µL/min. UV absorption was detected from 190 to 400 nm using a diode array detector.

^{1}H-, ^{13}C- and ^{31}P-NMR spectra were recorded on a Bruker Avance 500 and 600 MHz spectrometers. DMSO-d_6, D_2O or $CDCl_3$ was employed as a solvent at 25 or 30 °C. Chemical shifts are reported in parts per million (ppm) relative to deuterated solvents (DMSO-d_6: δ ^{1}H, 2.50 ppm; ^{13}C, 39.52 ppm. D_2O: δ ^{1}H, 4.79 ppm. $CDCl_3$: δ ^{1}H, 7.26 ppm; ^{13}C, 77.16 ppm.).[168] Coupling constants J are given in Hertz, and spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet).

8.2 Purification by semi-preparative HPLC

The UV absorption was detected from 220 to 400 nm. After purification by semi-preparative HPLC, fractions were collected, and appropriate fractions pooled, evaporated to remove the organic solvents, and finally lyophilized to remove aqueous solvents, yielding the desired product.

8.2.1 Purification of some ticlopidine derivatives and analogs by semi-preparative HPLC

Even after the purification by silica gel column chromatography or/and flash chromatography, some ticlopidine derivatives and analogs were still impure. Then they were dissolved in about 3 mL of MeOH or THF and injected into the semi-preparative HPLC instrument. The sample was eluted with a solvent gradient of 30-100% MeCN (+ 0.05% TFA) in H_2O (+ 0.05% TFA) in 16 min and with a flow rate of 25 mL/min.

8.2.2 Purification of nucleotides by semi-preparative HPLC

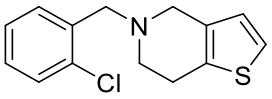
Lyophilized crude nucleotide was dissolved in about 3 mL of deionized water and its pH value was adjust to 6-7 by low concentration of aqueous TFA before injected into a semi-preparative HPLC instrument. The sample was eluted with a solvent gradient of 0-3 min, 10%; 3-12 min, 10-80%; 12-15.5 min, 80%; 15.5-16 min, 80-10%; 16-20 min, 10% of MeCN (+ 0.05% TFA) in H_2O (+ 0.05% TFA) in 20 min and with a flow rate of 20 mL/min.

8.3 Preparation of triethylammonium hydrogen carbonate (TEAC) buffer

This buffer was prepared by a reported method.[101] A 1 M solution of TEAC was prepared by adding crushed dry ice to a 1 M aqueous triethylamine solution for several hours until the pH was reached 7.4-7.6 indicated by a pH meter. Before each use, it was diluted with the same volume of H_2O in the final concentration of 0.5 M.

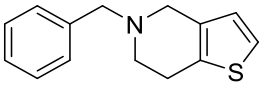
8.4 Synthesis

5-(2-Chlorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (ticlopidine, le, Bcy-Yazh-k381), CAS: 55142-85-3



To a solution of 4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (0.87 mL, 7.18 mmol, 1 eq.) in DMF (10 mL), 2-chlorobenzyl chloride (1.00 mL, 7.90 mmol, 1.1 eq.) and K_2CO_3 (2.48 g, 17.95 mmol, 2.5 eq.) were added. The reaction mixture was stirred at 80 °C for 1 h and the reaction progress was monitored by TLC (EtOAc/petroleum ether, 1:4). After the reaction was completed, the mixture was poured on ice water (20 mL) and extracted with ethyl acetate (50 mL × 3). The collected organic layers were washed with brine (20 mL), dried over Mg_2SO_4, and concentrated *in vacuum*. The crude product was purified by silica gel column chromatography using 10% ethyl acetate in petroleum ether. **Appearance**: brownish oil; $\mathbf{n_D^{20}}$: 1.6135. **Yield**: 1.33 g, 70%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.57 (dd, J = 7.6, 1.7 Hz, 1H), 7.39 – 7.35 (m, 1H), 7.28 – 7.23 (m, 1H), 7.20 (dd, J = 7.5, 1.8 Hz, 1H), 7.08 (d, J = 5.1 Hz, 1H), 6.72 (d, J = 5.1 Hz, 1H), 3.86 (s, 2H), 3.67 (d, J = 1.7 Hz, 2H), 2.92 (dd, J = 5.9, 3.8 Hz, 2H), 2.90 (dd, J = 6.5, 3.2 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 134.3, 133.7, 133.3, 130.7, 129.5, 128.3, 126.8, 125.2, 122.7, 58.3, 53.0, 50.7, 25.4. **LC-MS** (*m/z*): 263.8 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.3%.

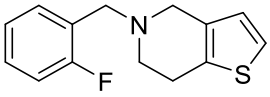
5-Benzyl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4a, Bcy-Yazh-k387), CAS: 55142-78-4



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), benzyl bromide (388 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 10% ethyl acetate in petroleum ether. **Appearance**: orange oil; $\mathbf{n_D^{20}}$: 1.6035. **Yield**: 395 mg, 61%. ^{1}H NMR (500 MHz,

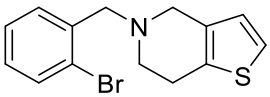
DMSO-d_6) δ 7.36 – 7.29 (m, 4H), 7.27 – 7.24 (m, 1H), 7.23 (d, J = 5.1 Hz, 1H), 6.74 (d, J = 5.1 Hz, 1H), 3.66 (s, 2H), 3.43 (t, J = 1.7 Hz, 2H), 2.83 – 2.74 (m, 2H), 2.75 – 2.67 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 138.5, 134.0, 132.8, 128.7, 128.2, 126.9, 125.4, 122.9, 61.2, 52.4, 50.2, 25.0. **LC-MS** (*m/z*): 230.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%.

5-(2-Fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4b, Bcy-Yazh-k406), CAS: 53885-46-4



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 2-fluorobenzyl bromide (428 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 10% ethyl acetate in petroleum ether. **Appearance**: colorless oil; $\mathbf{n_D^{20}}$: 1.5851. **Yield**: 180 mg, 34%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.49 – 7.42 (m, 1H), 7.32 (m, 1H), 7.23 (dd, J = 8.3, 5.1 Hz, 1H), 7.20 – 7.13 (m, 2H), 6.76 (d, J = 5.1 Hz, 1H), 3.71 (d, J = 1.3 Hz, 2H), 3.47 (d, J = 1.7 Hz, 2H), 2.79 (dd, J = 6.3, 4.1 Hz, 2H), 2.74 (dd, J = 6.3, 3.9 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.3, 141.7, 133.9, 132.7, 130.0, 125.4, 124.6, 122.9, 115.1, 113.8, 60.4, 52.3, 50.1, 24.9. **LC-MS** (*m/z*): 247.8 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%.

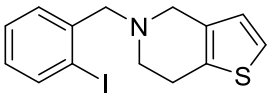
5-(2-Bromobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4c, Bcy-Yazh-k382), CAS: 72406-13-4



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.87 mL, 7.18 mmol), DMF (10 mL), 2-bromobenzyl chloride (0.97 mL, 7.91 mmol) and K_2CO_3 (2.48 g, 17.95 mmol) were used. The crude compound was purified by silica gel column chromatography using 10% ethyl acetate

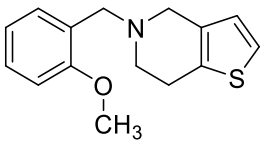
in petroleum ether. **Appearance**: brown oil; $\mathbf{n_D^{20}}$: 1.6251. **Yield**: 1.61 g, 73%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.60 (dd, J = 8.1, 1.2 Hz, 1H), 7.52 (dd, J = 7.7, 1.8 Hz, 1H), 7.41 – 7.32 (m, 1H), 7.25 (*d*, J = 5.1 Hz, 1H), 7.23 – 7.19 (m, 1H), 6.77 (d, J = 5.1 Hz, 1H), 3.73 (s, 2H), 3.52 (d, J = 1.7 Hz, 2H), 2.89 – 2.70 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 137.5, 133.9, 132.8, 132.5, 130.8, 128.9, 127.6, 125.4, 123.9, 122.9, 60.3, 52.5, 50.2, 25.0. **LC-MS** (*m/z*): 307.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.0%.

5-(2-Iodobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4d, Bcy-Yazh-k432)



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 2-iodobenzyl bromide (672 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 10% ethyl acetate in petroleum ether. **Appearance**: brownish oil; $\mathbf{n_D^{20}}$: 1.6510. **Yield**: 420 mg, 55%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.85 (dd, J = 7.9, 1.2 Hz, 1H), 7.45 (dd, J = 7.6, 1.7 Hz, 1H), 7.41 – 7.35 (m, 1H), 7.25 (d, J = 5.1 Hz, 1H), 7.08 – 6.99 (m, 1H), 6.77 (d, J = 5.1 Hz, 1H), 3.66 (s, 2H), 3.52 (d, J = 1.4 Hz, 2H), 2.79 (dt, J = 5.0, 2.5 Hz, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 140.4, 139.1, 133.9, 132.8, 130.3, 129.1, 128.2, 125.4, 122.9, 100.7, 65.0, 52.4, 50.1, 25.0. **LC-MS** (*m/z*): 355.24 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.9%.

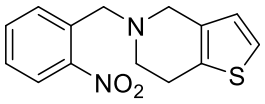
5-(2-Methoxybenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4e, Bcy-Yazh-k392), CAS: 54943-17-8



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 1-

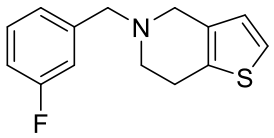
(bromomethyl)-2-methoxybenzene (456 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 10% ethyl acetate in petroleum ether. **Appearance**: yellowish solid; **mp**: 91-93 ℃ (*lit.*[169] 90 ℃). **Yield**: 225 mg, 40%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.34 (dd, J = 7.5, 1.7 Hz, 1H), 7.26 – 7.20 (m, 2H, 3H), 6.98 (dd, J = 8.2, 1.0 Hz, 1H), 6.94 – 6.89 (m, 1H), 6.75 (d, J = 5.1 Hz, 1H), 3.77 (s, 3H), 3.65 (s, 2H), 3.47 (t, J = 1.8 Hz, 2H), 2.78 (dd, J = 6.1, 4.2 Hz, 2H), 2.75 – 2.67 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.3, 134.2, 132.8, 129.6, 128.0, 126.0, 125.4, 122.8, 120.1, 110.8, 55.3, 54.6, 52.6, 50.2, 25.0. **LC-MS** (*m/z*): 259.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.5%.

5-(2-Nitrobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4f, Bcy-Yazh-k386), CAS: 55143-02-7



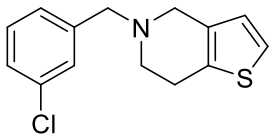
This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.44 mL, 3.59 mmol), DMF (10 mL), 2-nitrobenzyl bromide (0.85 g, 3.95 mmol) and K_2CO_3 (1.24 g, 8.98 mmol) were used. The crude compound was purified by silica gel column chromatography using 10% ethyl acetate in petroleum ether. **Appearance**: yellowish solid; **mp**: 79-81 ℃. **Yield**: 0.59 g, 60%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.90 – 7.84 (m, 1H), 7.69 – 7.65 (m, 2H), 7.52 (m, J = 8.2, 5.4, 3.6 Hz, 1H), 7.24 (d, J = 5.1 Hz, 1H), 6.75 (d, J = 5.1 Hz, 1H), 3.92 (s, 2H), 3.45 (t, J = 1.7 Hz, 2H), 2.71 (dd, J = 6.9, 5.1 Hz, 2H), 2.69 – 2.63 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 149.5, 133.6, 133.0, 132.7, 132.6, 131.1, 128.5, 125.4, 124.2, 122.9, 57.3, 52.6, 50.0, 24.8. **LC-MS** (*m/z*): 275.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.8%.

5-(3-Fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4g, Bcy-Yazh-k407), CAS: 2324649-06-9



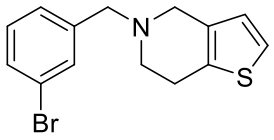
This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 3-fluorobenzyl bromide (428 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 10% ethyl acetate in petroleum ether. **Appearance**: colorless oil; $\mathbf{n_D^{20}}$: 1.5694. **Yield**: 190 mg, 36%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.40 – 7.33 (m, 1H), 7.24 (d, J = 5.1 Hz, 1H), 7.20 – 7.14 (m, 2H), 7.11 – 7.04 (m, 1H), 6.75 (d, J = 5.1 Hz, 1H), 3.68 (s, 2H), 3.44 (d, J = 1.8 Hz, 2H), 2.79 (t, J = 5.7 Hz, 2H), 2.73 (t, J = 5.6 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.6, 141.9, 134.1, 132.9, 125.6, 124.8, 124.8, 123.1, 115.3, 114.0, 60.6, 52.5, 50.3, 25.1. **LC-MS** (*m/z*): 247.8 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.2%.

5-(3-Chlorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4h, Bcy-Yazh-k420), CAS: 55142-86-4



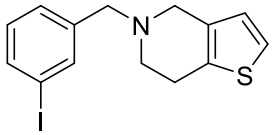
This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 3-chlorobenzyl bromide (466 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 10% ethyl acetate in petroleum ether. **Appearance**: gray oil; $\mathbf{n_D^{20}}$: 1.6091. **Yield**: 150 mg, 26%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.41 – 7.29 (m, 4H), 7.24 (d, J = 5.1 Hz, 1H), 6.76 (d, J = 5.1 Hz, 1H), 3.67 (s, 2H), 3.44 (d, J = 1.9 Hz, 2H), 2.79 (t, J = 5.8 Hz, 2H), 2.73 (t, J = 5.6 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 141.5, 134.0, 133.1, 132.9, 130.3, 128.5, 127.5, 127.1, 125.6, 123.1, 60.5, 52.5, 50.3, 25.1. **LC-MS** (*m/z*): 263.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.3%.

5-(3-Bromobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4i, Bcy-Yazh-k422), CAS: 1292689-48-5



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 3-bromobenzyl bromide (566 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 10% ethyl acetate in petroleum ether. **Appearance**: gray oil; $\mathbf{n_D^{20}}$: 1.6248. **Yield**: 340 mg, 51%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.54 (t, J = 1.7 Hz, 1H), 7.45 (dt, J = 7.8, 1.5 Hz, 1H), 7.35 (dt, J = 7.7, 1.4 Hz, 1H), 7.29 (t, J = 7.7 Hz, 1H), 7.24 (d, J = 5.1 Hz, 1H), 6.75 (d, J = 5.1 Hz, 1H), 3.66 (s, 2H), 3.44 (t, J = 1.7 Hz, 2H), 2.79 (t, J = 5.5 Hz, 2H), 2.73 (t, J = 5.9 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 141.6, 133.9, 132.7, 131.2, 129.8, 127.7, 125.4, 122.9, 121.6, 60.3, 52.3, 50.2, 24.9. **LC-MS** (*m/z*): 309.8 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.4%.

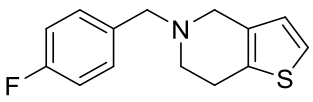
5-(3-Iodobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4j, Bcy-Yazh-k419)



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 3-iodobenzyl bromide (673 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 6% ethyl acetate in petroleum ether. **Appearance**: brownish viscous semi-solid. **Yield**: 270 mg, 35%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.72 (t, J = 1.7 Hz, 1H), 7.62 (dt, J = 7.9, 1.4 Hz, 1H), 7.40 – 7.34 (m, 1H), 7.24 (d, J = 5.2 Hz, 1H), 7.18 – 7.11 (m, 1H), 6.76 (d, J = 5.1 Hz, 1H), 3.63 (s, 2H), 3.43 (t, J = 1.7 Hz, 2H), 2.81 – 2.76 (m, 2H), 2.72 (t, J = 5.3 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 140.4, 139.1, 133.9, 132.8, 130.3, 129.1, 128.2,

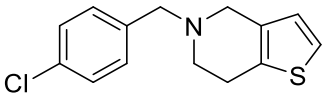
125.4, 122.9, 100.7, 65.0, 52.4, 50.1, 25.0. **LC-MS** (*m/z*): 355.8 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.5%.

5-(4-Fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4k, Bcy-Yazh-k408), CAS: 55142-84-2



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 4-fluorobenzyl bromide (428 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 10% ethyl acetate in petroleum ether. **Appearance**: colorless oil; $\mathbf{n_D^{20}}$: 1.5855. **Yield**: 200 mg, 38%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.39 – 7.35 (m, 2H), 7.24 (d, J = 5.1 Hz, 1H), 7.16 – 7.12 (m, 2H), 6.74 (d, J = 5.1 Hz, 1H), 3.64 (s, 2H), 3.42 (d, J = 1.8 Hz, 2H), 2.78 (t, J = 5.7 Hz, 2H), 2.71 (t, J = 5.6 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.1, 160.5, 134.7, 134.6, 133.9, 132.8, 130.5, 130.5, 125.4, 122.9, 115.0, 114.8, 60.3, 52.3, 50.1, 25.0. **LC-MS** (*m/z*): 247.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.2%.

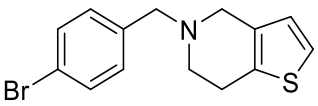
5-(4-Chlorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4l, Bcy-Yazh-k424), CAS: 55157-56-7



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 4-chlorobenzyl bromide (466 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 10% ethyl acetate in petroleum ether. **Appearance**: yellowish solid; **mp**: 72-74 °C. **Yield**: 230 mg, 40%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.37 (d, J = 1.0 Hz, 4H), 7.23 (d, J = 5.1 Hz, 1H), 6.74 (d, J = 5.1 Hz, 1H), 3.65 (s, 2H), 3.42 (d, J = 1.9 Hz, 2H), 2.82 – 2.75 (m, 2H), 2.72 (t, J = 5.3 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 137.6, 133.9, 132.7, 131.4,

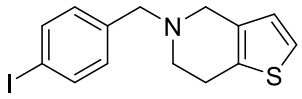
130.5, 128.2, 125.4, 122.9, 60.3, 52.3, 50.1, 24.9. **LC-MS** (*m/z*): 261.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.1%.

5-(4-Bromobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4m, Bcy-Yazh-k404), CAS: 1306900-16-2



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 4-bromobenzyl bromide (566 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 10% ethyl acetate in petroleum ether. **Appearance**: white solid; **mp**: 90-92 ℃. **Yield**: 340 mg, 51%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.53 – 7.48 (m, 2H), 7.34 – 7.27 (m, 2H), 7.24 (d, J = 5.0 Hz, 1H), 6.74 (d, J = 5.1 Hz, 1H), 3.63 (s, 2H), 3.42 (t, J = 1.7 Hz, 2H), 2.81 – 2.74 (m, 2H), 2.72 (t, J = 5.3 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 138.0, 133.9, 132.7, 131.1, 130.8, 125.4, 122.9, 119.9, 60.3, 52.3, 50.1, 24.9. **LC-MS** (*m/z*): 309.8 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.1%.

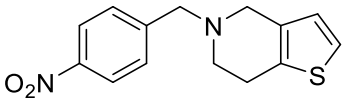
5-(4-Iodobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4n, Bcy-125), CAS: 1466482-18-7



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 4-iodobenzyl bromide (674 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 6% ethyl acetate in petroleum ether. **Appearance**: yellowish solid; **mp**: 70.0-72.0 ℃. **Yield**: 427 mg, 56%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.69 (d, J = 8.0 Hz, 2H), 7.25 (d, J = 5.1 Hz, 1H), 7.17 (d, J = 8.0 Hz, 2H), 6.75 (d, J = 5.0 Hz, 1H), 3.62 (s, 2H), 2.75 (dt, J = 41.6, 5.8 Hz, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 138.44, 136.97, 133.89, 132.71, 131.01,

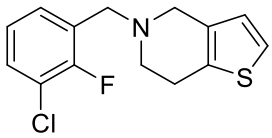
125.39, 122.90, 92.70, 60.46, 52.33, 50.14, 24.95. **LC-MS** (*m/z*): 355.8 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.9%.

5-(4-Nitrobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4o, Bcy-Yazh-k403), CAS: 60612-13-7



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 4-nitrobenzyl bromide (489 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 10% ethyl acetate in petroleum ether. **Appearance**: yellow solid; **mp**: 69-71 °C (*lit.*[169] 119-121 °C). **Yield**: 320 mg, 54%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.26 – 8.13 (m, 2H), 7.64 (d, J = 8.4 Hz, 2H), 7.25 (d, J = 5.1 Hz, 1H), 6.75 (d, J = 5.1 Hz, 1H), 3.81 (s, 2H), 3.48 (t, J = 1.6 Hz, 2H), 2.81 (d, J = 5.6 Hz, 2H), 2.75 (t, J = 5.8 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 147.0, 146.6, 133.7, 132.7, 129.6, 125.4, 123.4, 123.0, 60.2, 52.4, 50.2, 24.9. **LC-MS** (*m/z*): 275.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.6%.

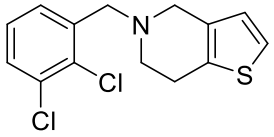
5-(3-Chloro-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4p, Bcy-135)



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 2-fluoro-3-chlorobenzyl bromide (0.31 mL, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 7% ethyl acetate in petroleum ether. **Appearance**: yellowish oil; $\mathbf{n_D^{20}}$: 1.5939. **Yield**: 265 mg, 44%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.55 – 7.40 (m, 2H), 7.29 – 7.17 (m, 2H), 6.77 (d, J = 5.1 Hz, 1H), 3.76 (d, J = 1.3 Hz, 2H), 3.48 (d, J = 1.6 Hz, 2H), 2.86 – 2.71 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 156.64, 133.74, 132.67, 130.11, 129.33,

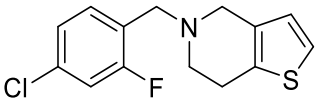
127.14, 125.39, 125.12, 122.96, 119.61, 53.81, 52.17, 50.06, 24.93. **LC-MS** (*m/z*): 281.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.1%.

5-(2,3-Dichlorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4q, Bcy-122)



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 2,3-dichlorobenzyl chloride (0.32 mL, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 5% ethyl acetate in petroleum ether. **Appearance**: yellowish oil; $\mathbf{n_D^{20}}$: 1.6086. **Yield**: 456 mg, 71%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.54 (ddd, J = 22.3, 7.8, 1.6 Hz, 2H), 7.37 (t, J = 7.8 Hz, 1H), 7.26 (d, J = 5.1 Hz, 1H), 6.78 (d, J = 5.1 Hz, 1H), 3.80 (s, 2H), 3.54 (d, J = 1.8 Hz, 2H), 2.80 (p, J = 4.7 Hz, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 138.77, 133.81, 132.73, 131.76, 131.10, 129.11, 127.95, 125.40, 122.96, 58.51, 52.46, 50.24, 24.92. **LC-MS** (*m/z*): 297.9 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%.

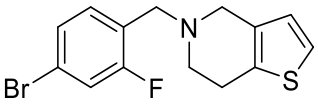
5-(4-Chloro-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4r, Bcy-134)



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 2-fluoro-4-chlorobenzyl bromide (0.31 mL, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 7% ethyl acetate in petroleum ether. **Appearance**: reddish oil; $\mathbf{n_D^{20}}$: 1.5783. **Yield**: 411 mg, 68%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.49 (t, J = 8.2 Hz, 1H), 7.40 (dd, J = 10.0, 2.1 Hz, 1H), 7.33 – 7.21 (m, 2H), 6.76 (d, J = 5.1 Hz, 1H), 3.70 (d, J = 1.2 Hz, 2H), 3.47 (d, J = 1.8 Hz, 2H), 2.85 – 2.70 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.77, 133.77, 132.67, 132.58, 132.42, 125.37, 124.53, 124.26, 122.95, 115.88, 53.22, 52.14,

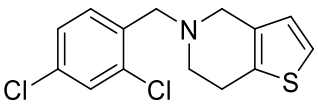
50.01, 24.93. **LC-MS** (*m/z*): 281.8 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.0%.

5-(4-Bromo-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4s, Bcy-141), CAS: 1306062-28-1



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 2-fluoro-4-bromobenzyl bromide (608 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 5% ethyl acetate in petroleum ether. **Appearance**: yellowish oil; $\mathbf{n_D^{20}}$: 1.5964. **Yield**: 339 mg, 48%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.52 (dd, J = 9.7, 1.8 Hz, 1H), 7.46 – 7.38 (m, 2H), 7.25 (d, J = 5.1 Hz, 1H), 6.76 (d, J = 5.1 Hz, 1H), 3.69 (d, J = 1.2 Hz, 2H), 3.47 (d, J = 1.8 Hz, 2H), 2.83 – 2.69 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.46, 133.76, 132.91, 132.66, 127.44, 125.37, 124.67, 122.95, 120.33, 118.46, 53.27, 52.15, 50.02, 24.93. **LC-MS** (*m/z*): 325.8 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.4%.

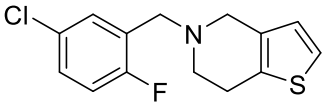
5-(2,4-Dichlorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4t, Bcy-137), CAS: 2329700-87-8



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 2,4-dichlorobenzyl chloride (0.32 mL, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 3.5% ethyl acetate in petroleum ether. **Appearance**: yellowish oil; $\mathbf{n_D^{20}}$: 1.6156. **Yield**: 213 mg, 33%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.60 (d, J = 2.2 Hz, 1H), 7.56 (d, J = 8.3 Hz, 1H), 7.42 (dd, J = 8.3, 2.2 Hz, 1H), 7.26 (d, J = 5.1 Hz, 1H), 6.77 (d, J = 5.1

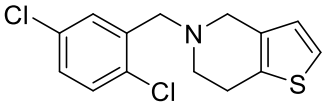
Hz, 1H), 3.74 (s, 2H), 3.52 (d, *J* = 1.7 Hz, 2H), 2.85 – 2.74 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 135.17, 134.08, 133.80, 132.71, 132.18, 131.98, 128.66, 127.26, 125.39, 122.96, 57.20, 52.41, 50.17, 24.93. **LC-MS** (*m/z*): 297.9 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.1%.

5-(5-Chloro-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4u, Bcy-123)



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 2-fluoro-5-chlorobenzyl bromide (0.31 mL, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 4% ethyl acetate in petroleum ether. **Appearance**: colorless oil; $\mathbf{n_D^{20}}$: 1.5998. **Yield**: 380 mg, 62%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.51 (dd, *J* = 6.3, 2.8 Hz, 1H), 7.39 (ddd, *J* = 8.8, 4.5, 2.9 Hz, 1H), 7.32 – 7.19 (m, 2H), 6.78 (d, *J* = 5.1 Hz, 1H), 3.72 (s, 2H), 3.49 (d, *J* = 1.7 Hz, 2H), 2.87 – 2.71 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.22, 133.73, 132.66, 130.62, 128.83, 127.45, 125.40, 122.97, 117.25, 53.28, 52.13, 50.04, 24.92. **LC-MS** (*m/z*): 281.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.3%.

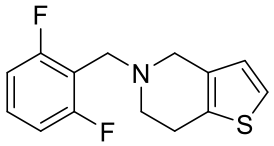
5-(2,5-Dichlorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4v, Bcy-138)



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 2,5-dichlorobenzyl bromide (545 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 3.5% ethyl acetate in petroleum ether. **Appearance**: colorless oil; $\mathbf{n_D^{20}}$: 1.6161. **Yield**: 415 mg, 64%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.57 (d, *J* = 2.7 Hz, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.38 (dd, *J* = 8.5, 2.7 Hz, 1H), 7.27 (d, *J* = 5.1 Hz, 1H), 6.79 (d, *J* = 5.1 Hz, 1H), 3.76 (s, 2H), 3.54 (s, 2H), 2.81 (dd, *J* = 10.3, 4.5 Hz, 4H). ^{13}C NMR (151 MHz,

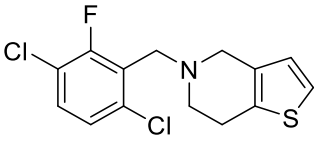
DMSO-d_6) δ 138.35, 133.76, 132.70, 131.78, 130.92, 129.99, 128.45, 125.41, 122.99, 57.37, 52.40, 50.21, 24.92. **LC-MS** (*m/z*): 297.9 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.5%.

5-(2,6-Difluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (4w, Bcy-170), CAS: 2327315-98-8



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 2,6-difluorobenzyl bromide (470 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 6% ethyl acetate in petroleum ether. **Appearance**: yellowish solid; **mp**: 60.0-62.0 °C. **Yield**: 269 mg, 47%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.54 – 7.35 (m, 1H), 7.24 (d, J = 5.1 Hz, 1H), 7.18 – 7.05 (m, 2H), 6.77 (d, J = 5.1 Hz, 1H), 3.76 (t, J = 1.5 Hz, 2H), 3.48 (s, 2H), 2.77 (q, J = 3.3, 2.8 Hz, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.25, 160.61, 133.70, 132.59, 130.09, 125.37, 122.96, 113.08, 111.52, 111.34, 51.76, 49.84, 47.60, 24.95. **LC-MS** (*m/z*): 265.8 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.9%.

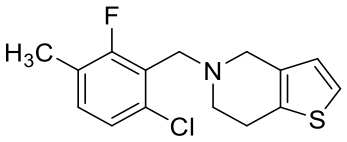
5-(3,6-Dichloro-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (5a, Bcy-142)



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 3,6-dichloro-2-fluorobenzyl bromide (585 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 4% ethyl acetate in petroleum ether. **Appearance**: yellowish solid; **mp**: 62.0-63.5 °C. **Yield**:

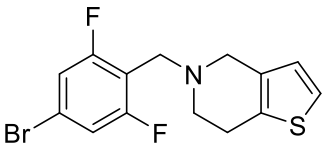
479 mg, 70%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.59 (dd, J = 8.7, 8.0 Hz, 1H), 7.40 (dd, J = 8.7, 1.5 Hz, 1H), 7.25 (d, J = 5.1 Hz, 1H), 6.78 (d, J = 5.1 Hz, 1H), 3.82 (d, J = 2.2 Hz, 2H), 3.53 (d, J = 1.6 Hz, 2H), 2.88 – 2.70 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 157.47, 134.25, 133.60, 132.61, 130.21, 126.26, 125.57, 125.41, 122.96, 118.73, 52.12, 51.74, 50.12, 24.88. **LC-MS** (*m/z*): 315.9 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.9%.

5-(6-Chloro-2-fluoro-3-methylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (5b, Bcy-169)



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 6-chloro-2-fluoro-3-methylbenzyl bromide (539 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 4% ethyl acetate in petroleum ether. **Appearance**: yellowish oil; $\mathbf{n_D^{20}}$: 1.5870. **Yield**: 498 mg, 78%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.37 – 7.14 (m, 3H), 6.77 (d, J = 5.1 Hz, 1H), 3.77 (d, J = 2.2 Hz, 2H), 3.52 (d, J = 1.5 Hz, 2H), 2.89 – 2.70 (m, 4H), 2.23 (d, J = 2.0 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.68, 133.79, 132.67, 131.27, 125.41, 124.83, 123.42, 123.29, 123.17, 122.95, 52.21, 51.46, 50.12, 24.94, 14.04. **LC-MS** (*m/z*): 295.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.1%.

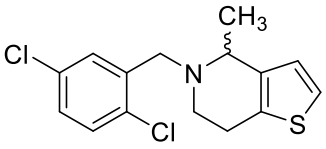
5-(4-Bromo-2,6-difluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (5c, Bcy-168)



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.44 mL, 3.59 mmol), DMF (15 mL), 4-bromo-2,6-

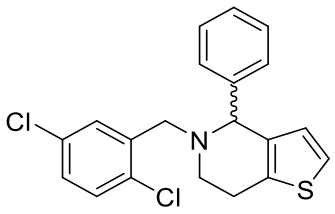
difluorobenzyl bromide (0.54 mL, 3.77 mmol) and K_2CO_3 (1.241 g, 8.98 mmol) were used. The crude compound was purified by silica gel column chromatography using 5% ethyl acetate in petroleum ether. **Appearance**: white solid; **mp**: 81.0-83.0 °C. **Yield**: 761 mg, 62%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.53 – 7.44 (m, 2H), 7.24 (d, J = 5.1 Hz, 1H), 6.77 (d, J = 5.2 Hz, 1H), 3.72 (t, J = 1.4 Hz, 2H), 3.46 (d, J = 1.8 Hz, 2H), 2.75 (dt, J = 9.4, 4.8 Hz, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.24, 160.58, 133.62, 132.57, 125.38, 122.99, 120.85, 115.42, 115.23, 113.00, 51.72, 49.81, 47.47, 24.94. **LC-MS** (*m/z*): 343.8 [M - H]$^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.6%.

5-(2,5-Dichlorobenzyl)-4-methyl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (5d, Bcy-276)



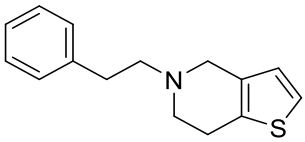
This compound was synthesized using the same procedure as for **8a**. 4-Methyl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine hydrochloride (248 mg, 1.31 mmol), 2,5-dichlorobenzyl bromide (377 mg, 1.57 mmol), K_2CO_3 (453 mg, 3.28 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 2% ethyl acetate in petroleum ether. **Appearance**: yellowish oil; $\mathbf{n_D^{20}}$: 1.5972. **Yield**: 381 mg, 93%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.61 (d, J = 2.8 Hz, 1H), 7.47 (d, J = 8.5 Hz, 1H), 7.36 (dd, J = 8.5, 2.8 Hz, 1H), 7.26 (d, J = 5.2 Hz, 1H), 6.87 (d, J = 5.2 Hz, 1H), 3.85 (d, J = 15.4 Hz, 1H), 3.80 – 3.67 (m, 2H), 3.04 – 2.93 (m, 1H), 2.80 (dt, J = 15.4, 5.3 Hz, 1H), 2.75 – 2.65 (m, 2H), 1.30 (d, J = 6.6 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 139.50, 138.68, 132.35, 131.83, 131.45, 130.83, 129.56, 128.17, 126.03, 122.62, 55.08, 53.74, 45.35, 23.44, 19.28. **LC-MS** (*m/z*): 312.0 [M - H]$^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.9%.

5-(2,5-Dichlorobenzyl)-4-phenyl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (5e, Bcy-273)



This compound was synthesized using the same procedure as for **8a**. 4-Phenyl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (100 mg, 0.46 mmol), 2,5-dichlorobenzyl bromide (132 mg, 0.55 mmol), K_2CO_3 (159 mg, 1.15 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 100% petroleum ether. **Appearance**: yellowish solid; **mp**: 64.0-66.0 ℃. **Yield**: 166 mg, 96%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.55 (d, J = 2.7 Hz, 1H), 7.42 (d, J = 8.5 Hz, 1H), 7.37 – 7.29 (m, 5H), 7.29 – 7.22 (m, 1H), 7.18 (d, J = 5.4 Hz, 1H), 6.31 (d, J = 5.4 Hz, 1H), 4.68 (d, J = 1.8 Hz, 1H), 3.64 – 3.53 (m, 2H), 3.05 – 2.92 (m, 2H), 2.91 – 2.82 (m, 1H), 2.71 – 2.60 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 142.44, 138.85, 137.02, 132.94, 131.37, 130.80, 129.23, 128.50, 128.28, 128.26, 127.43, 126.55, 122.86, 65.90, 54.14, 47.54, 24.44. **LC-MS** (*m/z*): 373.9 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.5%.

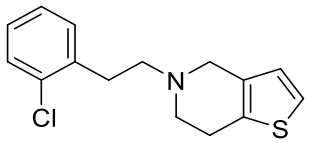
5-Phenethyl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (6a, Bcy-Yazh-k425b), CAS: 87403-75-6



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), (2-bromoethyl)benzene (419 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 10% ethyl acetate in petroleum ether. **Appearance**: colorless oil; $\mathbf{n_D^{20}}$: 1.5837. **Yield**: 100 mg, 19%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.29 – 7.20 (m, 5H), 7.17 (m, 1H), 6.78 (d, J = 5.1 Hz, 1H), 3.53 (d, J = 1.8 Hz, 2H), 2.84 – 2.74 (m, 6H), 2.74 – 2.67 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 140.4, 134.1, 132.8, 128.6, 128.2, 125.8, 125.4, 122.8,

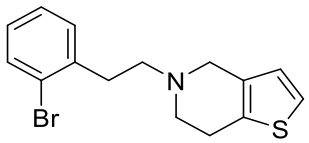
58.9, 52.4, 50.3, 33.0, 25.0. **LC-MS** (*m/z*): 244.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%.

5-(2-Chlorophenethyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (6b, Bcy-Yazh-k448)



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 2-(2-chlorophenyl)ethyl bromide (497 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 10% ethyl acetate in petroleum ether. **Appearance**: colorless oil; $\mathbf{n_D^{20}}$: 1.5834. **Yield**: 230 mg, 38%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.39 (dd, J = 7.7, 1.7 Hz, 2H), 7.29 – 7.18 (m, 3H), 6.78 (d, J = 5.1 Hz, 1H), 3.55 (d, J = 1.6 Hz, 2H), 2.97 – 2.91 (m, 2H), 2.79 (d, J = 1.2 Hz, 4H), 2.74 – 2.67 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 137.7, 134.0, 132.9, 132.8, 131.2, 129.1, 127.9, 127.2, 125.4, 122.8, 56.9, 52.3, 50.2, 30.7, 25.0. **LC-MS** (*m/z*): 277.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.1%.

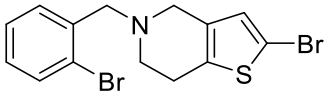
5-(2-Bromophenethyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (6c, Bcy-Yazh-k421)



This compound was synthesized using the same procedure as for **le**. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridine (0.26 mL, 2.16 mmol), DMF (10 mL), 1-bromo-2-(2-chloroethyl)benzene (497 mg, 2.27 mmol) and K_2CO_3 (746 mg, 5.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 6% ethyl acetate in petroleum ether. **Appearance**: colorless oil; $\mathbf{n_D^{20}}$: 1.5841. **Yield**: 110 mg, 18%.

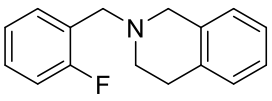
^{1}H NMR (500 MHz, DMSO-d_6) δ 7.41 (s, 2H), 7.32 (d, J = 5.1 Hz, 1H), 7.25 (s, 2H), 6.85 (s, 1H), 4.41 (t, J = 1.7 Hz, 2H), 4.25 (t, J = 6.5 Hz, 2H), 3.61 (s, 2H), 3.04 (t, J = 6.6 Hz, 2H), 2.72 (d, J = 18.9 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.6, 135.6, 133.2, 131.4, 129.2, 128.4, 127.2, 125.1, 123.5, 64.0, 43.8, 41.3, 41.2, 32.4, 24.4. **LC-MS** (*m/z*): 322.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.5%.

2-Bromo-5-(2-bromobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (7a, Bcy-Yazh-k433)



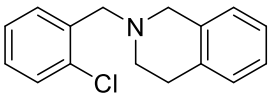
To a solution of **4c** (200 mg, 0.65 mmol, 1 eq.) in CH_3CO_2H (8 mL), Br_2 (0.035 mL, 0.68 mmol, 1.05 eq.) was added dropwise. The mixture was stirred at rt for 12 h and the reaction progress was monitored by TLC (EtOAc/petroleum ether, 1:9). After the reaction was completed, the mixture was poured on ice water (10 mL) and extracted with ethyl acetate (30 mL × 2). The collected organic layers were washed with H_2O (30 mL × 2) and brine (30 mL), dried over Mg_2SO_4, and concentrated *in vacuum*. The crude product was purified by silica gel column chromatography using 5% ethyl acetate in petroleum ether. **Appearance**: yellowish viscous solid. **Yield**: 130 mg, 52%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.60 (dd, J = 8.1, 1.2 Hz, 1H), 7.50 (dd, J = 7.6, 1.8 Hz, 1H), 7.44 – 7.29 (m, 1H), 7.29 – 7.14 (m, 1H), 6.90 (s, 1H), 3.71 (s, 2H), 3.55 – 3.40 (m, 2H), 2.80 – 2.75 (m, 2H), 2.72 (tt, J = 6.6, 1.5 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 137.3, 135.0, 134.9, 132.5, 130.8, 129.0, 128.6, 127.6, 123.9, 108.1, 60.1, 51.8, 49.8, 24.9. **LC-MS** (*m/z*): 387.8 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.9%.

2-(2-Fluorobenzyl)-1,2,3,4-tetrahydroisoquinoline (8a, Bcy-190), CAS: 827333-14-2



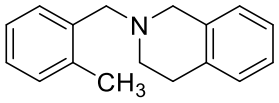
To a solution of 1,2,3,4-tetrahydroisoquinoline (0.19 mL, 1.50 mmol, 1 eq.) in EtOH (10 mL), 2-fluorobenzyl chloride (0.21 mL, 1.80 mmol, 1.2 eq.) and K_2CO_3 (518 mg, 3.75 mmol, 2.5 eq.) were added. The reaction mixture was refluxed for 1 h (or overnight) and the reaction progress was monitored by TLC (EtOAc/petroleum ether, 1:9). After the reaction was completed, the mixture was cooled to rt, K_2CO_3 was removed by filtration and the filter cake was washed with ethyl acetate (30 mL). The filtrate was then evaporated *in vacuum*. The crude product was purified by silica gel column chromatography using 6% ethyl acetate in petroleum ether. **Appearance**: yellowish oil; $\mathbf{n_D^{20}}$: 1.5632. **Yield**: 180 mg, 50%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.56 – 7.50 (m, 2H), 7.36 – 7.30 (m, 2H), 7.13 – 7.05 (m, 3H), 7.01 – 6.97 (m, 1H), 3.62 (s, 2H), 3.53 (s, 2H), 2.81 (t, J = 5.9 Hz, 2H), 2.67 (t, J = 5.9 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 137.93, 134.63, 133.99, 131.07, 130.80, 128.38, 126.29, 125.92, 125.41, 119.89, 60.87, 55.27, 50.12, 28.60. **LC-MS** (*m/z*): 241.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.0%.

2-(2-Chlorobenzyl)-1,2,3,4-tetrahydroisoquinoline (8b, Bcy-Yazh-k461), CAS: 72809-43-9



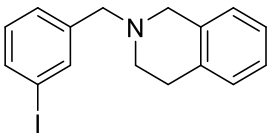
This compound was synthesized using the same procedure as for **8a**. 1,2,3,4-Tetrahydroisoquinoline (0.28 mL, 2.25 mmol), EtOH (10 mL), 2-chlorobenzyl chloride (380 mg, 2.36 mmol), K_2CO_3 (778 mg, 5.63 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 5% ethyl acetate in petroleum ether. **Appearance**: white solid; **mp**: 38-40 °C. **Yield**: 290 mg, 50%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.54 (dd, J = 7.5, 1.9 Hz, 1H), 7.44 (dd, J = 7.8, 1.5 Hz, 1H), 7.37 – 7.22 (m, 2H), 7.14 – 7.03 (m, 3H), 7.03 – 6.92 (m, 1H), 3.73 (s, 2H), 3.61 (s, 2H), 2.81 (t, J = 5.9 Hz, 2H), 2.72 (t, J = 5.9 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 135.8, 134.7, 134.0, 133.2, 130.7, 129.2, 128.6, 128.4, 127.1, 126.3, 126.0, 125.4, 58.4, 55.5, 50.3, 28.6. **LC-MS** (*m/z*): 257.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.3%.

2-(2-Methylbenzyl)-1,2,3,4-tetrahydroisoquinoline (8c, Bcy-198), CAS: 173034-80-5



This compound was synthesized using the same procedure as for **8a**. 1,2,3,4-Tetrahydroisoquinoline (0.28 mL, 2.25 mmol), 2-methylbenzyl chloride (0.36 mL, 2.70 mmol), K_2CO_3 (778 mg, 5.63 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 3% ethyl acetate in petroleum ether. **Appearance**: yellowish oil; $\mathbf{n_D^{20}}$: 1.5818. **Yield**: 315 mg, 59%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.32 – 7.25 (m, 1H), 7.19 – 7.13 (m, 3H), 7.11 – 7.05 (m, 3H), 6.99 (dd, J = 8.0, 2.0 Hz, 1H), 3.59 (s, 2H), 3.55 (s, 2H), 2.79 (t, J = 5.9 Hz, 2H), 2.67 (t, J = 5.9 Hz, 2H), 2.33 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 137.00, 136.32, 134.80, 134.12, 130.04, 129.42, 128.36, 126.91, 126.28, 125.88, 125.40, 125.38, 59.91, 55.61, 50.22, 28.71, 18.75. **LC-MS** (*m/z*): 237.8 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.9%.

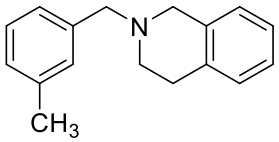
2-(3-Iodobenzyl)-1,2,3,4-tetrahydroisoquinoline (8d, Bcy-132), CAS: 1057279-29-4



This compound was synthesized using the same procedure as for **8a**. 1,2,3,4-Tetrahydroisoquinoline (0.28 mL, 2.25 mmol), DMF (10 mL), 3-iodobenzyl bromide (701 mg, 2.36 mmol) and K_2CO_3 (778 mg, 5.63 mmol) were used. The crude compound was purified by silica gel column chromatography using 7% ethyl acetate in petroleum ether. **Appearance**: white solid; **mp**: 73.0-75.0 °C. **Yield**: 310 mg, 39%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.73 (d, J = 1.8 Hz, 1H), 7.63 (dt, J = 7.8, 1.4 Hz, 1H), 7.38 (dt, J = 7.6, 1.3 Hz, 1H), 7.19 – 7.05 (m, 4H), 7.03 – 6.98 (m, 1H), 3.61 (s, 2H), 3.53 (s, 2H), 2.81 (t, J = 5.9 Hz, 2H), 2.67 (t, J = 5.9 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 141.34, 137.08, 135.68, 134.63, 133.99, 130.45, 128.42, 128.08, 126.33, 125.96,

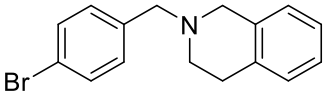
125.45, 94.79, 60.85, 55.28, 50.15, 28.63. **LC-MS** (*m/z*): 349.7 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.3%.

2-(3-Methylbenzyl)-1,2,3,4-tetrahydroisoquinoline (8e, Bcy-199), CAS: 885432-60-0



This compound was synthesized using the same procedure as for **8a**. 1,2,3,4-Tetrahydroisoquinoline (0.28 mL, 2.25 mmol), 3-methylbenzyl chloride (0.36 mL, 2.70 mmol), K_2CO_3 (778 mg, 5.63 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 5% ethyl acetate in petroleum ether. **Appearance**: colorless oil; $\mathbf{n_D^{20}}$: 1.5776. **Yield**: 237 mg, 44%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.22 (t, J = 7.5 Hz, 1H), 7.19 – 7.04 (m, 6H), 6.99 (d, J = 6.7 Hz, 1H), 3.60 (s, 2H), 3.52 (s, 2H), 2.80 (t, J = 5.9 Hz, 2H), 2.66 (t, J = 5.9 Hz, 2H), 2.30 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 138.29, 137.21, 134.78, 134.07, 129.28, 128.37, 128.05, 127.56, 126.28, 125.88, 125.75, 125.38, 61.84, 55.41, 50.20, 28.64, 20.95. **LC-MS** (*m/z*): 237.8 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.4%.

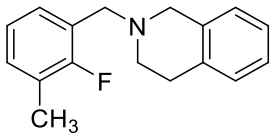
2-(4-Bromobenzyl)-1,2,3,4-tetrahydroisoquinoline (8f, Bcy-191), CAS: 885432-39-3



This compound was synthesized using the same procedure as for **8a**. 1,2,3,4-Tetrahydroisoquinoline (0.19 mL, 1.50 mmol), 4-bromobenzyl bromide (450 mg, 1.80 mmol), K_2CO_3 (518 mg, 3.75 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 5% ethyl acetate in petroleum ether. **Appearance**: white solid; **mp**: 68.0-70.0 °C (*lit.*[170] 70-72 °C). **Yield**: 392 mg, 86%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.56 – 7.49 (m, 2H), 7.32 (d, J = 8.3 Hz, 2H), 7.13 – 7.05 (m, 3H), 7.02 – 6.97 (m, 1H), 3.61 (s, 2H), 3.53 (s, 2H), 2.81 (t, J

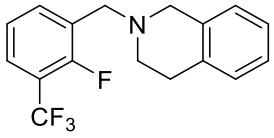
= 5.9 Hz, 2H), 2.66 (t, *J* = 5.9 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 137.93, 134.63, 133.99, 131.07, 130.80, 128.38, 126.29, 125.92, 125.41, 119.89, 60.87, 55.27, 50.12, 28.60. **LC-MS** (*m/z*): 301.8 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.7%.

2-(2-Fluoro-3-methylbenzyl)-1,2,3,4-tetrahydroisoquinoline (8g, Bcy-206)



This compound was synthesized using the same procedure as for **8a**. 1,2,3,4-Tetrahydroisoquinoline (0.28 mL, 2.25 mmol), 2-fluoro-3-methylbenzyl bromide (0.38 mL, 2.70 mmol), K_2CO_3 (778 mg, 5.63 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 4% ethyl acetate in petroleum ether. **Appearance**: colorless oil; $\mathbf{n_D^{20}}$: 1.5678. **Yield**: 345 mg, 60%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.27 (t, *J* = 7.3 Hz, 1H), 7.19 (t, *J* = 7.4 Hz, 1H), 7.14 – 7.03 (m, 4H), 7.00 (dd, *J* = 5.5, 3.8 Hz, 1H), 3.67 (s, 2H), 3.56 (s, 2H), 2.80 (t, *J* = 5.9 Hz, 2H), 2.73 – 2.65 (m, 2H), 2.24 (t, *J* = 1.6 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.28, 134.65, 133.95, 130.28, 128.69, 128.36, 126.28, 125.92, 125.39, 124.45, 124.33, 123.63, 55.26, 54.48, 50.10, 28.61, 14.18. **LC-MS** (*m/z*): 255.8 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.1%.

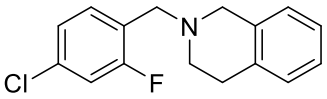
2-(2-Fluoro-3-(trifluoromethyl)benzyl)-1,2,3,4-tetrahydroisoquinoline (8h, Bcy-210)



This compound was synthesized using the same procedure as for **8a**. 1,2,3,4-Tetrahydroisoquinoline (0.19 mL, 1.50 mmol), 2-fluoro-3-(trifluoromethyl)benzyl bromide (462 mg, 1.80 mmol), K_2CO_3 (518 mg, 3.75 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 5%

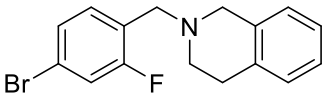
ethyl acetate in petroleum ether. **Appearance**: light orange oil; $\mathbf{n_D^{20}}$: 1.5205. **Yield**: 347 mg, 75%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.86 – 7.79 (m, 1H), 7.74 – 7.67 (m, 1H), 7.41 (t, J = 7.7 Hz, 1H), 7.15 – 7.06 (m, 3H), 7.05 – 6.98 (m, 1H), 3.78 (d, J = 1.3 Hz, 2H), 3.60 (s, 2H), 2.82 (t, J = 5.9 Hz, 2H), 2.71 (t, J = 5.9 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.25, 136.05, 134.44, 133.86, 128.41, 127.06, 126.97, 126.33, 126.03, 125.47, 124.69, 123.69, 116.65, 55.16, 53.69, 50.07, 28.56. **LC-MS** (*m/z*): 309.9 [M + H]$^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.5%.

2-(4-Chloro-2-fluorobenzyl)-1,2,3,4-tetrahydroisoquinoline (8i, Bcy-133)



This compound was synthesized using the same procedure as for **8a**. 1,2,3,4-Tetrahydroisoquinoline (0.28 mL, 2.25 mmol), DMF (10 mL), 2-fluoro-4-chlorobenzyl bromide (0.32 mL, 2.36 mmol) and K_2CO_3 (778 mg, 5.63 mmol) were used. The crude compound was purified by silica gel column chromatography using 6% ethyl acetate in petroleum ether. **Appearance**: yellowish oil; $\mathbf{n_D^{20}}$: 1.5760. **Yield**: 425 mg, 69%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.50 (t, J = 8.2 Hz, 1H), 7.41 (dd, J = 9.9, 2.1 Hz, 1H), 7.28 (dd, J = 8.2, 2.1 Hz, 1H), 7.13 – 7.05 (m, 3H), 7.01 (dd, J = 7.8, 1.7 Hz, 1H), 3.68 (d, J = 1.2 Hz, 2H), 3.56 (s, 2H), 2.80 (t, J = 5.9 Hz, 2H), 2.68 (t, J = 5.9 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.43, 134.52, 133.89, 132.56, 128.40, 126.32, 125.98, 125.44, 124.53, 124.15, 124.05, 115.88, 55.11, 53.75, 50.02, 28.59. **LC-MS** (*m/z*): 275.8 [M + H]$^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.1%.

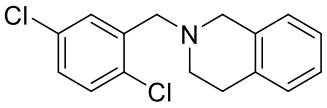
2-(4-Bromo-2-fluorobenzyl)-1,2,3,4-tetrahydroisoquinoline (8j, Bcy-140), CAS: 1283947-25-0



This compound was synthesized using the same procedure as for **8a**. 1,2,3,4-Tetrahydroisoquinoline (0.28 mL, 2.25 mmol), DMF (10 mL), 2-fluoro-4-bromobenzyl bromide (632 mg, 2.36 mmol) and K_2CO_3 (778 mg, 5.63 mmol) were used. The crude

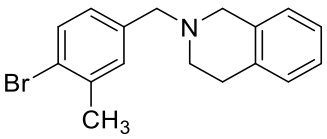
compound was purified by silica gel column chromatography using 5% ethyl acetate in petroleum ether. **Appearance**: yellowish oil; $\mathbf{n_D^{20}}$: 1.5889. **Yield**: 425 mg, 59%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.52 (dd, J = 9.6, 1.9 Hz, 1H), 7.47 – 7.38 (m, 2H), 7.14 – 7.05 (m, 3H), 7.01 (dd, J = 7.8, 1.8 Hz, 1H), 3.66 (d, J = 1.2 Hz, 2H), 3.56 (s, 2H), 2.80 (t, J = 5.9 Hz, 2H), 2.68 (t, J = 5.9 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.47, 134.52, 133.89, 132.89, 128.40, 127.44, 126.31, 125.98, 125.44, 124.56, 120.23, 118.63, 55.11, 53.80, 50.04, 28.59. **LC-MS** (*m/z*): 319.7 [M - H]$^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.9%.

2-(2,5-Dichlorobenzyl)-1,2,3,4-tetrahydroisoquinoline (8k, PSB-21139, Bcy-139), CAS: 1057279-72-7



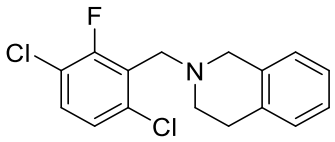
This compound was synthesized using the same procedure as for **8a**. 1,2,3,4-Tetrahydroisoquinoline (0.28 mL, 2.25 mmol), DMF (10 mL), 2,5-dichlorobenzyl bromide (566 mg, 2.36 mmol) and K_2CO_3 (778 mg, 5.63 mmol) were used. The crude compound was purified by silica gel column chromatography using 3.5% ethyl acetate in petroleum ether. **Appearance**: white solid; **mp**: 62.0-63.5 °C. **Yield**: 389 mg, 59%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.58 (d, J = 2.6 Hz, 1H), 7.49 (d, J = 8.5 Hz, 1H), 7.38 (dd, J = 8.5, 2.7 Hz, 1H), 7.16 – 7.07 (m, 3H), 7.06 – 7.00 (m, 1H), 3.73 (s, 2H), 3.63 (s, 2H), 2.83 (t, J = 5.9 Hz, 2H), 2.74 (t, J = 5.9 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 138.26, 134.51, 131.79, 130.92, 129.91, 128.46, 128.42, 126.36, 126.04, 125.49, 57.94, 55.33, 50.28, 28.61. **LC-MS** (*m/z*): 291.9 [M - H]$^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.0%.

2-(4-Bromo-3-methylbenzyl)-1,2,3,4-tetrahydroisoquinoline (8l, Bcy-207), CAS: 1896109-23-1



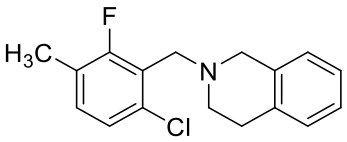
This compound was synthesized using the same procedure as for **8a**. 1,2,3,4-Tetrahydroisoquinoline (0.19 mL, 1.50 mmol), 4-bromo-3-methylbenzylbromide (475 mg, 1.80 mmol), K_2CO_3 (518 mg, 3.75 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 3% ethyl acetate in petroleum ether. **Appearance**: colorless oil; $\mathbf{n_D^{20}}$: 1.6034. **Yield**: 190 mg, 40%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.53 (d, J = 8.1 Hz, 1H), 7.34 (d, J = 2.4 Hz, 1H), 7.15 – 7.05 (m, 4H), 7.01 – 6.97 (m, 1H), 3.58 (s, 2H), 3.52 (s, 2H), 2.80 (t, J = 5.9 Hz, 2H), 2.66 (t, J = 5.9 Hz, 2H), 2.34 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 138.21, 136.94, 134.67, 134.02, 131.85, 131.36, 128.40, 128.13, 126.32, 125.94, 125.43, 60.98, 55.34, 50.19, 28.62, 22.33. **LC-MS** (*m/z*): 315.9 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.6%.

2-(3,6-Dichloro-2-fluorobenzyl)-1,2,3,4-tetrahydroisoquinoline (8m, Bcy-143)



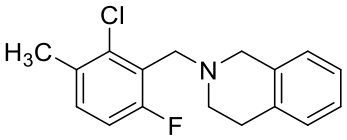
This compound was synthesized using the same procedure as for **8a**. 1,2,3,4-Tetrahydroisoquinoline (0.28 mL, 2.25 mmol), DMF (10 mL), 3,6-dichloro-2-fluorobenzyl bromide (609 mg, 2.36 mmol) and K_2CO_3 (778 mg, 5.63 mmol) were used. The crude compound was purified by silica gel column chromatography using 3% ethyl acetate in petroleum ether. **Appearance**: white solid; **mp**: 61.5-62.8 °C. **Yield**: 444 mg, 64%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.60 (t, J = 8.3 Hz, 1H), 7.40 (dd, J = 8.7, 1.6 Hz, 1H), 7.13 – 7.04 (m, 3H), 7.05 – 6.99 (m, 1H), 3.79 (d, J = 2.3 Hz, 2H), 3.63 (s, 2H), 2.81 – 2.70 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 157.50, 134.35, 134.30, 133.75, 130.21, 128.36, 126.35, 126.23, 126.02, 125.55, 125.46, 118.86, 55.11, 52.26, 50.08, 28.51. **LC-MS** (*m/z*): 310.0 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.1%.

2-(6-Chloro-2-fluoro-3-methylbenzyl)-1,2,3,4-tetrahydroisoquinoline (8n, Bcy-189)



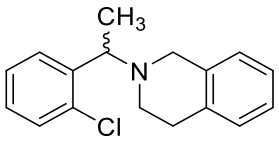
This compound was synthesized using the same procedure as for **8a**. 1,2,3,4-Tetrahydroisoquinoline (0.19 mL, 1.50 mmol), 6-chloro-2-fluoro-3-methylbenzyl bromide (428 mg, 1.80 mmol), K_2CO_3 (518 mg, 3.75 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 3% ethyl acetate in petroleum ether. **Appearance**: white solid; **mp**: 49.0-51.0 ℃. **Yield**: 242 mg, 56%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.30 – 7.20 (m, 2H), 7.12 – 7.04 (m, 3H), 7.03 – 6.99 (m, 1H), 3.74 (d, J = 2.1 Hz, 2H), 3.62 (s, 2H), 2.82 – 2.69 (m, 4H), 2.24 (d, J = 2.1 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.05, 134.53, 133.84, 132.67, 131.24, 128.35, 126.32, 125.97, 125.43, 124.81, 123.51, 123.01, 55.23, 51.97, 50.06, 28.55, 14.03. **LC-MS** (*m/z*): 289.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.8%.

2-(2-Chloro-6-fluoro-3-methylbenzyl)-1,2,3,4-tetrahydroisoquinoline (8o, Bcy-202)



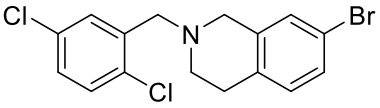
This compound was synthesized using the same procedure as for **8a**. 1,2,3,4-Tetrahydroisoquinoline (0.28 mL, 2.25 mmol), 2-chloro-6-fluoro-3-methylbenzyl bromide (641 mg, 2.70 mmol), K_2CO_3 (778 mg, 5.63 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 3% ethyl acetate in petroleum ether. **Appearance**: yellowish solid; **mp**: 101.5-103.0 ℃. **Yield**: 490 mg, 75%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.36 (dd, J = 8.5, 6.2 Hz, 1H), 7.15 (t, J = 8.9 Hz, 1H), 7.12 – 7.04 (m, 3H), 7.04 – 6.98 (m, 1H), 3.76 (d, J = 2.2 Hz, 2H), 3.62 (s, 2H), 2.82 – 2.68 (m, J = 3.3 Hz, 4H), 2.33 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.66, 134.53, 133.84, 132.16, 130.80, 128.33, 126.30, 125.95, 125.41, 123.51, 113.66, 113.48, 55.21, 52.19, 50.07, 28.54, 19.78. **LC-MS** (*m/z*): 289.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.7%.

2-(1-(2-Chlorophenyl)ethyl)-1,2,3,4-tetrahydroisoquinoline (8p, Bcy-205)



This compound was synthesized using the same procedure as for **8a**. 1,2,3,4-Tetrahydroisoquinoline (0.19 mL, 1.50 mmol), 1-chloro-2-(1-chloroethyl)benzene (0.26 mL, 1.80 mmol), K_2CO_3 (518 mg, 3.75 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 2% ethyl acetate in petroleum ether. **Appearance**: colorless oil; $\mathbf{n_D^{20}}$: 1.5840. **Yield**: 176 mg, 43%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.62 (dd, J = 7.5, 1.7 Hz, 1H), 7.43 (dt, J = 8.0, 1.1 Hz, 1H), 7.38 – 7.32 (m, 1H), 7.30 – 7.24 (m, 1H), 7.13 – 7.05 (m, 3H), 7.05 – 6.99 (m, 1H), 3.99 (q, J = 6.6 Hz, 1H), 3.79 (d, J = 14.9 Hz, 1H), 3.51 (d, J = 14.8 Hz, 1H), 2.87 – 2.56 (m, 4H), 1.34 (d, J = 6.6 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 141.87, 134.80, 134.20, 129.29, 128.25, 128.23, 127.42, 126.50, 125.87, 125.40, 59.33, 52.94, 47.70, 28.70, 19.26. **LC-MS** (*m/z*): 271.7 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.6%.

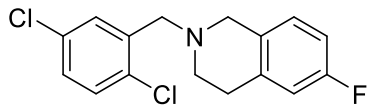
7-Bromo-2-(2,5-dichlorobenzyl)-1,2,3,4-tetrahydroisoquinoline (8q, Bcy-274)



This compound was synthesized using the same procedure as for **8a**. 7-Bromo-1,2,3,4-tetrahydroisoquinoline (200 mg, 0.94 mmol), 2,5-dichlorobenzyl bromide (271 mg, 1.13 mmol), K_2CO_3 (332 mg, 2.40 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 2% ethyl acetate in petroleum ether. **Appearance**: yellowish oil; $\mathbf{n_D^{20}}$: 1.6080. **Yield**: 338 mg, 97%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.56 (d, J = 2.6 Hz, 1H), 7.49 (d, J = 8.5 Hz, 1H), 7.38 (dd, J = 8.5, 2.7 Hz, 1H), 7.33 – 7.26 (m, 2H), 7.08 (d, J = 8.1 Hz, 1H), 3.73 (s, 2H), 3.63 (s, 2H), 2.79 (t, J = 5.9 Hz, 2H), 2.73 (dd, J = 6.4, 4.6 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 138.08, 137.28, 133.41, 131.79, 130.90, 130.64, 129.88, 128.96,

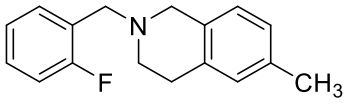
128.83, 128.43, 118.29, 57.63, 54.64, 49.84, 27.98. **LC-MS** (*m/z*): 371.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.2%.

2-(2,5-Dichlorobenzyl)-6-fluoro-1,2,3,4-tetrahydroisoquinoline (8r, Bcy-318)



This compound was synthesized using the same procedure as for **8a**. 6-Fluoro-1,2,3,4-tetrahydroisoquinoline (0.18 mL, 1.32 mmol), 2,5-dichlorobenzyl bromide (379 mg, 1.58 mmol), K_2CO_3 (456 mg, 3.30 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 1% ethyl acetate in petroleum ether and preparative HPLC. **Appearance**: yellowish oil; $\mathbf{n_D^{20}}$: 1.5855. **Yield**: 40 mg, 10%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.56 (d, J = 2.7 Hz, 1H), 7.49 (d, J = 8.5 Hz, 1H), 7.38 (dd, J = 8.5, 2.7 Hz, 1H), 7.08 (dd, J = 8.2, 5.9 Hz, 1H), 6.97 – 6.90 (m, 2H), 3.73 (s, 2H), 3.60 (s, 2H), 2.84 (t, J = 5.9 Hz, 2H), 2.72 (t, J = 5.9 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.71, 138.16, 136.38, 131.79, 130.92, 130.58, 129.91, 128.45, 128.18, 114.50, 112.59, 57.82, 54.68, 49.79, 28.68. **LC-MS** (*m/z*): 310.10 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.9%.

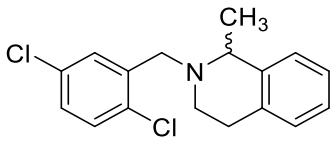
2-(2-Fluorobenzyl)-6-methyl-1,2,3,4-tetrahydroisoquinoline (8s, Bcy-121)



This compound was synthesized using the same procedure as for **8a**. 6-Methyl-1,2,3,4-tetrahydroisoquinoline (0.30 mL, 2.04 mmol), DMF (10 mL), 2-fluorobenzyl bromide (0.26 mL, 2.14 mmol) and K_2CO_3 (705 mg, 5.10 mmol) were used. The crude compound was purified by silica gel column chromatography using 3% ethyl acetate in petroleum ether. **Appearance**: colorless oil; $\mathbf{n_D^{20}}$: 1.5869. **Yield**: 182 mg, 35%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.33 – 7.23 (m, 1H), 7.23 – 7.16 (m, 2H), 7.16 – 7.04 (m, 1H), 6.78 – 6.64 (m, 2H), 6.34 (d, J = 8.2 Hz, 1H), 4.47 (s, 2H), 2.69 (t, J = 6.3 Hz, 2H), 2.10 (s, 3H), 1.96 – 1.82 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.58, 142.61, 129.47, 128.56, 128.48, 127.22, 125.49, 124.27, 123.93, 121.82, 115.31, 110.62, 49.33,

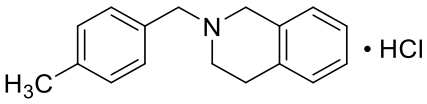
48.24, 27.50, 21.93, 19.85. **LC-MS** (*m/z*): 255.7 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.6%.

2-(2,5-Dichlorobenzyl)-1-methyl-1,2,3,4-tetrahydroisoquinoline (8t, Bcy-268)



This compound was synthesized using the same procedure as for **8a**. 1-Methyl-1,2,3,4-tetrahydroisoquinoline (0.21 mL, 1.36 mmol), 2,5-dichlorobenzyl bromide (391 mg, 1.63 mmol), K_2CO_3 (470 mg, 3.40 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 2% ethyl acetate in petroleum ether and preparative HPLC. **Appearance**: colorless oil; $\mathbf{n_D^{20}}$: 1.5952. **Yield**: 295 mg, 71%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.60 (d, J = 2.7 Hz, 1H), 7.47 (d, J = 8.5 Hz, 1H), 7.36 (dd, J = 8.5, 2.7 Hz, 1H), 7.14 – 7.07 (m, 4H), 3.87 (q, J = 6.7 Hz, 1H), 3.78 (s, 2H), 3.06 – 2.97 (m, 1H), 2.90 – 2.80 (m, 1H), 2.71 – 2.61 (m, 2H), 1.32 (d, J = 6.6 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 139.86, 139.34, 133.60, 131.81, 131.51, 130.82, 129.55, 128.61, 128.16, 127.19, 125.73, 125.55, 56.19, 54.22, 43.44, 26.92, 19.62. **LC-MS** (*m/z*): 306.10 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.8%.

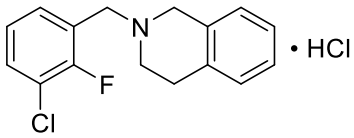
2-(4-Methylbenzyl)-1,2,3,4-tetrahydroisoquinoline hydrochloride (9a, Bcy-200H), CAS: 1452863-51-2



The precursor of **9a** was synthesized using the same procedure as for **8a**. 1,2,3,4-Tetrahydroisoquinoline (0.28 mL, 2.25 mmol), 4-methylbenzyl bromide (500 mg, 2.70 mmol), K_2CO_3 (778 mg, 5.63 mmol) and EtOH (10 mL) were used for generating the precursor of **9a**. The crude compound was purified by silica gel column chromatography using 6% ethyl acetate in petroleum ether. The oily precursor of **9a** (1 eq.) was dissolved in 3 mL ethyl acetate, then 1.78 mL HCl solution (1 M in ethyl

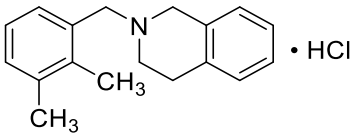
acetate, 1.2 eq.) was added dropwise at 0 ℃. The reaction mixture was stirred at 0 ℃ for 5 min. After the reaction was completed, **9a** was obtained by filtration and washed with cold ethyl acetate (1 mL). **Appearance**: white solid; **mp**: 231-233 ℃ (*lit.*[171], 232-233 ℃). **Yield**: 352 mg, 66%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.23 (d, J = 8.0 Hz, 2H), 7.16 – 7.04 (m, 5H), 7.00 – 6.95 (m, 1H), 3.58 (s, 2H), 3.51 (s, 2H), 2.79 (t, J = 5.9 Hz, 2H), 2.65 (t, J = 5.9 Hz, 2H), 2.29 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 135.91, 135.24, 134.80, 134.08, 128.74, 128.64, 128.36, 126.26, 125.86, 125.37, 61.55, 55.36, 50.09, 28.64, 20.64. **LC-MS** (*m/z*): 237.8 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.4%.

2-(3-Chloro-2-fluorobenzyl)-1,2,3,4-tetrahydroisoquinoline hydrochloride (9b, Bcy-197H)



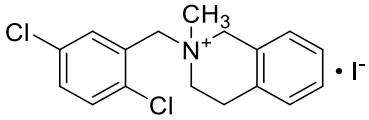
This compound was synthesized using the same procedure as for **9a**. 1,2,3,4-Tetrahydroisoquinoline (0.28 mL, 2.25 mmol), 3-chloro-2-fluorobenzyl bromide (603 mg, 2.70 mmol), K_2CO_3 (778 mg, 5.63 mmol) and EtOH (10 mL) were used for generating the precursor of **9b**. The crude compound was purified by silica gel column chromatography using 4.5% ethyl acetate in petroleum ether. Then 1.90 mL HCl solution (1 M in ethyl acetate) and 3 mL ethyl acetate were used for generating **9b**. **Appearance**: white solid; **mp**: 216.0-218.0 ℃. **Yield**: 435 mg, 70%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.56 – 7.40 (m, 2H), 7.24 – 7.20 (m, 1H), 7.12 – 7.07 (m, 3H), 7.01 (dd, J = 7.8, 1.8 Hz, 1H), 3.74 (d, J = 1.3 Hz, 2H), 3.58 (s, 2H), 2.81 (t, J = 5.9 Hz, 2H), 2.70 (t, J = 5.9 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 156.65, 134.50, 133.88, 130.08, 129.31, 128.40, 127.04, 126.33, 126.00, 125.45, 125.13, 119.61, 55.14, 54.33, 50.07, 28.59. **LC-MS** (*m/z*): = 276. 0 $[M + H]^+$, Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.1%.

2-(2,3-Dimethylbenzyl)-1,2,3,4-tetrahydroisoquinoline hydrochloride (9c, Bcy-204H)



This compound was synthesized using the same procedure as for **9a**. 1,2,3,4-Tetrahydroisoquinoline (0.28 mL, 2.25 mmol), 2,3-dimethylbenzyl bromide (538 mg, 2.70 mmol), K_2CO_3 (778 mg, 5.63 mmol) and EtOH (10 mL) were used for generating the precursor of **9c**. The crude compound was purified by silica gel column chromatography using 2% ethyl acetate in petroleum ether. Then 1.44 mL HCl solution (1 M in ethyl acetate) and 3 mL ethyl acetate were used for generating **9c**. **Appearance**: white powder; **mp**: 206.0-208.0 °C. **Yield**: 302 mg, 53%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.14 – 7.01 (m, 6H), 6.98 (dd, J = 7.8, 1.7 Hz, 1H), 3.59 (s, 2H), 3.53 (s, 2H), 2.78 (t, J = 5.9 Hz, 2H), 2.66 (t, J = 5.9 Hz, 2H), 2.23 (d, J = 4.6 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 136.40, 136.04, 135.75, 134.83, 134.16, 128.67, 128.37, 127.76, 126.31, 125.89, 125.39, 124.79, 60.71, 55.61, 50.21, 28.73, 20.06, 14.64. **LC-MS** (*m/z*): 251.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.5%.

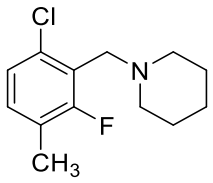
2-(2,5-Dichlorobenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-ium iodide (10, Bcy-360)



To the solution of **8k** (50 mg, 0.17 mmol, 1 eq.) in MeCN (8 mL), CH_3I (0.03 mL, 0.51 mmol, 3 eq.) was added. The reaction mixture was stirred at 80 °C for 3 h and the reaction progress was monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, the mixture was cooled to rt, and the solvent was evaporated *in vacuum*. The crude product was purified by silica gel column chromatography using 5% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 118-119 °C. **Yield**: 56 mg, 76%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.85 (d, J = 2.2 Hz, 1H), 7.74 – 7.66 (m, 2H), 7.40 – 7.28 (m, 3H), 7.22 (d, J = 7.4 Hz, 1H), 4.80 (d, J = 13.1 Hz, 3H), 4.57 (d, J = 15.1 Hz, 1H), 3.84

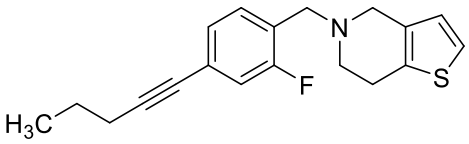
(dd, *J* = 8.5, 5.6 Hz, 2H), 3.27 – 3.15 (m, 2H), 3.04 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 135.27, 134.90, 132.30, 132.15, 129.55, 128.73, 128.23, 127.58, 127.15, 127.03, 126.82, 63.31, 60.63, 57.47, 45.80, 23.15. **LC-MS** (*m/z*): 308.20 $[M - I + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%.

1-(6-Chloro-2-fluoro-3-methylbenzyl)piperidine (11, Bcy-213)



This compound was synthesized using the same procedure as for **8a**. Piperidine (0.12 mL, 1.26 mmol), 6-chloro-2-fluoro-3-mehylbenzyl bromide (359 mg, 1.51 mmol), K_2CO_3 (435 mg, 3.15 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 4% ethyl acetate in petroleum ether. **Appearance**: colorless oil; $\mathbf{n_D^{20}}$: 1.5268. **Yield**: 176 mg, 58%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.26 – 7.17 (m, 2H), 3.52 (s, 2H), 2.38 (t, *J* = 5.2 Hz, 4H), 2.21 (d, *J* = 2.3 Hz, 3H), 1.44 (p, *J* = 5.5 Hz, 4H), 1.40 – 1.29 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.76, 130.97, 130.92, 124.67, 123.34, 123.19, 53.74, 52.90, 25.47, 23.75, 13.98, 13.95. **LC-MS** (*m/z*): 241.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.3%.

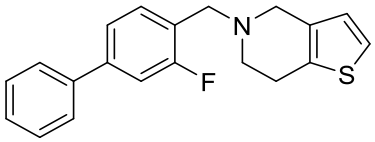
5-(2-Fluoro-4-(pent-1-yn-1-yl)benzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (12a, Bcy-165)



To the solution of **4s** (400 mg, 1.23 mmol, 1 eq.) in DMF (10 mL), 1-pentyne (0.30 mL, 3.08 mmol, 2.5 eq.), $Pd(PPh_3)_2Cl_2$ (35 mg, 4%), CuI (19 mg, 8%) and Et_3N (0.68 mL, 4.92 mmol, 4 eq.) were added. The reaction mixture was stirred at 90 ℃ under argon for 6 h and the reaction progress was monitored by TLC (EtOAc/petroleum ether, 1:9). After the reaction was completed, the mixture was cooled to rt, 3 g silica gel was added, and the solvent was evaporated *in vacuum*. The crude product was purified by silica gel

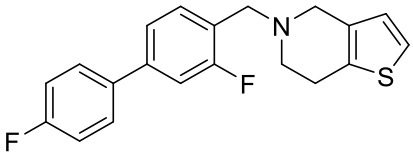
column chromatography and then flash chromatography using 4% and 8% ethyl acetate in petroleum ether, respectively. **Appearance**: yellowish oil; $\mathbf{n_D^{20}}$: 1.5858. **Yield**: 135 mg, 35%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.42 (t, J = 8.0 Hz, 1H), 7.28 – 7.17 (m, 3H), 6.76 (d, J = 5.1 Hz, 1H), 3.71 (s, 2H), 3.46 (d, J = 1.9 Hz, 2H), 2.77 (dt, J = 29.0, 5.9 Hz, 4H), 2.40 (t, J = 7.0 Hz, 2H), 1.56 (h, J = 7.2 Hz, 2H), 1.00 (t, J = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.10, 132.67, 131.49, 127.37, 127.35, 125.37, 122.95, 117.74, 117.58, 91.55, 79.53, 53.49, 52.17, 50.07, 24.95, 21.51, 20.50, 13.29. **LC-MS** (*m/z*): 314.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.0%.

5-((3-Fluoro-[1,1'-biphenyl]-4-yl)methyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (12b, Bcy-163)



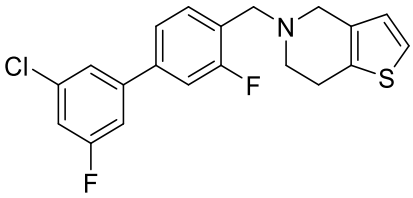
To the solution of **4s** (200 mg, 0.61 mmol, 1 eq.) in dioxane/H_2O (2:1, 9 mL), phenylboronic acid (112 mg, 0.92 mmol, 1.5 eq.), $Pd(PPh_3)_2Cl_2$ (42 mg, 0.06 mmol, 0.1 eq.) and K_2CO_3 (253 mg, 1.83 mmol, 3 eq.) were added. The reaction mixture was stirred at 90 °C under argon for 1 h and the reaction progress was monitored by TLC (EtOAc/petroleum ether, 1:9). After the reaction was completed, the mixture was cooled to rt, 3 g silica gel was added, and the solvent was evaporated *in vacuum*. The crude product was purified by silica gel column chromatography and then flash chromatography using 6% and 10% ethyl acetate in petroleum ether, respectively. **Appearance**: yellowish oil; $\mathbf{n_D^{20}}$: 1.6285. **Yield**: 81 mg, 41%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.78 – 7.65 (m, 2H), 7.59 – 7.43 (m, 5H), 7.43 – 7.35 (m, 1H), 7.26 (d, J = 5.1 Hz, 1H), 6.78 (d, J = 5.1 Hz, 1H), 3.76 (s, 2H), 3.51 (s, 2H), 2.80 (dt, J = 9.4, 4.8 Hz, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.97, 141.26, 138.58, 133.87, 132.71, 131.87, 128.96, 127.93, 126.65, 125.41, 123.82, 122.94, 122.37, 113.30, 53.59, 52.25, 50.11, 24.99. **LC-MS** (*m/z*): 324.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.1%.

5-((3,4'-Difluoro-[1,1'-biphenyl]-4-yl)methyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (12c, Bcy-164)



This compound was synthesized using the same procedure as for **12b**. Compound **4s** (200 mg, 0.61 mmol), dioxane/H_2O (2:1, 9 mL), 4-fluorophenylboronic acid (129 mg, 0.92 mmol), $Pd(PPh_3)_2Cl_2$ (42 mg, 0.06 mmol) and K_2CO_3 (253 mg, 1.83 mmol) were used. The crude compound was purified by silica gel column chromatography using 6% ethyl acetate in petroleum ether. **Appearance**: colorless oil; $\mathbf{n_D^{20}}$: 1.6010. **Yield**: 111 mg, 53%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.81 – 7.71 (m, 2H), 7.57 – 7.46 (m, 3H), 7.34 – 7.20 (m, 3H), 6.78 (d, J = 5.1 Hz, 1H), 3.76 (s, 2H), 3.51 (d, J = 1.7 Hz, 2H), 2.80 (dt, J = 9.6, 5.1 Hz, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.91, 160.32, 140.18, 135.06, 133.86, 132.71, 131.90, 128.76, 125.40, 123.91, 123.81, 122.95, 122.34, 115.83, 115.69, 113.32, 53.56, 52.24, 50.09, 24.98. **LC-MS** (*m/z*): 341.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.9%.

5-((3'-Chloro-3,5'-difluoro-[1,1'-biphenyl]-4-yl)methyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (12d, Bcy-167)



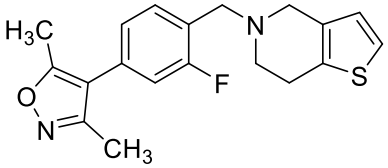
This compound was synthesized using the same procedure as for **12b**. Compound **4s** (200 mg, 0.61 mmol), 3-chloro-5-fluorobenzeneboronic acid (160 mg, 0.92 mmol), $Pd(PPh_3)_2Cl_2$ (42 mg, 0.06 mmol), K_2CO_3 (253 mg, 1.83 mmol) and dioxane/H_2O (2:1, 9 mL) were used. The crude compound was purified by silica gel column chromatography using 4% ethyl acetate in petroleum ether and preparative HPLC. **Appearance**: yellow oil; $\mathbf{n_D^{20}}$: 1.6170. **Yield**: 72 mg, 31%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.69 (t, J = 1.7 Hz, 1H), 7.65 – 7.63 (m, 1H), 7.63 – 7.59 (m, 2H), 7.56 (t, J = 7.8 Hz, 1H), 7.47 – 7.43 (m, 1H), 7.26 (d, J = 5.1 Hz, 1H), 6.78 (d, J = 5.1 Hz, 1H), 3.77

(s, 2H), 3.51 (d, *J* = 1.7 Hz, 2H), 2.85 – 2.75 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.54, 161.57, 142.17, 138.24, 134.54, 133.80, 132.68, 131.85, 125.42, 125.36, 122.94, 122.85, 122.67, 115.31, 113.64, 112.68, 53.49, 52.24, 50.08, 24.95. **LC-MS** (*m/z*): 376.20 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.8%.

5-(2-Fluoro-4-(thiophen-3-yl)benzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (12e, Bcy-172)

This compound was synthesized using the same procedure as for **12b**. Compound **4s** (200 mg, 0.61 mmol), dioxane/H_2O (2:1, 9 mL), 3-thiopheneboronic acid (118 mg, 0.92 mmol), $Pd(PPh_3)_2Cl_2$ (42 mg, 0.06 mmol) and K_2CO_3 (253 mg, 1.83 mmol) were used. The crude compound was purified by silica gel column chromatography using 10% ethyl acetate in petroleum ether. **Appearance**: yellowish solid; **mp**: 88.0-89.5 °C. **Yield**: 201 mg, 100%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.97 (dd, *J* = 2.9, 1.3 Hz, 1H), 7.65 (dd, *J* = 5.0, 2.9 Hz, 1H), 7.62 – 7.53 (m, 3H), 7.48 (t, *J* = 7.9 Hz, 1H), 7.25 (d, *J* = 5.1 Hz, 1H), 6.77 (d, *J* = 5.1 Hz, 1H), 3.73 (s, 2H), 3.50 (d, *J* = 1.8 Hz, 2H), 2.85 – 2.73 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.36, 140.04, 136.25, 133.88, 132.71, 131.84, 127.22, 126.13, 125.39, 123.43, 122.94, 121.80, 112.70, 53.61, 52.22, 50.06, 24.98. **LC-MS** (*m/z*): 329.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.1%.

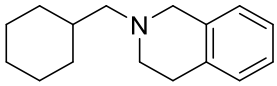
4-(4-((6,7-Dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)methyl)-3-fluorophenyl)-3,5-dimethylisoxazole (12f, Bcy-173)



This compound was synthesized using the same procedure as for **12b**. Compound **4s** (200 mg, 0.61 mmol), 3,5-dimethylisoxazole-4-boronic acid (130 mg, 0.92 mmol), $Pd(PPh_3)_2Cl_2$ (42 mg, 0.06 mmol), K_2CO_3 (253 mg, 1.83 mmol) and dioxane/H_2O (2:1,

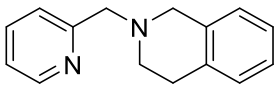
9 mL) were used. The crude compound was purified by silica gel column chromatography using 10% ethyl acetate in petroleum ether and preparative HPLC. **Appearance**: yellowish oil. **Yield**: 9 mg, 4%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.55 (t, J = 7.9 Hz, 1H), 7.30 – 7.24 (m, 2H), 7.22 (dd, J = 7.8, 1.7 Hz, 1H), 6.79 (d, J = 5.1 Hz, 1H), 3.76 (d, J = 3.3 Hz, 2H), 3.52 (s, 2H), 2.84 – 2.77 (m, 4H), 2.42 (s, 3H), 2.24 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.49, 159.76, 158.00, 133.80, 132.69, 131.69, 130.90, 125.39, 124.73, 124.03, 122.93, 115.56, 53.56, 52.24, 50.12, 24.93, 11.33, 10.39. **LC-MS** (*m/z*): 343.20 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.4%.

2-(Cyclohexylmethyl)-1,2,3,4-tetrahydroisoquinoline (14, Bcy-203), CAS: 1226112-04-4



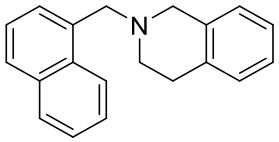
This compound was synthesized using the same procedure as for **8a**. 1,2,3,4-Tetrahydroisoquinoline (0.28 mL, 2.25 mmol), cyclohexylmethyl bromide (0.38 mL, 2.70 mmol), K_2CO_3 (778 mg, 5.63 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 3.5% ethyl acetate in petroleum ether. **Appearance**: colorless oil; $\mathbf{n_D^{20}}$: 1.5386. **Yield**: 278 mg, 45%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.12 – 7.05 (m, 3H), 7.05 – 7.00 (m, 1H), 3.49 (s, 2H), 2.78 (t, J = 5.9 Hz, 2H), 2.60 (t, J = 5.8 Hz, 2H), 2.24 (d, J = 7.2 Hz, 2H), 1.76 (dd, J = 13.3, 3.7 Hz, 2H), 1.71 – 1.53 (m, 4H), 1.30 – 1.09 (m, 3H), 0.93 – 0.81 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 135.02, 134.20, 128.27, 126.29, 125.78, 125.30, 64.78, 56.10, 50.90, 34.61, 31.27, 28.72, 26.36, 25.51. **LC-MS** (*m/z*): 230.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.8%.

2-(Pyridin-2-ylmethyl)-1,2,3,4-tetrahydroisoquinoline (15, Bcy-293), CAS: 5666-61-5



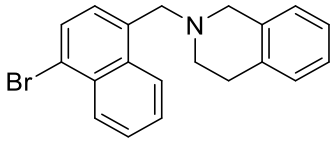
This compound was synthesized using the same procedure as for **8a**. 2-(Chloromethyl)pyridine hydrochloride (0.19 mL, 1.44 mmol), 1,2,3,4-tetrahydroisoquinoline (284 mg, 1.73 mmol), K_2CO_3 (498 mg, 3.60 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 3% MeOH in DCM. **Appearance**: yellow oil; $\mathbf{n_D^{20}}$: 1.5842. **Yield**: 265 mg, 82%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.54 – 8.48 (m, 1H), 7.80 – 7.73 (m, 1H), 7.48 (dd, J = 7.9, 1.2 Hz, 1H), 7.31 – 7.25 (m, 1H), 7.14 – 7.05 (m, 3H), 7.00 (dd, J = 6.8, 1.3 Hz, 1H), 3.77 (s, 2H), 3.60 (s, 2H), 2.83 (t, J = 5.8 Hz, 2H), 2.73 (t, J = 5.9 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.53, 148.74, 136.49, 134.70, 133.97, 128.39, 126.29, 125.92, 125.40, 122.58, 122.11, 63.49, 55.44, 50.37, 28.64. **LC-MS** (*m/z*): 225.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.1%.

2-(Naphthalen-1-ylmethyl)-1,2,3,4-tetrahydroisoquinoline (16a, Bcy-209), CAS: 258504-30-2



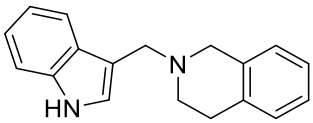
This compound was synthesized using the same procedure as for **8a**. 1,2,3,4-Tetrahydroisoquinoline (0.28 mL, 2.25 mmol), 1-(bromomethyl)naphthalene (597 mg, 2.70 mmol), K_2CO_3 (778 mg, 5.63 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 2.5% ethyl acetate in petroleum ether. **Appearance**: white solid; **mp**: 144.0-146.0 °C (*lit.*[172] 210-212 °C). **Yield**: 562 mg, 91%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.36 – 8.29 (m, 1H), 7.97 – 7.90 (m, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.56 – 7.42 (m, 4H), 7.16 – 7.03 (m, 3H), 7.03 – 6.94 (m, 1H), 4.05 (s, 2H), 3.62 (s, 2H), 2.77 (dq, J = 9.8, 5.0 Hz, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 134.76, 134.13, 133.95, 133.47, 132.03, 128.38, 128.19, 127.79, 127.32, 126.34, 125.92, 125.73, 125.65, 125.42, 125.22, 124.79, 60.20, 55.66, 50.30, 28.71. **LC-MS** (*m/z*): 274.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.7%.

2-((4-Bromonaphthalen-1-yl)methyl)-1,2,3,4-tetrahydroisoquinoline (16b, Bcy-212)



This compound was synthesized using the same procedure as for **8a**. 1,2,3,4-Tetrahydroisoquinoline (0.19 mL, 1.50 mmol), 1-bromo-4-(bromomethyl)naphthalene (540 mg, 1.80 mmol), K_2CO_3 (518 mg, 3.75 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 4% ethyl acetate in petroleum ether. **Appearance**: yellowish oil; $\mathbf{n_D^{20}}$: 1.6454. **Yield**: 327 mg, 62%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.54 – 8.30 (m, 1H), 8.17 (dd, J = 8.4, 1.2 Hz, 1H), 7.86 (d, J = 7.5 Hz, 1H), 7.72 – 7.59 (m, 2H), 7.46 (d, J = 7.6 Hz, 1H), 7.14 – 7.03 (m, 3H), 6.99 (dd, J = 7.1, 2.2 Hz, 1H), 4.05 (s, 2H), 3.62 (s, 2H), 2.83 – 2.71 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 134.65, 134.06, 131.31, 129.40, 128.38, 128.03, 127.55, 126.80, 126.68, 126.35, 125.96, 125.63, 125.44, 121.49, 59.73, 55.56, 50.29, 28.67. **LC-MS** (*m/z*): 354.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.3%.

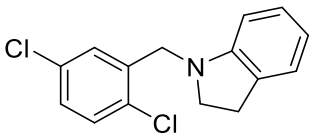
2-((1*H*-Indol-3-yl)methyl)-1,2,3,4-tetrahydroisoquinoline (17, Bcy-201), CAS: 159390-88-2



To a solution of 1,2,3,4-tetrahydroisoquinoline (0.28 mL, 2.25 mmol, 1 eq.) in anhydrous toluene (10 mL), gramine (589 mg, 3.38 mmol, 1.5 eq.) was added. The mixture was refluxed overnight, and the reaction progress was monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, the mixture was cooled to rt, and the solvent was evaporated *in vacuum*. The crude product was purified by silica gel column chromatography using 4% MeOH in DCM. **Appearance**: light orange solid; **mp**: 135.5-137.5 ℃ (*lit.*[173] 141 ℃). **Yield**: 200 mg, 22%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.92 (s, 1H), 7.65 (d, J = 7.9 Hz, 1H), 7.35 (d, J = 8.1 Hz, 1H), 7.28 (d, J = 2.3 Hz, 1H), 7.13 – 7.02 (m, 4H), 7.01 – 6.93 (m, 2H), 3.80 (s, 2H), 3.31 (s, 2H), 2.78 (t, J

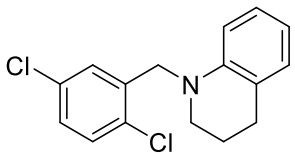
= 5.9 Hz, 2H), 2.70 (t, *J* = 5.9 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 136.37, 135.11, 134.26, 128.34, 127.53, 126.32, 125.80, 125.35, 124.51, 120.92, 119.08, 118.35, 111.32, 110.87, 55.47, 53.14, 50.07, 28.82. **LC-MS** (*m/z*): 263.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.7%.

1-(2,5-Dichlorobenzyl)indoline (19, Bcy-295)



This compound was synthesized using the same procedure as for **8a**. Indoline (0.19 mL, 1.68 mmol), 2,5-dichlorobenzyl bromide (485 mg, 2.02 mmol), K_2CO_3 (580 mg, 4.20 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 2% ethyl acetate in petroleum ether and preparative HPLC. **Appearance**: colorless oil; $\mathbf{n_D^{20}}$: 1.6228. **Yield**: 43 mg, 9%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.53 (d, *J* = 8.5 Hz, 1H), 7.48 (d, *J* = 2.6 Hz, 1H), 7.40 (dd, *J* = 8.5, 2.7 Hz, 1H), 7.07 (dd, *J* = 7.1, 1.3 Hz, 1H), 7.01 – 6.95 (m, 1H), 6.66 – 6.59 (m, 1H), 6.48 (d, *J* = 7.6 Hz, 1H), 4.31 (s, 2H), 3.35 (t, *J* = 8.3 Hz, 2H), 2.95 (t, *J* = 8.3 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 151.79, 138.08, 131.87, 131.24, 131.04, 129.42, 129.00, 128.57, 127.10, 124.28, 117.67, 106.73, 53.35, 50.62, 27.98. **LC-MS** (*m/z*): 278.10 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.6%.

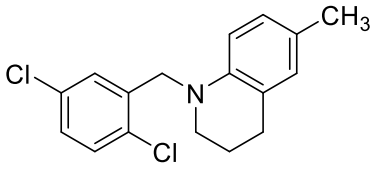
1-(2,5-Dichlorobenzyl)-1,2,3,4-tetrahydroquinoline (20a, Bcy-294)



This compound was synthesized using the same procedure as for **8a**. 1,2,3,4-Tetrahydroquinoline (0.19 mL, 1.50 mmol), 2,5-dichlorobenzyl bromide (432 mg, 1.80 mmol), K_2CO_3 (518 mg, 3.75 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 2% ethyl acetate in petroleum ether. **Appearance**: reddish solid; **mp**: 73.0-75.0 °C. **Yield**: 336 mg, 77%.

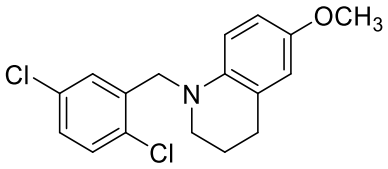
^{1}H NMR (500 MHz, DMSO-d_6) δ 7.53 (d, J = 8.4 Hz, 1H), 7.37 (dd, J = 8.5, 2.7 Hz, 1H), 7.12 (d, J = 2.6 Hz, 1H), 6.94 (dd, J = 7.3, 1.6 Hz, 1H), 6.91 – 6.84 (m, 1H), 6.55 – 6.47 (m, 1H), 6.21 (dd, J = 8.2, 1.0 Hz, 1H), 4.47 (s, 2H), 3.43 – 3.34 (m, 2H), 2.77 (t, J = 6.3 Hz, 2H), 2.01 – 1.89 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 144.51, 138.08, 132.02, 131.26, 130.69, 128.88, 128.28, 127.02, 121.99, 116.01, 110.28, 52.67, 49.51, 27.40, 21.87. **LC-MS** (*m/z*): 291.9 [M - H]$^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.8%.

1-(2,5-Dichlorobenzyl)-6-methyl-1,2,3,4-tetrahydroquinoline (20b, Bcy-269)



This compound was synthesized using the same procedure as for **8a**. 6-Methyl-1,2,3,4-tetrahydroquinoline (200 mg, 1.36 mmol), 2,5-dichlorobenzyl bromide (391 mg, 1.63 mmol), K_2CO_3 (470 mg, 3.40 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 2% ethyl acetate in petroleum ether. **Appearance**: colorless oil; $\mathbf{n_D^{20}}$: 1.5930. **Yield**: 400 mg, 96%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.60 (d, J = 2.7 Hz, 1H), 7.47 (d, J = 8.5 Hz, 1H), 7.36 (dd, J = 8.5, 2.7 Hz, 1H), 7.13 – 7.07 (m, 4H), 3.87 (q, J = 6.6 Hz, 1H), 3.78 (s, 2H), 3.08 – 2.95 (m, 1H), 2.91 – 2.79 (m, 1H), 2.70 – 2.61 (m, 2H), 1.32 (d, J = 6.6 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 139.87, 139.35, 133.61, 131.82, 131.52, 130.83, 129.55, 128.62, 128.17, 127.19, 125.74, 125.56, 56.19, 54.22, 43.44, 26.92, 19.62. **LC-MS** (*m/z*): 306.0 [M - H]$^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.7%.

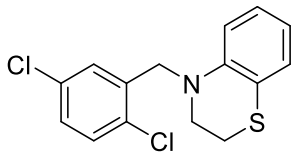
1-(2,5-Dichlorobenzyl)-6-methoxy-1,2,3,4-tetrahydroquinoline (20c, Bcy-275)



This compound was synthesized using the same procedure as for **8a**. 6-Methoxy-1,2,3,4-tetrahydroquinoline (200 mg, 1.23 mmol), 2,5-dichlorobenzyl bromide (355 mg,

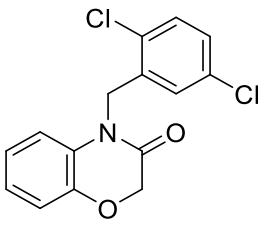
1.48 mmol), K_2CO_3 (426 mg, 3.08 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 2% ethyl acetate in petroleum ether. **Appearance**: yellow solid; **mp**: 58.0-60.0 °C. **Yield**: 361 mg, 91%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.52 (d, J = 8.5 Hz, 1H), 7.36 (dd, J = 8.5, 2.6 Hz, 1H), 7.17 (d, J = 2.7 Hz, 1H), 6.60 (d, J = 3.0 Hz, 1H), 6.53 (dd, J = 8.8, 3.0 Hz, 1H), 6.17 (d, J = 8.8 Hz, 1H), 4.40 (s, 2H), 3.62 (s, 3H), 3.31 (d, J = 5.6 Hz, 2H), 2.76 (t, J = 6.3 Hz, 2H), 2.00 – 1.88 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 150.68, 138.99, 138.50, 131.98, 131.18, 130.64, 128.20, 127.33, 123.43, 114.97, 112.31, 111.47, 55.16, 53.29, 49.60, 27.57, 22.07. **LC-MS** (*m/z*): 322.1 [M - H]$^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.7%.

4-(2,5-Dichlorobenzyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]thiazine (20d, Bcy-290)



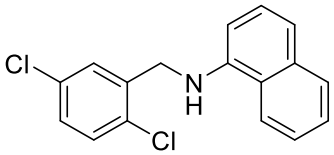
This compound was synthesized using the same procedure as for **8a**. 3,4-Dihydro-2*H*-1,4-benzothiazine (200 mg, 1.32 mmol), 2,5-dichlorobenzyl bromide (379 mg, 1.58 mmol), K_2CO_3 (456 mg, 3.30 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 2% ethyl acetate in petroleum ether and preparative HPLC. **Appearance**: white solid. **Yield**: 4 mg, 1%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 (d, J = 8.1 Hz, 1H), 7.31 (s, 1H), 7.24 (d, J = 2.5 Hz, 1H), 7.15 (dd, J = 7.7, 1.5 Hz, 1H), 7.02 – 6.95 (m, 1H), 6.71 (t, J = 7.5 Hz, 1H), 6.46 (d, J = 8.3 Hz, 1H), 4.58 (s, 2H), 3.82 – 3.77 (m, 2H), 3.21 – 3.14 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 143.11, 137.33, 133.44, 131.20, 130.98, 128.53, 128.24, 127.60, 126.45, 118.19, 117.92, 113.27, 55.06, 51.11, 25.89. **LC-MS** (*m/z*): 310.10 [M - H]$^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.9%.

4-(2,5-Dichlorobenzyl)-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one (21, Bcy-288)



This compound was synthesized using the same procedure as for **8a**. 2*H*-1,4-Benzoxazin-3(4*H*)-one (200 mg, 1.34 mmol), 2,5-dichlorobenzyl bromide (386 mg, 1.61 mmol), K_2CO_3 (463 mg, 3.35 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 5% ethyl acetate in petroleum ether. **Appearance**: white solid; **mp**: 112.0-114.0 ℃. **Yield**: 388 mg, 94%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.57 (dd, J = 7.8, 2.0 Hz, 1H), 7.40 (dd, J = 8.5, 2.6 Hz, 1H), 7.15 (d, J = 2.5 Hz, 1H), 7.09 – 6.94 (m, 3H), 6.82 (dd, J = 7.9, 1.5 Hz, 1H), 5.12 (s, 2H), 4.86 (d, J = 3.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.66, 144.99, 135.49, 132.27, 131.28, 130.42, 128.90, 128.36, 126.72, 123.95, 122.74, 116.64, 115.33, 67.14, 42.29. **LC-MS** (*m/z*): 308.1 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.2%.

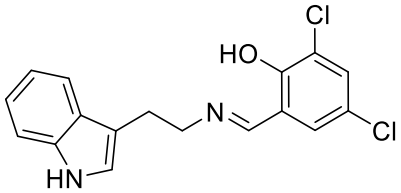
_N_-(2,5-Dichlorobenzyl)naphthalen-1-amine (22, Bcy-333), CAS: 1486983-50-9



This compound was synthesized using the same procedure as for **8a**. 1-Naphthylamine (200 mg, 1.40 mmol), 2,5-dichlorobenzyl bromide (403 mg, 1.68 mmol), K_2CO_3 (484 mg, 3.50 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 2% ethyl acetate in petroleum ether and preparative HPLC. **Appearance**: white solid; **mp**: 85.5-87.5 ℃. **Yield**: 72 mg, 17%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.29 – 8.21 (m, 1H), 7.83 – 7.75 (m, 1H), 7.58 – 7.51 (m, 1H), 7.51 – 7.44 (m, 2H), 7.36 (dd, J = 6.0, 2.8 Hz, 2H), 7.21 (t, J = 7.8 Hz, 1H), 7.14 (d, J = 8.0 Hz, 1H), 6.98 (t, J = 5.9 Hz, 1H), 6.24 (d, J = 7.4 Hz, 1H), 4.54 (d, J = 5.8 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 142.97, 139.12, 134.02, 131.88, 130.95, 130.84, 128.22, 128.04, 127.75, 126.62, 125.77, 124.30, 122.96, 121.44, 116.19,

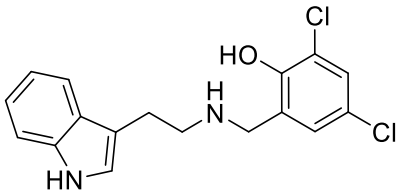
103.29, 44.16. **LC-MS** (*m/z*): 302.10 [M - H]$^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.4%.

(*E*)-2-(((2-(1*H*-Indol-3-yl)ethyl)imino)methyl)-4,6-dichlorophenol (1d, Bcy-106), CAS: 299420-53-4



To a solution of tryptamine (200 mg, 1.25 mmol, 1 eq.) in MeOH (10 mL), 3,5-dichlorosalicyladehyde (250 mg, 1.31 mmol, 1.05 eq.) was added. The reaction mixture was stirred at rt for 24 h and the reaction progress was monitored by TLC (MeOH/DCM, 5:95). After the reaction was completed, the solvent was dried *in vacuum*. The residue was then washed with hexane and the pure product was obtained after crystallization using MeOH. **Appearance**: bright yellow solid; **mp**: 108.5-110.0 °C (*lit.*[64] 160-162 °C). **Yield**: 101 mg, 24%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 14.62 (s, 1H), 10.86 (s, 1H), 8.45 (s, 1H), 7.58 (dd, J = 7.8, 1.0 Hz, 1H), 7.53 (d, J = 2.7 Hz, 1H), 7.36 – 7.29 (m, 2H), 7.16 (d, J = 2.3 Hz, 1H), 7.07 (ddd, J = 8.0, 6.9, 1.1 Hz, 1H), 6.97 (ddd, J = 7.9, 6.9, 1.0 Hz, 1H), 3.92 (t, J = 6.9 Hz, 2H), 3.10 (t, J = 6.9 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.21, 163.91, 136.25, 132.83, 130.24, 126.90, 124.72, 123.22, 121.04, 118.33, 118.26, 116.53, 116.50, 111.42, 110.48, 53.97, 25.79. **LC-MS** (*m/z*): 333.0 [M + H]$^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 93.9%.

2-(((2-(1*H*-Indol-3-yl)ethyl)amino)methyl)-4,6-dichlorophenol (24, Bcy-390)



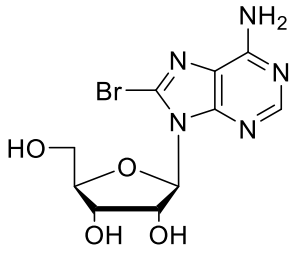
To a solution of **1d** (30 mg, 0.09 mmol, 1 eq.) in MeOH (5 mL), $NaBH_4$ (9 mg, 0.23 mmol, 2.5 eq.) was added. The mixture was stirred at 50 °C for 3 h and the reaction progress was monitored by TLC (MeOH/DCM, 5:95). After the reaction was completed,

the solvent was evaporated *in vacuum*. The crude mixture was purified by silica gel column chromatography using 3% MeOH in DCM. **Appearance**: white solid; **mp**: 167-169 °C. **Yield**: 31 mg, >100%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.82 (s, 1H), 7.50 (d, J = 7.9 Hz, 1H), 7.33 (d, J = 8.2 Hz, 1H), 7.30 (d, J = 2.6 Hz, 1H), 7.17 (d, J = 2.4 Hz, 1H), 7.09 – 7.04 (m, 2H), 7.00 – 6.94 (m, 1H), 4.68 (s, 2H), 3.98 (s, 2H), 3.01 – 2.78 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.65, 136.24, 127.16, 127.00, 126.61, 125.66, 122.74, 120.89, 120.36, 120.18, 118.18, 118.07, 111.34, 111.27, 50.55, 47.98, 24.31. **LC-MS** (*m/z*): 335.1 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.9%.

General procedure for the synthesis of AMP derivatives and analogs

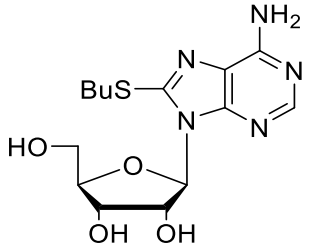
Nucleoside (1 eq.) was dissolved in $PO(OCH_3)_3$ (5 mL), and proton sponge (1.5 eq.) was added. The mixture was cooled to 0 °C under argon, $POCl_3$ (4 eq.) was added 5 min later. The mixture was stirred at 0 °C for 3-4 h and monitored by TLC (2-propanol/NH_4OH (25% in H_2O)/H_2O, 6:3:1). After the reaction was completed, the mixture was quenched by a cold 10 mL 0.5 M aqueous TEAC buffer (pH 7.4-7.6), or H_2O, or saturated aqueous NH_4HCO_3 solution, and stirred at 0 °C for several minutes. The solution was allowed to reach rt and left standing for 1 h. $PO(OCH_3)_3$ and proton sponge were extracted by *tert*-butylmethylether (500 mL), and the inorganic layer was lyophilized. The crude product was finally purified by semi-preparative HPLC. Appropriate fractions were collected and lyophilized to yield the desired nucleoside monophosphate.

8-Bromoadenosine (26a, Bcy-1), CAS, 2946-39-6



To adenosine (3.00 g, 11.23 mmol, 1 eq.) in 1 M sodium acetate buffer (pH 4.0, 5 mL) and H_2O (15 mL), Br_2 (1.44 mL, 28.08 mmol, 2.5 eq.) was added dropwise. The mixture was stirred at rt overnight and monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, the mixture was decolorized by the addition of 1 M $NaHSO_3$ solution, and then neutralized with 2 M NaOH. The solvent was concentrated *in vacuum* and cooled to 4 °C for crystallization, then filtered and washed with 5 mL H_2O. **Appearance**: orange solid; **mp**: 228.0-230.0 °C (*lit.*[174] >200 °C). **Yield**: 2.29 g, 59%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.11 (s, 1H), 7.53 (s, 2H), 5.82 (d, J = 6.7 Hz, 1H), 5.48 (dd, J = 3.9, 8.4 Hz, 1H), 5.45 (d, J = 7.2 Hz, 1H), 5.21 (d, J = 4.9 Hz, 1H), 5.07 (dd, J = 3.8, 6.8 Hz, 1H), 4.18 (m, 1H), 3.97 (d, J = 3.2 Hz, 1H), 3.67-3.51 (m, 2H). ^{13}C NMR (126 MHz, DMSO- d_6) δ 155.34, 152.61, 150.05, 127.35, 119.86, 90.59, 86.90, 71.30, 71.06, 62.30. **LC-MS** (*m/z*): 346.1 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.8%.

8-(Butylthio)adenosine (27a, Bcy-67), CAS: 68807-84-1

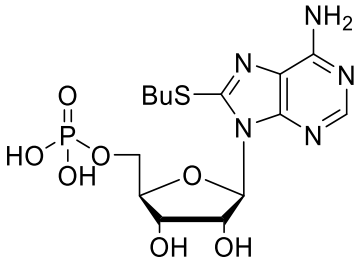


Method 1: To a solution of **26a** (1.00 g, 2.89 mmol, 1 eq.) in EtOH (10 mL), thiourea (440 mg, 5.78 mmol, 2 eq.) was added. The mixture was refluxed overnight. Then butylbromide (0.24 mL, 5.78 mmol, 2 eq.) and H_2O (10 mL) were added and basified slightly with 2 M NaOH. The mixture was refluxed for 3 h and monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, cooled to rt, 5 g silica gel was added, and the solvent was evaporated *in vacuum*. The crude mixture was purified by silica gel column chromatography using 7% MeOH in DCM. **Yield**: 838 mg, 82%. **LC-MS** (*m/z*): 356.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.0%.

Method 2: To a solution of **26a** (1.00 g, 2.89 mmol, 1 eq.) in 10 mL EtOH, NaOMe (468 mg, 8.67 mmol, 3eq.) and 1-butanethiol (0.93 mL, 8.67 mmol, 3 eq.) were added. The mixture was refluxed overnight, and the reaction progress was monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, cooled to rt, 5 g silica gel was

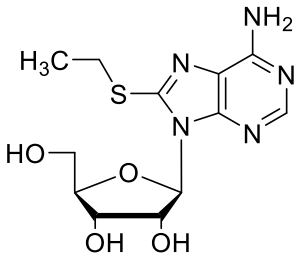
added, and the solvent was evaporated *in vacuum*. The crude mixture was purified by silica gel column chromatography using 8% MeOH in DCM. **Appearance**: white solid; **mp**: 160.0-162.0 °C (*lit.*[175] 171.5 °C). **Yield**: 723 mg, 70%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.05 (s, 1H), 7.26 (s, 2H), 5.77 (d, J = 6.9 Hz, 1H), 5.62 (dd, J = 9.0, 3.7 Hz, 1H), 5.38 (d, J = 6.4 Hz, 1H), 5.16 (d, J = 4.4 Hz, 1H), 5.00 (q, J = 6.3 Hz, 1H), 4.21 – 4.12 (m, 1H), 4.04 – 3.93 (m, 1H), 3.74 – 3.62 (m, 1H), 3.60 – 3.46 (m, 1H), 3.37 – 3.32 (m, 1H), 3.30 – 3.25 (m, 1H), 1.68 (p, J = 7.3 Hz, 2H), 1.42 (h, J = 7.4 Hz, 2H), 0.90 (t, J = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.54, 151.26, 150.41, 148.69, 119.61, 88.86, 86.59, 71.26, 70.99, 62.22, 32.06, 30.88, 21.18, 13.42. **LC-MS** (*m/z*): 356.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.1%.

8-BuS-AMP (1i, PSB-20112, Bcy-112), CAS: 344402-39-7



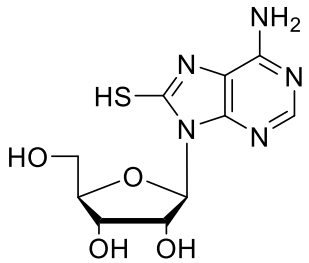
Compound **27a** (400 mg, 1.13 mmol), $PO(OCH_3)_3$ (10 mL), proton sponge (364 mg, 1.70 mmol) and $POCl_3$ (0.42 mL, 4.52 mmol) were used. **Appearance**: white powder; **mp**: 182.5-183.5 °C (*lit.*[79] 152 °C). **Yield**: 116 mg, 24%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.11 (s, 2H), 5.76 (d, J = 5.9 Hz, 1H), 5.11 (t, J = 5.7 Hz, 2H), 4.27 (dd, J = 5.5, 3.8 Hz, 2H), 4.10 – 3.86 (m, 3H), 3.83 – 3.56 (m, 2H), 3.41 – 3.16 (m, 2H), 1.79 – 1.60 (m, 2H), 1.52 – 1.34 (m, 2H), 0.90 (t, J = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.26, 151.67, 151.13, 148.68, 119.30, 88.50, 83.61, 70.75, 70.35, 64.36, 31.87, 30.90, 21.24, 13.44. ^{31}P NMR (243 MHz, DMSO-d_6) δ 0.99. **LC-MS** (*m/z*): 436.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.4%.

8-(Ethylthio)adenosine (27b, Bcy-181), CAS: 63614-44-8



To a solution of 8-bromoadenosine (**26a**, 400 mg, 1.16 mmol, 1 eq.) in anhydrous DMF (10 mL), sodium ethanethiolate (293 mg, 3.48 mmol, 3 eq.) was added. The mixture was stirred at rt overnight and the reaction progress was monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, 5 g silica gel was added, and the solvent was evaporated *in vacuum*. The crude compound was purified by silica gel column chromatography using 8% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 175.0-177.0 °C (*lit.*[154] 176 °C). **Yield**: 266 mg, 70%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.05 (s, 1H), 7.27 (s, 2H), 5.76 (d, J = 6.9 Hz, 1H), 5.62 (dd, J = 8.9, 3.7 Hz, 1H), 5.38 (d, J = 6.5 Hz, 1H), 5.16 (d, J = 4.4 Hz, 1H), 5.07 – 4.92 (m, 1H), 4.25 – 4.07 (m, 1H), 4.00 – 3.90 (m, 1H), 3.72 – 3.44 (m, 2H), 2.89 (s, 1H), 2.73 (s, 1H), 1.35 (q, J = 6.8, 6.2 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.56, 151.27, 150.39, 148.48, 119.63, 88.85, 86.58, 71.25, 70.97, 62.21, 26.76, 14.81. **LC-MS** (*m/z*): 328.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.7%.

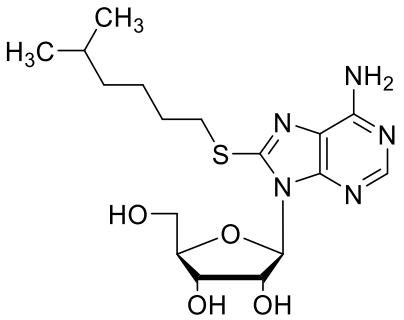
8-Thioadenosine (or 8-mercaptoadenosine, 26b, CS-389), CAS: 3001-45-4



To a solution of 8-bromoadenosine (**26a**, 500 mg, 1.44 mmol, 1 eq.) in DMF (5 mL), NaHS (810 mg, 14.40 mmol, 10 eq.) and 1 mL H_2O were added. The mixture was refluxed overnight and monitored by TLC (MeOH/DCM, 1:4). After the reaction was completed, the mixture was cooled down to rt and treated with MeOH followed by filtration. The filtrate was evaporated and co-evaporated with MeOH. The remaining

residue was taken up in H_2O, neutralized with 1 M HCl and lyophilized. The crude product was taken up in MeOH and DCM, then filtered and the residue was washed by MeOH and DCM. The filtrate was evaporated *in vacuum* and purified by silica gel column chromatography using 8% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 216 °C (*lit.*[154] 169-170 °C). **Yield**: 257 mg, 59%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.52 (s, 1H), 8.11 (s, 1H), 6.95 (br s, 2H), 6.33 (d, 1H, J = 6.3 Hz), 5.22 (d, 1H, J = 6.1 Hz), 5.18 (dd, 1H, J = 4.1, 8.2 Hz), 5.08 (d, 1H, J = 4.7 Hz), 4.99 (q, 1H, J = 5.9 Hz), 4.21 (m, 1H), 3.89 (q, 1H, J = 3.9 Hz), 3.65-3.49 (d m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.21, 152.20, 148.44, 148.18, 107.33, 88.95, 85.90, 71.01, 70.95, 62.44. **LC-MS** (*m/z*): 300.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.1%. This compound was co-synthesized with Dr. Constanze Cerine Schmies.

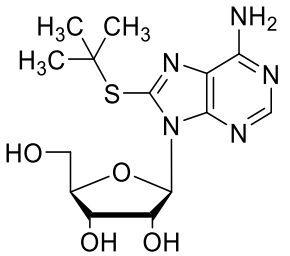
8-(5-Methylhexylthio)adenosine (27h, CS-390)



To a solution of 8-thioadenosine (**26b,** 200 mg, 0.67 mmol, 1 eq.) in EtOH/H_2O (1:1, 10 mL), 1-bromo-5-methylhexane (0.22 mL, 1.34 mmol, 2 eq.) was added and then basified slightly with 2 M NaOH. The mixture was refluxed for 3 h and monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, the mixture was cooled down to rt and extracted by ethyl acetate (30 mL × 3). The organic layers were combined, dried over $MgSO_4$, and evaporated *in vacuum*. The crude compound was purified by silica gel column chromatography using 6% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 180 °C. **Yield**: 80 mg, 30%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.04 (s, 1H), 7.22 (s, 2H), 5.77 (d, 1H, J = 6.9 Hz), 5.59 (dd, 1H, J = 3.7, 8.9 Hz), 5.35 (d, 1H, J = 6.2 Hz), 5.14 (d, 1H, J = 4.3 Hz), 4.99 (q, 1H, J = 6.2 Hz), 4.15 (m, 1H), 3.95 (td, 1H, J = 2.2, 3.8 Hz), 3.66-3.51 (d m, 2H), 3.28 (m, 2H, SCH_2 overlapping with

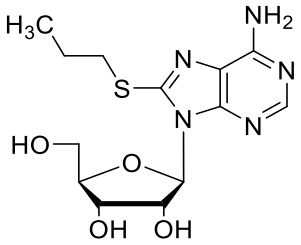
H_2O), 1.67 (m, 2H), 1.49 (m, 1H), 1.39 (m, 2H), 1.16 (m, 2H), 0.84 (s, 3H), 0.83 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.69, 151.41, 150.56, 148.86, 119.76, 89.03, 86.74, 71.43, 71.14, 62.37, 37.95, 32.58, 29.25, 27.47, 25.98, 22.58. **LC-MS** (*m/z*): 398.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%. This compound was co-synthesized with Dr. Constanze Cerine Schmies.

8-(*Tert*-butylthio)adenosine (27c, Bcy-227), CAS: 127236-49-1



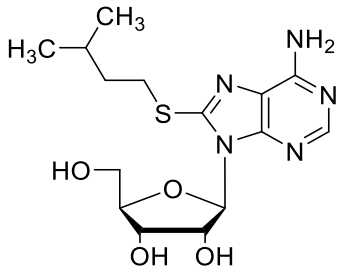
This compound was synthesized using the same procedure as for **27a** (Method 2). 8-Bromoadenosine (**26a**, 600 mg, 1.73 mmol), 10 mL EtOH, NaOMe (280 mg, 5.19 mmol) and 2-methyl-2-propanethiol (0.59 mL, 5.19 mmol) were used. The crude compound was purified by silica gel column chromatography using 10% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 217.0-218.0 °C. **Yield**: 125 mg, 20%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.44 (s, 2H), 6.06 (d, J = 6.9 Hz, 1H), 5.65 (dd, J = 9.2, 3.5 Hz, 1H), 5.27 (d, J = 6.5 Hz, 1H), 5.12 (d, J = 4.1 Hz, 1H), 5.07 – 4.97 (m, 1H), 4.23 – 4.14 (m, 1H), 4.01 – 3.89 (m, 1H), 3.73 – 3.62 (m, 1H), 3.58 – 3.47 (m, 1H), 1.45 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.48, 152.08, 149.44, 145.73, 119.81, 89.17, 86.51, 71.33, 71.11, 62.30, 50.32, 30.80. **LC-MS** (*m/z*): 356.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.6%.

8-(Propylthio)adenosine (27d, Bcy-233)



This compound was synthesized using the same procedure as for **27a** (Method 2). 8-Bromoadenosine (**26a**, 500 mg, 1.44 mmol), 10 mL EtOH, NaOMe (233 mg, 4.32 mmol) and 1-propanethiol (0.39 mL, 4.32 mmol) were used. The crude compound was purified by silica gel column chromatography using 8% MeOH in DCM. **Appearance**: milk white solid; **mp**: 189.5-191.0 ℃. **Yield**: 183 mg, 37%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.05 (d, J = 1.2 Hz, 1H), 7.24 (s, 2H), 5.78 (dd, J = 6.9, 1.1 Hz, 1H), 5.66 – 5.56 (m, 1H), 5.36 (dd, J = 6.6, 1.2 Hz, 1H), 5.15 (dd, J = 4.4, 1.2 Hz, 1H), 5.09 – 4.94 (m, 1H), 4.22 – 4.10 (m, 1H), 3.96 (dd, J = 4.1, 2.5 Hz, 1H), 3.74 – 3.63 (m, 1H), 3.58 – 3.48 (m, 1H), 3.30 – 3.21 (m, 2H), 1.84 – 1.63 (m, 2H), 1.05 – 0.95 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.52, 151.24, 150.40, 148.65, 119.58, 88.85, 86.57, 71.24, 70.96, 62.20, 34.23, 22.26, 12.99. **LC-MS** (*m/z*): 342.1[M + H]$^{+}$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%.

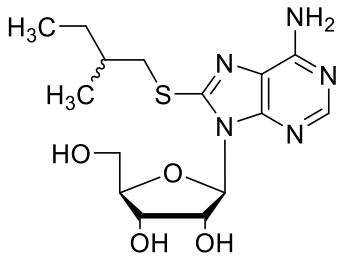
8-(Isopentylthio)adenosine (27e, Bcy-234)



This compound was synthesized using the same procedure as for **27a** (Method 2). 8-Bromoadenosine (**26a**, 500 mg, 1.44 mmol), 10 mL EtOH, NaOMe (233 mg, 4.32 mmol) and 3-methyl-1-butanethiol (0.54 mL, 4.32 mmol) were used. The crude compound was purified by silica gel column chromatography using 7% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 155.0-156.0 ℃. **Yield**: 208 mg, 39%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.06 (s, 1H), 7.23 (s, 2H), 5.77 (d, J = 6.9 Hz, 1H), 5.60 (dd, J = 8.9, 3.8 Hz, 1H), 5.36 (d, J = 6.4 Hz, 1H), 5.15 (d, J = 4.4 Hz, 1H), 5.06 – 4.92 (m, 1H), 4.24 – 4.10 (m, 1H), 4.02 – 3.93 (m, 1H), 3.67 (dt, J = 12.2, 3.8 Hz, 1H), 3.59 – 3.47 (m, 1H), 3.36 – 3.30 (m, 1H), 3.29 – 3.24 (m, 1H), 1.70 (dq, J = 13.3, 6.7 Hz, 1H), 1.63 – 1.54 (m, 2H), 0.91 (d, J = 6.6 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.53, 151.25, 150.38, 148.62, 119.60, 88.85, 86.56, 71.24, 70.96, 62.19, 37.67, 30.57, 26.78,

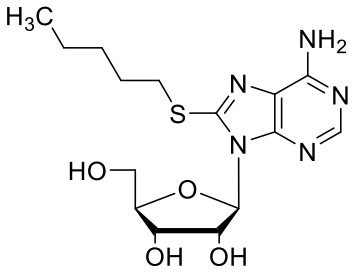
22.05, 22.02. **LC-MS** (*m/z*): 370.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%.

8-(2-Methylbutylthio)adenosine (27f, Bcy-235)



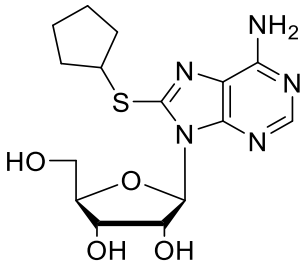
This compound was synthesized using the same procedure as for **27a** (Method 2). 8-Bromoadenosine (**26a**, 500 mg, 1.44 mmol), 10 mL EtOH, NaOMe (233 mg, 4.32 mmol) and 2-methyl-1-butanethiol (0.53 mL, 4.32 mmol) were used. The crude compound was purified by silica gel column chromatography using 8% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 165.0-166.5 °C. **Yield**: 254 mg, 48%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.05 (s, 1H), 7.24 (s, 2H), 5.79 (d, J = 6.9 Hz, 1H), 5.62 (dd, J = 8.9, 3.7 Hz, 1H), 5.38 (d, J = 6.4 Hz, 1H), 5.17 (d, J = 4.3 Hz, 1H), 5.05 – 4.96 (m, 1H), 4.23 – 4.12 (m, 1H), 4.03 – 3.92 (m, 1H), 3.67 (dt, J = 12.1, 3.8 Hz, 1H), 3.59 – 3.44 (m, 1H), 3.35 – 3.31 (m, 1H), 3.25 (dd, J = 12.7, 7.0 Hz, 1H), 1.85 – 1.73 (m, 1H), 1.62 – 1.44 (m, 1H), 1.33 – 1.21 (m, 1H), 0.99 (d, J = 6.7 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.49, 151.22, 150.43, 148.99, 119.54, 88.85, 86.60, 71.26, 71.00, 62.21, 38.97, 34.10, 27.85, 18.46, 11.05. **LC-MS** (*m/z*): 370.3 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%.

8-(Pentylthio)adenosine (27g, Bcy-184), CAS: 68807-85-2



This compound was synthesized using the same procedure as for **27a** (Method 2). 8-Bromoadenosine (**26a**, 400 mg, 1.16 mmol), 10 mL EtOH, NaOMe (188 mg, 3.48 mmol) and 1-pentanethiol (0.43 mL, 3.48 mmol) were used. The crude compound was purified by silica gel column chromatography using 7% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 172.5-174.5 °C. **Yield**: 194 mg, 45%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.05 (s, 1H), 7.25 (s, 2H), 5.77 (d, J = 6.9 Hz, 1H), 5.62 (dd, J = 8.9, 3.7 Hz, 1H), 5.37 (d, J = 6.4 Hz, 1H), 5.16 (d, J = 4.3 Hz, 1H), 5.09 – 4.94 (m, 1H), 4.22 – 4.11 (m, 1H), 4.00 – 3.92 (m, 1H), 3.67 (dt, J = 12.2, 3.7 Hz, 1H), 3.59 – 3.45 (m, 1H), 3.36 – 3.31 (m, 1H), 3.29 – 3.24 (m, 1H), 1.70 (p, J = 7.3 Hz, 2H), 1.41 – 1.28 (m, 4H), 0.87 (t, J = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.53, 151.25, 150.40, 148.69, 119.60, 88.85, 86.58, 71.25, 70.98, 62.21, 32.32, 30.19, 28.49, 21.59, 13.80. **LC-MS** (*m/z*): 370.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.8%.

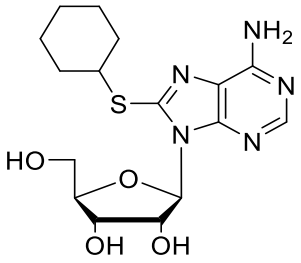
8-(Cyclopentylthio)adenosine (27i, Bcy-66)



This compound was synthesized using the same procedure as for **27a** (Method 1). 8-Bromoadenosine (**26a**, 500 mg, 1.44 mmol), EtOH (10 mL), thiourea (219 mg, 2.88 mmol) and bromocyclopentane (0.15 mL, 2.88 mmol) were used. The crude compound was purified by silica gel column chromatography using 7% MeOH in DCM. **Appearance**: white solid; **mp**: 66.0-68.0 °C. **Yield**: 90 mg, 17%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.06 (s, 1H), 7.30 (s, 2H), 6.55 (s, 1H), 5.81 (d, J = 6.9 Hz, 1H), 5.64 (ddd, J = 9.1, 3.6, 1.5 Hz, 1H), 5.37 (d, J = 6.4 Hz, 1H), 5.16 (d, J = 4.3 Hz, 1H), 5.00 (td, J = 6.7, 5.2 Hz, 1H), 4.16 (td, J = 4.8, 2.2 Hz, 1H), 3.96 (td, J = 3.8, 2.1 Hz, 1H), 3.67 (dt, J = 12.1, 3.7 Hz, 1H), 3.52 (ddd, J = 12.5, 9.1, 3.9 Hz, 1H), 2.25 – 2.05 (m, 2H), 1.84 – 1.54 (m, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.76, 151.46, 150.08, 148.36,

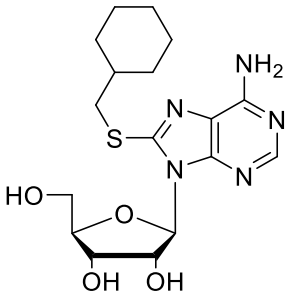
119.73, 88.99, 86.61, 71.27, 71.03, 62.24, 46.21, 33.35, 32.90, 24.28, 24.17. **LC-MS** (*m/z*): 367.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.0%.

8-(Cyclohexylthio)adenosine (27j, Bcy-188), CAS: 171502-16-2



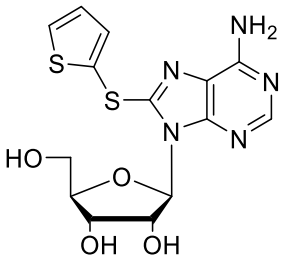
This compound was synthesized using the same procedure as for **27a** (Method 2). 8-Bromoadenosine (**26a**, 600 mg, 1.73 mmol), 10 mL EtOH, NaOMe (280 mg, 5.19 mmol) and cyclohexanethiol (0.63 mL, 5.19 mmol) were used. The crude compound was purified by silica gel column chromatography using 8% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 149.5-151.5 °C (*lit.*[146] 207-208 °C). **Yield**: 124 mg, 19%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.06 (s, 1H), 7.32 (s, 2H), 5.85 (d, J = 6.9 Hz, 1H), 5.65 (ddd, J = 9.1, 3.6, 1.2 Hz, 1H), 5.36 (d, J = 6.4 Hz, 1H), 5.16 (d, J = 4.2 Hz, 1H), 5.00 (q, J = 6.3 Hz, 1H), 4.23 – 4.10 (m, 1H), 3.96 (q, J = 3.4 Hz, 1H), 3.85 – 3.74 (m, 1H), 3.67 (dt, J = 12.1, 3.7 Hz, 1H), 3.52 (ddd, J = 12.5, 9.0, 3.9 Hz, 1H), 2.16 – 1.92 (m, 2H), 1.78 – 1.66 (m, 2H), 1.59 – 1.25 (m, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.79, 151.49, 150.04, 147.42, 119.71, 88.96, 86.60, 71.27, 71.04, 62.24, 46.62, 32.95, 32.55, 25.28, 25.17, 25.00. **LC-MS** (*m/z*): 381.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.9%.

8-(Cyclohexylmethylthio)adenosine (27k, Bcy-62)



This compound was synthesized using the same procedure as for **27a** (Method 1). 8-Bromoadenosine (**26a**, 300 mg, 0.87 mmol), EtOH (10 mL), thiourea (132 mg, 1.74 mmol) and cyclohexylmethyl bromide (0.24 mL, 1.74 mmol) were used. The crude compound was purified by silica gel column chromatography using 7% MeOH in DCM. **Appearance**: white solid; **mp**: 183.8-185.8 °C (*lit.*[176] 210-213 °C). **Yield**: 195 mg, 57%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.05 (s, 1H), 7.25 (s, 2H), 5.78 (d, J = 6.9 Hz, 1H), 5.62 (dd, J = 8.9, 3.7 Hz, 1H), 5.38 (d, J = 6.4 Hz, 1H), 5.16 (d, J = 4.3 Hz, 1H), 5.00 (td, J = 6.7, 5.2 Hz, 1H), 4.16 (td, J = 4.7, 2.2 Hz, 1H), 3.96 (td, J = 3.8, 2.2 Hz, 1H), 3.67 (dt, J = 12.2, 3.8 Hz, 1H), 3.52 (ddd, J = 12.5, 9.0, 3.9 Hz, 1H), 3.30 – 3.19 (m, 2H), 1.91 – 1.77 (m, 2H), 1.74 – 1.56 (m, 4H), 1.26 – 1.11 (m, 3H), 1.07 – 0.98 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.47, 151.20, 150.45, 149.03, 119.54, 88.83, 86.59, 71.26, 70.99, 62.21, 37.12, 31.81, 31.76, 25.73, 25.39. **LC-MS** (*m/z*): 396.0 [M + H]$^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.0%.

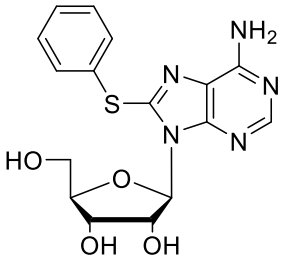
8-(2-Thienylthio)adenosine (27l, Bcy-244)



This compound was synthesized using the same procedure as for **27a** (Method 2). 8-Bromoadenosine (**26a**, 500 mg, 1.44 mmol), 10 mL EtOH, NaOMe (233 mg, 4.32 mmol) and 2-thiophenethiol (0.40 mL, 4.32 mmol) were used. The crude compound was purified by silica gel column chromatography using 12% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 121.0-123.0 °C. **Yield**: 453 mg, 82%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.11 (s, 1H), 7.79 (dd, J = 5.3, 1.3 Hz, 1H), 7.49 – 7.42 (m, 3H), 7.13 (dd, J = 5.4, 3.6 Hz, 1H), 6.13 (d, J = 6.9 Hz, 1H), 5.58 (dd, J = 8.8, 3.7 Hz, 1H), 5.42 (d, J = 6.3 Hz, 1H), 5.22 (d, J = 4.5 Hz, 1H), 5.11 – 4.98 (m, 1H), 4.25 – 4.15 (m, 1H), 4.04 – 3.95 (m, 1H), 3.74 – 3.64 (m, 1H), 3.60 – 3.51 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.47, 152.46, 149.89, 145.68, 135.88, 132.70, 128.13, 126.28,

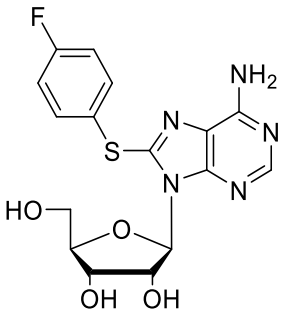
119.57, 89.39, 86.73, 70.96, 62.15, 56.00. **LC-MS** (*m/z*): 382.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.4%.

8-(Phenylthio)adenosine (27m, Bcy-284)



This compound was synthesized using the same procedure as for **27a** (Method 2). 8-Bromoadenosine (**26a**, 300 mg, 0.87 mmol), thiophenol (0.27 mL, 2.61 mmol), NaOMe (141 mg, 2.61 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 8% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 115.0-117.0 °C. **Yield**: 120 mg, 37%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.14 (d, J = 1.0 Hz, 1H), 7.53 (s, 2H), 7.43 – 7.35 (m, 4H), 7.35 – 7.31 (m, 1H), 6.10 (d, J = 6.9 Hz, 1H), 5.60 (dd, J = 8.9, 3.6 Hz, 1H), 5.36 (dd, J = 6.3, 1.0 Hz, 1H), 5.18 – 5.12 (m, 1H), 5.06 (q, J = 6.2 Hz, 1H), 4.23 – 4.17 (m, 1H), 3.96 (d, J = 3.0 Hz, 1H), 3.75 – 3.65 (m, 1H), 3.58 – 3.49 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.65, 152.62, 149.82, 144.29, 132.03, 129.81, 129.57, 127.81, 119.99, 89.56, 86.67, 71.37, 71.01, 62.16. **LC-MS** (*m/z*): 376.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.7%.

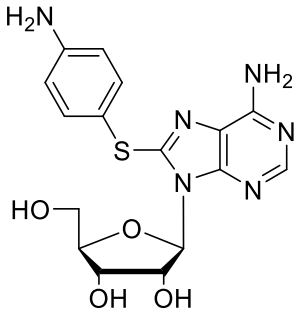
8-(4-Fluorophenylthio)adenosine (27n, Bcy-300)



This compound was synthesized using the same procedure as for **27a** (Method 2). 8-Bromoadenosine (**26a**, 300 mg, 0.87 mmol), 4-fluorothiophenol (0.56 mL, 5.22 mmol),

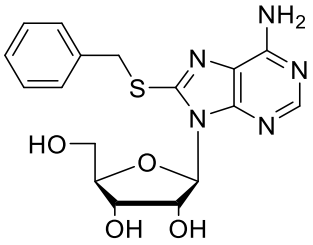
NaOMe (282 mg, 5.22 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 10% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 107.0-109.0 °C. **Yield**: 192 mg, 56%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.13 (s, 1H), 7.59 – 7.43 (m, 4H), 7.32 – 7.20 (m, 2H), 6.07 (d, J = 6.9 Hz, 1H), 5.57 (dd, J = 8.8, 3.8 Hz, 1H), 5.37 (d, J = 6.2 Hz, 1H), 5.16 (d, J = 4.4 Hz, 1H), 5.05 (q, J = 6.2 Hz, 1H), 4.25 – 4.15 (m, 1H), 4.04 – 3.92 (m, 1H), 3.69 (dt, J = 12.2, 3.8 Hz, 1H), 3.61 – 3.50 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.01, 155.52, 152.51, 149.90, 144.89, 133.18, 133.11, 126.94, 119.90, 116.77, 116.59, 89.46, 86.67, 71.34, 70.97, 62.14. **LC-MS** (*m/z*): 394.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.9%.

8-(4-Aminophenylthio)adenosine (27o, Bcy-297)



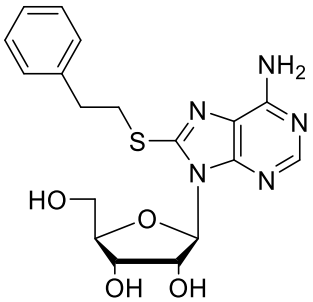
This compound was synthesized using the same procedure as for **27a** (Method 2). 8-Bromoadenosine (**26a**, 300 mg, 0.87 mmol), 4-aminothiophenol (653 mg, 5.22 mmol), NaOMe (282 mg, 5.22 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 10% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 122.0-124.0 °C. **Yield**: 184 mg, 54%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.07 (s, 1H), 7.30 (s, 2H), 7.26 – 7.20 (m, 2H), 6.60 – 6.53 (m, 2H), 6.09 (d, J = 6.7 Hz, 1H), 5.47 (s, 2H), 5.37 (d, J = 6.6 Hz, 1H), 5.17 (d, J = 4.3 Hz, 1H), 5.04 (q, J = 6.4 Hz, 1H), 4.32 (t, J = 5.1 Hz, 1H), 4.23 – 4.17 (m, 1H), 3.99 (d, J = 2.7 Hz, 1H), 3.69 (dt, J = 12.1, 3.7 Hz, 1H), 3.60 – 3.50 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.09, 151.78, 150.03, 149.96, 148.15, 134.97, 119.61, 114.58, 112.52, 89.21, 86.55, 71.34, 71.01, 62.22. **LC-MS** (*m/z*): 391.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.5%.

8-(Benzylthio)adenosine (27p, Bcy-254), CAS: 121059-93-6



This compound was synthesized using the same procedure as for **27a** (Method 2). 8-Bromoadenosine (**26a**, 400 mg, 1.16 mmol), NaOMe (188 mg, 3.48 mmol), phenylmethanethiol (0.41 mL, 3.48 mmol) and EtOH (10 mL) were used. The crude compound was purified by silica gel column chromatography using 8% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 210.0-212.0 °C (*lit.*[177] 204-206 °C). **Yield**: 314 mg, 70%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.06 (s, 1H), 7.51 – 7.47 (m, 2H), 7.35 – 7.29 (m, 4H), 7.29 – 7.24 (m, 1H), 5.74 (d, J = 6.8 Hz, 1H), 5.58 (dd, J = 8.8, 3.8 Hz, 1H), 5.38 (d, J = 6.5 Hz, 1H), 5.16 (d, J = 4.6 Hz, 1H), 5.04 – 4.92 (m, 1H), 4.58 (q, J = 13.1 Hz, 2H), 4.19 – 4.12 (m, 1H), 4.00 – 3.92 (m, 1H), 3.66 (dt, J = 12.2, 3.8 Hz, 1H), 3.56 – 3.47 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.58, 151.36, 150.52, 148.08, 137.05, 129.14, 128.42, 127.43, 119.50, 88.86, 86.59, 71.32, 70.90, 62.15, 35.95. **LC-MS** (*m/z*): 390.3 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.9%.

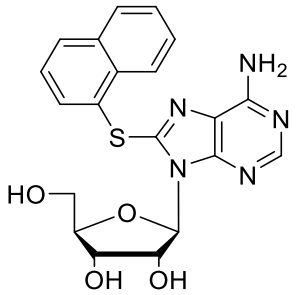
8-(Phenethylthio)adenosine (27q, Bcy-256)



This compound was synthesized using the same procedure as for **27a** (Method 2). 8-Bromoadenosine (**26a**, 400 mg, 1.16 mmol), 10 mL EtOH, NaOMe (188 mg, 3.48 mmol) and 2-phenylethanethiol (0.47 mL, 3.48 mmol) were used. The crude compound was purified by silica gel column chromatography using 9% MeOH in DCM. **Appearance**: white solid; **mp**: 176.0-177.5 °C. **Yield**: 426 mg, 91%. ^{1}H NMR (500

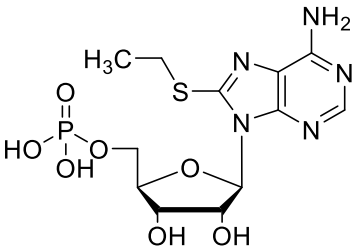
MHz, DMSO-d_6) δ 8.07 (s, 1H), 7.36 – 7.30 (m, 4H), 7.27 (s, 2H), 7.26 – 7.20 (m, 1H), 5.75 (d, J = 6.9 Hz, 1H), 5.60 (dd, J = 8.8, 3.7 Hz, 1H), 5.37 (d, J = 6.4 Hz, 1H), 5.15 (d, J = 4.4 Hz, 1H), 5.04 – 4.97 (m, 1H), 4.21 – 4.12 (m, 1H), 4.00 – 3.94 (m, 1H), 3.67 (dt, J = 12.2, 3.8 Hz, 1H), 3.62 – 3.47 (m, 3H), 3.03 (t, J = 7.6 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.53, 151.25, 150.44, 148.52, 139.72, 128.65, 128.35, 126.38, 119.65, 88.84, 86.58, 71.25, 70.94, 62.19, 34.84, 33.50. **LC-MS** (*m/z*): 404.3 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.9%.

8-(1-Naphthylthio)adenosine (27r, Bcy-239)



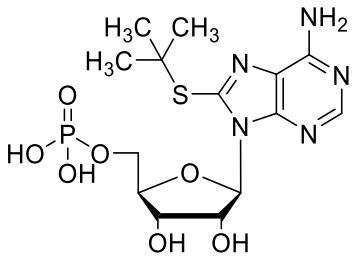
This compound was synthesized using the same procedure as for **27a** (Method 2). 8-Bromoadenosine (**26a**, 400 mg, 1.16 mmol), 10 mL EtOH, NaOMe (188 mg, 3.48 mmol) and 1-thionaphthol (0.49 mL, 3.48 mmol) were used. The crude compound was purified by silica gel column chromatography using 9% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 134.0-136.0 °C. **Yield**: 161 mg, 33%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.31 – 8.25 (m, 1H), 8.12 (s, 1H), 8.04 – 7.99 (m, 2H), 7.67 – 7.59 (m, 3H), 7.53 (dd, J = 8.2, 7.3 Hz, 1H), 7.37 (s, 2H), 6.18 (d, J = 6.9 Hz, 1H), 5.63 (dd, J = 9.0, 3.7 Hz, 1H), 5.46 (d, J = 6.4 Hz, 1H), 5.19 (d, J = 4.4 Hz, 1H), 5.14 – 5.09 (m, 1H), 4.24 – 4.18 (m, 1H), 4.04 – 3.97 (m, 1H), 3.76 – 3.67 (m, 1H), 3.60 – 3.52 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.28, 152.30, 150.06, 145.06, 133.81, 132.01, 131.44, 129.48, 128.77, 127.76, 127.44, 126.73, 126.18, 124.28, 119.99, 89.56, 86.78, 71.43, 71.05, 62.19. **LC-MS** (*m/z*): 426.4 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.1%.

8-Ethylthio-AMP (28b, Bcy-229), CAS: 81609-36-1



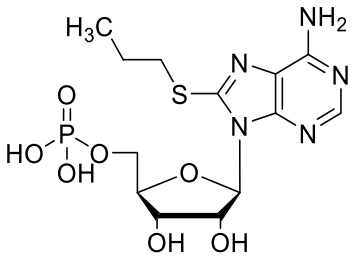
Compound **27b** (100 mg, 0.31 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (101 mg, 0.47 mmol) and $POCl_3$ (0.12 mL, 1.24 mmol) were used. **Appearance**: white powder; **mp**: 166.0-168.0 °C. **Yield**: 7 mg, 6%. ^{1}H NMR (600 MHz, D_2O) δ 8.35 (d, J = 1.0 Hz, 1H), (N*H*$_2$ is missing due to it`s exchangeable with D_2O), 6.07 (dd, J = 5.7, 0.9 Hz, 1H), 5.19 – 5.13 (m, 1H), 4.82 – 4.79 (m, 1H), 4.79 (s, 3H), 4.60 (t, J = 5.1 Hz, 1H), 4.29 (q, J = 4.7 Hz, 1H), 4.24 – 4.12 (m, 2H), 3.41 – 3.27 (m, 2H), 1.45 – 1.39 (m, 3H). ^{13}C NMR (151 MHz, D_2O) δ 157.78, 152.91, 150.82, 146.17, 121.89, 91.57, 86.75, 74.34, 72.64, 67.29, 30.06, 16.59. ^{31}P NMR (243 MHz, D_2O) δ 0.57. **LC-MS** (*m/z*): 408.20 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.9%.

8-(*Tert*-butyl)thio-AMP (28c, Bcy-232)



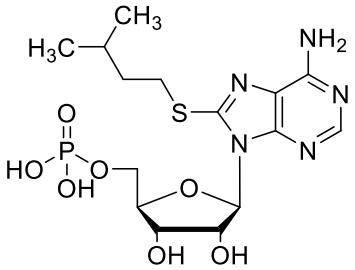
Compound **27c** (100 mg, 0.28 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (90 mg, 0.42 mmol) and $POCl_3$ (0.10 mL, 1.12 mmol) were used. **Appearance**: white powder; **mp**: >300 °C. **Yield**: 64 mg, 52%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.31 (s, 2H), 6.02 (d, J = 5.6 Hz, 1H), 5.17 (t, J = 5.5 Hz, 2H), 4.34 (dd, J = 5.6, 3.8 Hz, 2H), 4.10 – 3.90 (m, 3H), 3.78 – 3.59 (m, 2H), 1.46 (s, 9H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.23, 152.55, 150.06, 145.73, 119.66, 89.05, 83.47, 70.92, 70.58, 64.42, 50.23, 30.82. ^{31}P NMR (243 MHz, DMSO-d_6) δ 0.81. **LC-MS** (*m/z*): 436.4 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.9%.

8-Propylthio-AMP (28d, Bcy-237)



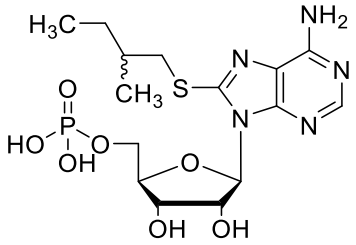
Compound **27d** (100 mg, 0.29 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (94 mg, 0.44 mmol) and $POCl_3$ (0.11 mL, 1.16 mmol) were used. **Appearance**: white powder; **mp**: 76.0-78.0 °C. **Yield**: 24 mg, 20%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.10 (s, 2H), 5.77 (d, J = 5.8 Hz, 1H), 5.10 (t, J = 5.7 Hz, 3H), 4.28 (dd, J = 5.6, 3.9 Hz, 2H), 4.09 – 3.83 (m, 3H), 3.74 – 3.64 (m, 1H), 3.37 – 3.18 (m, 2H), 1.73 (h, J = 7.3 Hz, 2H), 0.99 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.24, 151.65, 151.11, 148.60, 119.26, 88.51, 83.56, 70.80, 70.42, 64.29, 34.05, 22.26, 13.04. ^{31}P NMR (202 MHz, DMSO-d_6) δ 0.83. **LC-MS** (*m/z*): 422.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.5%.

8-Isopentylthio-AMP (28e, Bcy-238)



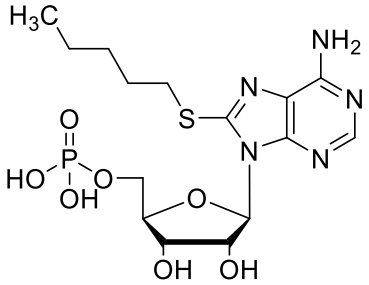
Compound **27e** (100 mg, 0.27 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (88 mg, 0.41 mmol) and $POCl_3$ (0.10 mL, 1.08 mmol) were used. **Appearance**: white powder; **mp**: 72.0-74.0 °C. **Yield**: 49 mg, 40%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.10 (s, 2H), 5.75 (d, J = 5.9 Hz, 1H), 5.11 (t, J = 5.7 Hz, 2H), 4.27 (dd, J = 5.5, 3.8 Hz, 2H), 4.07 – 3.87 (m, 3H), 3.75 – 3.63 (m, 2H), 3.35 – 3.24 (m, 2H), 1.76 – 1.65 (m, 1H), 1.65 – 1.55 (m, 2H), 0.91 (d, J = 6.6 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.27, 151.69, 151.11, 148.64, 119.31, 88.51, 83.55, 70.77, 70.37, 64.35, 45.25, 37.69, 30.37, 26.86, 22.07. ^{31}P NMR (243 MHz, DMSO-d_6) δ 0.78. **LC-MS** (*m/z*): 450.3 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.5%.

8-(2-Methylbutyl)thio-AMP (28f, Bcy-246)



Compound **27f** (100 mg, 0.27 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (88 mg, 0.41 mmol) and $POCl_3$ (0.10 mL, 1.08 mmol) were used. **Appearance**: white powder; **mp**: 168.0-170.0 °C. **Yield**: 84 mg, 62%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.08 (s, 2H), 5.76 (d, J = 5.9 Hz, 1H), 5.13 (t, J = 5.7 Hz, 1H), 4.28 (dd, J = 5.6, 3.8 Hz, 4H), 4.04 – 3.89 (m, 3H), 3.73 – 3.65 (m, 1H), 3.33 (dd, J = 12.7, 6.0 Hz, 1H), 3.23 (dd, J = 12.7, 7.1 Hz, 1H), 1.83 – 1.71 (m, 1H), 1.56 – 1.44 (m, 1H), 1.33 – 1.20 (m, 1H), 0.99 (d, J = 6.9 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.20, 151.63, 151.12, 148.97, 119.24, 88.56, 83.53, 70.81, 70.38, 64.28, 38.79, 34.08, 27.90, 18.47, 11.04. ^{31}P NMR (202 MHz, DMSO-d_6) δ 0.77 (d, J = 9.5 Hz). **LC-MS** (*m/z*): 450.3 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.9%.

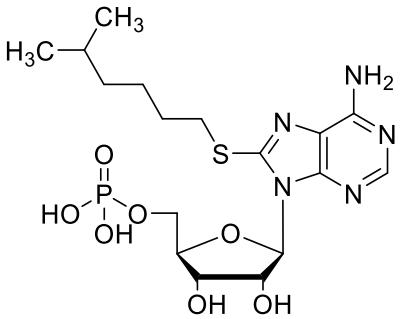
8-Pentylthio-AMP (28g, Bcy-223), CAS: 724414-61-3



Compound **27g** (100 mg, 0.27 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (88 mg, 0.41 mmol) and $POCl_3$ (0.10 mL, 1.08 mmol) were used. **Appearance**: white powder; **mp**: 114.0-116.0 °C. **Yield**: 66 mg, 54%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.10 (s, 2H), 5.75 (d, J = 5.8 Hz, 1H), 5.10 (t, J = 5.7 Hz, 1H), 4.28 (dd, J = 5.5, 3.9 Hz, 2H), 4.05 – 3.84 (m, 4H), 3.73 – 3.62 (m, 2H), 3.35 – 3.23 (m, 2H), 1.71 (p, J = 7.3 Hz, 2H), 1.44 – 1.25 (m, 4H), 0.87 (t, J = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.25, 151.67, 151.12, 148.68, 119.29, 88.52, 83.57, 70.83, 70.42, 64.31, 32.12, 30.24,

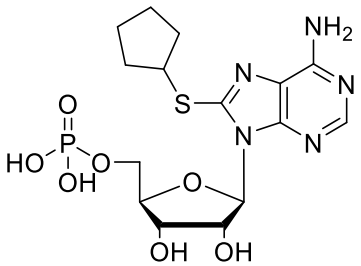
28.51, 21.60, 13.81. ^{31}P NMR (243 MHz, DMSO-d_6) δ 0.82. **LC-MS** (*m/z*): 450.3 [M + H]$^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.4%.

8-(5-Methylhexyl)thio-AMP (28h, CS-402)



Compound **27h** (80 mg, 0.20 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (64 mg, 0.30 mmol) and $POCl_3$ (0.10 mL, 1.10 mmol) were used. **Appearance**: white powder; **mp**: degradation >180 ℃. **Yield**: 30 mg, 24%. ^{1}H NMR (600 MHz, D_2O) δ 8.15 (s, 1H), 6.09 (d, *J* = 6.15 Hz, 1H), 5.17 (t, 1H, *J* = 6.13 Hz), 4.54 (m, 1H), 4.26 (q, 1H, *J* = 4.91 Hz), 4.16 (m, 2H), 3.27 (m, 2H), 1.71 (m, 2H), 1.46 (m, 1H), 1.40 (m, 2H), 1.15 (q, 2H, *J* = 7.11 Hz), 0.81 (d, 6H, *J* = 6.60 Hz). ^{13}C NMR (126 MHz, D_2O) δ 156.46, 154.58, 154.36, 153.54, 121.87, 90.94, 86.44, 73.59, 72.64, 67.30, 40.39, 35.88, 31.63, 29.98, 28.40, 24.67, 21.83. ^{31}P NMR (202 MHz, D_2O) δ 1.43. **LC-MS** (*m/z*): 478.2 [M + H]$^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.5%. This compound was co-synthesized with Dr. Constanze Cerine Schmies.

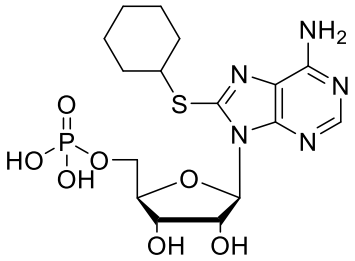
8-Cyclopentylthio-AMP (28i, Bcy-88)



Compound **27i** (80 mg, 0.22 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (92 mg, 0.38 mmol) and $POCl_3$ (0.08 mL, 0.88 mmol) were used. **Appearance**: white solid; **mp**: 118.3-119.5 ℃. **Yield**: 25 mg, 25%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.16 (s, 2H), 5.79 (d, *J* = 5.9 Hz, 1H), 5.14 (t, *J* = 5.7 Hz, 1H), 4.26 (dd, *J* = 5.6, 3.3 Hz,

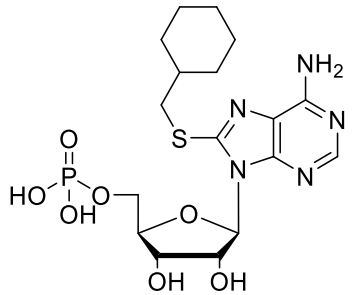
3H), 4.04 (q, *J* = 6.7 Hz, 2H), 3.81 – 3.70 (m, 2H), 2.96 (q, *J* = 7.3 Hz, 2H), 2.16 (dtd, *J* = 14.8, 8.2, 3.1 Hz, 2H), 1.80 – 1.57 (m, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.49, 151.86, 150.75, 148.39, 119.47, 88.67, 83.40, 70.66, 70.29, 64.61, 46.04, 45.11, 33.24, 24.26. ^{31}P NMR (243 MHz, DMSO-d_6) δ 0.77. **LC-MS** (*m/z*): 448.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.2%.

8-Cyclohexylthio-AMP (28j, PSB-20231, Bcy-231)



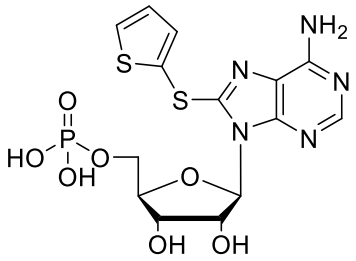
Compound **27j** (100 mg, 0.26 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (84 mg, 0.39 mmol) and $POCl_3$ (0.10 mL, 1.04 mmol) were used. **Appearance**: white powder; **mp**: 157.0-159.0 °C. **Yield**: 32 mg, 27%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.10 (s, 1H), 7.16 (s, 2H), 5.80 (d, *J* = 5.7 Hz, 1H), 5.13 (t, *J* = 5.7 Hz, 2H), 4.29 (dd, *J* = 5.5, 3.9 Hz, 2H), 4.02 – 3.90 (m, 3H), 3.85 – 3.76 (m, 1H), 3.71 – 3.64 (m, 1H), 2.57 (d, *J* = 7.1 Hz, 1H), 2.11 – 1.96 (m, 2H), 1.78 – 1.66 (m, 2H), 1.58 – 1.48 (m, 3H), 1.45 – 1.25 (m, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.49, 151.90, 150.75, 147.50, 119.46, 88.70, 83.55, 70.85, 70.40, 64.33, 46.40, 32.83, 32.66, 25.28, 25.01. ^{31}P NMR (243 MHz, DMSO-d_6) δ -0.37. **LC-MS** (*m/z*): 462.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.9%.

8-Cyclohexylmethylthio-AMP (28k, Bcy-87)



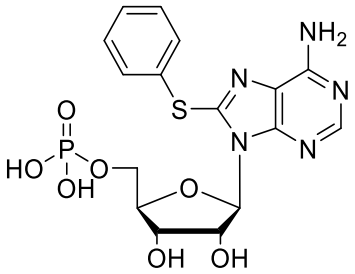
Compound **27k** (100 mg, 0.25 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (92 mg, 0.38 mmol) and $POCl_3$ (0.09 mL, 1.00 mmol) were used. **Appearance**: white power; **mp**: 171.0-173.0 ℃. **Yield**: 26 mg, 21%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.08 (s, 1H), 7.11 (s, 2H), 5.76 (d, J = 5.8 Hz, 1H), 5.13 (t, J = 5.7 Hz, 1H), 4.26 (dd, J = 5.5, 3.7 Hz, 2H), 4.11 – 3.89 (m, 3H), 3.76 – 3.67 (m, 1H), 3.37 – 3.15 (m, 2H), 2.75 (s, 1H), 1.83 (ddt, J = 13.0, 9.1, 3.6 Hz, 2H), 1.75 – 1.51 (m, 4H), 1.30 – 0.90 (m, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.21, 151.62, 151.15, 149.02, 119.25, 88.51, 83.52, 70.72, 70.30, 64.43, 45.04, 37.11, 31.83, 25.74, 25.40. ^{31}P NMR (243 MHz, DMSO-d_6) δ 0.77. **LC-MS** (*m/z*): 476.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%.

8-(2-Thienyl)thio-AMP (28l, Bcy-248)



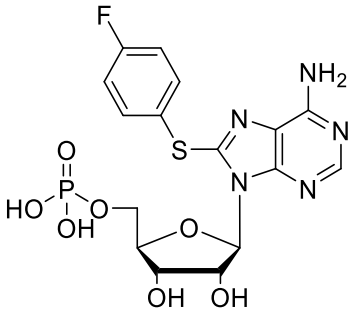
Compound **27l** (100 mg, 0.26 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (84 mg, 0.39 mmol) and $POCl_3$ (0.10 mL, 1.04 mmol) were used. **Appearance**: white powder; **mp**: 180.0-182.0 ℃. **Yield**: 40 mg, 33%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.78 (dd, J = 5.4, 1.3 Hz, 1H), 7.48 (dd, J = 3.8, 1.2 Hz, 1H), 7.28 (s, 2H), 7.12 (dd, J = 5.4, 3.7 Hz, 1H), 6.09 (d, J = 5.8 Hz, 1H), 5.13 (t, J = 5.7 Hz, 1H), 4.33 (dd, J = 5.6, 3.9 Hz, 2H), 4.10 – 3.89 (m, 4H), 3.81 – 3.66 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.16, 152.81, 150.57, 145.68, 135.93, 132.63, 128.08, 126.25, 119.30, 88.97, 83.78, 70.84, 70.79, 64.25. ^{31}P NMR (202 MHz, DMSO-d_6) δ 0.85 (t, J = 8.2 Hz). **LC-MS** (*m/z*): 462.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.4%.

8-Phenylthio-AMP (28m, Bcy-301), CAS: 78710-83-5



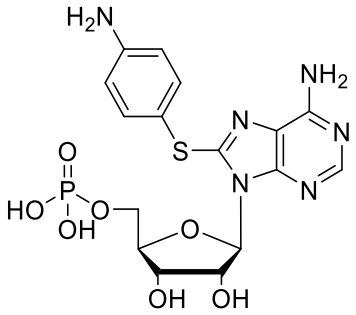
Compound **27m** (80 mg, 0.21 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (69 mg, 0.32 mmol) and $POCl_3$ (0.08 mL, 0.84 mmol) were used. **Appearance**: white powder; **mp**: 149.0-151.0 °C. **Yield**: 27 mg, 28%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.23 (d, J = 1.3 Hz, 1H), 7.86 (s, 2H), 7.47 – 7.42 (m, 2H), 7.42 – 7.37 (m, 2H), 7.37 – 7.33 (m, 1H), 6.10 (dd, J = 5.6, 1.3 Hz, 1H), 5.50 (s, 2H), 5.18 – 5.11 (m, 1H), 4.32 (s, 1H), 4.19 – 4.10 (m, 1H), 4.04 (q, J = 5.5 Hz, 1H), 3.99 – 3.89 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.88, 151.00, 150.12, 145.46, 131.43, 130.35, 129.62, 128.10, 119.74, 89.45, 83.24, 70.74, 70.36, 65.38. ^{31}P NMR (202 MHz, DMSO-d_6) δ -0.15. **LC-MS** (*m/z*): 456.20 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.4%.

8-(4-Fluorophenyl)thio-AMP (28n, Bcy-303)



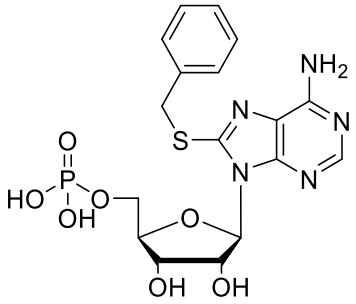
Compound **27n** (60 mg, 0.15 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (49 mg, 0.23 mmol) and $POCl_3$ (0.06 mL, 0.60 mmol) were used. **Appearance**: white powder; **mp**: 143.0-145.0 °C. **Yield**: 38 mg, 54%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.21 (s, 1H), 7.80 (s, 2H), 7.58 – 7.51 (m, 2H), 7.27 (t, J = 8.8 Hz, 2H), 6.07 (d, J = 5.7 Hz, 1H), 5.14 (t, J = 5.6 Hz, 1H), 4.31 (dd, J = 5.3, 3.9 Hz, 2H), 4.20 – 4.10 (m, 2H), 4.08 – 4.00 (m, 2H), 3.98 – 3.86 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.17, 154.06, 151.31, 150.28, 145.91, 133.73, 133.66, 126.51, 119.68, 116.91, 116.73, 89.34, 83.21, 70.71, 70.39, 65.38. ^{31}P NMR (202 MHz, DMSO-d_6) δ -0.13. **LC-MS** (*m/z*): 474.10 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.7%.

8-(4-Aminophenyl)thio-AMP (28o, Bcy-302)



Compound **27o** (100 mg, 0.26 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (84 mg, 0.39 mmol) and $POCl_3$ (0.10 mL, 1.04 mmol) were used. **Appearance**: white powder; **mp**: 146.0-148.0 ℃. **Yield**: 31 mg, 25%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.25 (d, J = 1.6 Hz, 1H), 8.18 (s, 2H), 7.32 – 7.24 (m, 2H), 6.65 (dd, J = 8.4, 1.4 Hz, 2H), 6.07 (dd, J = 5.8, 1.4 Hz, 1H), 5.40 (s, 5H), 5.09 (t, J = 5.6 Hz, 2H), 4.30 (s, 1H), 4.17 – 4.10 (m, 1H), 4.06 (q, J = 5.3, 4.4 Hz, 1H), 3.98 – 3.89 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.65, 152.09, 150.33, 150.18, 148.66, 135.45, 119.55, 115.81, 89.44, 83.48, 71.04, 70.50, 65.60. ^{31}P NMR (202 MHz, DMSO-d_6) δ -0.14. **LC-MS** (*m/z*): 471.20 [M + H]$^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.5%.

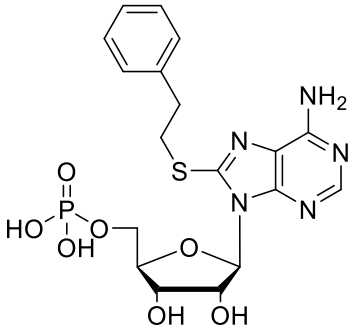
8-Benzylthio-AMP (28p, Bcy-258), CAS: 78710-85-7



Compound **27p** (100 mg, 0.26 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (84 mg, 0.39 mmol) and $POCl_3$ (0.10 mL, 1.04 mmol) were used. **Appearance**: white powder; **mp**: 149.0-151.0 ℃. **Yield**: 100 mg, 82%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.53 – 7.47 (m, 2H), 7.31 (dd, J = 8.2, 6.8 Hz, 2H), 7.27 – 7.23 (m, 1H), 7.19 (s, 2H), 5.73 (d, J = 5.8 Hz, 1H), 5.05 (t, J = 5.7 Hz, 1H), 4.61 – 4.53 (m, 2H), 4.25 (dd, J = 5.5, 3.7 Hz, 2H), 4.03 – 3.88 (m, 4H), 3.75 – 3.66 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.34, 151.79, 151.21, 148.04, 137.15, 129.19, 128.43, 127.39, 119.20, 88.50,

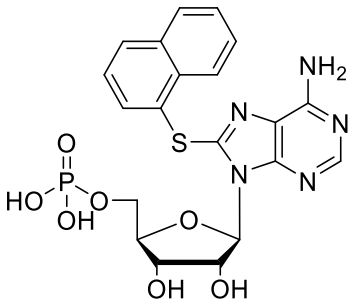
83.53, 70.71, 70.46, 64.43, 35.77. ^{31}P NMR (243 MHz, DMSO-d_6) δ 0.80. **LC-MS** (*m/z*): 470.4 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%.

8-Phenethylthio-AMP (28q, Bcy-259), CAS: 78710-95-9



Compound **27q** (100 mg, 0.25 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (81 mg, 0.38 mmol) and $POCl_3$ (0.09 mL, 1.00 mmol) were used. **Appearance**: white powder; **mp**: 141.0-143.0 °C. **Yield**: 56 mg, 46%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.35 – 7.28 (m, 4H), 7.24 – 7.19 (m, 1H), 7.13 (s, 2H), 5.75 (d, J = 5.9 Hz, 1H), 5.12 (t, J = 5.7 Hz, 1H), 4.26 (dd, J = 5.5, 3.3 Hz, 1H), 4.04 – 3.97 (m, 3H), 3.80 – 3.75 (m, 3H), 3.59 – 3.49 (m, 3H), 3.03 (t, J = 7.7 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.28, 151.67, 151.12, 148.49, 139.80, 128.68, 128.36, 126.36, 119.37, 88.48, 83.33, 70.58, 70.32, 64.71, 34.86, 33.39. ^{31}P NMR (202 MHz, DMSO-d_6) δ 0.79 (d, J = 7.1 Hz). **LC-MS** (*m/z*): 484.5 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.5%.

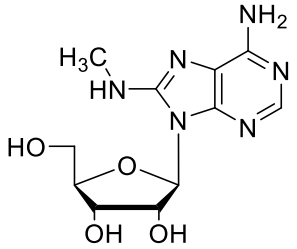
8-(1-Naphthyl)thio-AMP (28r, Bcy-247)



Compound **27r** (100 mg, 0.24 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (77 mg, 0.36 mmol) and $POCl_3$ (0.09 mL, 0.96 mmol) were used. **Appearance**: white powder; **mp**: 188.0-190.0 °C. **Yield**: 13 mg, 10%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.32 – 8.24 (m, 1H), 8.14 (s, 1H), 8.04 – 7.98 (m, 2H), 7.70 – 7.57 (m, 3H), 7.52 (dd, J = 8.2, 7.3 Hz,

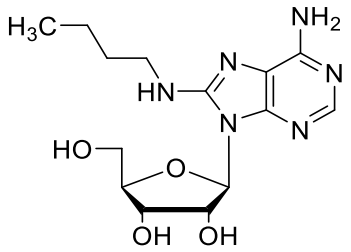
1H), 7.21 (s, 2H), 6.14 (d, *J* = 5.8 Hz, 1H), 5.26 (t, *J* = 5.7 Hz, 1H), 4.33 (dd, *J* = 5.5, 3.4 Hz, 1H), 4.10 – 3.99 (m, 3H), 3.87 – 3.76 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.02, 152.68, 150.69, 145.17, 133.81, 132.14, 131.72, 129.54, 128.73, 127.70, 127.47, 126.70, 126.18, 124.41, 119.76, 89.22, 83.55, 70.76, 70.55, 64.60. ^{31}P NMR (243 MHz, DMSO-d_6) δ 0.84. **LC-MS** (*m/z*): 506.5 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.7%.

8-(Methylamino)adenosine (29a, Bcy-6), CAS: 13389-13-4



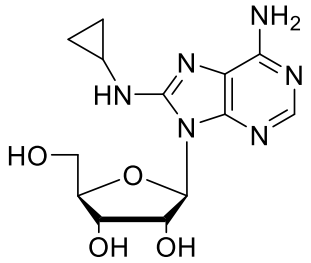
To a solution of **26a** (600 mg, 1.73 mmol, 1 eq.) in 40% aqueous methylamine (20 mL), Et_3N (2.40 mL, 17.30 mmol, 10 eq.) was added. The mixture was refluxed overnight and monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, cooled to rt and the solvent was evaporated *in vacuum*. The crude compound was purified by silica gel column chromatography using 6% MeOH in DCM. **Appearance**: white solid; **mp**: 215.0-217.0 ℃ (*lit.*[79] 215 ℃). **Yield**: 230 mg, 45%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.89 (s, 1H), 6.93 (q, *J* = 4.6 Hz, 1H), 6.51 (s, 2H), 5.88 (dd, *J* = 24.5, 6.2 Hz, 2H), 5.23 (d, *J* = 6.6 Hz, 1H), 5.13 (d, *J* = 4.1 Hz, 1H), 4.66 (td, *J* = 6.9, 5.3 Hz, 1H), 4.18 – 4.09 (m, 1H), 3.96 (d, *J* = 2.4 Hz, 1H), 3.62 (dtt, *J* = 14.3, 8.1, 2.9 Hz, 2H), 2.88 (d, *J* = 4.5 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 152.39, 152.05, 149.82, 148.48, 117.12, 86.50, 85.68, 70.95, 70.72, 61.64, 29.11. **LC-MS** (*m/z*): 297.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.9%.

8-(Butylamino)adenosine (29b, Bcy-10), CAS: 65456-84-0



This compound was synthesized using the same procedure as for **29a**. 8-Bromoadenosine (**26a**, 400 mg, 1.16 mmol), butylamine (10 mL) and Et_3N (1.61 mL, 11.60 mmol) were used. The crude compound was purified by silica gel column chromatography using 15% MeOH in DCM. **Appearance**: yellow solid. **Yield**: 152 mg, 39%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.89 (s, 1H), 6.87 (t, J = 5.4 Hz, 1H), 6.47 (s, 2H), 5.90 (d, J = 7.4 Hz, 1H), 5.17 (dd, J = 46.1, 5.4 Hz, 2H), 4.62 (td, J = 7.1, 5.4 Hz, 1H), 4.18 – 4.08 (m, 1H), 3.96 (q, J = 2.3 Hz, 1H), 3.63 (s, 2H), 1.57 (p, J = 7.2 Hz, 2H), 1.33 (dq, J = 14.6, 7.4 Hz, 3H), 0.89 (dt, J = 12.9, 7.4 Hz, 5H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 152.16, 151.35, 149.76, 148.32, 117.01, 86.33, 85.66, 70.95, 70.67, 61.60, 42.02, 30.84, 19.64, 13.77. **LC-MS** (*m/z*): 339.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 93.0%.

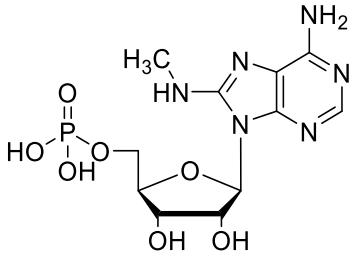
8-(Cyclopropylamino)adenosine (29c, Bcy-175)



This compound was synthesized using the same procedure as for **29a**. 8-Bromoadenosine (**26a**, 400 mg, 1.16 mmol), cyclopropylamine (10 mL) and Et_3N (2.42 mL, 17.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 15% MeOH in DCM. **Appearance**: brownish solid; **mp**: 200.0-202.0 °C. **Yield**: 100 mg, 27%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.90 (s, 1H), 6.53 (s, 2H), 5.87 (dd, J = 8.0, 4.0 Hz, 2H), 5.26 – 5.06 (m, 2H), 4.56 (q, J = 5.6 Hz, 1H), 4.09 (d, J = 5.3 Hz, 1H), 3.94 (q, J = 2.3 Hz, 1H), 3.62 (q, J = 2.8 Hz, 2H), 2.99 (q, J = 7.3 Hz, 1H), 1.15 (t, J = 7.3 Hz, 1H), 0.69 – 0.66 (m, 2H), 0.57 – 0.47 (m, 2H). ^{13}C NMR

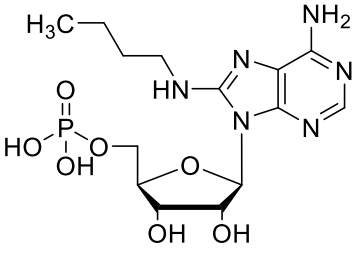
(151 MHz, DMSO-d_6) δ 152.56, 151.56, 149.81, 148.63, 117.05, 86.32, 85.60, 70.90, 70.63, 61.57, 24.86, 6.69, 6.03. **LC-MS** (*m/z*): 323.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.2%.

8-Methylamino-AMP (30a, PSB-20148, Bcy-148), CAS: 61370-73-8



Compound **29a** (300 mg, 1.01 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (326 mg, 1.52 mmol) and $POCl_3$ (0.38 mL, 4.04 mmol) were used. **Appearance**: white powder; **mp**: 140.0-142.0 °C. **Yield**: 20 mg, 5%. ^{1}H NMR (600 MHz, D_2O) δ 8.38 – 8.24 (m, 1H), (N*H* and NH_2 are missing due to it`s exchangeable with D_2O), 6.09 (dd, *J* = 26.7, 7.4 Hz, 1H), 4.87 – 4.80 (m, 2H), 4.78 – 4.71 (m, 2H), 4.47 (dd, *J* = 5.6, 2.7 Hz, 1H), 4.42 – 4.36 (m, 1H), 4.25 – 4.10 (m, 2H), 3.90 (s, 1H), 3.10 (dd, *J* = 11.2, 0.7 Hz, 3H). ^{13}C NMR (151 MHz, D_2O) δ 156.02, 151.82, 149.09, 146.50, 117.17, 89.77, 87.49, 73.76, 73.03, 67.56, 31.95. ^{31}P NMR (243 MHz, D_2O) δ 0.20. **LC-MS** (*m/z*): 377.20 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%.

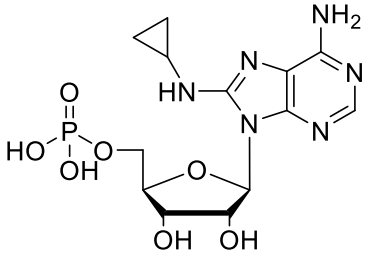
8-Butylamino-AMP (30b, CS-383A), CAS: 344402-40-0



Compound **29b** (150 mg, 0.44 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (160 mg, 0.66 mmol) and $POCl_3$ (0.16 mL, 1.76 mmol) were used. **Appearance**: white powder; **mp**: 180 °C. **Yield**: 20 mg, 11%. ^{1}H NMR (600 MHz, D_2O) δ 7.99 (d, 1H, *J* = 1.08 Hz), 6.00 (d, 1H, *J* = 7.70 Hz), 4.73 (dd, 1H, *J* = 5.94, 7.82 Hz), 4.45 (dd, 1H, *J* = 2.50, 5.86 Hz), 4.33 (t, 1H, *J* = 2.41 Hz), 4.13 (m, 2H), 3.45 (m, 2H), 1.65 (m, 2H), 1.37 (q, 2H, *J*

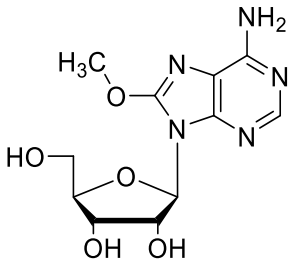
= 7.51 Hz), 0.91 (m, 3H). ^{13}C NMR (126 MHz, D_2O) δ 155.20, 153.89, 152.36, 151.55, 119.14, 89.14, 87.33, 73.34, 73.02, 67.33, 45.24, 33.52, 22.37, 16.12. ^{31}P NMR (202 MHz, D_2O) δ 1.14. **LC-MS** (*m/z*): 419.4 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%. This compound was co-synthesized with Dr. Constanze Cerine Schmies.

8-Cyclopropylamino-AMP (30c, Bcy-225)



Compound **29c** (100 mg, 0.31 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (101 mg, 0.47 mmol) and $POCl_3$ (0.12 mL, 1.24 mmol) were used. **Appearance**: white powder; **mp**: 163.0-165.0 °C. **Yield**: 26 mg, 21%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.90 (s, 1H), 6.68 (s, 1H), 6.46 (s, 2H), 5.77 (d, *J* = 6.6 Hz, 1H), 4.76 (t, *J* = 6.1 Hz, 1H), 4.26 (dd, *J* = 5.6, 3.3 Hz, 2H), 3.94 (q, *J* = 3.9 Hz, 2H), 3.85 – 3.77 (m, 4H), 2.80 – 2.71 (m, 1H), 0.91 – 0.76 (m, 1H), 0.70 – 0.51 (m, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 152.44, 151.90, 150.05, 148.80, 117.07, 86.11, 83.58, 70.39, 70.14, 64.40, 25.24, 6.84, 6.13. ^{31}P NMR (243 MHz, DMSO-d_6) δ 0.85. **LC-MS** (*m/z*): 403.4 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.6%.

8-Methoxyadenosine (31a, Bcy-155), CAS: 3969-27-5



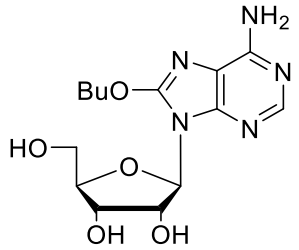
To a solution of **26a** (500 mg, 1.44 mmol, 1 eq.) in MeOH (15 mL), NaOMe (778 mg, 14.40 mmol, 10 eq.) was added. The mixture was refluxed overnight, and the reaction

progress was monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, cooled to rt, 5 g silica gel was added, and the solvent was evaporated *in vacuum*. The crude compound was purified by silica gel column chromatography using 12% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 166.5-168.5 ℃ (*lit.*[178] 206-208 ℃). **Yield**: 195 mg, 46%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.02 (s, 1H), 6.95 (s, 2H), 5.71 (d, J = 6.6 Hz, 1H), 5.47 – 5.28 (m, 2H), 5.10 (d, J = 4.7 Hz, 1H), 4.96 – 4.83 (m, 1H), 4.20 – 4.07 (m, 4H), 3.97 – 3.86 (m, 1H), 3.62 (dt, J = 12.0, 4.1 Hz, 1H), 3.58 – 3.41 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.39, 154.03, 150.53, 148.69, 114.87, 86.67, 85.96, 71.04, 70.84, 62.18, 57.20. **LC-MS** (*m/z*): 298.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%.

General procedure for the synthesis of 8-oxy-substituted adenosine derivatives (31b-c)

To **26a** (1 eq.) in a 10 mL appropriate alkyl alcohol solution, NaOH (3 eq.) was added. The mixture was stirred at 50 ℃ for 2 h and monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, cooled to rt, 5 g silica gel was added, and the solvent was evaporated *in vacuum*. The crude mixture was purified by silica gel column chromatography using 10% MeOH in DCM.

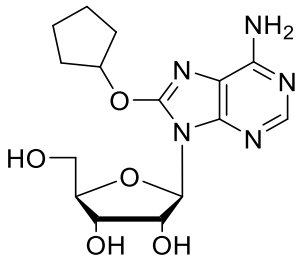
8-Butoxyadenosine (31b, Bcy-252), CAS: 255716-03-1



8-Bromoadenosine (**26a**, 200 mg, 0.58 mmol), butanol (10 mL) and NaOH (70 mg, 1.74 mmol) were used. **Appearance**: yellowish solid; **mp**: 180.0-182.0 ℃ (*lit.*[175] 173 ℃). **Yield**: 112 mg, 57%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.02 (s, 1H), 6.90 (s, 2H), 5.72 (d, J = 6.6 Hz, 1H), 5.38 (dd, J = 8.2, 4.3 Hz, 1H), 5.31 (d, J = 6.2 Hz, 1H), 5.10 (d, J = 4.6 Hz, 1H), 4.87 (q, J = 6.1 Hz, 1H), 4.52 – 4.45 (m, 2H), 4.17 – 4.08 (m,

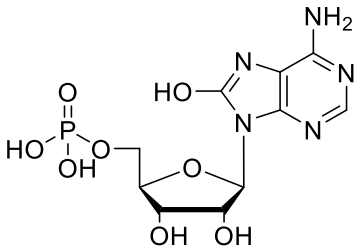
1H), 3.90 (q, *J* = 3.9 Hz, 1H), 3.63 (dt, *J* = 12.0, 4.1 Hz, 1H), 3.53 – 3.43 (m, 1H), 1.85 – 1.71 (m, 2H), 1.44 (h, *J* = 7.4 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.91, 153.85, 150.42, 148.57, 114.88, 86.64, 85.85, 71.04, 70.82, 69.85, 62.18, 30.24, 18.48, 13.53. **LC-MS** (*m/z*): 340.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.4%.

8-Cyclopentyloxyadenosine (31c, Bcy-249)



8-Bromoadenosine (**26a**, 300 mg, 0.87 mmol), cyclopentanol (10 mL) and NaOH (104 mg, 2.61 mmol) were used. **Appearance**: yellowish solid; **mp**: 63.0-65.0 °C. **Yield**: 86 mg, 28%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.01 (s, 1H), 6.89 (s, 2H), 5.70 (d, *J* = 6.4 Hz, 1H), 5.51 – 5.44 (m, 1H), 5.31 (d, *J* = 6.2 Hz, 1H), 5.11 (d, *J* = 4.7 Hz, 1H), 4.85 – 4.78 (m, 1H), 4.15 – 4.06 (m, 1H), 3.91 – 3.85 (m, 1H), 3.62 (dt, *J* = 12.0, 4.2 Hz, 1H), 3.53 – 3.40 (m, 2H), 2.03 – 1.83 (m, 4H), 1.70 – 1.55 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 153.85, 153.33, 150.35, 148.54, 114.99, 86.61, 85.69, 82.98, 71.07, 70.78, 62.17, 34.98, 32.18, 23.10. **LC-MS** (*m/z*): 352.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.2%.

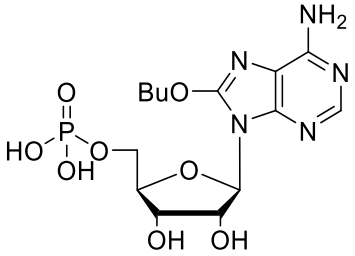
8-Hydroxy-AMP (32a, Bcy-219), CAS: 25030-04-0



Compound **31a** (120 mg, 0.40 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (129 mg, 0.60 mmol) and $POCl_3$ (0.15 mL, 1.60 mmol) were used. **Appearance**: white powder; **mp**: 258-260 °C. **Yield**: 19 mg, 13%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.32 (s, 1H), 8.03

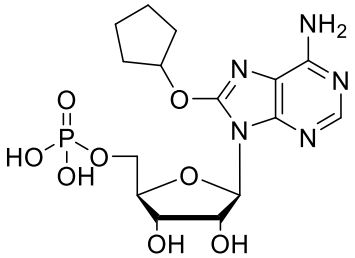
(s, 1H), 6.48 (s, 2H), 5.68 (d, *J* = 5.2 Hz, 1H), 5.27 (s, 2H), 4.90 (t, *J* = 5.2 Hz, 1H), 4.24 (t, *J* = 5.0 Hz, 1H), 4.18 – 3.99 (m, 2H), 3.98 – 3.91 (m, 1H), 3.89 – 3.71 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 151.38, 150.85, 147.01, 146.74, 103.41, 85.78, 82.06, 70.43, 69.90, 65.81. ^{31}P NMR (202 MHz, DMSO-d_6) δ -0.13. **LC-MS** (*m/z*): 364.10 [M + H]$^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%.

8-Butoxy-AMP (32b, Bcy-263)



Compound **31b** (100 mg, 0.29 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (94 mg, 0.44 mmol) and $POCl_3$ (0.11 mL, 1.16 mmol) were used. **Appearance**: white powder; **mp**: 236.0-238.0 ℃. **Yield**: 55 mg, 45%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.04 (s, 1H), 6.81 (s, 2H), 5.71 (d, *J* = 5.6 Hz, 1H), 4.91 (t, *J* = 5.5 Hz, 1H), 4.54 – 4.41 (m, 3H), 4.21 (dd, *J* = 5.5, 4.2 Hz, 2H), 4.02 – 3.82 (m, 3H), 3.75 – 3.55 (m, 2H), 1.86 – 1.72 (m, 2H), 1.43 (h, *J* = 7.4 Hz, 2H), 0.94 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.88, 153.71, 150.78, 149.11, 114.67, 86.46, 83.09, 70.80, 70.56, 69.80, 64.46, 30.19, 18.50, 13.51. ^{31}P NMR (243 MHz, DMSO-d_6) δ 0.76. **LC-MS** (*m/z*): 420.20 [M + H]$^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.7%.

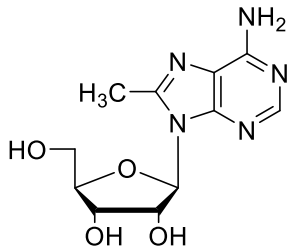
8-Cyclopentyloxy-AMP (32c, Bcy-264)



Compound **31c** (80 mg, 0.23 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (75 mg, 0.35 mmol) and $POCl_3$ (0.09 mL, 0.92 mmol) were used. **Appearance**: white powder; **mp**:

96.0-98.0 °C. **Yield**: 17 mg, 17%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.03 (s, 1H), 6.77 (s, 2H), 5.69 (d, J = 5.3 Hz, 1H), 5.50 – 5.41 (m, 1H), 4.81 (t, J = 5.4 Hz, 1H), 4.18 (t, J = 5.0 Hz, 2H), 3.92 (q, J = 5.8 Hz, 2H), 3.87 – 3.82 (m, 3H), 3.66 – 3.61 (m, 1H), 2.02 – 1.84 (m, 4H), 1.81 – 1.70 (m, 2H), 1.67 – 1.56 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 153.67, 153.37, 150.74, 149.09, 114.78, 86.44, 82.98, 82.91, 70.94, 70.74, 64.51, 32.30, 32.22, 23.16, 23.13. ^{31}P NMR (243 MHz, DMSO-d_6) δ 0.60. **LC-MS** (*m/z*): 432.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.1%.

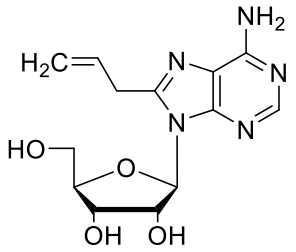
8-Methyladenosine (33a, Bcy-20), CAS: 56973-12-7



8-Bromoadenosine (**26a**, 500 mg, 1.44 mmol, 1 eq.) was dissolved in hexamethyldisilazane (10 mL) and dry dioxane (20 mL) in a three-necked flask. A catalytic amount of $(NH_4)_2SO_4$ (10%, 50 mg) was added to the suspension and the mixture was refluxed at 125 °C for 3 h. Then the mixture was dried *in vacuum* and redissolved in dry THF under argon without purification. Triphenylphosphine (39 mg, 0.14 mmol, 0.1 eq.), $PdCl_2$ (13 mg, 0.07 mmol, 0.05 eq.) and 2 M trimethylaluminum in toluene (1.45 mL, 2.89 mmol, 2 eq.) were subsequently added. The mixture was refluxed under argon for 3 h and dried *in vacuum* to yield a green residue. The residue was dissolved in MeOH (50 mL) and refluxed overnight with a small amount of NH_4Cl (10%, 50 mg) for the deprotection of 2′-, 3′- and 5′-trimethylsilyl groups. The reaction progress was monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, cooled to rt, 5 g silica gel was added, and the solvent was evaporated *in vacuum*. The crude compound was purified by silica gel column chromatography using 20% MeOH in DCM. **Appearance**: milk white solid; **mp**: 204.5-206.3 °C (*lit.*[156] 208 °C). **Yield**: 124 mg, 31%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.05 (s, 1H), 7.21 (s, 2H), 5.85 (dd, J = 9.2, 3.4 Hz, 1H), 5.78 (d, J = 7.1 Hz, 1H), 5.35 (d, J = 6.9 Hz, 1H), 5.19 (d, J = 4.5 Hz, 1H), 4.84 (td, J = 7.0, 5.2 Hz, 1H), 4.15 (td, J = 4.7, 2.1 Hz, 1H), 3.99 (td, J = 3.3,

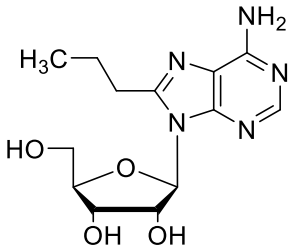
2.0 Hz, 1H), 3.68 (dt, *J* = 12.3, 3.4 Hz, 1H), 3.54 (ddd, *J* = 12.4, 9.2, 3.4 Hz, 1H), 2.55 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.39, 151.27, 149.64, 148.94, 118.10, 88.52, 86.57, 72.03, 70.93, 62.15, 14.31. **LC-MS** (*m/z*): 282.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.6%.

8-Allyladenosine (33b, Bcy-45), CAS: 73340-77-9



8-Bromoadenosine (**26a**, 1.00 g, 2.89 mmol, 1 eq.) was dissolved in hexamethyldisilazane (12 mL) in a three-necked flask. A catalytic amount of $(NH_4)_2SO_4$ (10%, 100 mg) was added to the suspension and the mixture was refluxed at 125 ℃ for 3 h. Then the mixture was dried *in vacuum*. In NMP (10 mL) under argon, PPh_3 (78 mg, 0.29 mmol, 0.1 eq.), $PdCl_2$ (27 mg, 0.15 mmol, 0.05 eq.) and allyltributyltin (1.79 mL, 5.78 mmol, 2 eq.) were added. The reaction was refluxed at 125 ℃ under argon for 3 h, then the crude mixture was dried *in vacuum*. The residue was dissolved in MeOH (50 mL) and refluxed overnight with a small amount of NH_4Cl (10%, 100 mg). The reaction progress was monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, cooled to rt, 5 g silica gel was added, and the solvent was evaporated *in vacuum*. The crude compound was purified by silica gel column chromatography using 8% MeOH in DCM. **Appearance**: yellowish solid; **mp**:110-112 ℃. **Yield**: 557 mg, 63%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.07 (s, 1H), 7.28 (d, *J* = 8.8 Hz, 2H), 6.12 – 6.04 (m, 1H), 5.95 – 5.89 (m, 1H), 5.77 (d, *J* = 7.1 Hz, 1H), 5.34 (d, *J* = 7.1 Hz, 1H), 5.23 – 5.12 (m, 3H), 4.90 – 4.85 (m, 1H), 4.19 – 4.13 (m, 1H), 4.01 – 3.97 (m, 1H), 3.77 – 3.65 (m, 3H), 3.57 – 3.50 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.63, 151.44, 150.10, 149.56, 133.06, 118.36, 117.62, 88.54, 86.74, 72.08, 71.00, 62.23, 31.91. **LC-MS** (*m/z*): 308.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.5%.

8-Propyladenosine (33c, Bcy-113), CAS: 101904-46-5

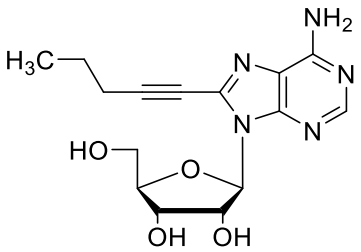


To a solution of **33b** (200 mg, 0.65 mmol, 1 eq.) in MeOH/THF (1:1, 10 mL), 10 wt. % Pd/C (10%, 20 mg) was added. The mixture was shaken with hydrogen (45 psi) at rt for 2 h in a Parr apparatus and the reaction progress was monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, the suspension was filtered on celite. The filter cake was washed with THF (10 mL) and MeOH (10 mL), and the filtrate was evaporated *in vacuum*. **Appearance**: white solid. **Yield**: 167 mg, 83%. **LC-MS** (*m/z*): 310.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 90.1%.

General procedure for the synthesis of 33d-e by Sonogashira coupling

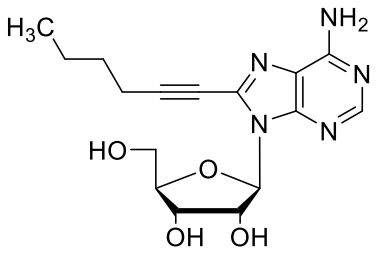
To a solution of **26a** (1 eq.) in anhydrous DMF (10 mL) under argon, Pd $(PPh_3)_2Cl_2$ (4%), CuI (8%), Et_3N (4 eq.) and 1-pentyne or 1-hexyne (5 or 8 eq.) were added. The mixture was stirred at 100 °C under argon for 6 h and monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, cooled to rt and the mixture was concentrated *in vacuum*. The residue was dissolved in $CHCl_3$ (150 mL), the organic layer was extracted with H_2O (70 mL), brine (70 mL) and dried over anhydrous Mg_2SO_4, then evaporated *in vacuum*. The crude compound was purified by silica gel column chromatography using 6% MeOH in DCM.

8-(1-Pentynyl)adenosine (33d, Bcy-76)



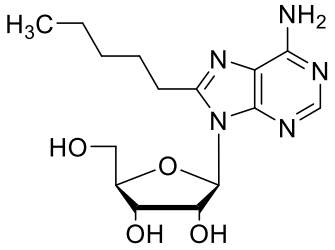
8-Bromoadenosine (**26a**, 500 mg, 1.44 mmol), anhydrous DMF (10 mL), 1-pentyne (0.71 mL, 7.20 mmol), Pd $(PPh_3)_2Cl_2$ (40 mg), CuI (22 mg) and Et_3N (0.80 mL, 5.76 mmol) were used. **Appearance**: yellowish solid. **Yield**: 79 mg, 16%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.54 (s, 2H), 5.94 (d, J = 6.7 Hz, 1H), 5.52 (dd, J = 8.7, 3.9 Hz, 1H), 5.39 (d, J = 6.2 Hz, 1H), 5.16 (d, J = 4.3 Hz, 1H), 5.00 (td, J = 6.5, 5.2 Hz, 1H), 4.19 (td, J = 4.7, 2.4 Hz, 1H), 3.97 (td, J = 3.9, 2.4 Hz, 1H), 3.68 (dt, J = 12.1, 3.9 Hz, 1H), 3.52 (ddd, J = 12.4, 8.7, 4.2 Hz, 1H), 2.55 (t, J = 6.9 Hz, 2H), 1.62 (h, J = 7.2 Hz, 2H), 1.03 (t, J = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.93, 152.98, 148.31, 134.02, 119.08, 97.35, 89.28, 86.51, 71.46, 70.99, 70.32, 62.21, 21.05, 20.47, 13.25. **LC-MS** (*m/z*): 334.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.4%.

8-(1-Hexynyl)adenosine (33e, Bcy-74), CAS: 364602-95-9



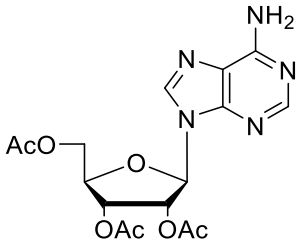
8-Bromoadenosine (**26a**, 300 mg, 0.87 mmol), anhydrous DMF (10 mL), 1-hexyne (0.80 mL, 6.96 mmol), Pd $(PPh_3)_2Cl_2$ (24 mg), CuI (13 mg) and Et_3N (0.48 mL, 3.48 mmol) were used. **Appearance**: yellowish solid; **mp**: 192.7-194.7 ℃. **Yield**: 50 mg, 17%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.53 (s, 2H), 5.93 (d, J = 6.7 Hz, 1H), 5.52 (dd, J = 8.7, 3.9 Hz, 1H), 5.38 (d, J = 6.3 Hz, 1H), 5.15 (d, J = 4.3 Hz, 1H), 5.00 (q, J = 6.1 Hz, 1H), 4.19 (td, J = 4.7, 2.5 Hz, 1H), 4.06 – 3.88 (m, 1H), 3.68 (dt, J = 12.1, 3.8 Hz, 1H), 3.52 (ddd, J = 12.4, 8.6, 4.2 Hz, 1H), 2.58 (t, J = 7.0 Hz, 2H), 1.62 – 1.55 (m, 2H), 0.93 (t, J = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.92, 152.98, 148.31, 134.02, 119.08, 97.46, 89.27, 86.51, 71.44, 70.98, 70.20, 62.20, 21.36, 18.24, 13.36. **LC-MS** (*m/z*): 348.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.0%.

8-Pentyladenosine (33f, Bcy-83)



This compound was synthesized using the same procedure as for **33c**. Compound **33d** (66 mg, 0.20 mmol, 1 eq.), MeOH/THF (1:1, 10 mL) and 10 wt. % Pd/C (10%, 7 mg) were used. The crude compound was purified by silica gel column chromatography using 7% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 75.0-77.0 °C. **Yield**: 63 mg, 95%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.05 (s, 1H), 7.25 (s, 2H), 5.91 (d, J = 8.5 Hz, 1H), 5.76 (d, J = 7.2 Hz, 1H), 5.35 (d, J = 7.0 Hz, 1H), 5.24 – 5.13 (m, 1H), 4.91 (q, J = 6.2 Hz, 1H), 4.15 (d, J = 5.2 Hz, 1H), 4.00 (q, J = 3.1 Hz, 1H), 3.68 (dd, J = 12.3, 3.3 Hz, 1H), 3.54 (d, J = 12.2 Hz, 1H), 2.86 (p, J = 7.4 Hz, 2H), 1.83 – 1.70 (m, 2H), 1.35 (dddd, J = 15.7, 10.3, 8.3, 4.1 Hz, 4H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.45, 152.47, 151.13, 149.52, 118.27, 88.37, 86.75, 71.80, 71.09, 62.28, 30.85, 27.23, 27.14, 21.79, 13.83. **LC-MS** (*m/z*): 338.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.0%.

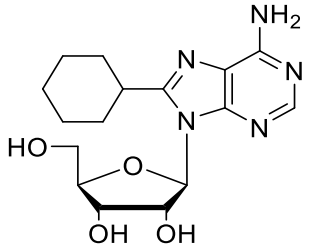
2′,3′,5′-Tri-*O*-acetyladenosine (25b, Bcy-291), CAS: 7387-57-7



To a solution of adenosine (1.00 g, 3.74 mmol, 1 eq.) in MeCN (10 mL), Et_3N (2.34 mL, 16.83 mmol, 4.5 eq.), DMAP (68 mg, 0.56 mmol, 0.15 eq.) and acetic anhydride (1.24 mL, 13.09 mmol, 3.5 eq.) were added at 0 °C. The mixture was stirred at rt for 30 min and monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, the solvent volume was reduced *in vacuum*. Then 5 mL EtOH was added, and the crude mixture was stirred at rt for 1 h. The precipitate was filtrated and washed by EtOH (5 mL) and dried in oven at 60 °C overnight. **Appearance**: white powder; **mp**: 176.0-178.0 °C (*lit.*[179] 173-174 °C). **Yield**: 1.10 g, 75%. ^{1}H NMR (500 MHz, DMSO-d_6) δ

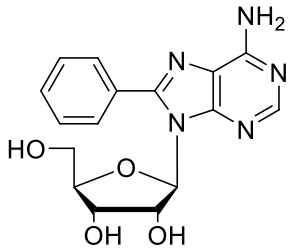
8.34 (s, 1H), 8.17 (s, 1H), 7.34 (s, 2H), 6.20 (d, *J* = 5.3 Hz, 1H), 6.03 (t, *J* = 5.7 Hz, 1H), 5.63 (t, *J* = 5.4 Hz, 1H), 4.49 – 4.32 (m, 2H), 4.24 (dd, *J* = 11.8, 5.5 Hz, 1H), 2.12 (s, 3H), 2.02 (d, *J* = 14.6 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.97, 169.40, 169.22, 156.15, 152.81, 149.07, 139.99, 119.17, 85.56, 79.36, 71.85, 70.05, 62.77, 20.42, 20.32, 20.15. **LC-MS** (*m/z*): 394.3 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.9%.

8-Cyclohexyladenosine (33g, Bcy-292)



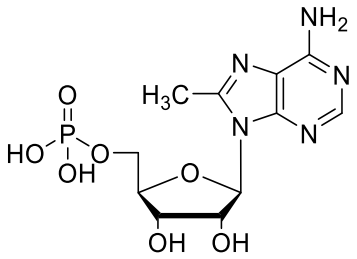
To a solution of **25b** (300 mg, 0.76 mmol, 1 eq.) in cyclohexane (10 mL), di-*tert*-butyl peroxide (0.28 mL, 1.52 mmol, 2 eq.) was added. The mixture was stirred in an autoclave at 140 °C for 24 h and monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, the solvent volume was reduced *in vacuum*. Then the 2'-, 3'- and 5'-*O*-acetyl groups were removed by stirring the mixture in a solution of 7 N NH_3 in MeOH (10 mL) at rt overnight. After the reaction was completed, 5 g silica gel was added, and the solvent was evaporated *in vacuum*. The crude compound was purified by silica gel column chromatography using 8% MeOH in DCM. **Appearance**: brownish solid; **mp**: 86.0-88.0 °C. **Yield**: 130 mg, 49%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.05 (d, *J* = 1.2 Hz, 1H), 7.17 (s, 2H), 5.93 – 5.84 (m, 1H), 5.79 (d, *J* = 7.2 Hz, 1H), 5.35 (dd, *J* = 6.9, 1.2 Hz, 1H), 5.18 (dd, *J* = 4.4, 1.2 Hz, 1H), 5.04 – 4.93 (m, 1H), 4.20 – 4.13 (m, 1H), 4.03 – 3.97 (m, 1H), 3.73 – 3.64 (m, 1H), 3.61 – 3.50 (m, 1H), 3.02 – 2.93 (m, 1H), 1.85 – 1.77 (m, 2H), 1.75 (d, *J* = 1.1 Hz, 2H), 1.67 – 1.58 (m, 2H), 1.47 – 1.37 (m, 2H), 1.30 – 1.19 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.12, 155.54, 151.11, 149.41, 118.29, 87.99, 86.74, 71.56, 71.09, 62.31, 35.34, 31.67, 31.39, 25.52, 25.48, 22.44. **LC-MS** (*m/z*): 350.30 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.0%.

8-Phenyladenosine (33h, Bcy-53), CAS: 73340-78-0



To a solution of **26a** (200 mg, 0.58 mmol, 1 eq.) in dioxane/H_2O (2:1, 9 mL), benzeneboronic acid (106 mg, 0.87 mmol, 1.5 eq.), $Pd(PPh_3)_2Cl_2$ (41 mg, 0.06 mmol, 0.1 eq.) and K_2CO_3 (240 mg, 1.74 mmol, 3 eq.) were added. The mixture was stirred at 90 °C under argon for 2 h, and monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, cooled to rt, 5 g silica gel was added, and the solvent was evaporated *in vacuum*. The crude mixture was purified by silica gel column chromatography using 12% MeOH in DCM. **Appearance**: yellow solid; **mp**: 156.0-158.0 °C (*lit.*[162] 142-143 °C). **Yield**: 189 mg, 95%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.16 (s, 1H), 7.76 (dd, J = 6.7, 2.9 Hz, 2H), 7.59 (p, J = 3.6, 3.1 Hz, 3H), 7.48 (s, 2H), 5.87 – 5.71 (m, 2H), 5.46 (d, J = 6.4 Hz, 1H), 5.29 – 5.07 (m, 2H), 4.17 (t, J = 4.7 Hz, 1H), 3.94 (q, J = 3.3 Hz, 1H), 3.70 (dt, J = 12.1, 3.5 Hz, 1H), 3.55 (ddd, J = 12.5, 9.2, 3.7 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 156.24, 152.03, 150.98, 149.83, 130.14, 129.68, 129.42, 128.76, 119.14, 89.13, 86.73, 71.25, 71.11, 62.33. **LC-MS** (*m/z*): 344.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.8%.

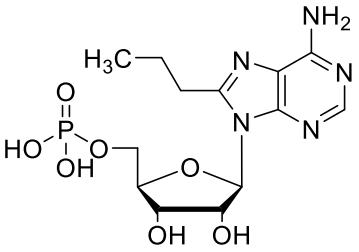
8-Methyl-AMP (34a, Bcy-86), CAS: 68045-12-5



Compound **33a** (60 mg, 0.21 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (69 mg, 0.32 mmol) and $POCl_3$ (0.08 mL, 0.84 mmol) were used. **Appearance**: white powder; **mp**: 87.0-89.0 °C (*lit.*[156] 208 °C). **Yield**: 21 mg, 28%. ^{1}H NMR (600 MHz, D_2O) δ 8.38 (d, J = 0.9 Hz, 1H), (N*H*$_2$ is missing due to it`s exchangeable with D_2O), 6.08 (d, J = 5.9

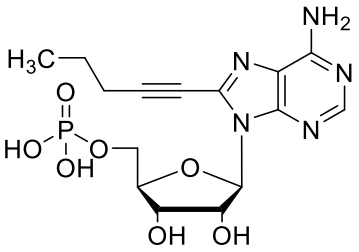
Hz, 1H), 5.19 – 5.12 (m, 1H), 4.84 – 4.79 (m, 2H), 4.79 – 4.73 (m, 2H), 4.62 (dd, *J* = 5.6, 4.4 Hz, 1H), 4.35 – 4.28 (m, 1H), 4.21 – 4.12 (m, 2H), 2.72 (d, *J* = 0.9 Hz, 3H). ^{13}C NMR (151 MHz, D_2O) *δ* 157.69, 152.23, 151.77, 146.39, 120.29, 91.40, 86.59, 74.73, 72.67, 67.40, 16.97. ^{31}P NMR (243 MHz, D_2O) *δ* 0.37. **LC-MS** (*m/z*): 362.10 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.2%.

8-Propyl-AMP (34c, Bcy-118)



Compound **33c** (150 mg, 0.49 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (159 mg, 0.74 mmol) and $POCl_3$ (0.18 mL, 1.96 mmol) were used. **Appearance**: white powder; **mp**: >300 ℃. **Yield**: 14 mg, 7%. ^{1}H NMR (600 MHz, DMSO-d_6) *δ* 8.09 (s, 1H), 7.07 (s, 2H), 5.76 (d, *J* = 6.0 Hz, 1H), 5.09 (t, *J* = 5.8 Hz, 1H), 4.27 (dd, *J* = 5.5, 3.1 Hz, 2H), 3.99 (d, *J* = 6.4 Hz, 3H), 3.87 – 3.72 (m, 3H), 2.91 – 2.76 (m, 2H), 1.77 (h, *J* = 7.4 Hz, 2H), 0.98 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) *δ* 155.21, 152.37, 151.68, 150.33, 118.02, 87.99, 83.34, 70.65, 70.57, 64.63, 29.30, 20.91, 13.71. ^{31}P NMR (243 MHz, DMSO-d_6) *δ* 0.95. **LC-MS** (*m/z*): 390.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.0%.

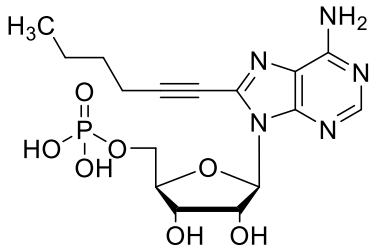
8-(1-Pentynyl)-AMP (34d, Bcy-81)



Compound **33d** (70 mg, 0.21 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (77 mg, 0.32 mmol) and $POCl_3$ (0.08 mL, 0.84 mmol) were used. **Appearance**: white power; **mp**: 175.6-177.6 ℃. **Yield**: 38 mg, 44%. ^{1}H NMR (600 MHz, DMSO-d_6) *δ* 8.16 (s, 1H), 7.42 (s, 2H), 5.93 (d, *J* = 5.8 Hz, 1H), 5.10 (t, *J* = 5.6 Hz, 1H), 4.30 (t, *J* = 4.5 Hz, 2H),

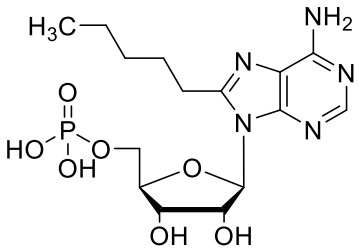
4.03 – 3.95 (m, 4H), 3.75 – 3.67 (m, 2H), 2.57 (t, *J* = 6.9 Hz, 2H), 1.62 (h, *J* = 7.2 Hz, 2H), 1.02 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.73, 153.43, 148.95, 133.82, 118.83, 97.34, 88.74, 83.51, 70.74, 70.61, 64.53, 64.50, 21.05, 20.45, 13.27. ^{31}P NMR (243 MHz, DMSO-d_6) δ 0.80. **LC-MS** (*m/z*): 414.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.1%.

8-(1-Hexynyl)-AMP (34e, Bcy-109)



Compound **33e** (50 mg, 0.14 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (51 mg, 0.21 mmol) and $POCl_3$ (0.05 mL, 0.56 mmol) were used. **Appearance**: white powder; **mp**: 197.0-199.0 °C. **Yield**: 2 mg, 3%. ^{1}H NMR (600 MHz, D_2O) δ 8.24 (s, 1H), 6.17 (d, *J* = 6.1 Hz, 1H), 5.27 (t, *J* = 6.1 Hz, 1H), 4.83 (s, 4H), 4.75 (dd, *J* = 4.1, 2.4 Hz, 2H), 4.53 (t, *J* = 5.4 Hz, 1H), 4.26 (q, *J* = 5.2 Hz, 1H), 4.11 (dt, *J* = 11.3, 5.6 Hz, 1H), 4.02 (dt, *J* = 11.9, 6.3 Hz, 1H), 2.61 (t, *J* = 7.0 Hz, 2H), 1.67 (p, *J* = 7.2 Hz, 2H), 1.50 (h, *J* = 7.4 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, D_2O) δ 157.95, 156.35, 151.63, 138.55, 121.31, 104.53, 91.16, 86.55, 73.58, 72.70, 71.45, 66.99, 32.02, 24.35, 21.31, 15.68. ^{31}P NMR (243 MHz, D_2O) δ 2.54. **LC-MS** (*m/z*): 428.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.6%.

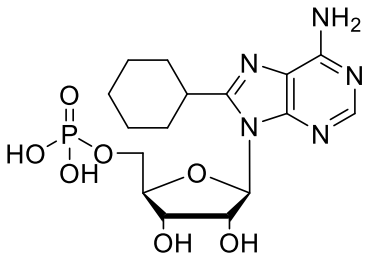
8-Pentyl-AMP (34f, Bcy-96)



Compound **33f** (60 mg, 0.18 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (58 mg, 0.27 mmol) and $POCl_3$ (0.07 mL, 0.72 mmol) were used. **Appearance**: white powder; **mp**: 137.0-139.0 °C. **Yield**: 9 mg, 12%. ^{1}H NMR (600 MHz, D_2O) δ 8.40 (d, *J* = 0.9 Hz,

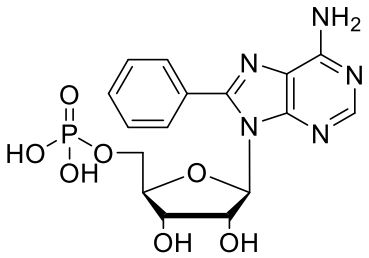
1H), (N*H*$_2$ is missing due to it`s exchangeable with D_2O), 6.07 (d, *J* = 5.9 Hz, 1H), 5.26 (t, *J* = 5.7 Hz, 1H), 4.85 – 4.81 (m, 2H), 4.80 – 4.75 (m, 2H), 4.65 (dd, *J* = 5.6, 4.2 Hz, 1H), 4.32 (q, *J* = 4.6 Hz, 1H), 4.17 (hept, *J* = 5.8 Hz, 2H), 3.10 – 3.02 (m, 2H), 1.84 (p, *J* = 7.2 Hz, 2H), 1.45 – 1.31 (m, 4H), 0.89 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, D_2O) *δ* 161.19, 152.13, 151.89, 146.29, 120.55, 91.41, 86.72, 74.57, 72.84, 67.34, 33.28, 30.08, 29.52, 24.51, 16.02. ^{31}P NMR (243 MHz, D_2O) *δ* 0.58. **LC-MS** (*m/z*): 418.20 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.3%.

8-Cyclohexyl-AMP (34g, Bcy-334)



Compound **33g** (60 mg, 0.17 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (56 mg, 0.26 mmol) and $POCl_3$ (0.06 mL, 0.68 mmol) were used. **Appearance**: white powder; **mp**: 119-121 ℃. **Yield**: 13 mg, 18%. ^{1}H NMR (600 MHz, D_2O) *δ* 8.38 (s, 1H), (N*H*$_2$ is missing due to it`s exchangeable with D_2O), 6.10 (d, *J* = 5.9 Hz, 1H), 5.28 (t, *J* = 5.8 Hz, 1H), 4.83 – 4.80 (m, 2H), 4.64 (dd, *J* = 5.7, 4.2 Hz, 1H), 4.35 – 4.27 (m, 1H), 4.23 – 4.11 (m, 2H), 3.81 (d, *J* = 11.1 Hz, 1H), 3.70 (d, *J* = 2.1 Hz, 1H), 3.17 – 3.07 (m, 1H), 2.10 – 1.98 (m, 2H), 1.92 – 1.81 (m, 2H), 1.76 (d, *J* = 13.5 Hz, 1H), 1.68 – 1.55 (m, 2H), 1.54 – 1.41 (m, 2H), 1.35 – 1.24 (m, 1H). ^{13}C NMR (151 MHz, D_2O) *δ* 164.75, 152.07, 151.87, 146.20, 120.62, 91.17, 86.71, 74.53, 72.85, 67.50, 39.04, 34.51, 34.02, 28.22, 28.09. ^{31}P NMR (243 MHz, D_2O) *δ* 0.40. **LC-MS** (*m/z*): 430.20 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.0%.

8-Phenyl-AMP (34h, Bcy-230), CAS: 1018828-70-0

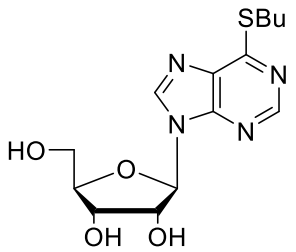


Compound **33h** (80 mg, 0.23 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (75 mg, 0.35 mmol) and $POCl_3$ (0.09 mL, 0.92 mmol) were used. **Appearance**: white powder; **mp**: 169.0-171.0 °C. **Yield**: 55 mg, 56%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.28 (s, 1H), 7.98 (s, 2H), 7.81 – 7.71 (m, 2H), 7.63 (q, J = 3.0 Hz, 3H), 5.75 (d, J = 5.8 Hz, 1H), 5.68 – 5.30 (m, 2H), 5.24 (t, J = 5.7 Hz, 1H), 4.28 (t, J = 4.6 Hz, 1H), 4.23 – 4.12 (m, 1H), 4.10 – 3.92 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.07, 151.78, 149.99, 149.89, 130.36, 129.47, 129.05, 128.84, 118.94, 89.46, 83.21, 70.42, 70.31, 65.32. ^{31}P NMR (202 MHz, DMSO-d_6) δ -0.09. **LC-MS** (*m/z*): 424.20 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.4%.

General procedure for the synthesis of 6-thiosubstituted nucleosides (35a-b)

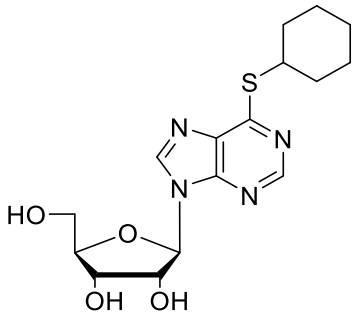
To a solution of 6-chloro-9-(*β-D*-ribofuranosyl)purine (1 eq.) in EtOH (10 mL), NaOMe (6 eq.) and appropriate thiol compound (6 eq.) were added. The mixture was refluxed overnight and monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, cooled to rt, 5 g silica gel was added, and the solvent was evaporated *in vacuum*. The crude mixture was purified by silica gel column chromatography using 6% MeOH in DCM.

6-*S*-Butyl-6-thioinosine (35a, Bcy-270), CAS: 70421-25-9



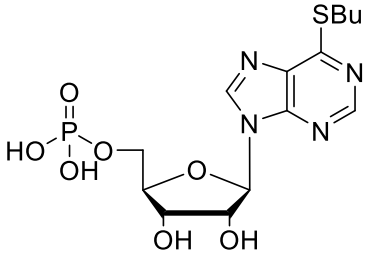
6-Chloro-9-(*β-D*-ribofuranosyl)purine (400 mg, 1.40 mmol), 1-butanethiol (0.90 mL, 8.40 mmol), NaOMe (454 mg, 8.40 mmol) and EtOH (10 mL) were used. **Appearance**: yellowish solid; **mp**: 51.0-53.0 ℃ (*lit*.[180] 60-63 ℃). **Yield**: 301 mg, 63%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.72 (s, 1H), 8.69 (s, 1H), 5.99 (d, J = 5.6 Hz, 1H), 5.48 (d, J = 5.9 Hz, 1H), 5.19 (d, J = 5.0 Hz, 1H), 5.08 (t, J = 5.6 Hz, 1H), 4.60 (q, J = 5.5 Hz, 1H), 4.23 – 4.16 (m, 1H), 4.01 – 3.93 (m, 1H), 3.74 – 3.64 (m, 1H), 3.63 – 3.53 (m, 1H), 3.42 – 3.33 (m, 2H), 1.76 – 1.65 (m, 2H), 1.44 (h, J = 7.4 Hz, 2H), 0.92 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.02, 151.44, 148.07, 143.08, 131.18, 87.76, 85.67, 73.72, 70.23, 61.22, 31.18, 27.49, 21.32, 13.45. **LC-MS** (*m/z*): 341.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.7%.

6-*S*-Cyclohexyl-6-thioinosine (35b, Bcy-271), CAS: 56964-71-7



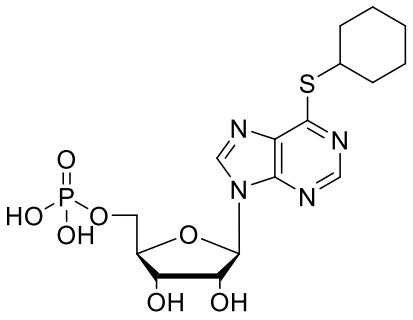
6-Chloro-9-(*β-D*-ribofuranosyl)purine (400 mg, 1.40 mmol), cyclohexylthiol (1.03 mL, 8.40 mmol), NaOMe (454 mg, 8.40 mmol) and EtOH (10 mL) were used. **Appearance**: yellowish solid; **mp**: 78.0-80.0 ℃ (*lit*.[181] 165 ℃). **Yield**: 240 mg, 47%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.72 (s, 1H), 8.68 (s, 1H), 5.98 (d, J = 5.6 Hz, 1H), 5.48 (d, J = 5.9 Hz, 1H), 5.18 (d, J = 5.0 Hz, 1H), 5.08 (dd, J = 6.0, 5.1 Hz, 1H), 4.59 (q, J = 5.6 Hz, 1H), 4.25 – 4.13 (m, 2H), 3.97 (q, J = 3.9 Hz, 1H), 3.73 – 3.65 (m, 1H), 3.61 – 3.53 (m, 1H), 2.18 – 2.02 (m, 2H), 1.79 – 1.69 (m, 2H), 1.66 – 1.27 (m, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.84, 151.50, 148.18, 143.08, 130.99, 87.75, 85.66, 73.72, 70.23, 61.22, 41.01, 32.71, 32.67, 25.42, 25.09. **LC-MS** (*m/z*): 367.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.1%.

6-Butylthiopurine-*β*-*D*-ribofuranosyl-5′-monophosphate (36a, Bcy-283), CAS: 81609-42-9



Compound **35a** (100 mg, 0.29 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (94 mg, 0.44 mmol) and $POCl_3$ (0.11 mL, 1.16 mmol) were used. **Appearance**: white powder; **mp**: 192.0-193.5 °C. **Yield**: 79 mg, 65%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.80 (s, 1H), 8.71 (s, 1H), 6.01 (d, J = 6.1 Hz, 1H), 5.07 (s, 3H), 4.73 (dd, J = 6.2, 4.7 Hz, 2H), 4.26 (dd, J = 4.9, 2.7 Hz, 1H), 4.08 (q, J = 3.5 Hz, 1H), 3.90 – 3.77 (m, 2H), 3.41 – 3.27 (m, 2H), 1.78 – 1.64 (m, 2H), 1.44 (h, J = 7.3 Hz, 2H), 0.92 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.72, 151.54, 148.54, 142.96, 130.79, 86.90, 84.82, 74.23, 71.25, 63.94, 31.17, 27.50, 21.34, 13.46. ^{31}P NMR (202 MHz, DMSO-d_6) δ 1.18 (d, J = 7.5 Hz). **LC-MS** (*m/z*): 421.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.5%.

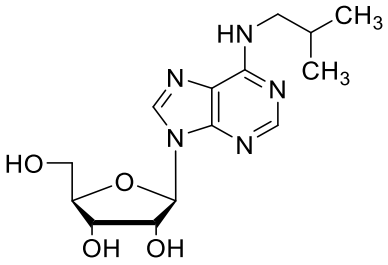
6-Cyclohexylthiopurine-*β*-*D*-ribofuranosyl-5′-monophosphate (36b, Bcy-286)



Compound **35b** (100 mg, 0.27 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (88 mg, 0.41 mmol) and $POCl_3$ (0.10 mL, 1.08 mmol) were used. **Appearance**: white powder; **mp**: 191.0-193.0 °C. **Yield**: 93 mg, 77%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.78 (s, 1H), 8.71 (s, 1H), 6.01 (d, J = 6.2 Hz, 1H), 4.72 (dd, J = 6.2, 4.8 Hz, 1H), 4.27 – 4.16 (m, 4H), 4.07 (q, J = 3.4 Hz, 2H), 3.88 – 3.79 (m, 3H), 2.14 – 2.02 (m, 2H), 1.74 (dt, J = 13.0, 4.3 Hz, 2H), 1.64 – 1.27 (m, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.54,

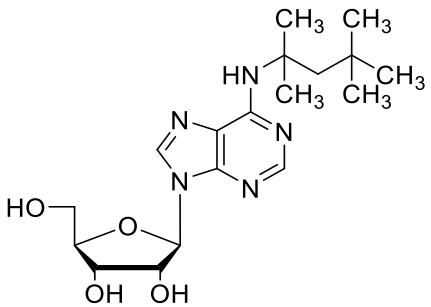
151.60, 148.65, 142.95, 130.60, 86.89, 84.75, 74.23, 71.24, 63.95, 40.97, 32.75, 32.70, 25.43, 25.11. ^{31}P NMR (202 MHz, DMSO-d_6) δ 1.06 (d, J = 7.2 Hz). **LC-MS** (*m/z*): 447.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.9%.

N^6-Isobutyladenosine (37a, Bcy-15), CAS: 36031-53-5



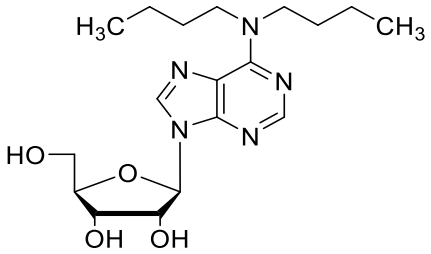
To a solution of 6-chloro-9-(*β-D*-ribofuranosyl)purine (500 mg, 1.74 mmol, 1 eq.) in absolute EtOH (10 mL), isobutylamine (0.26 mL, 2.61 mmol, 1.5 eq.) and Et_3N (0.48 mL, 3.48 mmol, 2 eq.) were added. The mixture was refluxed for 3 h and monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, cooled to rt and the solvent was evaporated *in vacuum*. The crude compound was purified by silica gel column chromatography using 8% MeOH in DCM. **Appearance**: white solid; **mp**: 151.5-153.5 °C (*lit.*[182] 166-167 °C). **Yield**: 653 mg, 96%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.33 (s, 1H), 8.19 (s, 1H), 7.86 (s, 1H), 5.88 (d, J = 6.2 Hz, 1H), 5.40 (t, J = 4.6 Hz, 2H), 5.14 (d, J = 4.7 Hz, 1H), 4.62 (q, J = 5.9 Hz, 1H), 4.15 (td, J = 4.8, 3.1 Hz, 1H), 3.97 (q, J = 3.4 Hz, 1H), 3.78 – 3.48 (m, 2H), 1.34 – 1.06 (m, 1H), 0.89 (d, J = 6.8 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.84, 152.25, 148.20, 139.55, 119.64, 87.94, 85.88, 73.43, 70.64, 61.66, 27.78, 20.05. **LC-MS** (*m/z*): 324.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.6%.

N^6-(1,1,3,3-Tetramethyl)butyladenosine (37b, CS-364)



This compound was synthesized using the same procedure as for **37a**. 6-Chloro-9-(*β*-*D*-ribofuranosyl)purine (500 mg, 1.74 mmol), EtOH (15 mL), 1,1,3,3-tetramethylbutylamine (337 mg, 2.61 mmol) and Et_3N (0.48 mL, 3.48 mmol) were used. The crude compound was purified by silica gel column chromatography using 3% MeOH in DCM. **Appearance**: white powder; **mp**: 110 °C. **Yield**: 224 mg, 34%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.31 (s, 1H), 8.21 (s, 1H), 6.69 (s, 1H), 5.86 (d, 1H, J = 6.24 Hz), 5.41 (br s, 1H), 5.37 (dd, 1H, J = 4.6, 7.2 Hz), 5.16 (d, 1H, J = 3.3 Hz), 4.62 (br s, 1H), 4.13 (br s, 1H), 3.95 (q, 1H, J = 3.5 Hz), 3.66-3.54 (d m, 2H), 2.00 (s, 2H), 1.54 (s, 6H), 0.92 (s, 9H). ^{13}C NMR (126 MHz, CD_3OD) δ 154.78, 151.89, 148.22, 139.77, 120.43, 88.13, 86.12, 73.57, 70.86, 61.88, 55.56, 50.23, 31.65, 31.40, 29.97. **LC-MS** (*m/z*): 379.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 94.5%. This compound was co-synthesized with Dr. Constanze Cerine Schmies.

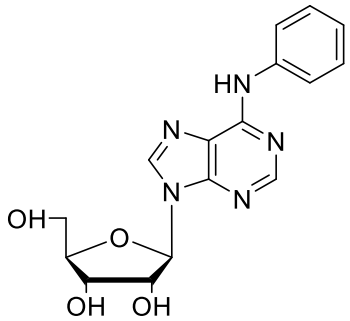
N^6,*N*^{6}-Dibutyladenosine (37c, Bcy-13), CAS: 81609-38-3



This compound was synthesized using the same procedure as for **37a**. 6-Chloro-9-(*β*-*D*-ribofuranosyl)purine (500 mg, 1.74 mmol), EtOH (10 mL), dibutylamine (0.44 mL, 2.61 mmol) and Et_3N (0.48 mL, 3.48 mmol) were used. The crude compound was purified by silica gel column chromatography using 5% MeOH in DCM. **Appearance**: light gray solid; **mp**: 141.0-143.0 °C (*lit.*[180] 149-151 °C). **Yield**: 590 mg, 89%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.35 (s, 1H), 8.19 (s, 1H), 5.90 (d, J = 6.0 Hz, 1H), 5.40 (d, J = 6.3 Hz, 1H), 5.33 (dd, J = 7.0, 4.5 Hz, 1H), 5.14 (d, J = 4.7 Hz, 1H), 4.59 (td, J = 6.0, 4.8 Hz, 1H), 4.15 (td, J = 4.8, 3.1 Hz, 1H), 3.96 (q, J = 3.5 Hz, 1H), 3.66 (dt, J = 12.1, 4.2 Hz, 1H), 3.55 (ddd, J = 12.0, 7.0, 3.7 Hz, 1H), 2.90 – 2.79 (m, 1H), 1.65 – 1.58 (m, 4H), 1.33 (qd, J = 7.5, 2.2 Hz, 5H), 0.90 (dt, J = 9.7, 7.4 Hz, 8H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.57, 151.71, 149.92, 138.62, 119.33, 87.72, 85.74, 73.41, 70.54, 61.56,

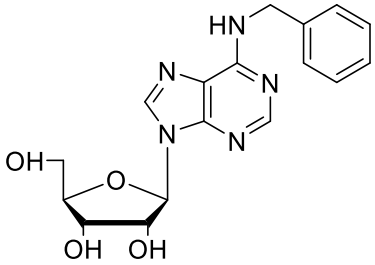
46.44, 27.47, 19.50, 19.24, 13.82, 13.42. **LC-MS** (*m/z*): 380.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.2%.

N^6-Phenyladenosine (37d, Bcy-26), CAS: 23589-16-4



This compound was synthesized using the same procedure as for **37a**. 6-Chloro-9-(*β*-*D*-ribofuranosyl)purine (300 mg, 1.05 mmol), EtOH (10 mL), aniline (0.14 mL, 1.58 mmol) and Et_3N (0.29 mL, 2.10 mmol) were used. The crude compound was purified by silica gel column chromatography using 6% MeOH in DCM. **Appearance**: white solid; **mp**: 192.3-194.3 ℃ (*lit.*[183] 199 ℃). **Yield**: 259 mg, 72%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.92 (s, 1H), 8.54 (s, 1H), 8.40 (s, 1H), 8.02 – 7.88 (m, 2H), 7.42 – 7.26 (m, 2H), 7.10 – 6.99 (m, 1H), 5.96 (d, J = 5.9 Hz, 1H), 5.47 (d, J = 6.1 Hz, 1H), 5.33 – 5.13 (m, 2H), 4.71 – 4.59 (m, 1H), 4.24 – 4.12 (m, 1H), 3.99 (q, J = 3.7 Hz, 1H), 3.78 – 3.50 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 152.17, 151.91, 149.33, 140.68, 139.51, 128.38, 122.72, 120.90, 120.35, 87.86, 85.84, 73.60, 70.53, 61.55. **LC-MS** (*m/z*): 344.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.8%.

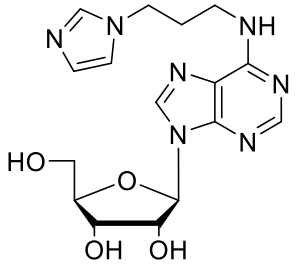
N^6-Benzyladenosine (37e, Bcy-27), CAS: 4294-16-0



This compound was synthesized using the same procedure as for **37a**. 6-Chloro-9-(*β*-*D*-ribofuranosyl)purine (500 mg, 1.74 mmol), EtOH (10 mL), benzylamine (0.27 mL, 2.61 mmol) and Et_3N (0.48 mL, 3.48 mmol) were used. The crude compound was

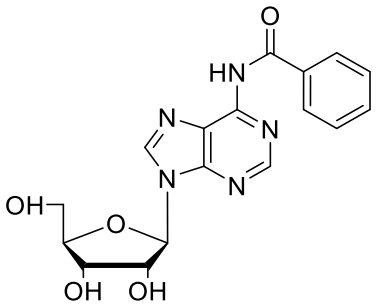
purified by silica gel column chromatography using 6% MeOH in DCM. **Appearance**: Light yellow solid; **mp**: 163.0-165.0 °C (*lit.*[182] 168-169 °C). **Yield**: 609 mg, 98%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.37 (s, 1H), 8.20 (s, 1H), 7.37 – 7.26 (m, 5H), 5.90 (d, J = 6.1 Hz, 1H), 5.27 (d, J = 122.2 Hz, 3H), 4.81 – 4.55 (m, 3H), 4.15 (dd, J = 5.0, 3.1 Hz, 1H), 3.97 (q, J = 3.5 Hz, 1H), 3.74 (s, 1H), 3.62 (ddd, J = 71.4, 12.1, 3.7 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.50, 152.33, 148.47, 142.92, 139.92, 128.19, 128.11, 127.20, 127.10, 126.59, 119.77, 87.94, 85.88, 73.48, 70.63, 61.65, 45.22. **LC-MS** (*m/z*): 358.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.6%.

N^6-(3-(Imidazol-1-yl))propyladenosine (37f, CS-365)



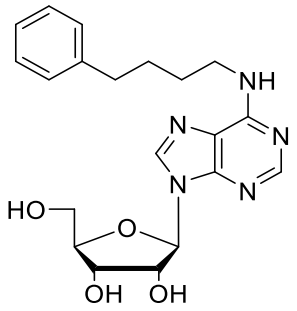
To a solution of 6-chloro-9-(*β-D*-ribofuranosyl)purine (500 mg, 1.74 mmol, 1 eq.) in absolute EtOH (10 mL), *N*-(3-aminopropyl)imidazole (327 mg, 2.61 mmol, 1.5 eq.) and Et_3N (0.48 mL, 3.48 mmol, 2 eq.) were added. The mixture was refluxed for 3 h and monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, cooled to rt and the solvent was concentrated *in vacuum*. Then the mixture was poured on H_2O (30 mL) and extracted with ethyl acetate (30 mL × 4). The aqueous layer was finally lyophilized. **Appearance**: brown solid; **mp**: 100 °C. **Yield**: 427 mg, 65%. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.35 (s, 1H), 8.20 (s, 1H), 7.98 (s, 1H), 7.71 (d, 1H, J = 27.30 Hz), 7.21 (d, 1H, J = 21.59 Hz), 6.91 (s, 1H), 5.88 (d, 1H, J = 6.14 Hz), 5.40 (br s, 1H), 5.18 (br s, 1H), 4.59 (t, 1H, J = 5.51 Hz), 4.14 (m, 1H), 4.07 (t, 2H, J = 6.86 Hz), 4.04 (t, 2H, J = 6.94 Hz), 3.95 (q, 1H, J = 3.40 Hz), 3.66-3.54 (d m, 2H), 2.69 (m, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 154.87, 152.50, 148.52, 139.93, 137.43, 128.03, 119.70, 119.51, 88.09, 86.07, 73.70, 70.82, 61.63, 43.23, 36.32, 28.84. **LC-MS** (*m/z*): 376.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.2%. This compound was co-synthesized with Dr. Constanze Cerine Schmies.

N^6-Benzoyladenosine (37g, Bcy-12), CAS: 4546-55-8



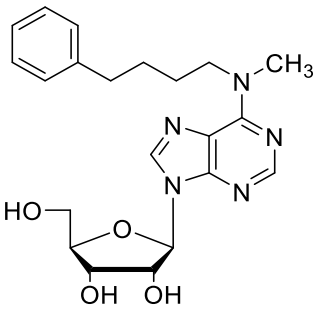
To a solution of adenosine (500 mg, 1.87 mmol, 1 eq.) in anhydrous pyridine (10 mL), chlorotrimethylsilane (1.78 mL, 14.03 mmol, 7.5 eq.) was added, and the mixture was stirred at rt for 15 min. Benzoyl chloride (1.39 mL, 9.35 mmol, 5 eq.) was subsequently added, and the mixture was stirred at rt for 2 h. The mixture was cooled to 0 ℃, and H_2O (2 mL) was added to quench the reaction. Aqueous ammonia (5 mL, 28% NH_3 in H_2O) was subsequently added for the deprotection of 2′-, 3′- and 5′-trimethylsilyl groups, and the mixture was stirred at rt for 30 min. The reaction progress was monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, the mixture was concentrated, and the residue was lyophilized. The crude compound was finally purified by silica gel column chromatography using 10% MeOH in DCM. **Appearance**: white solid; **mp**: 133.0-135.0 ℃ (*lit.*[184] 133-134 ℃). **Yield**: 688 mg, 99%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.20 (s, 1H), 8.73 (d, J = 23.5 Hz, 2H), 8.10 – 7.99 (m, 2H), 7.99 – 7.89 (m, 1H), 7.69 – 7.45 (m, 4H), 6.05 (d, J = 5.8 Hz, 1H), 5.13 (s, 1H), 4.65 (t, J = 5.4 Hz, 1H), 4.20 (dd, J = 4.8, 3.6 Hz, 1H), 3.99 (q, J = 3.9 Hz, 1H), 3.70 (dd, J = 11.9, 4.1 Hz, 1H), 3.59 (dd, J = 11.9, 4.0 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.72, 152.20, 151.57, 150.41, 143.15, 133.39, 132.48, 132.45, 129.23, 128.48, 128.42, 125.88, 87.62, 85.74, 73.69, 70.38, 61.34. **LC-MS** (*m/z*): 372.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.3%.

N^6-(4-Phenylbutyl)adenosine (37h, Bcy-28), CAS: 101565-58-6



This compound was synthesized using the same procedure as for **37a**. 6-Chloro-9-(*β*-*D*-ribofuranosyl)purine (2.00 g, 6.98 mmol), EtOH (15 mL), 4-phenylbutylamine (1.65 mL, 10.47 mmol) and Et_3N (1.94 mL, 13.96 mmol) were used. The crude compound was purified by silica gel column chromatography using 6% MeOH in DCM. **Appearance**: light gray solid; **mp**: 115.5-117.5 °C (*lit.*[79] 106 °C). **Yield**: 2.73 g, 98%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.31 (s, 1H), 8.18 (s, 1H), 7.85 (s, 1H), 7.42 (d, J = 8.4 Hz, 2H), 7.15 (d, J = 8.4 Hz, 2H), 5.86 (d, J = 6.2 Hz, 1H), 5.38 (m, 2H), 5.14 (d, J = 4.6 Hz, 1H), 4.60 (q, J = 5.8 Hz, 1H), 4.14 (q, J = 4.6 Hz, 1H), 3.95 (q, J = 3.5 Hz, 1H), 3.60-3.51 (dm, 2H), 3.50 (s, 2H), 2.57 (t, J = 6.8 Hz, 2H), 1.59 (s, 4H). ^{13}C NMR (151 MHz, CD_3OD) δ 154.85, 152.49, 148.39, 141.80, 139.75, 132.59, 131.18, 130.77, 118.76, 88.11, 86.05, 73.63, 70.81, 61.84, 34.27, 28.84, 28.30. **LC-MS** (*m/z*): 399.9 [M + H]$^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.8%.

N^6-Methyl-*N*^{6}-(4-phenylbutyl)adenosine (37i, Bcy-54)



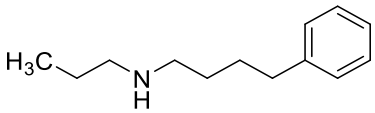
This compound was synthesized using the same procedure as for **37a**. 6-Chloro-9-(*β*-*D*-ribofuranosyl)purine (500 mg, 1.74 mmol), EtOH (10 mL), methyl(4-phenylbutyl)amine (0.38 mL, 2.09 mmol) and Et_3N (0.48 mL, 3.48 mmol) were used. The crude compound was purified by silica gel column chromatography using 5% MeOH in DCM. **Appearance**: white solid; **mp**: 168.0-169.0 °C. **Yield**: 614 mg, 85%.

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.37 (s, 1H), 8.20 (s, 1H), 7.25 (t, J = 7.5 Hz, 2H), 7.20 – 7.11 (m, 3H), 5.91 (d, J = 6.0 Hz, 1H), 5.42 (d, J = 6.2 Hz, 1H), 5.35 (dd, J = 6.9, 4.6 Hz, 1H), 5.16 (d, J = 4.7 Hz, 1H), 4.58 (q, J = 5.8 Hz, 1H), 4.15 (td, J = 4.8, 3.3 Hz, 3H), 3.96 (q, J = 3.6 Hz, 2H), 3.82 – 3.45 (m, 4H), 2.61 (t, J = 7.5 Hz, 2H), 1.73 – 1.54 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 153.97, 151.75, 149.92, 142.09, 138.64, 128.27, 128.24, 125.66, 119.56, 87.79, 85.77, 73.48, 70.55, 61.58, 34.89, 28.12. **LC-MS** (*m/z*): 414.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.4%.

General procedure for the synthesis of *N*-substituted 4-phenylbutan-1-amine derivatives (40c-e)

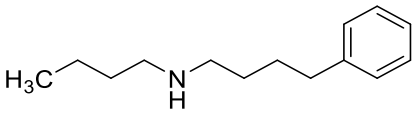
To a solution of 1-bromo-4-phenylbutane (1 eq.) in MeOH (10 mL), appropriate amine (5 eq.) was added. The mixture was refluxed overnight and monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, cooled to rt and the solvent was evaporated *in vacuum*. The crude compound was purified by silica gel column chromatography using 4% MeOH in DCM.

4-Phenyl-*N*-propylbutan-1-amine (40c, Bcy-47), CAS: 1094654-83-7



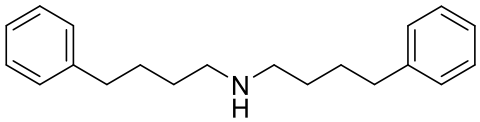
1-Bromo-4-phenylbutane (1.00 mL, 5.70 mmol), MeOH (10 mL) and propylamine (2.34 mL, 28.50 mmol) were used. **Appearance**: yellowish solid; **mp**: 183.0-184.8 ℃. **Yield**: 854 mg, 78%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.29 (t, J = 7.6 Hz, 2H), 7.23 – 7.15 (m, 3H), 2.89 (s, 2H), 2.85 – 2.79 (m, 2H), 2.60 (t, J = 6.9 Hz, 2H), 1.75 – 1.44 (m, 7H), 0.90 (t, J = 7.5 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 141.57, 128.32, 128.28, 125.81, 48.37, 46.62, 34.48, 27.76, 25.07, 18.97, 10.92. **LC-MS** (*m/z*): 191.8 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.7%.

***N*-Butyl-4-phenylbutan-1-amine (40d, Bcy-159), CAS: 143996-03-6**



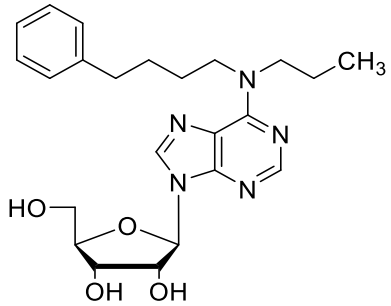
1-Bromo-4-phenylbutane (1.00 mL, 5.70 mmol), MeOH (10 mL) and butylamine (2.82 mL, 28.50 mmol) were used. **Appearance**: white solid; **mp**: 203.5-205.5 °C (*lit.*[185] oil). **Yield**: 888 mg, 76%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.40 – 7.08 (m, 5H), 3.02 – 2.77 (m, 4H), 2.60 (t, J = 7.0 Hz, 2H), 1.87 – 1.40 (m, 7H), 1.32 (h, J = 7.4 Hz, 2H), 0.88 (t, J = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 141.55, 128.25, 125.78, 46.61, 46.51, 34.45, 27.74, 27.47, 25.07, 19.23, 13.44. **LC-MS** (*m/z*): 205.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.9%.

Bis(4-phenylbutyl)amine (40e, Bcy-177), CAS: 94875-96-4



1-Bromo-4-phenylbutane (0.50 mL, 2.90 mmol), MeOH (10 mL) and 4-phenylbutylamine (2.29 mL, 14.50 mmol) were used. **Appearance**: yellowish oil. **Yield**: 690 mg, 85%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.36 – 7.03 (m, 10H), 2.60 – 2.53 (m, 4H), 2.51 (s, 5H), 1.64 – 1.49 (m, 4H), 1.41 (q, J = 7.4 Hz, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 142.20, 128.19, 128.12, 125.52, 48.96, 35.02, 28.85, 28.68. **LC-MS** (*m/z*): 281.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%.

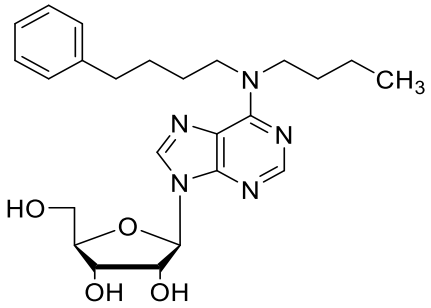
***N*^{6}-Propyl-*N*^{6}-(4-phenylbutyl)adenosine (37j, Bcy-55)**



This compound was synthesized using the same procedure as for **37a**. 6-Chloro-9-(*β*-*D*-ribofuranosyl)purine (500 mg, 1.74 mmol), EtOH (10 mL), compound **40c** (499 mg, 2.61 mmol) and Et_3N (0.48 mL, 3.48 mmol) were used. The crude compound was

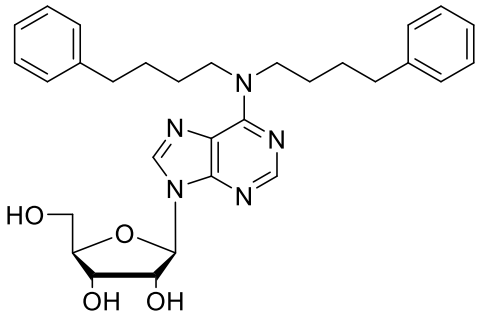
purified by silica gel column chromatography using 5% MeOH in DCM. **Appearance**: brownish solid; **mp**: 73.0-74.5 °C. **Yield**: 719 mg, 94%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.36 (s, 1H), 8.19 (s, 1H), 7.35 – 7.09 (m, 5H), 5.90 (d, J = 6.0 Hz, 1H), 5.47 – 5.30 (m, 2H), 5.16 (d, J = 4.6 Hz, 1H), 4.59 (q, J = 5.7 Hz, 1H), 4.15 (q, J = 4.3 Hz, 2H), 3.96 (q, J = 3.5 Hz, 2H), 3.67 (dt, J = 12.1, 3.9 Hz, 2H), 3.55 (ddd, J = 11.6, 6.6, 3.6 Hz, 1H), 2.93 – 2.75 (m, 1H), 2.61 (dt, J = 11.1, 7.4 Hz, 2H), 1.61 (ddp, J = 23.1, 15.2, 7.6 Hz, 6H), 0.88 (dt, J = 11.3, 7.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 153.63, 151.75, 149.96, 142.11, 138.75, 128.33, 128.29, 128.22, 125.65, 119.36, 87.78, 85.78, 73.45, 70.59, 61.60, 46.75, 34.93, 34.51, 28.21, 27.79, 19.16, 10.95. **LC-MS** (m/z): 442.4 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.0%.

N^6-Butyl-N^6-(4-phenylbutyl)adenosine (37k, Bcy-162)



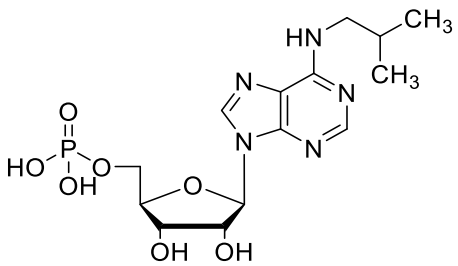
This compound was synthesized using the same procedure as for **37a**. 6-Chloro-9-(β-*D*-ribofuranosyl)purine (300 mg, 1.05 mmol), EtOH (10 mL), compound **40d** (325 mg, 1.58 mmol) and Et_3N (2.90 mL, 21.00 mmol) were used. The crude compound was purified by silica gel column chromatography using 4% MeOH in DCM. **Appearance**: gray viscous oil. **Yield**: 491 mg, >100%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.36 (s, 1H), 8.19 (s, 1H), 7.35 – 7.07 (m, 5H), 5.90 (d, J = 6.0 Hz, 1H), 5.47 – 5.28 (m, 2H), 5.15 (d, J = 4.7 Hz, 1H), 4.59 (q, J = 5.8 Hz, 1H), 4.35 – 4.03 (m, 3H), 3.96 (q, J = 3.5 Hz, 1H), 3.86 – 3.48 (m, 4H), 2.62 (t, J = 7.3 Hz, 2H), 1.74 – 1.50 (m, 6H), 1.31 (q, J = 7.5 Hz, 2H), 0.90 (t, J = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 153.57, 151.73, 149.93, 142.07, 138.69, 128.26, 128.18, 125.62, 119.33, 87.74, 85.75, 73.41, 70.56, 61.58, 34.88, 30.65, 28.16, 19.50, 13.83. **LC-MS** (m/z): 456.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.8%.

N^6,N^6-Di-(4-phenylbutyl)adenosine (37l, Bcy-178)



This compound was synthesized using the same procedure as for **37a**. 6-Chloro-9-(*β*-*D*-ribofuranosyl)purine (300 mg, 1.05 mmol), EtOH (10 mL), compound **40e** (445 mg, 1.58 mmol) and Et_3N (2.90 mL, 21.00 mmol) were used. The crude compound was purified by silica gel column chromatography using 4% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 110.0-111.5 °C. **Yield**: 517 mg, 93%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.36 (s, 1H), 8.19 (s, 1H), 7.31 – 7.11 (m, 10H), 5.90 (d, J = 6.0 Hz, 1H), 5.40 (d, J = 6.1 Hz, 1H), 5.32 (dd, J = 7.0, 4.6 Hz, 1H), 5.14 (d, J = 4.7 Hz, 1H), 4.59 (q, J = 5.7 Hz, 1H), 4.43 – 3.91 (m, 4H), 3.90 – 3.49 (m, 4H), 2.61 (t, J = 7.2 Hz, 4H), 1.73 – 1.56 (m, 8H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.55, 151.69, 149.93, 142.04, 138.68, 128.25, 128.22, 128.16, 125.59, 119.31, 87.74, 85.72, 73.40, 70.52, 61.55, 47.31, 34.87, 34.59, 30.62, 28.12, 27.93, 26.11. **LC-MS** (*m/z*): 532.5 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%.

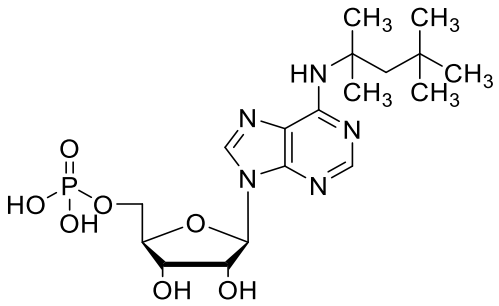
N^6-Isobutyl-AMP (38a, Bcy-39)



Compound **37a** (150 mg, 0.46 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (167 mg, 0.69 mmol) and $POCl_3$ (0.17 mL, 1.84 mmol) were used. **Appearance**: white powder; **mp**: 191.0-193.0 °C. **Yield**: 18 mg, 10%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.46 (s, 1H), 8.20 (s, 1H), 7.79 (s, 1H), 5.92 (d, J = 6.1 Hz, 1H), 4.66 (t, J = 5.5 Hz, 1H), 4.24 (t, J = 3.8 Hz, 2H), 4.04 (s, 1H), 3.83 (t, J = 5.0 Hz, 3H), 3.30 (s, 2H), 1.96 (s, 1H), 0.89 (d, J

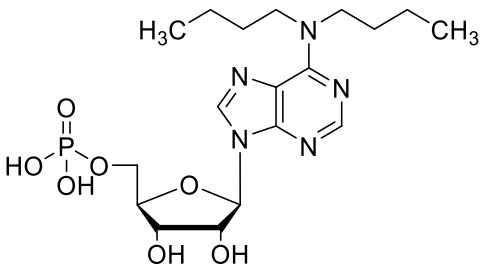
= 6.7 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.71, 152.54, 148.94, 139.02, 118.98, 86.56, 84.30, 73.99, 71.14, 64.11, 64.08, 27.82, 20.09. ^{31}P NMR (243 MHz, DMSO-d_6) δ 1.11. **LC-MS** (*m/z*): 404.3 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.9%.

N^6-(1,1,3,3-Tetramethyl)butyl-AMP (38b, CS-371)



Compound **37b** (100 mg, 0.26 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (84 mg, 0.39 mmol) and $POCl_3$ (0.13 mL, 1.43 mmol) were used. **Appearance**: white powder; **mp**: 183 ℃. **Yield**: 20 mg, 17%. ^{1}H NMR (600 MHz, D_2O) δ 8.44 (s, 1H), 8.22 (s, 1H), 6.10 (d, 1H, J = 5.9 Hz), 4.75 (t, 1H, J = 5.6 Hz), 4.49 (m, 1H), 4.37 (m, 1H), 4.07 (dd, 2H, J = 2.9, 4.8 Hz), 1.97 (s, 2H), 1.56 (s, 6H), 0.91 (s, 9H). ^{13}C NMR (126 MHz, D_2O) δ 157.23, 155.37, 150.39, 141.66, 122.35, 89.34, 87.20, 87.15, 77.25, 73.45, 66.96, 58.73, 52.52, 33.76, 33.67, 33.48, 32.12, 32.09. ^{31}P NMR (202 MHz, D_2O) δ 1.93. **LC-MS** (*m/z*): 460.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.0%. This compound was co-synthesized with Dr. Constanze Cerine Schmies.

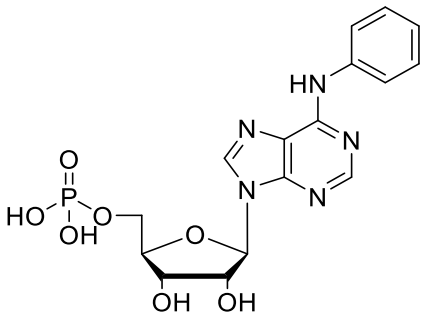
N^6,N^6-Dibutyl-AMP (38c, Bcy-24), CAS: 81609-40-7



Compound **37c** (150 mg, 0.40 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (145 mg, 0.60 mmol) and $POCl_3$ (0.15 mL, 1.60 mmol) were used. **Appearance**: white powder; **mp**: 177.5-179.0 ℃. **Yield**: 55 mg, 30%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.38 (s, 1H),

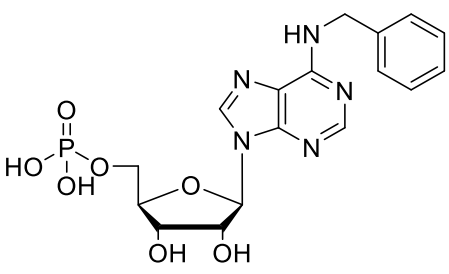
8.20 (s, 1H), 5.95 (d, *J* = 6.3 Hz, 1H), 4.63 (dd, *J* = 6.3, 4.9 Hz, 2H), 4.20 (dd, *J* = 4.9, 2.9 Hz, 2H), 4.05 (d, *J* = 3.6 Hz, 3H), 3.89 (dt, *J* = 6.8, 4.5 Hz, 3H), 3.83 – 3.40 (m, 3H), 1.61 (p, *J* = 7.5 Hz, 4H), 1.33 (h, *J* = 7.5 Hz, 4H), 0.91 (t, *J* = 7.4 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 153.49, 151.99, 150.58, 137.95, 118.82, 86.44, 83.93, 83.88, 73.77, 70.98, 64.57, 64.53, 19.53, 13.86. ^{31}P NMR (243 MHz, DMSO-d_6) δ 0.95. **LC-MS** (*m/z*): 460.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.0%.

N^6-Phenyl-AMP (38d, Bcy-266), CAS: 105740-46-3



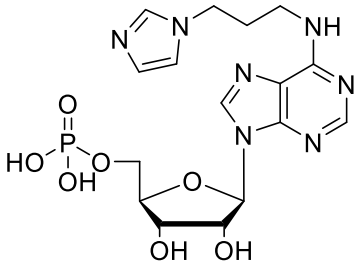
Compound **37d** (100 mg, 0.29 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (94 mg, 0.44 mmol) and $POCl_3$ (0.11 mL, 1.16 mmol) were used. **Appearance**: white powder; **mp**: 172.0-174.0 °C. **Yield**: 67 mg, 55%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.88 (s, 1H), 8.69 (s, 1H), 8.40 (s, 1H), 7.99 – 7.90 (m, 2H), 7.40 – 7.26 (m, 2H), 7.08 – 6.99 (m, 1H), 6.00 (d, *J* = 6.1 Hz, 1H), 4.72 (dd, *J* = 6.1, 4.8 Hz, 1H), 4.27 (dd, *J* = 4.8, 3.0 Hz, 2H), 4.08 (q, *J* = 3.6 Hz, 2H), 3.86 (dd, *J* = 6.7, 3.8 Hz, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 152.05, 149.90, 140.36, 139.67, 128.36, 122.52, 120.74, 119.79, 86.74, 84.53, 74.19, 71.20, 63.99. ^{31}P NMR (243 MHz, DMSO-d_6) δ 1.25. **LC-MS** (*m/z*): 424.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.4%.

N^6-Benzyl-AMP (38e, Bcy-267), CAS: 13484-66-7



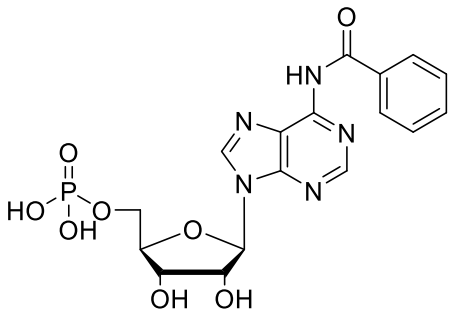
Compound **37e** (100 mg, 0.28 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (90 mg, 0.42 mmol) and $POCl_3$ (0.10 mL, 1.12 mmol) were used. **Appearance**: white powder; **mp**: 168.0-170.0 ℃. **Yield**: 62 mg, 51%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.50 (s, 1H), 8.20 (s, 1H), 7.33 (d, J = 7.5 Hz, 2H), 7.28 (t, J = 7.6 Hz, 2H), 7.22 – 7.17 (m, 1H), 5.93 (d, J = 6.2 Hz, 1H), 4.76 – 4.65 (m, 3H), 4.24 (dd, J = 4.9, 2.9 Hz, 2H), 4.04 (q, J = 3.6 Hz, 3H), 3.82 (dd, J = 6.9, 3.7 Hz, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.37, 152.54, 149.16, 140.10, 139.38, 128.18, 127.06, 126.54, 119.08, 86.60, 84.37, 74.07, 71.22, 64.02, 42.86. ^{31}P NMR (243 MHz, DMSO-d_6) δ 1.15. **LC-MS** (*m/z*): 438.3 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.5%.

N^6-(3-(Imidazol-1-yl))propyl-AMP (38f, CS-372)



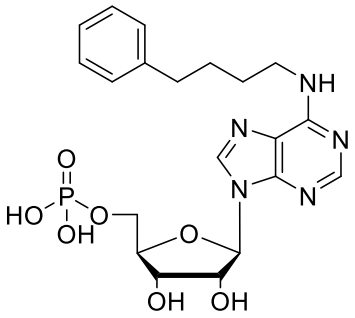
Compound **37f** (100 mg, 0.27 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (88 mg, 0.41 mmol) and $POCl_3$ (0.14 mL, 1.49 mmol) were used. **Appearance**: white powder; **mp**: 196 ℃. **Yield**: 21 mg, 17%. ^{1}H NMR (600 MHz, D_2O) δ 8.52 (s, 1H) 8.44 (s, 1H) 8.16 (s, 1H) 7.38 (s, 1H) 7.21 (s, 1H) 6.09 (m, 1H) 4.83 (br s, 1H) 4.53 (m, 1H) 4.38 (s, 1H) 4.34 (m, 2H) 4.07 (m, 2H) 3.69 (br s, 2H) 2.31 (m, 2H). ^{13}C NMR (126 MHz, D_2O) δ 157.11, 155.47, 142.36, 142.20, 137.55, 124.24, 122.97, 89.75, 87.44, 87.38, 77.29, 73.54, 66.68, 49.91, 40.62, 30.91. ^{31}P NMR (202 MHz, D_2O) δ 2.27. **LC-MS** (m/z): 456.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.7%. This compound was co-synthesized with Dr. Constanze Cerine Schmies.

N^6-Benzoyl-AMP (38g, Bcy-255), CAS: 40871-55-4



Compound **37g** (100 mg, 0.27 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (88 mg, 0.41 mmol) and $POCl_3$ (0.10 mL, 1.08 mmol) were used. **Appearance**: white powder; **mp**: 173.0-174.5 °C. **Yield**: 47 mg, 39%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.89 (s, 1H), 8.74 (s, 1H), 8.09 – 8.03 (m, 2H), 7.66 – 7.61 (m, 1H), 7.54 (t, J = 7.8 Hz, 2H), 6.07 (d, J = 6.2 Hz, 1H), 4.76 (dd, J = 6.2, 4.7 Hz, 2H), 4.27 (dd, J = 4.8, 2.8 Hz, 2H), 4.09 (q, J = 3.4 Hz, 2H), 3.85 (dd, J = 6.6, 3.6 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.68, 152.60, 151.60, 150.11, 143.11, 133.44, 132.35, 128.45, 128.42, 125.32, 86.91, 84.78, 74.22, 71.30, 63.92. ^{31}P NMR (243 MHz, DMSO-d_6) δ 1.09. **LC-MS** (*m/z*): 452.3 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.7%.

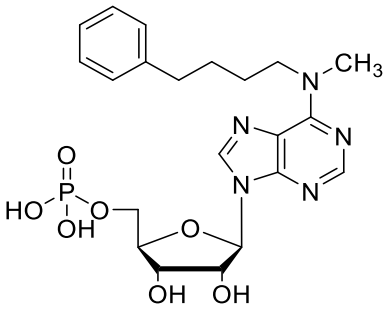
N^6-(4-Phenylbutyl)-AMP (38h, Bcy-308)



Compound **37h** (100 mg, 0.25 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (81 mg, 0.38 mmol) and $POCl_3$ (0.09 mL, 1.00 mmol) were used. **Appearance**: white powder; **mp**: 170.0-172.0 °C. **Yield**: 46 mg, 38%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.45 (s, 1H), 8.20 (s, 1H), 7.78 (s, 1H), 7.25 (t, J = 7.5 Hz, 2H), 7.21 – 7.17 (m, 2H), 7.16 – 7.12 (m, 1H), 5.92 (d, J = 6.1 Hz, 1H), 4.66 (t, J = 5.5 Hz, 3H), 4.24 (dd, J = 4.9, 3.0 Hz, 2H), 4.04 (q, J = 3.6 Hz, 1H), 3.82 (dd, J = 6.8, 3.8 Hz, 3H), 3.51 (s, 2H), 2.63 – 2.57 (m, 2H), 1.71 – 1.53 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.50, 152.55, 148.92, 142.20, 139.03, 128.27, 128.15, 125.54, 119.02, 86.54, 84.33, 74.02, 71.19, 64.05,

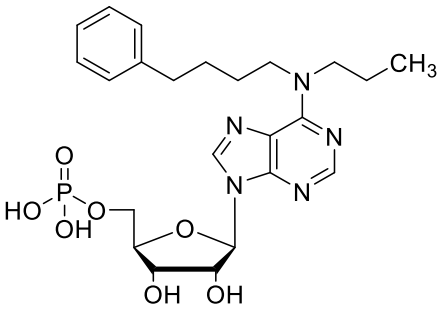
40.06, 34.87, 28.76, 28.44. ^{31}P NMR (243 MHz, DMSO-d_6) δ 1.18. **LC-MS** (*m/z*): 480.30 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.9%.

N^6-Methyl-N^6-(4-phenylbutyl)-AMP (38i, Bcy-70)



Compound **37i** (150 mg, 0.36 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (130 mg, 0.54 mmol) and $POCl_3$ (0.13 mL, 1.44 mmol) were used. **Appearance**: white powder; **mp**: 176.8-178.8 ℃. **Yield**: 83 mg, 47%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.42 (s, 1H), 8.21 (s, 1H), 7.24 (t, J = 7.6 Hz, 2H), 7.21 – 7.10 (m, 3H), 5.96 (d, J = 6.1 Hz, 1H), 4.63 (t, J = 5.5 Hz, 2H), 4.49 – 3.72 (m, 9H), 3.24 (s, 3H), 2.61 (t, J = 7.5 Hz, 2H), 1.72 – 1.53 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 153.84, 151.96, 150.54, 142.07, 137.98, 128.25, 128.20, 125.60, 119.00, 86.52, 83.93, 73.90, 70.96, 64.37, 34.89, 28.13. ^{31}P NMR (243 MHz, DMSO-d_6) δ 0.98. **LC-MS** (*m/z*): 494.3 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.8%.

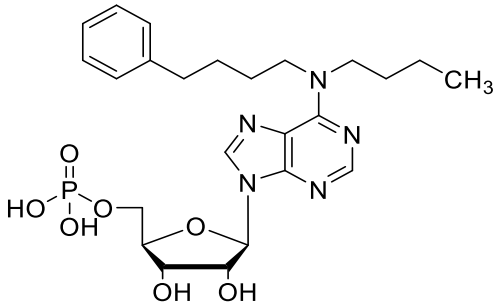
N^6-Propyl-N^6-(4-phenylbutyl)-AMP (38j, Bcy-71)



Compound **37j** (150 mg, 0.34 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (123 mg, 0.51 mmol) and $POCl_3$ (0.13 mL, 1.36 mmol) were used. **Appearance**: white powder; **mp**: 178.5-180.5 ℃. **Yield**: 59 mg, 33%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.41 (s, 1H), 8.20 (s, 1H), 7.31 – 7.16 (m, 5H), 5.95 (d, J = 6.2 Hz, 1H), 4.75 – 4.57 (m, 2H), 4.21

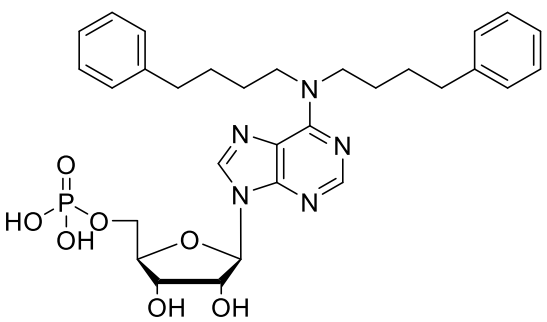
(dd, *J* = 4.9, 2.9 Hz, 3H), 3.87 (dd, *J* = 6.6, 3.9 Hz, 4H), 3.70 (s, 4H),2.61 (t, *J* = 7.3 Hz, 2H), 1.62 (td, *J* = 13.1, 9.3, 5.2 Hz, 6H), 0.87 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 153.50, 151.97, 150.61, 142.09, 138.07, 128.28, 128.18, 125.59, 118.79, 86.44, 84.02, 73.86, 71.09, 64.42, 34.92, 28.21, 11.01. ^{31}P NMR (243 MHz, DMSO-d_6) δ 0.99. **LC-MS** (*m/z*): 522.4 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.1%.

N^6-Butyl-N^6-(4-phenylbutyl)-AMP (38k, Bcy-221)



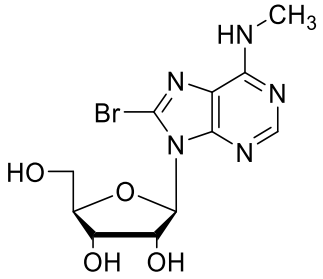
Compound **37k** (120 mg, 0.26 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (84 mg, 0.39 mmol) and $POCl_3$ (0.10 mL, 1.04 mmol) were used. **Appearance**: white powder; **mp**: 199.0-201.0 °C. **Yield**: 72 mg, 52%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.44 (s, 1H), 8.19 (s, 1H), 7.36 – 7.01 (m, 5H), 5.94 (d, *J* = 6.3 Hz, 1H), 4.68 (dd, *J* = 6.4, 4.8 Hz, 1H), 4.23 (dd, *J* = 4.8, 2.7 Hz, 3H), 4.03 (q, *J* = 3.4 Hz, 5H), 3.86 – 3.77 (m, 4H), 2.61 (t, *J* = 7.2 Hz, 2H), 1.73 – 1.52 (m, 6H), 1.31 (q, *J* = 7.5 Hz, 2H), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.48, 151.94, 150.65, 142.07, 138.12, 128.27, 125.58, 118.75, 86.37, 84.39, 74.00, 71.32, 64.11, 34.89, 28.19, 19.50, 13.84. ^{31}P NMR (243 MHz, DMSO-d_6) δ 1.19. **LC-MS** (*m/z*): 536.5 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.8%.

N^6,N^6-Di-(4-phenylbutyl)-AMP (38l, Bcy-226)



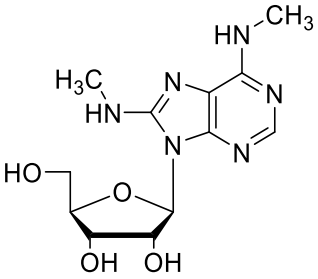
Compound **37l** (100 mg, 0.19 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (62 mg, 0.29 mmol) and $POCl_3$ (0.07 mL, 0.76 mmol) were used. **Appearance**: white powder; **mp**: 121.0-123.0 °C. **Yield**: 7 mg, 6%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.44 (s, 1H), 8.19 (s, 1H), 7.25 (t, J = 7.5 Hz, 4H), 7.21 – 7.11 (m, 6H), 5.94 (d, J = 6.2 Hz, 1H), 4.65 (dd, J = 6.1, 4.9 Hz, 1H), 4.22 (dd, J = 4.8, 2.9 Hz, 2H), 4.03 (q, J = 3.6 Hz, 2H), 3.83 (dt, J = 6.3, 3.2 Hz, 4H), 3.68 (s, 4H), 2.59 (t, J = 7.2 Hz, 4H), 1.77 – 1.50 (m, 8H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 153.45, 151.94, 150.61, 142.07, 138.16, 128.27, 128.18, 125.59, 118.76, 86.47, 84.25, 74.02, 71.27, 64.16, 45.22, 40.06, 34.91, 28.15. ^{31}P NMR (243 MHz, DMSO-d_6) δ 1.18. **LC-MS** (*m/z*): 612.7 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.2%.

8-Bromo-N^6-methyladenosine (42a, Bcy-370), CAS: 37116-71-5



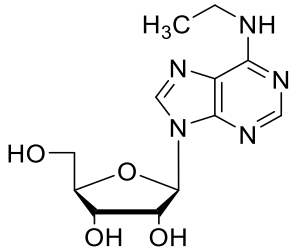
This compound was synthesized using the same procedure as for **26a**. N^6-Methyladenosine (**41a**,500 mg, 1.78 mmol), 1M sodium acetate buffer (pH 4.0, 10 mL), H_2O (20 mL) and bromine (0.23 mL, 4.45 mmol) were used. The crude product was purified by silica gel column chromatography using 10% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 226-228 °C (*lit.*[65] 228 °C). **Yield**: 270 mg, 42%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.21 (s, 1H), 8.04 (s, 1H), 5.84 (d, J = 6.9 Hz, 1H), 5.51 – 5.37 (m, 2H), 5.20 (d, J = 4.5 Hz, 1H), 5.13 – 5.05 (m, 1H), 4.23 – 4.16 (m, 1H), 4.02 – 3.95 (m, 1H), 3.73 – 3.64 (m, 1H), 3.58 – 3.48 (m, 1H), 2.95 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.94, 152.41, 148.86, 126.70, 120.22, 90.40, 86.68, 71.17, 70.82, 62.08, 26.94. **LC-MS** (*m/z*): 360.0 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.6%.

8-Methylamino-N^6-methyladenosine (43a, Bcy-373)



This compound was synthesized using the same procedure as for **29a**. Compound **42a** (92 mg, 0.26 mmol), 40% methylamine in MeOH (10 mL) and Et_3N (1.08 mL, 7.80 mmol, 30 eq.) were used. The crude product was purified by silica gel column chromatography using 10% MeOH in DCM. **Appearance**: brownish solid; **mp**: >300 °C. **Yield**: 81 mg, 100%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.97 (s, 1H), 6.92 (q, J = 4.6 Hz, 1H), 6.86 (q, J = 4.7 Hz, 1H), 5.92 (t, J = 5.2 Hz, 1H), 5.86 (d, J = 7.2 Hz, 1H), 5.23 (d, J = 6.5 Hz, 1H), 5.18 – 5.09 (m, 1H), 4.66 (q, J = 5.6 Hz, 1H), 4.13 (d, J = 5.3 Hz, 1H), 3.96 (q, J = 2.5 Hz, 1H), 3.68 – 3.57 (m, 2H), 2.92 (d, J = 4.7 Hz, 3H), 2.87 (d, J = 4.6 Hz, 3H). ^{13}C NMR (151 MHz, D_2O) δ 155.56, 149.93, 149.21, 146.64, 116.88, 89.81, 87.54, 73.79, 73.03, 67.56, 31.96, 31.04. **LC-MS** (*m/z*): 311.10 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.4%.

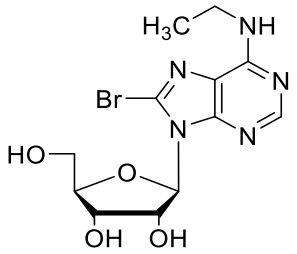
N^6-Ethyladenosine (41b, Bcy-9), CAS: 14357-08-5



This compound was synthesized using the same procedure as for **37a**. 6-Chloro-9-(*β*-*D*-ribofuranosyl)purine (2.00 g, 6.98 mmol), EtOH (15 mL), ethylamine (0.58 mL, 10.47 mmol) and Et_3N (1.94 mL, 13.96 mmol) were used. The crude compound was purified by silica gel column chromatography using 25% MeOH in DCM. **Appearance**: white solid; **mp**: 197.5-199.0 °C (*lit.*[186] 198-200 °C). **Yield**: 2.33 g, 93%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.32 (s, 1H), 8.20 (s, 1H), 7.80 (s, 1H), 5.88 (d, J = 6.2 Hz, 1H), 5.44 – 5.35 (m, 2H), 5.14 (d, J = 4.5 Hz, 1H), 4.65 – 4.55 (m, 1H), 4.15 (td, J = 4.7, 3.1 Hz, 1H), 3.97 (q, J = 3.5 Hz, 1H), 3.74 – 3.43 (m, 4H), 1.18 (t, J = 7.1 Hz, 3H). ^{13}C

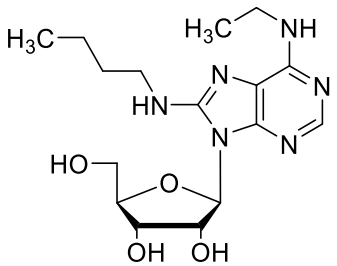
NMR (126 MHz, DMSO-d_6) δ 154.54, 152.35, 148.22, 139.60, 119.76, 87.95, 85.89, 73.47, 70.64, 61.67, 34.56, 14.79. **LC-MS** (*m/z*): 296.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.6%.

8-Bromo-N^6-ethyladenosine (42b, Bcy-16)



This compound was synthesized using the same procedure as for **26a**. Compound **41b** (1.92 g, 6.50 mmol), 1 M sodium acetate buffer (pH 4.0, 6 mL), H_2O (20 mL) and Br_2 (0.83 mL, 16.25 mmol) were used. The crude compound was purified by silica gel column chromatography using 4% MeOH in DCM. **Appearance**: yellow solid; **mp**: 173.3-175.0 °C. **Yield**: 92 mg, 4%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.15 (d, J = 49.3 Hz, 2H), 5.84 (d, J = 6.7 Hz, 1H), 5.53 – 5.41 (m, 2H), 5.22 (d, J = 4.4 Hz, 1H), 5.08 (td, J = 6.4, 5.1 Hz, 1H), 4.20 (td, J = 4.8, 2.4 Hz, 1H), 3.98 (td, J = 3.9, 2.3 Hz, 1H), 3.68 (dt, J = 12.1, 3.9 Hz, 1H), 3.58 – 3.41 (m, 3H), 1.19 – 1.13 (m, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 153.39, 152.44, 149.06, 126.77, 120.14, 90.44, 86.73, 71.21, 70.88, 62.12, 34.63, 14.61. **LC-MS** (*m/z*): 373.9 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.3%.

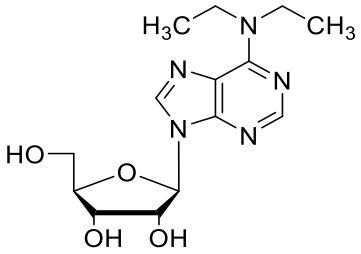
8-Butylamino-N^6-ethyl-adenosine (43b, CS-393A)



This compound was synthesized using the same procedure as for **29a**. Compound **42b** (150 mg, 0.40 mmol), butylamine (10 mL) and Et_3N (0.56 mL, 4.00 mmol) were used. The crude compound was purified by silica gel column chromatography using 7%

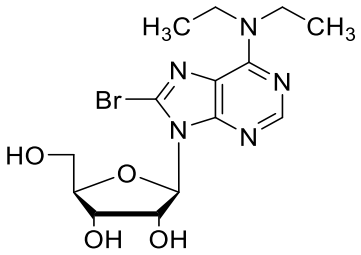
MeOH in DCM. **Appearance**: yellowish solid; **mp**: 229.8-231.8 °C. **Yield**: 53 mg, 36%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.93 (s, 1H), 6.81 (t, 1H, J = 5.4 Hz), 6.76 (t, 1H, J = 5.9 Hz), 5.89 (d, 1H, J = 7.4 Hz), 5.82 (s, 1H), 5.18 (s, 1H), 5.09 (s, 1H), 4.63 (t, 1H, J = 6.3 Hz), 4.11 (m, 1H), 3.95 (d, 1H, J = 2.2 Hz), 3.62 (br s, 2H), 3.48 (m, 2H), 3.35 (dt, 2H, J = 6.5, 12.9 Hz), 1.57 (m, 2H), 1.35 (m, 2H), 1.13 (t, 3H, J = 7.1 Hz), 0.90 (t, 3H, J = 7.4 Hz). ^{13}C NMR (126 MHz, CD_3OD) δ 151.42, 151.35, 148.56, 117.42, 86.48, 85.78, 71.09, 70.89, 61.80, 42.17, 39.94, 31.01, 19.78, 15.48, 13.89. **LC-MS** (*m/z*): 367.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.8%. This compound was co-synthesized with Dr. Constanze Cerine Schmies.

N^6,N^6-Diethyladenosine (41c, Bcy-8), CAS: 2139-60-8



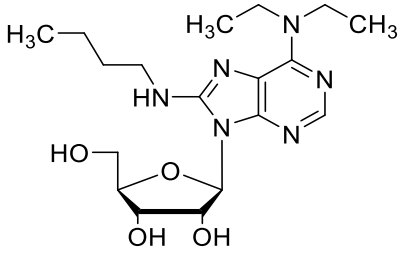
This compound was synthesized using the same procedure as for **37a**. 6-Chloro-9-(*β*-*D*-ribofuranosyl)purine (1.00 g, 3.49 mmol), EtOH (10 mL), diethylamine (0.54 mL, 5.24 mmol) and Et_3N (0.97 mL, 6.98 mmol) were used. The crude compound was purified by silica gel column chromatography using 10% MeOH in DCM. **Appearance**: white solid; **mp**: 177.5-179.5 °C (*lit.*[83] 178-180 °C). **Yield**: 1.01 g, 89%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.35 (s, 1H), 8.20 (s, 1H), 5.90 (d, J = 6.0 Hz, 1H), 5.46 – 5.30 (m, 2H), 5.14 (d, J = 4.7 Hz, 1H), 4.59 (td, J = 6.0, 4.9 Hz, 1H), 4.23 – 3.80 (m, 6H), 3.72 – 3.51 (m, 2H), 1.20 (t, J = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.14, 151.81, 149.92, 138.81, 119.33, 87.81, 85.77, 73.43, 70.55, 61.58, 42.41, 13.46. **LC-MS** (*m/z*): 324.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%.

8-Bromo-N^6,N^6-diethyladenosine (42c, Bcy-17)



This compound was synthesized using the same procedure as for **26a**. Compound **41c** (2.08 g, 6.43 mmol), 1 M sodium acetate buffer (pH 4.0, 6 mL), H_2O (20 mL) and Br_2 (0.82 mL, 16.08 mmol) were used. The crude compound was purified by silica gel column chromatography using 4% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 145.0-146.2 °C. **Yield**: 855 mg, 33%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.18 (s, 1H), 5.85 (d, J = 6.7 Hz, 1H), 5.52 – 5.38 (m, 2H), 5.21 (d, J = 4.5 Hz, 1H), 5.10 (q, J = 6.2 Hz, 1H), 4.20 (td, J = 4.8, 2.4 Hz, 1H), 3.98 (q, J = 3.7 Hz, 3H), 3.68 (dt, J = 12.1, 3.9 Hz, 3H), 3.53 (ddd, J = 12.3, 8.6, 4.2 Hz, 1H), 1.19 (t, J = 7.0 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 151.97, 151.71, 150.76, 126.19, 119.76, 90.53, 86.68, 70.91, 70.84, 62.12, 42.59, 13.27. **LC-MS** (*m/z*): 402.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 91.8%.

8-Butylamino-N^6,N^6-diethyladenosine (43c, Bcy-37)



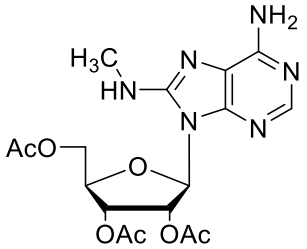
This compound was synthesized using the same procedure as for **29a**. Compound **42c** (400 mg, 1.00 mmol), butylamine (10 mL) and Et_3N (1.39 mL, 10.00 mmol) were used. The crude compound was purified by silica gel column chromatography using 5% MeOH in DCM. **Appearance**: brown oil. **Yield**: 341 mg, 86%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.95 (s, 1H), 6.84 (t, J = 5.5 Hz, 1H), 5.92 (d, J = 7.4 Hz, 1H), 5.23 – 5.06 (m, 2H), 4.63 (td, J = 7.1, 5.3 Hz, 1H), 4.11 (ddd, J = 5.7, 4.0, 2.0 Hz, 1H), 3.96 (q, J = 2.3 Hz, 1H), 3.86 (q, J = 7.2 Hz, 4H), 3.63 (dt, J = 5.2, 2.1 Hz, 2H), 1.59 (p, J = 7.2 Hz, 2H), 1.36 – 1.31 (m, 3H), 1.16 (t, J = 6.9 Hz, 7H), 0.90 (t, J = 7.4 Hz, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 150.38, 150.24, 148.08, 117.04, 86.25, 85.61, 70.95,

70.63, 61.63, 41.99, 41.75, 30.76, 19.54, 13.88, 13.68. **LC-MS** (*m/z*): 395.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.6%.

General procedure for the synthesis of 2′,3′,5′-tri-*O*-acetyl nucleosides (45a-b)

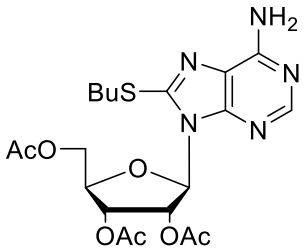
To a solution of 8-substituted nucleoside **29a** or **27a** (1 eq.) in MeCN (10 mL), acetic anhydride (3 eq.), DMAP (0.1 eq.) and DMEA (4 eq.) were added. The mixture was stirred at rt for 15 min and monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, the solvent was evaporated *in vacuum*, and the crude product was purified by silica gel column chromatography using 3% MeOH in DCM.

2′,3′,5′-Tri-*O*-acetyl-8-(methylamino)adenosine (45a, Bcy-144)



Compound **29a** (1.20 g, 4.05 mmol), MeCN (10 mL), acetic anhydride (1.15 mL, 12.15 mmol), DMAP (0.05 g, 0.41 mmol) and DMEA (1.76 mmL, 16.20 mmol) were used. **Appearance**: yellowish oil. **Yield**: 1.21 g, 71%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.92 (s, 1H), 6.91 (q, J = 4.6 Hz, 1H), 6.56 (s, 2H), 6.32 (dd, J = 6.3, 4.8 Hz, 1H), 5.98 (d, J = 4.7 Hz, 1H), 5.69 (t, J = 6.1 Hz, 1H), 4.38 (dd, J = 12.0, 3.5 Hz, 1H), 4.26 – 4.21 (m, 1H), 4.16 (dd, J = 12.0, 5.7 Hz, 1H), 2.90 (d, J = 4.6 Hz, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 1.94 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.96, 169.96, 169.38, 152.58, 151.95, 149.46, 148.84, 117.44, 84.62, 78.70, 70.57, 69.71, 62.67, 29.12, 21.03, 20.36, 20.25. **LC-MS** (*m/z*): 423.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.4%.

2′,3′,5′-Tri-*O*-acetyl-8-(butylthio)adenosine (45b, Bcy-145)

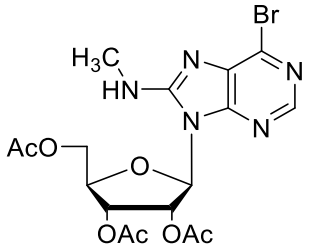


Compound **27a** (700 mg, 1.97 mmol), MeCN (10 mL), acetic anhydride (0.56 mL, 5.91 mmol), DMAP (24 mg, 0.20 mmol) and DMEA (0.85 mL, 7.88 mmol) were used. **Appearance**: yellowish oil. **Yield**: 717 mg, 76%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.11 (s, 1H), 7.25 (s, 2H), 6.24 (dd, J = 6.2, 4.9 Hz, 1H), 6.02 (d, J = 4.9 Hz, 1H), 5.69 (t, J = 6.0 Hz, 1H), 4.41 (dd, J = 12.1, 3.5 Hz, 1H), 4.38 – 4.33 (m, 1H), 4.18 (dd, J = 12.1, 5.5 Hz, 1H), 3.28 – 3.24 (m, 2H), 2.04 (s, 3H), 1.96 (s, 3H), 1.91 (s, 3H), 1.67 (p, J = 7.3 Hz, 2H), 1.46 – 1.36 (m, 2H), 0.90 (t, J = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.95, 169.92, 169.30, 154.45, 152.00, 150.69, 147.90, 119.12, 86.16, 79.12, 70.81, 69.67, 62.38, 32.31, 30.84, 21.14, 20.39, 20.31, 20.17, 13.38. **LC-MS** (*m/z*): 482.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.0%.

General procedure for the synthesis of 6-bromo-2′,3′,5′-tri-*O*-acetyl nucleosides (46a-b)

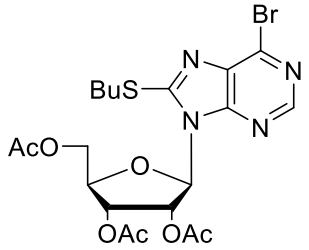
To a solution of appropriate 2′,3′,5′-tri-*O*-acetyl nucleoside **45a** or **45b** (1 eq.) in CH_2Br_2 (5 mL), $SbBr_3$ (1 eq.), BTEA-Br (1.5 eq.), $NaNO_2$ (20 eq.), DCA (1.5 eq.) and AcOH (0.5 eq.) were added. The mixture was stirred at rt overnight under argon and monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, celite (3 g) and $CHCl_3$ (10 mL) were added, and the suspension was stirred for 10 min. The mixture was filtered, and the filter cake was washed with $CHCl_3$ (120 mL). The filtrate was evaporated *in vacuum* and the crude compound was purified by silica gel column chromatography using 1% MeOH in DCM.

(2*R*,3*R*,4*R*,5*R*)-2-(Acetoxymethyl)-5-(6-bromo-8-(methylamino)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate (46a, Bcy-60)



Compound **45a** (1.50 g, 3.55 mmol), CH_2Br_2 (5 mL), $SbBr_3$ (1.28 g, 3.55 mmol), BTEA-Br (1.45 g, 5.33 mmol), $NaNO_2$ (4.90 g, 71.00 mmol), DCA (0.44 mL, 5.33 mmol) and AcOH (0.10 mL, 1.78 mmol) were used. **Appearance**: yellow solid; **mp**: 53.8-55.8 °C. **Yield**: 0.82 g, 47%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.86 (s, 1H), 6.23 (d, J = 3.6 Hz, 1H), 6.12 (dd, J = 6.3, 3.5 Hz, 1H), 5.79 (t, J = 6.7 Hz, 1H), 5.75 (s, 1H), 4.42 (dd, J = 12.1, 3.3 Hz, 1H), 4.37 – 4.32 (m, 1H), 4.26 (dd, J = 12.0, 5.8 Hz, 1H), 3.55 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 1.95 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.95, 169.25, 151.78, 150.76, 149.82, 132.08, 88.80, 79.00, 72.10, 69.18, 62.28, 40.06, 32.72, 20.37, 20.25, 20.17. **LC-MS** (*m/z*): 485.0 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.4%.

(2*R*,3*R*,4*R*,5*R*)-2-(Acetoxymethyl)-5-(6-bromo-8-(butylthio)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate (46b, Bcy-91)



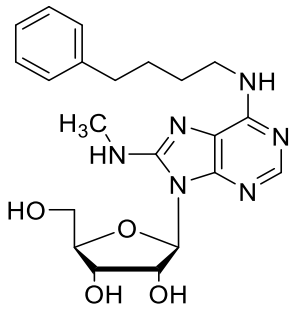
Compound **45b** (717 mg, 1.49 mmol), CH_2Br_2 (5 mL), $SbBr_3$ (539 g, 1.49 mmol), BTEA-Br (610 mg, 2.24 mmol), $NaNO_2$ (2.06 g, 29.80 mmol), DCA (0.19 mL, 2.24 mmol) and AcOH (0.04 mL, 0.75 mmol) were used. **Appearance**: yellow oil. **Yield**: 413 mg, 51%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.66 (s, 1H), 6.15 (dd, J = 6.3, 4.7 Hz, 1H), 6.06 (d, J = 4.7 Hz, 1H), 5.68 (t, J = 6.1 Hz, 1H), 4.46 – 4.38 (m, 2H), 4.24 – 4.18 (m, 1H), 3.50 – 3.39 (m, 2H), 2.12 (s, 3H), 2.04 (s, 3H), 1.94 (s, 3H), 1.80 – 1.73 (m, 2H), 1.50 – 1.41 (m, 2H), 0.93 (t, J = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.88, 169.38, 169.29, 157.11, 151.89, 150.57, 138.44, 134.05, 86.48, 79.38, 70.93,

69.37, 62.23, 31.64, 30.42, 21.10, 20.36, 20.29, 20.16, 13.35. **LC-MS** (*m/z*): 544.9 [M - H]$^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 92.9%.

General procedure for the synthesis of 8-substituted N^6-(4-phenylbutyl)adenosine derivatives (47a-b)

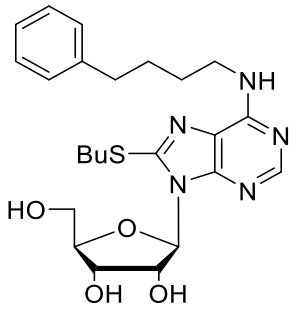
To a solution of appropriate acetyl-bromo-intermediate **46a** or **46b** (1 eq.) in absolute EtOH (10 mL), 4-phenylbutylamine (2 eq.) and Et_3N (2 eq.) were added. The mixture was refluxed for 3 h and monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, cooled to rt and the solvent was evaporated *in vacuum*. The residue was redissolved in MeOH (10 mL) and 20% NaOMe was added. The mixture was stirred at rt for 48 h and monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, 5 g silica gel was added, and the solvent was evaporated *in vacuum*. The crude mixture was purified by silica gel column chromatography using 4% MeOH in DCM.

8-Methylamino-N^6-(4-phenylbutyl)adenosine (47a, Bcy-93)



Compound **46a** (810 mg, 1.67 mmol), EtOH (10 mL) and 4-phenylbutylamine (0.53 mL, 3.34 mmol) were used. **Appearance**: brown viscous semi-solid. **Yield**: 405 mg, 57%. **LC-MS** (*m/z*): 429.1 [M + H]$^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 77.7%.

8-Butylthio-N^6-(4-phenylbutyl)adenosine (47b, Bcy-92)

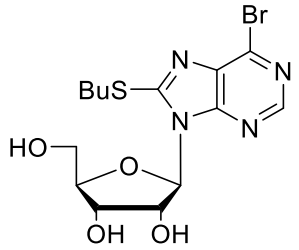


Compound **46b** (410 mg, 0.75 mmol), EtOH (10 mL) and 4-phenylbutylamine (0.24 mL, 1.50 mmol) were used. **Appearance**: yellowish solid; **mp**: 62.0-64.0 °C. **Yield**: 321 mg, 88%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.74 (s, 1H), 7.36 – 7.07 (m, 5H), 5.86 – 5.69 (m, 1H), 5.63 (dd, J = 8.9, 3.7 Hz, 1H), 5.37 (d, J = 6.4 Hz, 1H), 5.16 (d, J = 4.3 Hz, 1H), 5.06 – 4.90 (m, 1H), 4.21 – 4.11 (m, 1H), 4.04 – 3.78 (m, 2H), 3.74 – 3.42 (m, 4H), 3.30 – 3.19 (m, 2H), 2.68 – 2.57 (m, 2H), 1.75 – 1.54 (m, 6H), 1.41 (h, J = 7.3 Hz, 2H), 0.89 (t, J = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 153.16, 151.27, 148.27, 142.15, 128.26, 128.17, 125.59, 88.87, 86.61, 71.32, 71.00, 62.22, 34.87, 32.20, 30.90, 28.73, 28.41, 21.18, 13.41. **LC-MS** (*m/z*): 488.3 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.0%.

General procedure for the bromination of the N^6-position of 8-thio-substituted adenosine derivatives (46c-e)

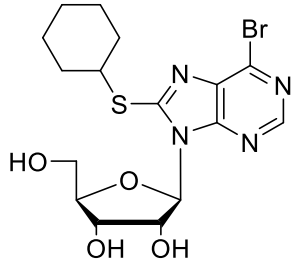
To a solution of 8-thio-substituted adenosine (1 eq.) in CH_2Br_2 (10 mL), TMSBr (5 eq.) and *tert*-butylnitrile (10 eq.) were added. The mixture was stirred overnight increasing the temperature from 0 °C to rt under argon and the reaction progress was monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, H_2O (20 mL) was added to quench the reaction. The mixture was extracted with EtOAc (100 mL × 2) and $CHCl_3$ (100 mL). The collected organic layers were dried over $MgSO_4$ and evaporated *in vacuum*. The crude compound was purified by silica gel column chromatography using 4% MeOH in DCM.

(2*R*,3*R*,4*S*,5*R*)-2-(6-Bromo-8-(butylthio)-9*H*-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (46c, Bcy-320)



Compound **27a** (700 mg, 1.41 mmol), CH_2Br_2 (10 mL), TMSBr (1.30 mL, 7.05 mmol) and *tert*-butylnitrile (2.35 mL, 14.10 mmol, 10 eq.) were used. **Appearance**: yellowish solid; **mp**: 63.0-65.0 °C. **Yield**: 540 mg, 91%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.61 (s, 1H), 5.79 (d, J = 6.1 Hz, 1H), 5.45 (d, J = 6.0 Hz, 1H), 5.24 (d, J = 4.8 Hz, 1H), 5.07 (q, J = 5.7 Hz, 1H), 4.86 (s, 1H), 4.23 (d, J = 4.5 Hz, 1H), 3.99 – 3.91 (m, 1H), 3.72 – 3.64 (m, 1H), 3.59 – 3.52 (m, 1H), 3.50 – 3.38 (m, 2H), 1.77 (p, J = 7.2 Hz, 2H), 1.46 (h, J = 7.4 Hz, 2H), 0.94 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.87, 152.11, 150.18, 138.27, 134.23, 89.01, 86.17, 70.75, 70.31, 61.65, 31.41, 30.46, 21.13, 13.34. **LC-MS** (*m/z*): 421.10 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.5%.

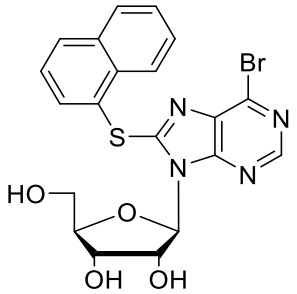
(2*R*,3*R*,4*S*,5*R*)-2-(6-Bromo-8-(cyclohexylthio)-9*H*-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (46d, Bcy-366)



Compound **27j** (163 mg, 0.42 mmol), CH_2Br_2 (10 mL), TMSBr (0.50 mL, 3.78 mmol, 9 eq.) and *tert*-butylnitrile (1.00 mL, 8.40 mmol, 20 eq.) were used. **Appearance**: white solid; **mp**: 77-79 °C; **Yield**: 70 mg, 30%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.61 (s, 1H), 5.78 (d, J = 6.2 Hz, 1H), 5.44 (d, J = 6.0 Hz, 1H), 5.23 (d, J = 5.0 Hz, 1H), 5.09 (q, J = 5.9 Hz, 1H), 4.85 (dd, J = 6.7, 5.2 Hz, 1H), 4.26 – 4.21 (m, 1H), 4.14 – 4.06 (m, 1H), 3.97 – 3.90 (m, 1H), 3.74 – 3.64 (m, 1H), 3.58 – 3.49 (m, 1H), 2.22 – 2.10 (m, 2H), 1.78 – 1.55 (m, 5H), 1.55 – 1.42 (m, 2H), 1.41 – 1.31 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.08, 151.81, 150.22, 138.42, 134.33, 89.06, 86.14, 70.72, 70.32,

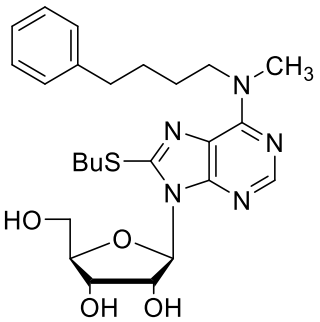
61.65, 45.93, 32.39, 32.26, 25.03, 24.97. **LC-MS** (*m/z*): 445.10 [M - H]$^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.3%.

(2*R*,3*R*,4*S*,5*R*)-2-(6-Bromo-8-(naphthalen-1-ylthio)-9*H*-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (46e, Bcy-369)



Compound **27r** (224 mg, 0.53 mmol), CH_2Br_2 (10 mL), TMSBr (0.70 mL, 5.30 mmol, 10 eq.) and *tert*-butylnitrile (0.63 mL, 5.30 mmol, 10 eq.) were used. **Appearance**: yellowish oil. **Yield**: 140 mg, 54%. **LC-MS** (*m/z*): 489.10 [M - H]$^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 83.1%.

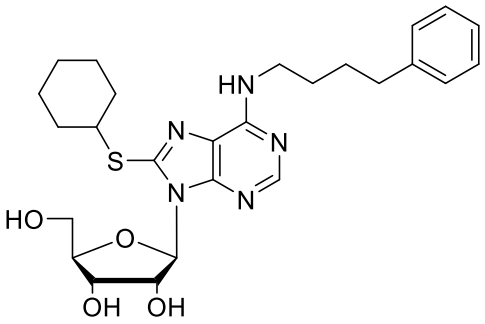
8-Butylthio-N^6-methyl-N^6-(4-phenylbutyl)adenosine (47c, Bcy-341)



This compound was synthesized using the same procedure as for **37a**. Compound **46c** (100 mg, 0.24 mmol), EtOH (10 mL), methyl(4-phenylbutyl)amine (0.07 mL, 0.36 mmol) and Et_3N (0.67 mL, 4.80 mmol) were used. The crude compound was purified by silica gel column chromatography using 4% MeOH in DCM. **Appearance**: yellowish semi-solid. **Yield**: 109 mg, 91%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.12 (s, 1H), 7.25 (dd, J = 8.2, 7.0 Hz, 2H), 7.15 (t, J = 7.4 Hz, 3H), 5.74 (d, J = 6.8 Hz, 1H), 5.56 (dd, J = 8.7, 3.7 Hz, 1H), 5.37 (d, J = 6.4 Hz, 1H), 5.17 (d, J = 4.4 Hz, 1H), 5.04 – 4.95 (m, 1H), 4.19 – 4.13 (m, 1H), 4.01 – 3.92 (m, 1H), 3.85 – 3.59 (m, 2H), 3.57 – 3.38 (m, 2H), (C*H*$_3$ is overlaid by H_2O), 3.29 – 3.22 (m, 2H), 2.62 (t, J = 7.5 Hz, 2H),

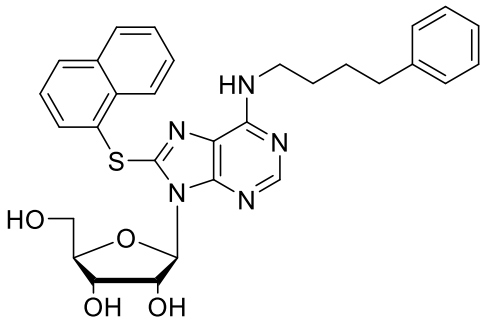
1.73 – 1.56 (m, 6H), 1.39 (h, *J* = 7.4 Hz, 2H), 0.87 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 152.32, 151.31, 150.52, 147.57, 142.02, 128.21, 128.16, 125.65, 119.80, 88.78, 86.54, 71.08, 70.92, 62.20, 34.98, 31.60, 30.97, 28.23, 21.22, 13.37. **LC-MS** (*m/z*): 502.30 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.8%.

8-Cyclohexylthio-*N*^{6}-(4-phenylbutyl)adenosine (47d, Bcy-376)



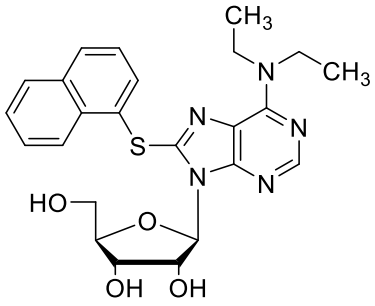
This compound was synthesized using the same procedure as for **37a**. Compound **46d** (65 mg, 0.15 mmol), EtOH (10 mL), 4-phenylbutylamine (0.04 mL, 0.23 mmol) and Et_3N (0.42 mL, 3.00 mmol) were used. The crude compound was purified by silica gel column chromatography using 4% MeOH in DCM. **Appearance**: brownish solid; **mp**: 58-60 ℃. **Yield**: 69 mg, 90%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.22 – 7.95 (m, 1H), 7.82 (s, 1H), 7.25 (t, *J* = 7.5 Hz, 2H), 7.21 – 7.13 (m, 3H), 5.86 (s, 1H), 5.63 (dd, *J* = 9.1, 3.6 Hz, 1H), 5.33 (d, *J* = 6.4 Hz, 1H), 5.14 (d, *J* = 4.3 Hz, 1H), 5.00 (q, *J* = 6.5 Hz, 1H), 4.21 – 4.14 (m, 1H), 3.96 (s, 1H), 3.81 – 3.71 (m, 1H), 3.72 – 3.63 (m, 1H), 3.59 – 3.42 (m, 2H), 2.60 (d, *J* = 7.0 Hz, 3H), 2.02 (d, *J* = 29.2 Hz, 2H), 1.76 – 1.67 (m, 2H), 1.64 – 1.60 (m, 3H), 1.58 – 1.46 (m, 3H), 1.43 – 1.20 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.37, 151.49, 146.95, 142.12, 128.24, 128.14, 125.57, 88.96, 86.58, 71.30, 71.02, 62.23, 46.73, 34.84, 32.92, 32.54, 28.70, 28.38, 25.27, 25.16, 24.98. **LC-MS** (*m/z*): 514.50 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.8%.

8-(1-Naphthylthio)-*N*^{6}-(4-phenylbutyl)adenosine (47e, Bcy-377)



This compound was synthesized using the same procedure as for **37a**. Compound **46e** (116 mg, 0.24 mmol), EtOH (10 mL), 4-phenylbutylamine (0.06 mL, 0.36 mmol) and Et_3N (1.00 mL, 7.20 mmol) were used. **Appearance**: brownish solid; **mp**: 74-75 °C. **Yield**: 55 mg, 41%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.29 – 8.23 (m, 1H), 8.19 (s, 1H), 8.05 – 7.95 (m, 2H), 7.89 (s, 1H), 7.62 (s, 3H), 7.51 (t, J = 7.7 Hz, 1H), 7.23 (t, J = 7.5 Hz, 2H), 7.14 (t, J = 7.2 Hz, 3H), 6.17 (s, 1H), 5.61 (dd, J = 9.0, 3.6 Hz, 1H), 5.43 (d, J = 6.4 Hz, 1H), 5.18 (d, J = 4.2 Hz, 1H), 5.11 (q, J = 6.2 Hz, 1H), 4.26 – 4.18 (m, 1H), 4.00 (s, 1H), 3.77 – 3.66 (m, 1H), 3.61 – 3.50 (m, 1H), 3.42 (s, 2H), 2.55 (s, 2H), 1.53 (s, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.70, 152.31, 149.22, 144.55, 142.08, 133.78, 131.91, 131.19, 129.36, 128.72, 128.21, 128.11, 127.95, 127.37, 126.68, 126.13, 125.53, 124.22, 120.41, 89.57, 86.77, 71.47, 71.02, 62.18, (NH*C*H$_2$ is overlaid by DMSO-d_6), 34.75, 28.50, 28.34. **LC-MS** (*m/z*): 558.30 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 93.8%.

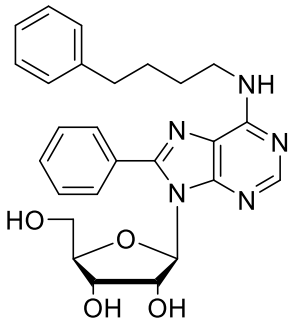
8-(1-Naphthylthio)-N^6,N^6-diethyladenosine (47f, Bcy-374)



This compound was synthesized using the same procedure as for **27a** (Method 2). Compound **42c** (310 mg, 0.77 mmol), EtOH (10 mL), NaOMe (499 mg, 9.24 mmol, 12 eq.) and 1-thionaphthol (1.29 mL, 9.24 mmol, 12 eq.) were used. The crude product was purified by silica gel column chromatography using 6% MeOH in DCM.

Appearance: white solid; **mp**: 93-95 °C. **Yield**: 328 mg, 88%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.36 – 8.26 (m, 1H), 8.13 (s, 1H), 8.08 – 7.97 (m, 2H), 7.83 – 7.77 (m, 1H), 7.64 – 7.50 (m, 3H), 6.09 (dd, J = 6.9, 1.4 Hz, 1H), 5.63 – 5.54 (m, 1H), 5.49 – 5.37 (m, 1H), 5.25 – 5.17 (m, 1H), 5.14 – 5.04 (m, 1H), 4.27 – 4.17 (m, 1H), 4.07 – 3.94 (m, 1H), 3.75 – 3.52 (m, 6H), 1.37 – 0.76 (m, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 151.87, 151.28, 151.03, 144.96, 133.85, 133.08, 132.98, 130.12, 128.52, 127.18, 126.65, 126.47, 125.90, 124.99, 119.68, 89.38, 86.73, 71.34, 70.99, 62.20, 55.99, 12.96. **LC-MS** (*m/z*): 482.20 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 92.7%.

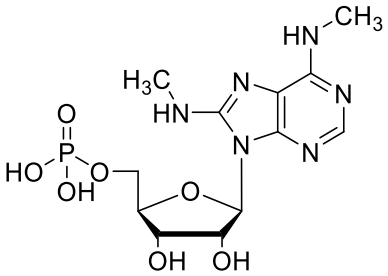
8-Phenyl-N^6-(4-phenylbutyl)adenosine (47g, Bcy-330)



To a solution of **37h** (400 mg, 1.00 mmol, 1 eq.) in dry DMF (10 mL), iodobenzene (0.22 mL, 2.00 mmol, 2 eq.), $Pd(OAc)_2$ (11 mg, 0.05 mmol, 0.05 eq.), CuI (571 mg, 3.00 mmol, 3 eq.) and Cs_2CO_3(815 mg, 2.50 mmol, 2.5 eq.) were added. The mixture was stirred in an autoclave at 120 °C under argon overnight. The reaction progress was monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, cooled to rt, 1 M HCl (aq., 10 mL) was added. The mixture was then neutralized with 2 M NaOH (aq.) and extracted with EtOAc (50 mL × 3). The collected organic layers were dried over $MgSO_4$ and evaporated *in vacuum*. The crude compound was purified by silica gel column chromatography using 4% MeOH in DCM. **Appearance**: brown solid; **mp**: 88.0-90.0 °C. **Yield**: 134 mg, 28%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.23 (s, 1H), 8.08 (s, 1H), 7.75 (dd, J = 6.8, 3.0 Hz, 2H), 7.61 – 7.56 (m, 3H), 7.32 – 7.09 (m, 5H), 5.77 (d, J = 7.0 Hz, 2H), 5.42 (s, 1H), 5.19 (t, J = 6.1 Hz, 1H), 5.10 (s, 1H), 4.18 (dd, J = 5.2, 2.0 Hz, 1H), 3.98 – 3.90 (m, 1H), 3.71 (dd, J = 12.2, 3.7 Hz, 1H), 3.64 – 3.44 (m, 3H), 2.67 – 2.56 (m, 2H), 1.63 (p, J = 3.6 Hz, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ

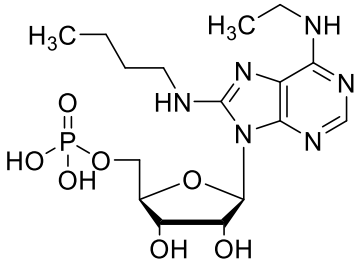
154.59, 151.93, 150.65, 148.95, 142.15, 130.03, 129.61, 129.39, 128.69, 128.25, 128.14, 125.55, 119.49, 89.11, 86.68, 71.27, 71.05, 62.28, ($NHCH_2$ is overlaid by DMSO-d_6), 34.83, 28.67, 28.39. **LC-MS** (*m/z*): 476.30 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.4%.

8-Methylamino-N^6-methyl-AMP (44a, Bcy-389)



Compound **43a** (81 mg, 0.26 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (84 mg, 0.39 mmol) and $POCl_3$ (0.10 mL, 1.04 mmol) were used. **Appearance**: white powder; **mp**: 144-146 °C. **Yield**: 14 mg, 14%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.05 (s, 1H), 6.59 (s, 1H), 5.80 (d, J = 6.1 Hz, 1H), 5.33 (s, 1H), 4.81 (t, J = 5.9 Hz, 1H), 4.21 (dd, J = 5.7, 3.9 Hz, 1H), 4.15 – 3.98 (m, 4H), 3.98 – 3.85 (m, 3H), 2.94 (d, J = 4.4 Hz, 3H), 2.92 (d, J = 2.9 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 157.88, 157.67, 152.16, 148.53, 116.19, 86.81, 82.77, 70.10, 70.02, 65.54, 29.29, 27.51. ^{31}P NMR (243 MHz, DMSO-d_6) δ -0.07. **LC-MS** (*m/z*): 391.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 94.0%.

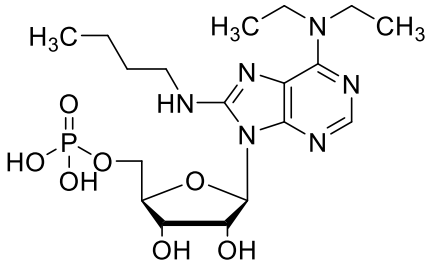
8-Butylamino-N^6-ethyl-AMP (44b, CS-401)



Compound **43b** (100 mg, 0.27 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (88 mg, 0.41 mmol) and $POCl_3$ (0.14 mL, 1.49 mmol) were used. **Appearance**: white powder; **mp**: 186 °C. **Yield**: 50 mg, 42%. ^{1}H NMR (600 MHz, D_2O) δ 7.48 (s, 1H), 5.99 (d, 1H, J =

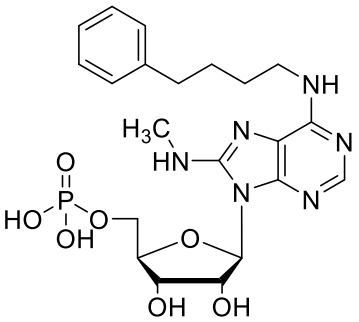
7.77 Hz), 4.70 (m, 1H), 4.44 (dd, 1H, *J* = 2.37, 5.78 Hz), 4.33 (br s, 1H), 4.15 (m, 2H), 3.43 (m, 4H), 1.64 (m, 2H), 1.37 (m, 2H), 1.26 (m, 3H), 0.91 (d, 3H, *J* = 7.36 Hz). ^{13}C NMR (126 MHz, D_2O) δ 154.72, 153.32, 151.47, 151.32, 150.65, 118.90, 89.09, 87.24, 87.18, 73.34, 72.99, 67.47, 45.26, 38.74, 33.52, 22.37, 16.62, 16.17. ^{31}P NMR (202 MHz, D_2O) δ 0.29. **LC-MS** (*m/z*): 447.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 94.8%. This compound was co-synthesized with Dr. Constanze Cerine Schmies.

8-Butylamino-*N*^{6},*N*^{6}-diethyl-AMP (44c, CS-382A)



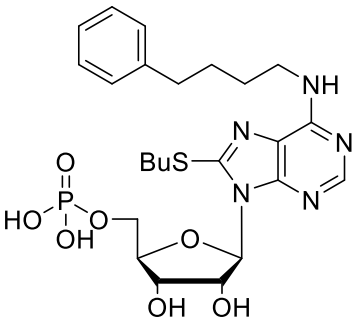
Compound **43c** (150 mg, 0.38 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (140 mg, 0.57 mmol) and $POCl_3$ (0.14 mL, 1.52 mmol) were used. **Appearance**: white powder; **mp**: 192 °C. **Yield**: 50 mg, 28%. ^{1}H NMR (600 MHz, D_2O) δ 7.99 (s, 1H), 6.03 (d, 1H, *J* = 7.79 Hz), 4.68 (m, 1H), 4.44 (dd, 1H, *J* = 2.31, 5.81 Hz), 4.34 (t, 1H, *J* = 2.38 Hz), 4.17 (d m, 2H), 3.83 (q, 4H, *J* = 7.28 Hz), 3.47 (d m, 2H), 1.64 (m, 2H), 1.33 (m, 2H), 1.19 (t, 6H, *J* = 6.99 Hz), 0.89 (t, 3H, *J* = 7.38 Hz). ^{13}C NMR (126 MHz, D_2O) δ 154.20, 152.19, 151.61, 149.41, 119.81, 89.00, 87.18, 87.13, 79.50, 73.01, 67.51, 67.48, 46.58, 45.15, 33.64, 22.32, 16.03, 15.65. ^{31}P NMR (202 MHz, D_2O) δ 0.48. **LC-MS** (*m/z*): 475.4 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.7%. This compound was co-synthesized with Dr. Constanze Cerine Schmies.

8-Methylamino-*N*^{6}-(4-phenylbutyl)-AMP (48a, PSB-20108, Bcy-108)



Compound **47a** (100 mg, 0.23 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (75 mg, 0.35 mmol) and $POCl_3$ (0.09 mL, 0.92 mmol) were used. **Appearance**: white powder; **mp**: 145.0-147.0 °C. **Yield**: 11 mg, 9%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.95 (s, 1H), 7.25 (t, J = 7.6 Hz, 2H), 7.20 – 7.17 (m, 2H), 7.17 – 7.13 (m, 1H), 6.84 (t, J = 6.1 Hz, 1H), 6.45 (d, J = 5.5 Hz, 1H), 5.89 (d, J = 7.4 Hz, 1H), 4.65 (dd, J = 7.4, 5.5 Hz, 1H), 4.20 (dd, J = 5.6, 2.5 Hz, 1H), 4.00 (p, J = 2.4 Hz, 2H), 3.96 – 3.80 (m, 5H), 3.51 (s, 2H), 2.96 (d, J = 3.0 Hz, 3H), 2.68 – 2.56 (m, 2H), 1.68 – 1.52 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 151.76, 151.37, 149.49, 148.65, 142.32, 128.29, 128.16, 125.53, 116.97, 85.90, 83.92, 70.59, 69.77, 64.44, 45.28, 34.92, 29.31, 28.50. ^{31}P NMR (243 MHz, DMSO-d_6) δ 0.82. **LC-MS** (*m/z*): 509.3 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.1%.

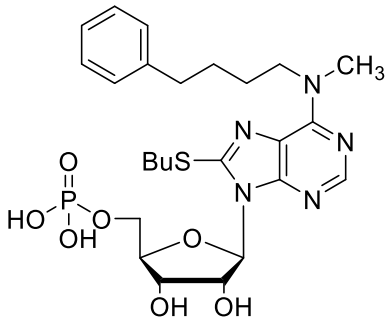
8-Butylthio-N^6-(4-phenylbutyl)-AMP (48b, PSB-20110, Bcy-110)



Compound **47b** (100 mg, 0.21 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (69 mg, 0.32 mmol) and $POCl_3$ (0.08 mL, 0.84 mmol) were used. **Appearance**: white powder; **mp**: 75.0-77.0 °C. **Yield**: 33 mg, 28%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.13 (q, J = 8.8, 7.6 Hz, 1H), 7.60 (t, J = 6.1 Hz, 1H), 7.25 (t, J = 7.5 Hz, 2H), 7.20 – 7.13 (m, 3H), 5.79 (d, J = 5.6 Hz, 1H), 5.42 (s, 1H), 5.13 (t, J = 5.7 Hz, 1H), 4.33 – 4.22 (m, 1H), 4.16 – 4.08 (m, 1H), 4.07 – 4.00 (m, 1H), 3.96 – 3.85 (m, 2H), 3.51 (s, 2H), 3.35 – 3.24 (m,

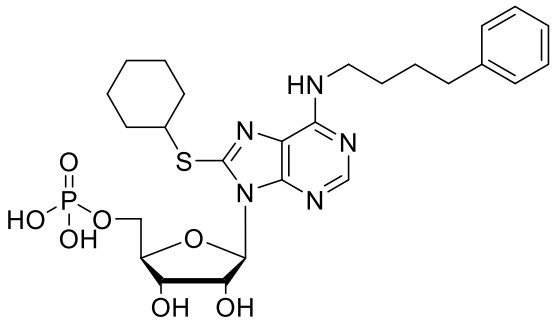
4H), 2.63 – 2.58 (m, 2H), 1.69 (p, *J* = 7.3 Hz, 2H), 1.65 – 1.58 (m, 4H), 1.41 (h, *J* = 7.5 Hz, 2H), 0.89 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.03, 151.60, 148.16, 142.15, 128.24, 128.14, 125.56, 88.62, 82.92, 70.34, 65.41, (NH*C*H$_2$ is overlaid by DMSO-d_6), 34.86, 32.00, 30.88, 28.40, 21.19, 13.39. ^{31}P NMR (202 MHz, DMSO-d_6) δ -0.04. **LC-MS** (*m/z*): 568.3 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.4%.

8-Butylthio-*N*^{6}-methyl-*N*^{6}-(4-phenylbutyl)-AMP (48c, Bcy-348)



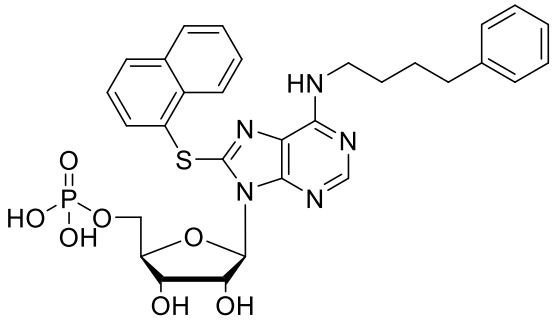
Compound **47c** (60 mg, 0.12 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (39 mg, 0.18 mmol) and $POCl_3$ (0.05 mL, 0.48 mmol) were used. **Appearance**: white powder; **mp**: 108-110 ℃. **Yield**: 7 mg, 10%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.29 – 7.23 (m, 2H), 7.16 (dd, *J* = 7.1, 1.8 Hz, 3H), 5.77 (d, *J* = 5.7 Hz, 1H), 5.40 (s, 1H), 5.09 (t, *J* = 5.6 Hz, 1H), 4.26 (dd, *J* = 5.5, 4.0 Hz, 1H), 4.17 – 4.09 (m, 2H), 4.05 – 3.98 (m, 2H), 3.90 (dt, *J* = 10.8, 6.8 Hz, 2H), 3.44 (s, 7H), 2.62 (t, *J* = 7.5 Hz, 2H), 1.74 – 1.57 (m, 6H), 1.39 (q, *J* = 7.4 Hz, 2H), 0.87 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 152.15, 151.87, 150.86, 147.45, 142.04, 128.21, 128.17, 125.65, 119.52, 88.58, 82.95, 82.90, 70.30, 65.47, 65.44, 39.94, 39.80, 34.99, 31.54, 30.98, 28.25, 21.25, 13.38. ^{31}P NMR (243 MHz, DMSO-d_6) δ -0.12. **LC-MS** (*m/z*): 582.50 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.7%.

8-Cyclohexylthio-*N*^{6}-(4-phenylbutyl)-AMP (48d, Bcy-378)



Compound **47d** (60 mg, 0.12 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (39 mg, 0.18 mmol) and $POCl_3$ (0.04 mL, 0.48 mmol) were used. **Appearance**: white powder; **mp**: 185-187 °C. **Yield**: 13 mg, 18%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.69 (s, 1H), 7.25 (t, J = 7.5 Hz, 2H), 7.21 – 7.14 (m, 2H), 5.85 (s, 1H), 5.40 (s, 1H), 5.14 (t, J = 5.6 Hz, 1H), 4.29 (t, J = 4.7 Hz, 1H), 4.07 – 3.98 (m, 1H), 3.95 – 3.86 (m, 1H), 3.82 – 3.71 (m, 2H), 3.50 (s, 5H), 3.18 (s, 1H), 2.61 (s, 2H), 2.03 (s, 2H), 1.77 – 1.16 (m, 12H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.21, 151.86, 146.99, 142.15, 128.24, 128.15, 125.57, 88.80, 82.95, 70.38, 65.48, 46.65, 34.86, 32.81, 32.65, 28.41, 25.25, 24.98. ^{31}P NMR (202 MHz, DMSO-d_6) δ -0.09. **LC-MS** (*m/z*): 594.40 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.3%.

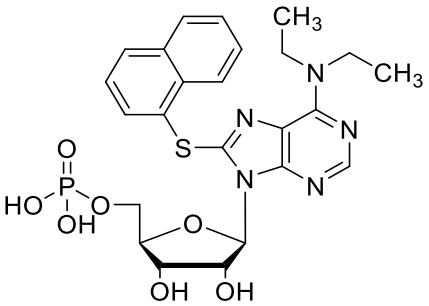
8-(1-Naphthylthio)-N^6-(4-phenylbutyl)-AMP (48e, Bcy-379)



Compound **47e** (50 mg, 0.09 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (30 mg, 0.14 mmol) and $POCl_3$ (0.03 mL, 0.36 mmol) were used. **Appearance**: white powder; **mp**: 82-84 °C. **Yield**: 3 mg, 5%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.27 (d, J = 8.2 Hz, 1H), 8.21 (s, 1H), 8.01 (dd, J = 13.1, 7.7 Hz, 2H), 7.82 (s, 1H), 7.62 (t, J = 8.8 Hz, 3H), 7.51 (t, J = 7.7 Hz, 1H), 7.23 (t, J = 7.5 Hz, 2H), 7.20 – 7.11 (m, 3H), 6.25 – 6.08 (m, 1H), 5.42 (s, 2H), 5.25 (t, J = 5.7 Hz, 1H), 4.34 (t, J = 4.6 Hz, 1H), 4.21 – 4.12 (m, 1H), 4.06 (q, J = 5.6 Hz, 1H), 4.00 – 3.93 (m, 1H), 3.41 (s, 4H), 2.55 (s, 2H), 1.53 (s, 4H). ^{13}C

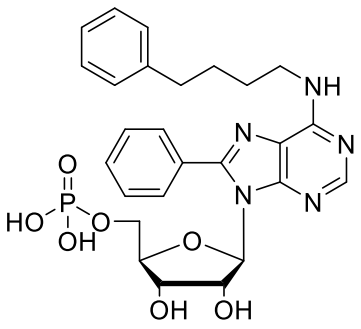
NMR (151 MHz, DMSO-d_6) δ 159.50, 153.43, 152.56, 144.63, 142.12, 133.80, 131.38, 129.46, 128.74, 128.25, 128.13, 127.43, 126.70, 126.17, 125.55, 124.30, 89.38, 83.14, 70.61, 70.45, 65.45, 40.06, 34.78, 28.52, 28.39. ^{31}P NMR (243 MHz, DMSO-d_6) δ -0.10. **LC-MS** (*m/z*): 638.20 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 92.3%.

8-(1-Naphthylthio)-N^6,N^6-diethyl-AMP (48f, Bcy-375)



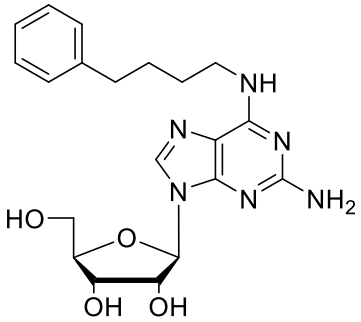
Compound **47f** (100 mg, 0.21 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (69 mg, 0.32 mmol) and $POCl_3$ (0.08 mL, 0.84 mmol) were used. **Appearance**: white powder; **mp**: 120-122 ℃. **Yield**: 11 mg, 9%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.30 (dd, *J* = 7.6, 1.8 Hz, 1H), 8.15 (s, 1H), 8.06 (d, *J* = 8.3 Hz, 1H), 8.03 – 8.00 (m, 1H), 7.82 (dd, *J* = 7.2, 1.2 Hz, 1H), 7.62 – 7.58 (m, 2H), 7.55 (dd, *J* = 8.3, 7.2 Hz, 1H), 6.10 (d, *J* = 5.8 Hz, 1H), 5.19 (t, *J* = 5.6 Hz, 2H), 4.34 (dd, *J* = 5.5, 3.9 Hz, 2H), 4.24 – 4.13 (m, 2H), 4.12 – 4.07 (m, 1H), 4.03 – 3.92 (m, 2H), 3.56 (q, *J* = 6.7, 6.2 Hz, 4H), 1.22 – 0.66 (m, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.51, 151.73, 151.66, 144.87, 133.86, 133.22, 133.05, 130.19, 128.54, 127.23, 126.60, 126.49, 125.93, 125.07, 119.40, 89.04, 83.17, 70.59, 70.40, 65.46, 40.06, 12.87. ^{31}P NMR (243 MHz, DMSO-d_6) δ -0.07. **LC-MS** (*m/z*): 562.20 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.3%.

8-Phenyl-N^6-(4-phenylbutyl)-AMP (48g, Bcy-355)



Compound **47g** (60 mg, 0.13 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (43 mg, 0.20 mmol) and $POCl_3$ (0.05 mL, 0.52 mmol) were used. **Appearance**: white powder; **mp**: 130-132 °C. **Yield**: 10 mg, 14%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.28 (d, J = 3.4 Hz, 1H), 7.79 – 7.70 (m, 2H), 7.65 – 7.57 (m, 3H), 7.25 (q, J = 7.8 Hz, 2H), 7.19 (d, J = 7.5 Hz, 2H), 7.17 – 7.13 (m, 1H), 5.74 (d, J = 5.8 Hz, 1H), 5.28 (t, J = 5.6 Hz, 4H), 4.29 (dd, J = 5.4, 3.6 Hz, 1H), 4.24 – 4.10 (m, 2H), 4.06 – 3.91 (m, 3H), 3.52 (s, 2H), 2.61 (q, J = 5.3, 3.5 Hz, 2H), 1.70 – 1.57 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 157.93, 153.74, 150.94, 149.32, 142.16, 130.20, 129.45, 129.30, 128.82, 128.29, 128.17, 125.59, 119.34, 89.38, 83.14, 70.39, 70.30, 65.43, 40.06, 34.84, 28.59, 28.40. ^{31}P NMR (243 MHz, DMSO-d_6) δ -0.10. **LC-MS** (*m/z*): 556.50 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.3%.

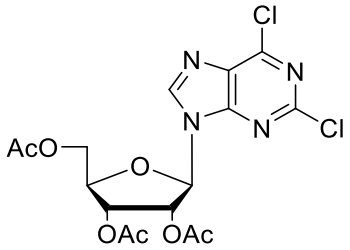
N^6-(4-Phenylbutyl)-2-aminoadenosine (49a, Bcy-187)



This compound was synthesized using the same procedure as for **37a**. 2-Amino-6-chloropurine riboside (300 mg, 0.99 mmol), EtOH (10 mL), 4-phenylbutylamine (0.24 mL, 1.49 mmol) and Et_3N (0.27 mL, 1.98 mmol) were used. The crude compound was purified by silica gel column chromatography using 6% MeOH in DCM. **Appearance**: yellow solid; **mp**: 72.0-74.0 °C. **Yield**: 305 mg, 74%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.89 (s, 1H), 7.45 – 7.02 (m, 6H), 5.72 (d, J = 6.3 Hz, 3H), 5.44 (t, J = 5.5 Hz, 1H), 5.33 (d, J = 6.2 Hz, 1H), 5.07 (d, J = 4.5 Hz, 1H), 4.51 (q, J = 5.9 Hz, 1H), 4.18 – 4.03 (m, 1H), 3.90 (q, J = 3.5 Hz, 1H), 3.64 (dt, J = 12.0, 3.9 Hz, 1H), 3.58 – 3.49 (m, 1H), 3.49 – 3.36 (m, 2H), 2.60 (t, J = 7.1 Hz, 2H), 1.59 (dhept, J = 6.2, 4.0, 3.4 Hz, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.45, 155.50, 151.05, 142.76, 136.33, 128.78, 128.67,

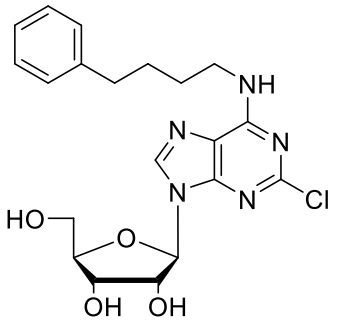
126.06, 114.23, 87.52, 85.99, 73.69, 71.19, 62.23, 35.40, 31.16, 29.44, 28.95. **LC-MS** (*m/z*): 415.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.5%.

2,6-Dichloro-9-(2,3,5-tri-*O*-acetyl-*β*-*D*-ribofuranosyl)-9*H*-purine (26e, Bcy-100), CAS: 3056-18-6



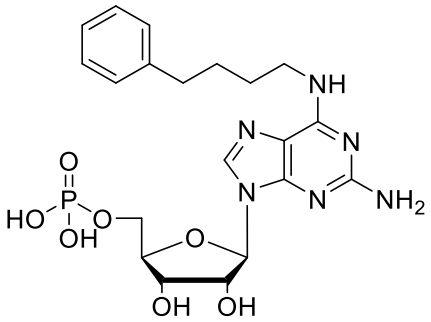
Tetraacetylribose (3.37 g, 10.58 mmol, 1 eq.) was melted at 110 ℃, then 2,6-dichloropurine (2.00 g, 10.58 mmol, 1 eq.) and triflic acid (0.05 mL, 0.53 mmol, 0.05 eq.) were added. The mixture was stirred at 110 ℃ and 0.09 MPa for removing the CH_3CO_2H which was produced during the reaction. The reaction was monitored by TLC (MeOH/DCM, 1:9). After 1 h, the reaction was completed, cooled to rt, MeOH (10 mL) and 5 g silica gel were added, and the mixture was concentrated *in vacuum*. The crude mixture was purified by silica gel column chromatography using 1.5% MeOH in DCM. **Appearance**: brown semi-solid. **Yield**: 2.44 g, 52%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.91 (s, 1H), 6.32 (d, J = 5.0 Hz, 1H), 5.90 (t, J = 5.4 Hz, 1H), 5.62 (t, J = 5.5 Hz, 1H), 4.46 – 4.37 (m, 2H), 4.30 (dd, J = 12.2, 5.5 Hz, 1H), 2.12 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.93, 169.28, 169.14, 152.74, 151.28, 150.23, 146.79, 131.20, 86.19, 79.82, 72.32, 69.73, 62.57, 20.41, 20.29, 20.15. **LC-MS** (*m/z*): 447.0 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 90.0%.

***N*^{6}-(4-Phenylbutyl)-2-chloroadenosine (49b, Bcy-117)**



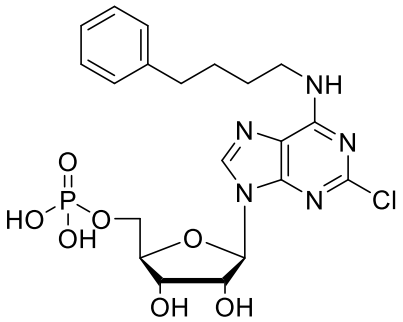
To a solution of **26e** (2.00 g, 4.47 mmol, 1 eq.) in EtOH (10 mL), 4-phenylbutylamine (1.41 mL, 8.94 mmol, 2 eq.) and Et_3N (1.24 mL, 8.94 mmol, 2 eq.) were added. The mixture was refluxed overnight and monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, cooled to rt and the solvent was evaporated *in vacuum*. The crude compound was purified by silica gel column chromatography using 3% MeOH in DCM. **Appearance**: brown viscous semi-solid. **Yield**: 0.74 g, 38%. **LC-MS** (*m/z*): 434.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.2%.

N^6-(4-Phenylbutyl)-2-amino-AMP (50a, Bcy-220)



Compound **49a** (100 mg, 0.24 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (77 mg, 0.36 mmol) and $POCl_3$ (0.09 mL, 0.96 mmol) were used. **Appearance**: white powder; **mp**: 117.0-119.0 °C. **Yield**: 37 mg, 26%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.98 (s, 1H), 7.34 – 7.08 (m, 6H), 5.83 (s, 2H), 5.74 (d, J = 6.2 Hz, 1H), 4.55 (t, J = 5.6 Hz, 1H), 4.20 (dd, J = 4.9, 3.1 Hz, 2H), 3.97 (q, J = 3.7 Hz, 2H), 3.90 – 3.74 (m, 3H), 3.51 (s, 1H), 3.42 (d, J = 13.6 Hz, 2H), 2.60 (t, J = 7.1 Hz, 2H), 1.68 – 1.50 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.22, 154.89, 142.28, 135.42, 128.29, 128.16, 125.55, 85.94, 83.80, 73.55, 71.05, 64.10, 45.32, 34.92, 29.02, 28.48. ^{31}P NMR (243 MHz, DMSO-d_6) δ 1.09. **LC-MS** (*m/z*): 495.3 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.8%.

N^6-(4-Phenylbutyl)-2-chloro-AMP (50b, Bcy-224)

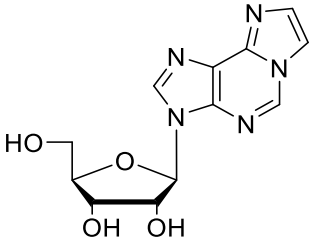


Compound **49b** (100 mg, 0.23 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (75 mg, 0.35 mmol) and $POCl_3$ (0.09 mL, 0.92 mmol) were used. **Appearance**: white powder; **mp**: 159.5-161.0 ℃. **Yield**: 39 mg, 33%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.50 (s, 1H), 7.31 – 7.10 (m, 5H), 5.82 (d, J = 6.2 Hz, 2H), 4.78 – 4.52 (m, 3H), 4.21 (dd, J = 4.8, 2.7 Hz, 2H), 4.05 (q, J = 3.4 Hz, 1H), 3.95 – 3.74 (m, 3H), 3.46 (s, 2H), 2.60 (s, 2H), 1.61 (q, J = 4.6, 3.2 Hz, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.99, 153.31, 149.88, 142.11, 139.41, 128.27, 128.14, 125.55, 117.96, 86.47, 84.64, 74.25, 71.19, 63.96, 45.59, 34.72, 28.35, 28.28. ^{31}P NMR (202 MHz, DMSO-d_6) δ 1.23. **LC-MS** (*m/z*): 514.4 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.6%.

General procedure for the synthesis of 1,N^6-ethenoadenosine derivatives (52a-b)

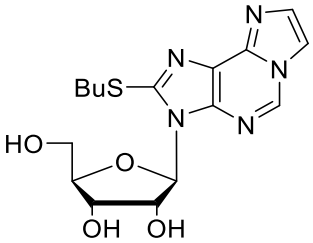
To a solution of appropriate adenosine derivative in 2 M aqueous chloroacetaldehyde (1 eq.), CH_3CO_2Na (5 eq.) was added. The mixture was stirred at 50 ℃ for 5 h and the reaction progress was monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, the solvent was evaporated *in vacuum.* Then the crude product was suspended in MeOH (10 mL), 5 g silica gel was added, and the mixture was evaporated *in vacuum*. Finally, the mixture was purified by silica gel column chromatography.

1,N^6-Ethenoadenosine (52a, Bcy-305), CAS: 39007-51-7



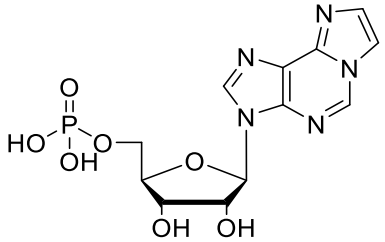
Adenosine (600 mg, 2.25 mmol), 2 M aqueous chloroacetaldehyde (15 mL) and CH_3CO_2Na (923 mg, 11.25 mmol) were used. The crude compound was purified by silica gel column chromatography using 12% MeOH in DCM. **Appearance**: brown solid. **Yield**: 605 mg, 92%. **LC-MS** (*m/z*): 292.20 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 78.4%.

8-Butylthio-1,N^6-ethenoadenosine (52b, Bcy-329)



Compound **27a** (100 mg, 0.28 mmol), 2 M aqueous chloroacetaldehyde (10 mL) and CH_3CO_2Na (115 mg, 1.40 mmol) were used. The crude compound was purified by silica gel column chromatography using 4% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 180.0-182.0 °C. **Yield**: 122 mg, >100%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.24 (s, 1H), 8.06 (s, 1H), 7.58 (s, 1H), 5.90 (d, J = 6.3 Hz, 1H), 5.42 (d, J = 6.2 Hz, 1H), 5.25 – 5.17 (m, 1H), 5.05 (q, J = 5.6 Hz, 1H), 4.88 (s, 1H), 4.23 (s, 1H), 3.94 (d, J = 4.1 Hz, 1H), 3.70 (dd, J = 11.9, 5.1 Hz, 1H), 3.56 (d, J = 11.7 Hz, 1H), 3.36 (dd, J = 11.5, 7.1 Hz, 2H), 1.73 (p, J = 7.2 Hz, 2H), 1.45 (h, J = 7.4 Hz, 2H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 149.47, 139.58, 139.41, 135.60, 132.82, 123.52, 112.00, 88.99, 85.88, 71.18, 70.46, 61.87, 31.92, 30.95, 21.20, 13.40. **LC-MS** (*m/z*): 380.20 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.9%.

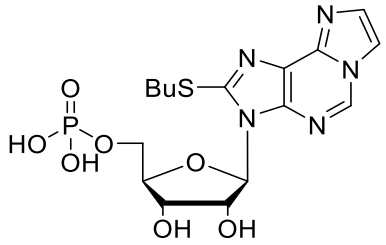
1,N^6-Etheno-AMP (53a, Bcy-326), CAS: 37482-16-9



Compound **52a** (60 mg, 0.21 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (69 mg, 0.32 mmol) and $POCl_3$ (0.08 mL, 0.84 mmol) were used. **Appearance**: white powder; **mp**:

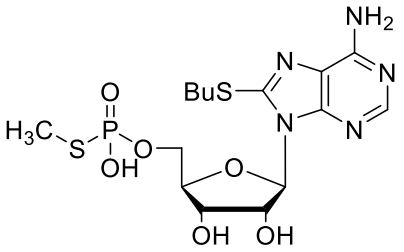
96.0-99.0 °C (*lit.*[187] 194-198 °C). **Yield**: 12 mg, 15%. ^{1}H NMR (600 MHz, D_2O) δ 9.43 (s, 1H), 8.84 (s, 1H), 8.30 (d, J = 2.3 Hz, 1H), 7.95 (d, J = 2.2 Hz, 1H), 6.37 (d, J = 5.1 Hz, 1H), 4.88 (t, J = 5.1 Hz, 1H), 4.79 – 4.73 (m, 4H), 4.57 (t, J = 4.6 Hz, 1H), 4.43 (p, J = 3.2 Hz, 1H), 4.24 – 4.12 (m, 2H). ^{13}C NMR (151 MHz, D_2O) δ 146.10, 145.69, 140.44, 139.59, 124.56, 121.29, 116.95, 91.21, 86.99, 77.37, 72.95, 66.94. ^{31}P NMR (243 MHz, D_2O) δ 0.46. **LC-MS** (*m/z*): 372.20 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.6%.

8-Butylthio-1,N^6-etheno-AMP (53b, Bcy-335)



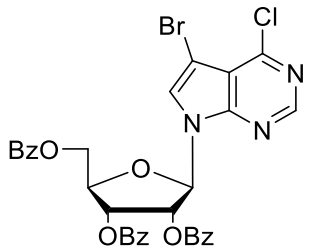
Compound **52b** (60 mg, 0.16 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (51 mg, 0.24 mmol) and $POCl_3$ (0.06 mL, 0.64 mmol) were used. **Appearance**: brownish powder; **mp**: 109.0-111.0 °C. **Yield**: 22 mg, 30%. ^{1}H NMR (600 MHz, D_2O) δ 9.32 (d, J = 1.0 Hz, 1H), 8.25 (d, J = 2.2 Hz, 1H), 7.92 (d, J = 2.1 Hz, 1H), 6.26 – 6.19 (m, 1H), 5.32 (t, J = 5.6 Hz, 1H), 4.84 – 4.79 (m, 2H), 4.79 – 4.74 (m, 2H), 4.69 (dd, J = 5.6, 4.4 Hz, 1H), 4.33 (q, J = 4.8 Hz, 1H), 4.20 (t, J = 5.7 Hz, 2H), 3.48 – 3.32 (m, 2H), 1.77 (p, J = 7.4 Hz, 2H), 1.47 (h, J = 7.4 Hz, 2H), 0.91 (t, J = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, D_2O) δ 159.47, 147.39, 138.99, 138.25, 124.78, 122.06, 116.82, 92.06, 86.75, 74.50, 72.85, 67.44, 35.68, 33.55, 24.05, 15.64. ^{31}P NMR (243 MHz, D_2O) δ 0.39. **LC-MS** (*m/z*): 460.30 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.8%.

8-(Butylthio)adenosine-5′-*S*-methylthiophosphate (54, Bcy-260)



Compound **27a** (80 mg, 0.23 mmol, 1 eq.) was dissolved in $PO(OCH_3)_3$ (5 mL), and 2,6-dimethylpyridine (0.08 mL, 0.69 mmol, 3 eq.) was added. The mixture was cooled to 0 °C under argon, and $PSCl_3$ (0.07 mL, 0.69 mmol, 3 eq.) was added 5 min later. The mixture was stirred at 0 °C for 3 h and monitored by TLC (2-propanol: NH_4OH (25% in H_2O): H_2O, 6:3:1). After the reaction was completed, a cold 0.5 M aqueous TEAC buffer (10 mL) was poured into the mixture and stirred at 0 °C for several minutes. The solution was allowed to reach rt upon stirring and left standing for 1 h. The pH was subsequently adjusted by saturated aqueous NH_4HCO_3 till 8. 2,6-Dimethylpyridine and $PO(OCH_3)_3$ were extracted by *tert*-butylmethylether (1 L), and the aqueous solution was lyophilized. The crude mixture was finally purified by preparative HPLC. **Appearance**: white solid; **mp**: 163.5-165.5 °C. **Yield**: 36 mg, 34%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.07 (s, 1H), 7.09 (s, 2H), 6.29 (s, 2H), 5.77 (d, J = 6.0 Hz, 1H), 5.31 (s, 1H), 5.18 (t, J = 5.8 Hz, 1H), 4.23 (dd, J = 5.5, 3.1 Hz, 1H), 4.00 – 3.91 (m, 2H), 3.75 – 3.68 (m, 1H), 3.28 (s, 2H), 1.93 (d, J = 11.4 Hz, 3H), 1.69 (p, J = 7.2 Hz, 2H), 1.43 (q, J = 7.4 Hz, 2H), 0.91 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.26, 151.61, 151.14, 148.70, 119.29, 88.32, 83.64, 70.78, 70.14, 64.64, 31.90, 30.86, 21.20, 13.42, 12.76. ^{31}P NMR (202 MHz, DMSO-d_6) δ 15.05 (t, J = 13.0 Hz). **LC-MS** (*m/z*): 466.4 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.2%.

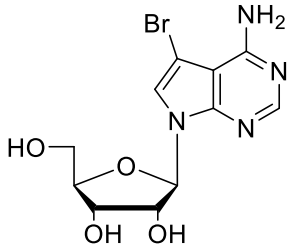
2′,3′,5′-Tri-*O*-benzoyl-6-chloro-7-bromo-9-*β*-*D*-ribofuranosyl-7-deazapurine (55a, Bcy-114), CAS: 952429-11-7



To a solution of 7-bromo-6-chloro-7-deazapurine (1.00 g, 4.30 mmol, 1 eq.) in anhydrous MeCN, BSA (1.37 mL, 5.59 mmol, 1.3 eq.) was added and stirred at rt for 10 min under argon till the mixture solution became clear. Then 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-*D*-ribofuranose (3.25 g, 6.45 mmol, 1.5 eq.) and TMSOTf (1.17 mL, 6.45 mmol, 1.5 eq.) were added and stirred at rt for 15 min under argon. Finally, the mixture

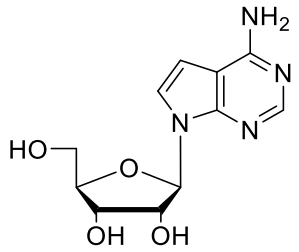
was stirred at 80 ℃ for 1 h under argon and the reaction progress was monitored by TLC (MeOH/DCM, 5:95). After the reaction was completed, cooled to rt, and 40 mL H_2O was poured on to quench the reaction. The mixture was extracted with EtOAc (40 mL × 3). The collected organic layers were extracted with saturated aqueous $NaHCO_3$ (40 mL) and brine solution (40 mL), dried over $MgSO_4$, and evaporated *in vacuum*. The crude compound was purified by silica gel column chromatography using 0.5% MeOH in DCM. **Appearance**: white solid. **Yield**: 1.81 g, 62%. **LC-MS** (*m/z*): 678.2 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 86.0%.

7-Bromo-7-deazaadenosine (55b, Bcy-116), CAS: 21193-80-6



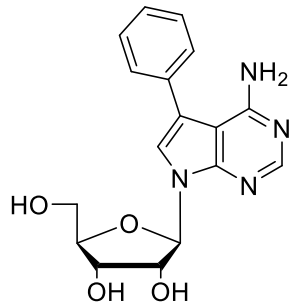
Compound **55a** (590 mg, 0.87 mmol, 1 eq.) in 20 mL ammonia solution (7 N in MeOH) was stirred in an autoclave at 120 ℃ overnight and monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, cooled to rt, and evaporated *in vacuum*. The crude compound was purified by silica gel column chromatography using 10% MeOH in DCM. **Appearance**: yellow solid; **mp**: 148.0-150.0 ℃ (*lit.*[166] 231-232 ℃). **Yield**: 251 mg, 84%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.10 (s, 1H), 7.65 (s, 1H), 6.78 (s, 2H), 6.05 (d, J = 6.1 Hz, 1H), 5.30 (d, J = 6.4 Hz, 1H), 5.20 – 5.03 (m, 2H), 4.35 (q, J = 5.9 Hz, 1H), 4.13 – 4.00 (m, 1H), 3.88 (q, J = 3.6 Hz, 1H), 3.73 – 3.46 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 156.94, 152.41, 149.61, 121.79, 101.05, 86.82, 86.67, 85.16, 73.90, 70.46, 61.50. **LC-MS** (*m/z*): 347.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%.

Tubercidin (56, Bcy-129), CAS: 69-33-0



To a solution of **55b** (220 mg, 0.64 mmol, 1 eq.) in THF/MeOH (1:1, 10 mL), 20 wt. % $Pd(OH)_2/C$ (20%, 44 mg) was added. The mixture was shaken with hydrogen (45 psi) at rt for 3 h in a Parr apparatus and the reaction progress was monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, the solution was filtered on celite, and the filter cake was washed with THF (10 mL) and MeOH (10 mL). The filtrate was evaporated *in vacuum*. The crude compound was purified by silica gel column chromatography using 15% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 82.0-84.0 °C (*lit.*[166] 249-250 °C). **Yield**: 165 mg, 97%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.03 (s, 1H), 7.33 (d, J = 3.6 Hz, 1H), 7.02 (s, 2H), 6.59 (d, J = 3.5 Hz, 1H), 5.98 (d, J = 6.2 Hz, 1H), 5.39 – 5.20 (m, 2H), 5.07 (d, J = 4.6 Hz, 1H), 4.52 – 4.38 (m, 1H), 4.20 – 4.01 (m, 1H), 3.89 (q, J = 3.6 Hz, 1H), 3.67 – 3.49 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 157.52, 151.51, 149.90, 122.23, 103.08, 99.53, 87.57, 85.04, 73.63, 70.69, 61.82. **LC-MS** (*m/z*): 267.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.9%.

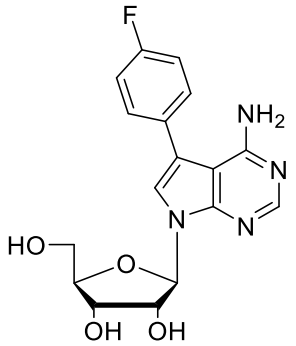
7-Phenyl-7-deazaadenosine (55c, Bcy-236) CAS: 1252857-91-2



This compound was synthesized using the same procedure as for **33h**. Compound **55b** (200 mg, 0.58 mmol), dioxane/H_2O (2:1, 9 mL), benzeneboronic acid (106 mg, 0.87 mmol), Pd $(PPh_3)_2Cl_2$ (42 mg, 0.06 mmol) and K_2CO_3 (240 mg, 1.74 mmol) were used. The crude compound was purified by silica gel column chromatography using 5% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 96.5-98.0 °C (*lit.*[188] 119 °C). **Yield**:

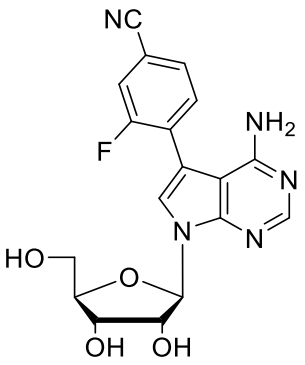
68 mg, 34%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.15 (s, 1H), 7.54 (s, 1H), 7.49 (d, J = 5.8 Hz, 4H), 7.41 – 7.34 (m, 1H), 6.12 (d, J = 6.2 Hz, 3H), 5.29 (d, J = 6.4 Hz, 1H), 5.15 (t, J = 5.6 Hz, 1H), 5.08 (d, J = 4.7 Hz, 1H), 4.46 (q, J = 5.9 Hz, 1H), 4.12 (q, J = 4.7 Hz, 1H), 3.91 (q, J = 3.6 Hz, 1H), 3.68 – 3.59 (m, 1H), 3.59 – 3.49 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.27, 151.63, 150.82, 134.44, 128.92, 128.40, 126.86, 121.10, 116.24, 100.48, 87.06, 85.07, 73.76, 70.58, 61.64. **LC-MS** (*m/z*): 343.1 [M + H]$^{+}$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.7%.

7-(4-Fluorophenyl)-7-deazaadenosine (55d, Bcy-319), CAS: 2307607-25-4



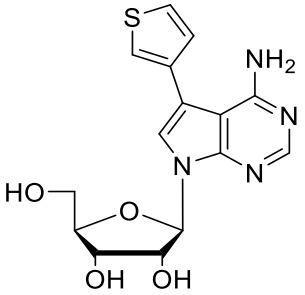
This compound was synthesized using the same procedure as for **33h**. Compound **55b** (150 mg, 0.43 mmol), dioxane/H_2O (2:1, 9 mL), 4-fluorobenzeneboronic acid (91 mg, 0.65 mmol), $Pd(PPh_3)_2Cl_2$ (28 mg, 0.04 mmol) and K_2CO_3 (178 mg, 1.29 mmol) were used. The crude compound was purified by silica gel column chromatography using 12% MeOH in DCM. **Appearance**: brownish solid. **Yield**: 80 mg, 52%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.64 – 7.59 (m, 2H), 7.51 (s, 1H), 7.50 – 7.45 (m, 2H), 7.32 – 7.26 (m, 2H), 6.10 (d, J = 6.2 Hz, 1H), 5.27 (d, J = 6.2 Hz, 1H), 5.17 – 4.99 (m, 2H), 4.44 (q, J = 6.2 Hz, 1H), 4.14 – 4.03 (m, 1H), 3.90 (q, J = 3.7 Hz, 1H), 3.69 – 3.59 (m, 1H), 3.57 – 3.47 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.40, 157.28, 151.65, 150.80, 131.47, 130.34, 128.74, 121.13, 115.60, 115.21, 100.48, 87.03, 85.06, 73.75, 70.56, 61.63. **LC-MS** (*m/z*): 361.10 [M + H]$^{+}$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 85.1%.

7-(4-Cyano-2-fluorophenyl)-7-deazaadenosine (55e, Bcy-381)



This compound was synthesized using the same procedure as for **33h**. Compound **55b** (150 mg, 0.43 mmol), dioxane/H_2O (2:1, 9 mL), (4-cyano-2-fluorophenyl)boronic acid (107 mg, 0.65 mmol), $Pd(PPh_3)_2Cl_2$ (28 mg, 0.04 mmol) and K_2CO_3 (178 mg, 1.29 mmol) were used. The crude compound was purified by silica gel column chromatography using 10% MeOH in DCM. **Appearance**: yellowish oil. **Yield**: 45 mg, 27%. **LC-MS** (*m/z*): 386.10 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 90.2%.

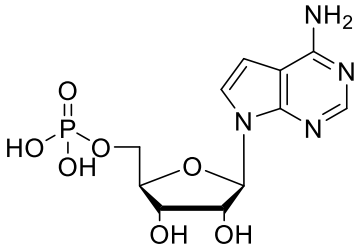
7-(3-Thienyl)-7-deazaadenosine (55f, Bcy-347), CAS: 1252858-10-8



This compound was synthesized using the same procedure as for **33h**. Compound **55b** (150 mg, 0.43 mmol), dioxane/H_2O (2:1, 9 mL), 3-thienylboronic acid (83 mg, 0.65 mmol), $Pd(PPh_3)_2Cl_2$ (28 mg, 0.04 mmol) and K_2CO_3 (178 mg, 1.29 mmol) were used. The crude compound was purified by silica gel column chromatography using 15% MeOH in DCM. **Appearance**: brownish solid; **mp**: 71-73 °C (*lit.*[189] 197 °C). **Yield**: 85 mg, 57%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.99 – 7.92 (m, 1H), 7.71 (dd, J = 4.9, 2.9 Hz, 1H), 7.54 (s, 1H), 7.51 (dd, J = 3.0, 1.3 Hz, 1H), 7.49 (t, J = 7.8 Hz, 1H), 7.27 (dd, J = 4.9, 1.3 Hz, 1H), 6.10 (d, J = 6.2 Hz, 1H), 5.31 (d, J = 10.7 Hz, 1H), 5.14 (d, J = 38.5 Hz, 2H), 4.44 (t, J = 5.7 Hz, 1H), 4.10 (dd, J = 5.2, 3.2 Hz, 1H), 3.90 (p, J = 3.7 Hz, 1H), 3.63 (dd, J = 11.9, 3.8 Hz, 1H), 3.56 – 3.50 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.35, 151.67, 150.54, 134.68, 129.18, 128.43, 127.29, 121.95,

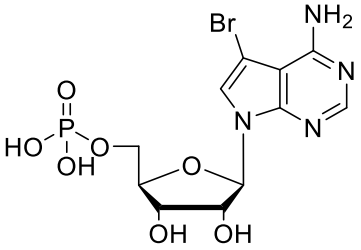
121.02, 110.90, 87.00, 85.05, 73.75, 70.56, 61.64. **LC-MS** (*m/z*): 347.20 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.8%.

7-Deaza-AMP (57a, Bcy-136) CAS: 16719-46-3



Compound **56** (150 mg, 0.56 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (180 mg, 0.84 mmol) and $POCl_3$ (0.21 mL, 2.24 mmol) were used. **Appearance**: white powder; **mp**: >300 °C (*lit.*[190] 250-260 °C). **Yield**: 21 mg, 11%. ^{1}H NMR (600 MHz, D_2O) δ 7.92 (d, J = 1.0 Hz, 1H), 7.28 (d, J = 3.8 Hz, 1H), (NH_2 is missing due to it`s exchangeable with D_2O), 6.43 (d, J = 3.7 Hz, 1H), 5.82 (d, J = 6.2 Hz, 1H), 4.83 – 4.81 (m, 2H), 4.77 – 4.73 (m, 2H), 4.38 – 4.31 (m, 1H), 3.94 (q, J = 2.3 Hz, 2H), 3.76 – 3.70 (m, 1H), 3.68 – 3.61 (m, 1H). ^{13}C NMR (151 MHz, D_2O) δ 160.02, 154.19, 152.58, 125.04, 105.90, 103.07, 90.18, 86.97, 77.91, 74.65, 67.39. ^{31}P NMR (243 MHz, D_2O) δ 4.40. **LC-MS** (*m/z*): 347.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%.

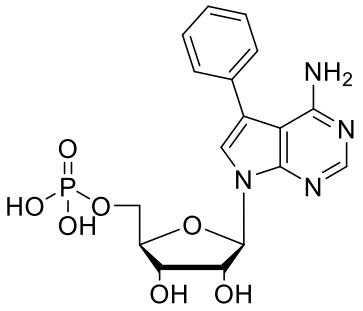
7-Bromo-7-deaza-AMP (57b, Bcy-128)



Compound **55b** (120 mg, 0.35 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (114 mg, 0.53 mmol) and $POCl_3$ (0.13 mL, 1.40 mmol) were used. **Appearance**: white powder; **mp**: >300 °C. **Yield**: 43 mg, 29%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.10 (s, 1H), 7.65 (s, 1H), 6.75 (s, 2H), 6.11 (d, J = 6.5 Hz, 1H), 4.44 (dd, J = 6.5, 5.0 Hz, 1H), 4.15 (dd, J = 5.0, 2.7 Hz, 1H), 3.98 (q, J = 3.6 Hz, 2H), 3.81 (dt, J = 10.2, 6.7 Hz, 5H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 156.87, 152.45, 150.06, 121.58, 100.87, 87.02, 85.89, 83.83,

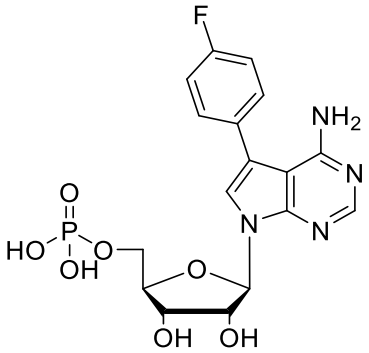
73.82, 71.13, 64.40. ^{31}P NMR (243 MHz, DMSO-d_6) δ 1.15. **LC-MS** (*m/z*): 425.0 [M - H]$^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%.

7-Phenyl-7-deaza-AMP (57c, Bcy-243), CAS: 1252974-10-9



Compound **55c** (60 mg, 0.18 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (58 mg, 0.27 mmol) and $POCl_3$ (0.07 mL, 0.72 mmol) were used. **Appearance**: white powder; **mp**: 178.0-180.0 °C. **Yield**: 10 mg, 13%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.16 (s, 1H), 7.57 (s, 1H), 7.52 – 7.49 (m, 2H), 7.46 (t, *J* = 7.7 Hz, 2H), 7.36 – 7.32 (m, 1H), 7.24 (s, 2H), 6.19 (d, *J* = 6.3 Hz, 1H), 4.50 (dd, *J* = 6.3, 5.1 Hz, 1H), 4.17 (dd, *J* = 5.1, 3.1 Hz, 1H), 4.01 (q, *J* = 4.0 Hz, 2H), 3.91 – 3.81 (m, 5H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 157.19, 151.73, 151.36, 134.45, 128.91, 128.45, 126.72, 120.63, 116.58, 100.24, 86.08, 83.30, 73.67, 71.00, 64.69. ^{31}P NMR (243 MHz, DMSO-d_6) δ 1.00. **LC-MS** (*m/z*): 423.2 [M + H]$^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.3%.

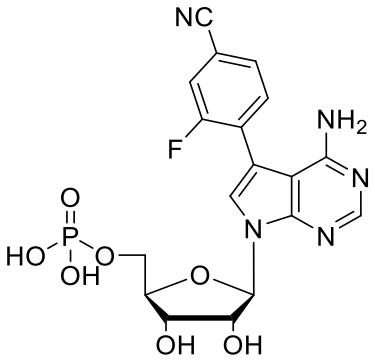
7-(4-Fluorophenyl)-7-deaza-AMP (57d, Bcy-323), CAS: 1252974-11-0



Compound **55d** (65 mg, 0.18 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (58 mg, 0.27 mmol) and $POCl_3$ (0.07 mL, 0.72 mmol) were used. **Appearance**: white powder; **mp**: >300 °C. **Yield**: 29 mg, 37%. ^{1}H NMR (600 MHz, D_2O) δ 8.24 (s, 1H), 7.62 (s,

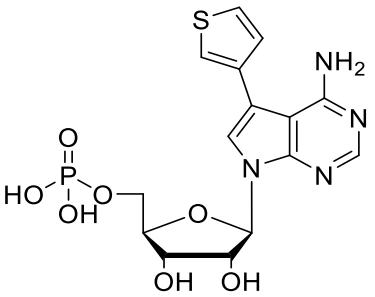
1H), 7.60 (dd, *J* = 8.4, 5.5 Hz, 2H), 7.33 (t, *J* = 8.7 Hz, 2H), (N*H*$_2$ is missing due to it`s exchangeable with D_2O), 6.32 (d, *J* = 6.8 Hz, 1H), 4.90 – 4.86 (m, 4H), 4.81 – 4.76 (m, 1H), 4.46 (dd, *J* = 5.5, 3.0 Hz, 1H), 4.35 (q, *J* = 4.1 Hz, 1H), 4.02 (t, *J* = 4.8 Hz, 2H). ^{13}C NMR (151 MHz, D_2O) δ 164.06, 159.99, 154.24, 153.18, 133.40, 132.27, 122.89, 120.18, 118.38, 103.87, 88.74, 86.89, 76.60, 73.88, 66.74. ^{31}P NMR (243 MHz, D_2O) δ 4.31. **LC-MS** (*m/z*): 441.30 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.1%

7-(4-Cyano-2-fluorophenyl)-7-deaza-AMP (57e, Bcy-393)



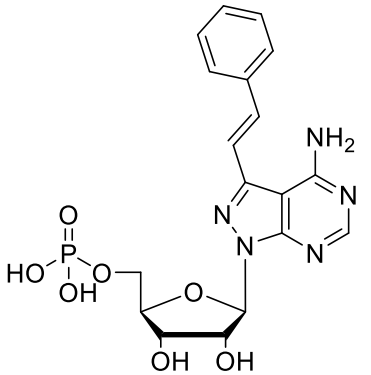
Compound **55e** (45 mg, 0.12 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (39 mg, 0.18 mmol) and $POCl_3$ (0.04 mL, 0.48 mmol) were used. **Appearance**: white powder; **mp**: 236-238 ℃. **Yield**: 7 mg, 13%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.19 (s, 1H), 7.93 (dd, *J* = 9.8, 1.6 Hz, 1H), 7.76 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.66 (s, 1H), 7.60 (t, *J* = 7.8 Hz, 1H), 6.45 (s, 2H), 6.20 (d, *J* = 6.1 Hz, 1H), 5.42 (s, 2H), 4.44 (t, *J* = 5.6 Hz, 1H), 4.12 (dd, *J* = 5.2, 3.3 Hz, 1H), 4.08 – 3.99 (m, 3H), 3.99 – 3.89 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.64, 157.99, 156.94, 151.18, 132.78, 128.82, 127.53, 127.43, 122.97, 120.07, 119.89, 117.98, 108.19, 100.67, 86.57, 73.49, 70.49, 65.53. ^{31}P NMR (243 MHz, DMSO-d_6) δ 0.01. **LC-MS** (*m/z*): 466.30 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 93.4%

7-(3-Thienyl)-7-deaza-AMP (57f, Bcy-356), CAS: 1252974-15-4



Compound **55f** (60 mg, 0.17 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (56 mg, 0.26 mmol) and $POCl_3$ (0.06 mL, 0.68 mmol) were used. **Appearance**: white powder; **mp**: 210-212 °C. **Yield**: 26 mg, 36%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.18 (s, 1H), 7.69 (dd, J = 5.0, 2.9 Hz, 1H), 7.56 – 7.50 (m, 2H), 7.30 (dd, J = 4.8, 1.4 Hz, 1H), 6.38 (s, 2H), 6.18 (d, J = 6.1 Hz, 1H), 5.35 (s, 3H), 4.45 – 4.38 (m, 1H), 4.12 (dd, J = 5.1, 3.2 Hz, 1H), 4.07 – 3.86 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.61, 150.86, 150.67, 134.41, 128.41, 127.26, 122.02, 120.65, 111.70, 100.39, 86.36, 82.66, 73.59, 70.55, 65.47. ^{31}P NMR (202 MHz, DMSO-d_6) δ 0.00. **LC-MS** (*m/z*): 429.10 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.1%

((2*R*,3*S*,4*R*,5*R*)-5-(4-Amino-3-((*E*)-styryl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl phosphoric acid (59, Bcy-357)



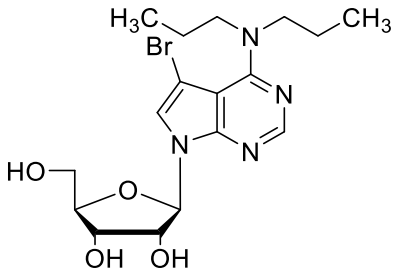
Compound **58e** (60 mg, 0.16 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (51 mg, 0.24 mmol) and $POCl_3$ (0.06 mL, 0.64 mmol) were used. **Appearance**: white powder; **mp**: 238-240 °C. **Yield**: 43 mg, 60%. ^{1}H NMR (600 MHz, D_2O) δ 7.94 (s, 1H), 7.33 (d, J = 7.3 Hz, 2H), 7.19 (dd, J = 15.9, 7.1 Hz, 4H), (NH_2 is missing due to it`s exchangeable with D_2O), 6.94 (d, J = 16.2 Hz, 1H), 6.15 (d, J = 4.7 Hz, 1H), 4.88 (t, J = 5.2 Hz, 1H), 4.83 – 4.79 (m, 2H), 4.78 – 4.75 (m, 2H), 4.59 (t, J = 5.2 Hz, 1H), 4.30 (q, J = 5.1 Hz, 1H), 4.09 – 4.02 (m, 1H), 4.01 – 3.95 (m, 1H). ^{13}C NMR (151 MHz, D_2O) δ 160.63,

158.15, 157.09, 147.53, 137.84, 137.63, 131.35, 131.25, 129.37, 118.83, 101.52, 89.83, 85.94, 75.57, 73.35, 67.24. ^{31}P NMR (243 MHz, D_2O) δ 3.30. **LC-MS** (*m/z*): 450.20 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.5%.

General procedure for the synthesis of 7-bromo-7-deaza-*N*^{6}-substituted adenosine derivatives (60a-f)

To a solution of **55a** (1 eq.) in absolute EtOH (10 mL), appropriate amine (2 eq.) and Et_3N (20 eq.) were added. The mixture was refluxed overnight, and the reaction progress was monitored by TLC (MeOH/DCM, 1:9). After the reaction was completed, cooled to rt and the solvent was evaporated *in vacuum*. The crude compound was then dissolved in 7 N NH_3 in MeOH (10 mL) and stirred in an autoclave at 60 °C overnight. After the reaction was completed, cooled to rt and the solvent was evaporated *in vacuum*. The crude compound was purified by silica gel column chromatography using 3% MeOH in DCM.

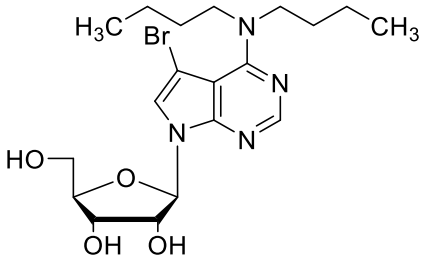
7-Bromo-7-deaza-*N*^{6},*N*^{6}-dipropyladenosine (60a, Bcy-327)



Compound **55a** (200 mg, 0.30 mmol), EtOH (10 mL), dipropylamine (0.08 mL, 0.60 mmol), Et_3N (0.83 mL, 6.00 mmol) and 7 N NH_3 in MeOH (10 mL) were used. **Appearance**: brownish semi-solid. **Yield**: 66 mg, 51%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.23 (s, 1H), 7.79 (s, 1H), 6.13 (s, 1H), 5.30 (s, 1H), 5.08 (s, 2H), 4.36 (t, J = 5.6 Hz, 1H), 4.08 (dd, J = 5.1, 3.4 Hz, 1H), 3.89 (q, J = 3.7 Hz, 1H), 3.68 – 3.48 (m, 6H), 1.60 (h, J = 7.2 Hz, 4H), 0.82 (t, J = 7.4 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.28, 151.40, 150.45, 123.56, 103.94, 87.43, 86.62, 85.11, 73.87, 70.39, 61.41, 51.84,

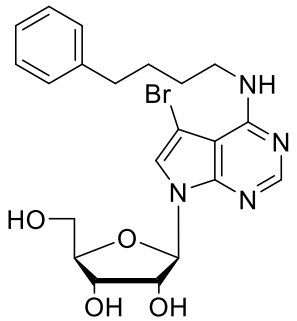
20.45, 11.10. **LC-MS** (*m/z*): 429.10 [M - H]$^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 90.6%.

7-Bromo-7-deaza-*N*^{6},*N*^{6}-dibutyladenosine (60b, Bcy-328)



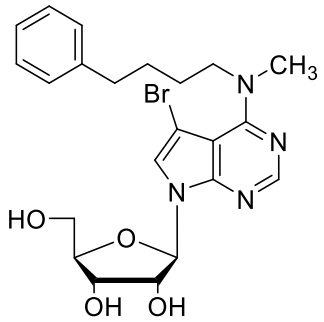
Compound **55a** (400 mg, 0.59 mmol), EtOH (10 mL), dibutylamine (0.20 mL, 1.18 mmol), Et_3N (1.64 mL, 11.80 mmol) and 7 N NH_3 in MeOH (10 mL) were used. **Appearance**: brownish semi-solid. **Yield**: 223 mg, 83%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.23 (s, 1H), 7.79 (s, 1H), 6.13 (d, J = 6.1 Hz, 1H), 5.30 (d, J = 6.3 Hz, 1H), 5.19 – 5.03 (m, 2H), 4.36 (q, J = 5.6 Hz, 1H), 4.09 (s, 1H), 3.89 (q, J = 3.7 Hz, 1H), 3.75 – 3.46 (m, 6H), 1.58 – 1.53 (m, 4H), 1.29 – 1.20 (m, 4H), 0.84 (t, J = 7.4 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.67, 151.39, 150.45, 123.55, 104.09, 87.43, 86.60, 85.11, 73.86, 70.38, 61.40, 49.84, 46.47, 29.32, 27.52, 19.49, 19.24, 13.68, 13.42. **LC-MS** (*m/z*): 457.20 [M - H]$^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.5%.

7-Bromo-7-deaza-*N*^{6}-(4-phenylbutyl)adenosine (60c, Bcy-115)



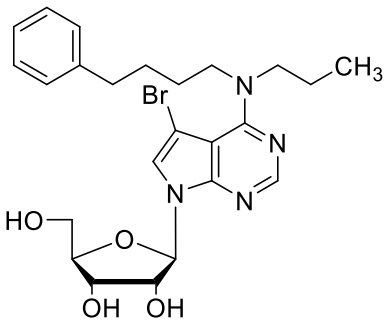
This compound was synthesized using the same procedure as for **37a**. Compound **55a** (730 mg, 1.08 mmol), EtOH (10 mL), 4-phenylbutylamine (0.32 mL, 2.16 mmol) and Et_3N (1.50 mL, 10.80 mmol) were used. **Appearance**: Brown semi-solid. **Yield**: 648 mg, >100%. **LC-MS** (*m/z*): 477.1 [M - H]$^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.3%.

7-Bromo-7-deaza-N^6-methyl-N^6-(4-phenylbutyl)adenosine (60d, Bcy-342)



Compound **55a** (200 mg, 0.30 mmol), EtOH (10 mL), methyl(4-phenylbutyl)amine (0.11 mL, 0.60 mmol), Et_3N (0.83 mL, 6.00 mmol) and 7 N NH_3 in MeOH (10 mL) were used. **Appearance**: yellowish solid; **mp**:58-60 °C. **Yield**: 147 mg, 100%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.21 (s, 1H), 7.79 (s, 1H), 7.24 (t, J = 7.5 Hz, 2H), 7.14 (t, J = 8.1 Hz, 3H), 6.14 (d, J = 6.0 Hz, 1H), 5.30 (s, 1H), 5.08 (s, 2H), 4.35 (t, J = 5.5 Hz, 1H), 4.13 – 4.04 (m, 1H), 3.90 (d, J = 3.5 Hz, 1H), 3.73 – 3.51 (m, 4H), 3.17 (s, 3H), 2.56 (q, J = 6.7, 5.9 Hz, 2H), 1.71 – 1.60 (m, 2H), 1.54 (p, J = 7.5 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.64, 151.29, 150.40, 141.94, 129.19, 128.47, 128.19, 127.38, 125.60, 123.50, 103.32, 87.35, 86.66, 85.08, 73.92, 70.35, 61.38, 52.12, 34.72, 28.08, 26.18. **LC-MS** (*m/z*): 493.30 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.9%.

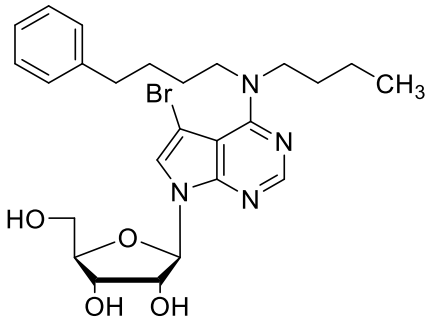
7-Bromo-7-deaza-N^6-propyl-N^6-(4-phenylbutyl)adenosine (60e, Bcy-331)



Compound **55a** (200 mg, 0.30 mmol), EtOH (10 mL), compound **40c** (86 mg, 0.45 mmol), Et_3N (0.83 mL, 6.00 mmol) and 7 N NH_3 in MeOH (10 mL) were used. **Appearance**: brownish semi-solid. **Yield**: 65 mg, 42%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.22 (s, 1H), 7.80 (s, 1H), 7.23 (t, J = 7.5 Hz, 2H), 7.17 – 7.09 (m, 3H), 6.14 (d, J = 6.0 Hz, 1H), 5.31 (d, J = 6.3 Hz, 1H), 5.14 – 5.01 (m, 2H), 4.36 (q, J = 5.6 Hz, 1H),

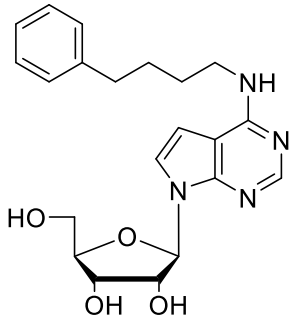
4.08 (p, *J* = 3.9 Hz, 1H), 3.89 (q, *J* = 3.7 Hz, 1H), 3.73 – 3.49 (m, 6H), 2.54 (t, *J* = 7.5 Hz, 2H), 1.56 (dp, *J* = 23.5, 7.9 Hz, 6H), 0.80 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.62, 151.40, 150.45, 141.95, 128.19, 128.15, 125.60, 123.59, 104.04, 87.44, 86.62, 85.11, 73.89, 70.39, 61.40, 51.87, 49.80, 34.73, 28.13, 26.65, 20.44, 11.11. **LC-MS** (*m/z*): 519.20 [M - H]$^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.8%.

7-Bromo-7-deaza-*N*^{6}-butyl-*N*^{6}-(4-phenylbutyl)adenosine (60f, Bcy-332)



Compound **55a** (200 mg, 0.30 mmol), EtOH (10 mL), compound **40d** (92 mg, 0.45 mmol), Et_3N (0.83 mL, 6.00 mmol) and 7 N NH_3 in MeOH (10 mL) were used. **Appearance**: brownish semi-solid. **Yield**: 43 mg, 27%. **LC-MS** (*m/z*): 533.20 [M - H]$^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 75.8%.

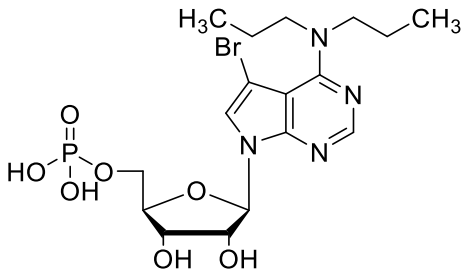
7-Deaza-*N*^{6}-(4-phenylbutyl)adenosine (62, Bcy-147)



This compound was synthesized using the same procedure as for **56**. Compound **60c** (380 mg, 0.80 mmol), THF/MeOH (1:1, 15 mL) and 20 wt. % $Pd(OH)_2$/C (20%, 76 mg) were used. The crude compound was purified by silica gel column chromatography using 4% MeOH in DCM. **Appearance**: yellowish solid; **mp**: 61.5-63.5 °C. **Yield**: 70 mg, 22%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.10 (s, 1H), 7.32 (d, *J* = 3.6 Hz, 1H), 7.26 (t, *J* = 7.5 Hz, 2H), 7.23 – 7.13 (m, 4H), 6.60 (d, *J* = 3.6 Hz, 1H), 5.98 (d, *J* = 6.2 Hz,

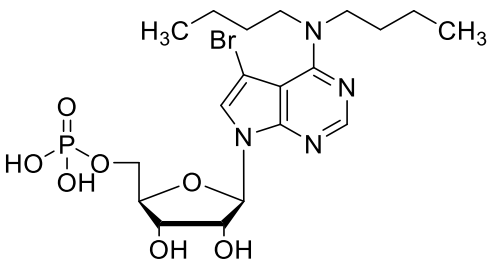
1H), 5.30 (dd, *J* = 6.7, 4.7 Hz, 1H), 5.22 (d, *J* = 6.4 Hz, 1H), 5.06 (d, *J* = 4.7 Hz, 1H), 4.42 (q, *J* = 6.0 Hz, 1H), 4.11 – 4.06 (m, 1H), 3.89 (q, *J* = 3.6 Hz, 1H), 3.66 – 3.45 (m, 4H), 2.62 (t, *J* = 7.3 Hz, 2H), 1.68 – 1.57 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 156.19, 151.37, 149.24, 142.14, 128.27, 128.19, 125.59, 122.08, 103.36, 99.16, 87.65, 85.04, 73.66, 70.69, 61.83, 34.87, 28.85, 28.49. **LC-MS** (*m/z*): 399.0 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.1%.

7-Bromo-7-deaza-N^6,N^6-dipropyl-AMP (61a, Bcy-336)



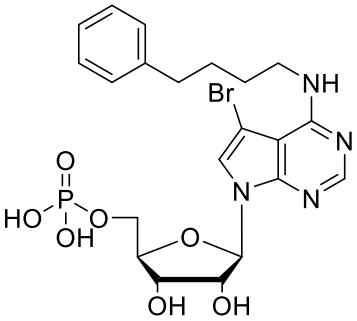
Compound **60a** (60 mg, 0.14 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (45 mg, 0.21 mmol) and $POCl_3$ (0.05 mL, 0.56 mmol) were used. **Appearance**: white powder; **mp**: 89.0-91.0 ℃. **Yield**: 15 mg, 21%. ^{1}H NMR (600 MHz, D_2O) δ 8.28 (t, *J* = 0.9 Hz, 1H), 7.95 (s, 1H), 6.35 (d, *J* = 6.2 Hz, 1H), 4.86 – 4.80 (m, 2H), 4.78 – 4.73 (m, 2H), 4.59 (t, *J* = 5.9 Hz, 1H), 4.45 (s, 1H), 4.36 (s, 1H), 4.11 (s, 2H), 3.90 – 3.79 (m, 4H), 1.77 (h, *J* = 7.4 Hz, 4H), 0.94 – 0.85 (m, 6H). ^{13}C NMR (151 MHz, D_2O) δ 154.39, 151.88, 145.71, 128.71, 106.89, 94.67, 89.72, 86.99, 77.41, 73.46, 67.48, 57.00, 22.99, 12.90. ^{31}P NMR (243 MHz, D_2O) δ 0.45. **LC-MS** (*m/z*): 509.20 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.2%.

7-Bromo-7-deaza-N^6,N^6-dibutyl-AMP (61b, Bcy-343)



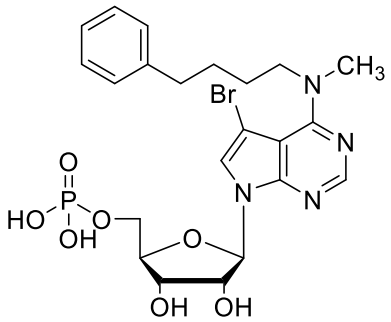
Compound **60b** (60 mg, 0.13 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (43 mg, 0.20 mmol) and $POCl_3$ (0.05 mL, 0.52 mmol) were used. **Appearance**: white powder; **mp**: 85.0-87.0 °C. **Yield**: 32 mg, 46%. ^{1}H NMR (600 MHz, D_2O) δ 7.95 (s, 1H), 7.38 (s, 1H), 5.94 – 5.82 (m, 1H), 4.83 – 4.81 (m, 2H), 4.77 – 4.75 (m, 2H), 4.20 (s, 1H), 3.95 (s, 2H), 3.80 (s, 1H), 3.74 – 3.61 (m, 1H), 3.38 (s, 4H), 1.33 (s, 4H), 0.99 (q, J = 7.3 Hz, 4H), 0.57 (t, J = 7.3 Hz, 6H). ^{13}C NMR (151 MHz, D_2O) δ 171.03, 165.63, 162.03, 153.55, 119.86, 117.93, 107.43, 91.82, 52.76, 31.78, 22.13, 15.82. ^{31}P NMR (243 MHz, D_2O) δ 4.49. **LC-MS** (*m/z*): 539.30 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.2%.

7-Bromo-7-deaza-N^6-(4-phenylbutyl)-AMP (61c, Bcy-131)



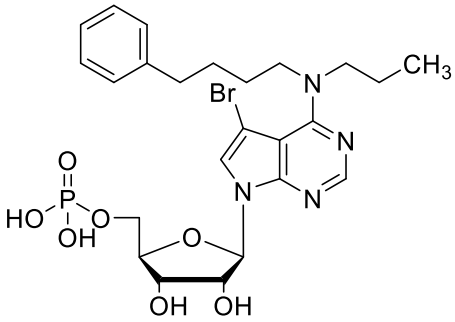
Compound **60c** (150 mg, 0.31 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (113 mg, 0.47 mmol) and $POCl_3$ (0.12 mL, 1.24 mmol) were used. **Appearance**: white powder; **mp**: 175.0-176.5 °C. **Yield**: 14 mg, 8%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.17 (s, 1H), 7.65 (s, 1H), 7.26 (t, J = 7.5 Hz, 2H), 7.21 – 7.18 (m, 2H), 7.17 – 7.13 (m, 1H), 6.52 (t, J = 5.9 Hz, 1H), 6.10 (d, J = 6.4 Hz, 1H), 4.45 (dd, J = 6.5, 5.0 Hz, 1H), 4.17 (dd, J = 4.9, 2.7 Hz, 1H), 3.97 (q, J = 3.5 Hz, 1H), 3.80 – 3.72 (m, 4H), 3.56 (dt, J = 6.5, 3.2 Hz, 4H), 2.67 – 2.58 (m, 2H), 1.68 – 1.56 (m, J = 3.8, 3.2 Hz, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.72, 152.36, 149.43, 142.14, 128.29, 128.21, 125.61, 121.42, 100.91, 86.45, 85.93, 83.93, 73.96, 71.24, 64.18, 40.06, 34.84, 28.65, 28.39. ^{31}P NMR (243 MHz, DMSO-d_6) δ 1.18. **LC-MS** (*m/z*): 559.1 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.2%.

7-Bromo-7-Deaza-N^6-methyl-N^6-(4-phenylbutyl)-AMP (61d, Bcy-349)



Compound **60d** (60 mg, 0.12 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (39 mg, 0.18 mmol) and $POCl_3$ (0.05 mL, 0.48 mmol) were used. **Appearance**: white powder; **mp**: 99-101 ℃. **Yield**: 33 mg, 48%. ^{1}H NMR (600 MHz, D_2O) δ 8.18 (s, 1H), 7.75 (s, 1H), 6.96 (t, J = 29.0 Hz, 5H), 6.18 (s, 1H), 4.83 (s, 2H), 4.78 – 4.74 (m, 2H), 4.47 (s, 1H), 4.39 (s, 1H), 4.29 (s, 1H), 4.10 (s, 2H), 3.51 (s, 2H), 3.26 – 3.08 (m, 3H), 2.41 (s, 2H), 1.51 (d, J = 64.1 Hz, 4H). ^{13}C NMR (151 MHz, D_2O) δ 165.78, 155.60, 151.48, 145.01, 131.19, 131.06, 128.48, 105.87, 93.47, 89.86, 86.78, 77.57, 73.38, 67.55, 56.59, 43.84, 37.63, 29.98, 28.84. ^{31}P NMR (243 MHz, D_2O) δ 0.58. **LC-MS** (*m/z*): 573.30 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.4%.

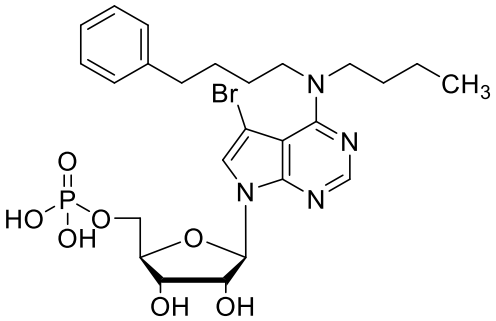
7-Bromo-7-deaza-N^6-propyl-N^6-(4-phenylbutyl)-AMP (61e, Bcy-344)



Compound **60e** (60 mg, 0.10 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (32 mg, 0.15 mmol) and $POCl_3$ (0.04 mL, 0.40 mmol) were used. **Appearance**: white powder; **mp**: 80-82 ℃. **Yield**: 21 mg, 35%. ^{1}H NMR (600 MHz, D_2O) δ 8.17 (s, 1H), 7.96 (s, 1H), 7.17 – 7.07 (m, 4H), 7.03 (t, J = 7.0 Hz, 1H), 6.35 (d, J = 6.3 Hz, 1H), 4.82 – 4.81 (m, 2H), 4.78 – 4.76 (m, 2H), 4.64 (t, J = 5.7 Hz, 1H), 4.48 – 4.44 (m, 1H), 4.37 (d, J = 3.2 Hz, 1H), 4.11 (t, J = 4.0 Hz, 2H), 3.83 – 3.66 (m, 4H), 2.68 – 2.54 (m, 2H), 1.82 – 1.62 (m, 6H), 0.80 (t, J = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, D_2O) δ 155.38, 152.17, 146.36, 145.37, 131.36, 131.13, 128.60, 128.46, 107.62, 94.70, 89.54, 87.13, 77.37, 73.62,

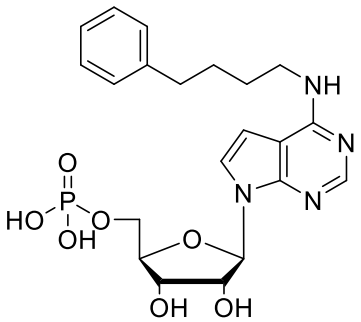
67.56, 58.08, 53.30, 37.27, 29.42, 28.26, 23.17, 12.93. ^{31}P NMR (243 MHz, D_2O) δ 0.51. **LC-MS** (*m/z*): 600.10 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.7%.

7-Bromo-7-deaza-*N*^{6}-butyl-*N*^{6}-(4-phenylbutyl)-AMP (61f, Bcy-338)



Compound **60f** (35 mg, 0.07 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (21 mg, 0.10 mmol) and $POCl_3$ (0.02 mL, 0.26 mmol) were used. **Appearance**: white powder; **mp**: 91-93 °C. **Yield**: 8 mg, 20%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.23 (s, 1H), 7.72 (s, 1H), 7.23 (t, *J* = 7.6 Hz, 2H), 7.16 – 7.09 (m, 3H), 6.19 (d, *J* = 6.3 Hz, 1H), 5.27 (d, *J* = 108.7 Hz, 2H), 4.37 (t, *J* = 5.7 Hz, 1H), 4.09 (dd, *J* = 5.2, 2.9 Hz, 1H), 4.06 – 3.99 (m, 2H), 3.98 – 3.92 (m, 1H), 3.69 – 3.57 (m, 6H), 2.54 (s, 2H), 1.62 – 1.51 (m, 6H), 1.23 (q, *J* = 7.3 Hz, 2H), 0.83 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.66, 151.72, 150.58, 141.92, 128.18, 128.13, 125.58, 123.19, 104.06, 87.96, 86.10, 82.73, 73.39, 70.45, 65.48, 49.88, 49.83, 34.70, 29.27, 28.07, 26.63, 19.49, 13.68. ^{31}P NMR (202 MHz, DMSO-d_6) δ -0.01. **LC-MS** (*m/z*): 615.40 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.2%.

7-Deaza-*N*^{6}-(4-phenylbutyl)-AMP (61g, Bcy-153)



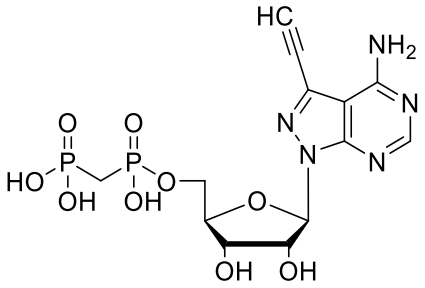
Compound **62** (55 mg, 0.14 mmol), $PO(OCH_3)_3$ (5 mL), proton sponge (45 mg, 0.21 mmol) and $POCl_3$ (0.05 mL, 0.56 mmol) were used. **Appearance**: white powder; **mp**:

128.0-130.0 °C. **Yield**: 16 mg, 24%. ^{1}H NMR (600 MHz, D_2O) δ 8.02 (d, J = 1.1 Hz, 1H), 7.50 (d, J = 3.7 Hz, 1H), 7.24 – 7.06 (m, 5H), 6.60 (d, J = 3.7 Hz, 1H), 6.21 (d, J = 6.6 Hz, 1H), 4.65 (t, J = 6.0 Hz, 1H), 4.44 (dd, J = 5.4, 2.8 Hz, 1H), 4.31 (t, J = 2.8 Hz, 1H), 4.11 – 3.98 (m, 2H), 3.40 (t, J = 6.6 Hz, 2H), 2.58 (t, J = 7.1 Hz, 2H), 1.79 – 1.49 (m, 4H) (5 protons are overlaid by D_2O). ^{13}C NMR (151 MHz, D_2O) δ 157.21, 151.65, 150.99, 145.55, 131.30, 131.25, 128.63, 124.90, 105.97, 104.21, 88.85, 86.73, 76.91, 73.78, 67.38, 43.75, 37.41, 30.53, 30.29. ^{31}P NMR (243 MHz, D_2O) δ 1.51. **LC-MS** (*m/z*): 479.3 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.4%.

General procedure for the synthesis of AMPCP derivatives and analogs

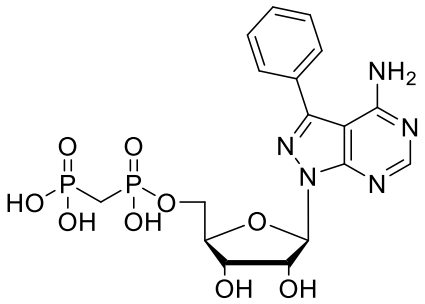
The nucleoside (1 eq.) in $PO(OCH_3)_3$ (4 mL) was cooled to 0 °C, a solution (2 mL) of methylenediphosphonic dichloride (5 eq.) was added, and the mixture was stirred at 0 °C under argon for 1 h. The reaction progress was monitored by TLC (2-propanol/NH_4OH (25% in H_2O)/H_2O, 6:3:1). After the reaction was completed, saturated aqueous NH_4HCO_3 (5 mL) and H_2O (5 mL) were poured into the mixture. The solution was allowed to reach rt upon stirring and left standing for 1 h. $PO(OCH_3)_3$ was finally removed by extracting with *tert*-butylmethylether (1 L), and the aqueous solution was lyophilized. The crude mixture was purified by preparative HPLC.

(((((2*R*,3*S*,4*R*,5*R*)-5-(4-Amino-3-ethynyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)methyl)phosphonic acid (63a, PSB-21310, Bcy-310)



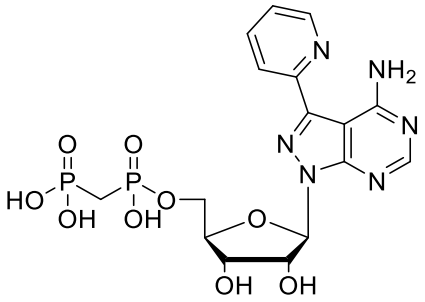
Compound **58a** (45 mg, 0.15 mmol), $PO(OCH_3)_3$ (6 mL) and methylenediphosphonic dichloride (187 mg, 0.75 mmol) were used. **Appearance**: white powder; **mp**: 200.0-202.0 °C. **Yield**: 8 mg, 12%. ^{1}H NMR (600 MHz, D_2O) δ 8.40 (s, 1H), (N*H*$_2$ is missing due to it`s exchangeable with D_2O), 6.31 (d, *J* = 4.0 Hz, 1H), 4.81 – 4.79 (m, 2H), 4.79 – 4.76 (m, 4H), 4.62 (t, *J* = 5.2 Hz, 1H), 4.38 – 4.33 (m, 1H), 4.26 – 4.20 (m, 1H), 4.18 (s, 1H), 4.16 – 4.10 (m, 1H), 2.31 (t, *J* = 20.2 Hz, 2H). ^{13}C NMR (151 MHz, D_2O) δ 154.49, 151.51, 132.81, 103.73, 91.35, 90.56, 86.49, 76.49, 74.93, 73.03, 66.81, 29.53. ^{31}P NMR (243 MHz, D_2O) δ 18.74, 17.93. **LC-MS** (*m/z*): 450.20 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 100%.

(((((2*R*,3*S*,4*R*,5*R*)-5-(4-Amino-3-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)methyl)phosphonic acid (63b, Bcy-309)



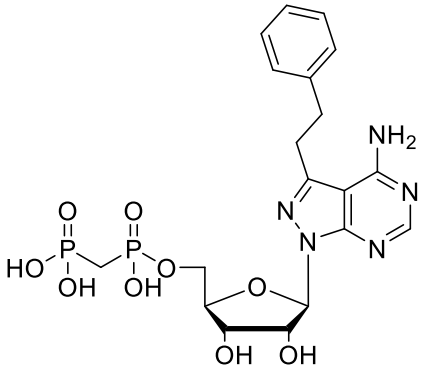
Compound **58b** (45 mg, 0.13 mmol), $PO(OCH_3)_3$ (6 mL) and methylenediphosphonic dichloride (162 mg, 0.65 mmol) were used. **Appearance**: white powder; **mp**: 178.0-180.0 °C. **Yield**: 15 mg, 23%. ^{1}H NMR (600 MHz, D_2O) δ 8.47 (s, 1H), 7.68 – 7.62 (m, 2H), 7.58 (d, *J* = 6.7 Hz, 3H), (N*H*$_2$ is missing due to it`s exchangeable with D_2O), 6.38 (d, *J* = 4.2 Hz, 1H), 4.88 (t, *J* = 4.8 Hz, 1H), 4.84 – 4.80 (m, 2H), 4.78 – 4.76 (m, 3H), 4.64 (t, *J* = 5.2 Hz, 1H), 4.41 – 4.36 (m, 1H), 4.26 – 4.20 (m, 1H), 4.19 – 4.11 (m, 1H), 2.32 – 2.12 (m, 2H). ^{13}C NMR (151 MHz, D_2O) δ 155.70, 154.99, 152.03, 150.77, 133.40, 132.64, 132.60, 131.21, 100.53, 91.14, 86.47, 76.23, 73.21, 67.16, 29.72. ^{31}P NMR (243 MHz, D_2O) δ 18.16, 17.58. **LC-MS** (*m/z*): 502.20 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 96.6%.

(((((2*R*,3*S*,4*R*,5*R*)-5-(4-Amino-3-(pyridin-2-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)methyl)phosphonic acid (63c, Bcy-307)



Compound **58c** (50 mg, 0.15 mmol), $PO(OCH_3)_3$ (6 mL) and methylenediphosphonic dichloride (187, 0.75 mmol) were used. **Appearance**: white powder; **mp**: 275.0-277.0 ℃. **Yield**: 4 mg, 5%. ^{1}H NMR (600 MHz, D_2O) δ 8.38 (s, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.94 (s, 1H), 7.73 (t, J = 7.7 Hz, 1H), 7.24 (s, 1H), (NH_2 is missing due to it`s exchangeable with D_2O), 6.03 (s, 1H), 4.73 – 4.70 (m, 4H), 4.59 (s, 2H), 4.33 (s, 1H), 4.07 (d, J = 15.2 Hz, 2H), 3.82 (s, 1H), 1.82 (t, J = 19.2 Hz, 2H). ^{31}P NMR (243 MHz, D_2O) δ 23.44, 12.59. **LC-MS** (*m/z*): 503.30 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.3%.

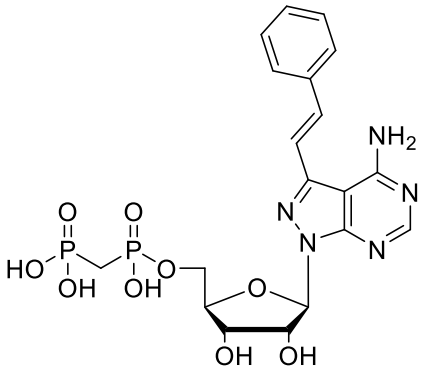
(((((2*R*,3*S*,4*R*,5*R*)-5-(4-Amino-3-phenethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)methyl)phosphonic acid (63d, Bcy-312)



Compound **58d** (35 mg, 0.09 mmol), $PO(OCH_3)_3$ (6 mL) and methylenediphosphonic dichloride (112 mg, 0.45 mmol) were used. **Appearance**: white powder; **mp**: 176.0-178.0 ℃. **Yield**: 5 mg, 10%. ^{1}H NMR (600 MHz, D_2O) δ 8.33 (s, 1H), 7.33 – 7.19 (m,

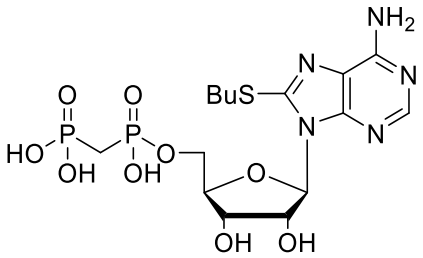
3H), 7.15 – 7.06 (m, 2H), (N*H*$_2$ is missing due to it`s exchangeable with D_2O), 6.22 (d, *J* = 4.0 Hz, 1H), 4.84 – 4.80 (m, 2H), 4.78 – 4.75 (m, 1H), 4.59 – 4.48 (m, 1H), 4.41 (q, *J* = 5.4 Hz, 1H), 4.33 – 4.24 (m, 1H), 4.17 – 4.08 (m, 1H), 4.04 – 3.95 (m, 1H), 3.70 (s, 1H), 3.65 – 3.52 (m, 1H), 3.45 – 3.27 (m, 2H), 3.20 – 3.09 (m, 1H), 3.08 – 2.99 (m, 1H), 2.30 – 2.14 (m, 2H). ^{13}C NMR (151 MHz, D_2O) *δ* 155.17, 154.58, 152.35, 150.12, 142.93, 131.72, 131.38, 129.34, 101.58, 90.92, 86.17, 76.21, 73.15, 67.18, 36.60, 31.97, 29.66. ^{31}P NMR (243 MHz, D_2O) *δ* 19.83, 17.90. **LC-MS** (*m/z*): 530.20 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 99.2%.

(((((2*R*,3*S*,4*R*,5*R*)-5-(4-Amino-3-((*E*)-styryl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)methyl)phosphonic acid (63e, Bcy-306)



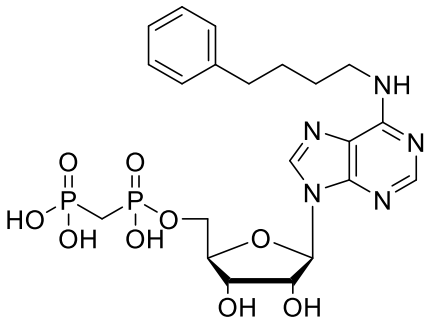
Compound **58e** (50 mg, 0.14 mmol), $PO(OCH_3)_3$ (6 mL) and methylenediphosphonic dichloride (140 mg, 0.56 mmol) were used. **Appearance**: white powder; **mp**: 157.0-159.0 °C. **Yield**: 14 mg, 19%. ^{1}H NMR (500 MHz, DMSO-d_6) *δ* 8.20 (s, 1H), 7.87 – 7.81 (m, 2H), 7.66 (d, *J* = 16.0 Hz, 2H), 7.56 (d, *J* = 15.9 Hz, 1H), 7.40 (t, *J* = 7.5 Hz, 3H), 7.31 (t, *J* = 7.3 Hz, 1H), 6.16 (d, *J* = 4.1 Hz, 1H), 4.64 – 4.60 (m, 1H), 4.43 (t, *J* = 4.7 Hz, 2H), 4.08 – 3.99 (m, 5H), 3.87 – 3.82 (m, 1H), 3.51 (s, 1H), 1.93 (t, *J* = 19.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) *δ* 158.05, 155.84, 155.26, 142.00, 136.35, 132.51, 128.46, 128.15, 127.45, 117.87, 98.25, 87.91, 82.87, 73.25, 71.08, 64.45 (*C*H_2 is overlaid by DMSO-d_6). ^{31}P NMR (202 MHz, DMSO-d_6) *δ* 17.75, 16.35. **LC-MS** (*m/z*): 528.30 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.3%.

8-BuS-AMPCP (64a, Bcy-298)



Compound **27a** (80 mg, 0.23 mmol), $PO(OCH_3)_3$ (6 mL) and methylenediphosphonic dichloride (287 mg, 1.15 mmol) were used. **Appearance**: white powder; **mp**: 124.0-126.0 °C. **Yield**: 30 mg, 25%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.19 (s, 1H), 7.79 (s, 2H), 5.80 (d, J = 5.7 Hz, 1H), 5.60 (s, 5H), 5.07 (t, J = 5.6 Hz, 1H), 4.30 (s, 1H), 4.25 – 4.17 (m, 1H), 4.10 – 3.98 (m, 2H), 3.42 – 3.26 (m, 2H), 2.19 (t, J = 20.4 Hz, 2H), 1.75 – 1.65 (m, 2H), 1.50 – 1.36 (m, 2H), 0.94 – 0.86 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 152.22, 150.61, 150.00, 149.05, 119.27, 88.76, 83.18, 70.57, 70.18, 64.47, (CH$_2$ is overlaid by DMSO-d_6), 31.94, 30.78, 21.18, 13.40. ^{31}P NMR (202 MHz, DMSO-d_6) δ 19.70, 15.84. **LC-MS** (m/z): 514.20 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.7%.

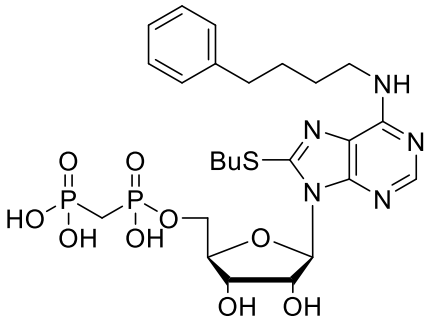
N^6-(4-Phenylbutyl)-AMPCP (64b, PSB-21282, Bcy-282)



Compound **37h** (100 mg, 0.25 mmol), $PO(OCH_3)_3$ (6 mL) and methylenediphosphonic dichloride (312 mg, 1.25 mmol) were used. **Appearance**: white powder; **mp**: 160.0-162.0 °C. **Yield**: 18 mg, 13%. ^{1}H NMR (600 MHz, D_2O) δ 8.65 (s, 1H), 8.33 (s, 1H), 7.32 – 7.19 (m, 4H), 7.13 (s, 1H), 6.18 (d, J = 5.4 Hz, 1H), (NH is missing due to it`s exchangeable with D_2O), 4.78 – 4.75 (m, 6H), 4.56 (t, J = 4.5 Hz, 1H), 4.41 (d, J = 3.5 Hz, 1H), 4.22 (s, 2H), 3.57 (s, 2H), 2.69 (s, 2H), 2.29 (t, J = 19.6 Hz, 2H), 1.80 (s, 4H). ^{13}C NMR (151 MHz, D_2O) δ 151.38, 147.47, 145.40, 144.85, 131.42, 131.24, 128.68, 90.72, 87.19, 77.35, 73.14, 66.53, 44.70, 37.28, 30.07, 29.20. ^{31}P NMR (243 MHz, D_2O)

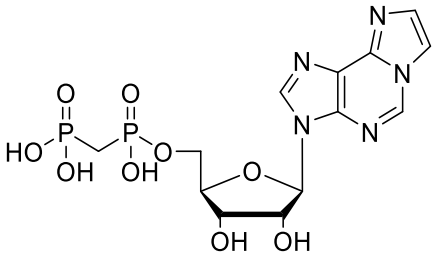
δ 18.43, 17.44. **LC-MS** (*m/z*): 558.30 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.8%.

8-Butylthio-N^6-(4-phenylbutyl)-AMPCP (64c, Bcy-364)



Compound **47b** (60 mg, 0.12 mmol), $PO(OCH_3)_3$ (6 mL) and methylenediphosphonic dichloride (150 mg, 0.60 mmol) were used. **Appearance**: white powder. **Yield**: 16 mg, 21%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.15 (s, 1H), 7.72 (s, 1H), 7.36 – 7.08 (m, 5H), 5.79 (d, *J* = 5.6 Hz, 1H), 5.12 (t, *J* = 5.6 Hz, 2H), 4.84 (s, 4H), 4.31 (t, *J* = 4.4 Hz, 1H), 4.21 (s, 1H), 4.10 – 3.96 (m, 2H), 3.52 (s, 2H), 3.29 (p, *J* = 6.3, 5.8 Hz, 2H), 2.66 – 2.56 (m, 2H), 2.19 (t, *J* = 20.4 Hz, 2H), 1.74 – 1.57 (m, 6H), 1.41 (q, *J* = 7.4 Hz, 2H), 0.89 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 152.63, 148.45, 142.13, 128.24, 128.15, 125.57, 88.63, 83.07, 70.41, 70.26, 64.55, 34.84, 30.87, 28.37, 21.20, 13.39. ^{31}P NMR (202 MHz, DMSO-d_6) δ 19.70, 15.77. **LC-MS** (*m/z*): 646.30 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 95.3%.

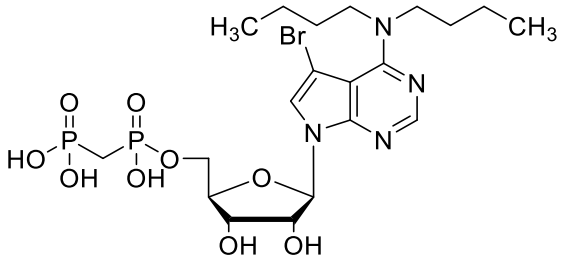
1,N^6-Etheno-AMPCP (64d, Bcy-350)



Compound **52a** (60 mg, 0.21 mmol), $PO(OCH_3)_3$ (6 mL) and methylenediphosphonic dichloride (262 mg, 1.05 mmol) were used. **Appearance**: white powder; **mp**: 187.0-189.0 °C. **Yield**: 14 mg, 15%. ^{1}H NMR (600 MHz, D_2O) δ 9.43 (s, 1H), 8.87 (s, 1H), 8.30 (d, *J* = 2.3 Hz, 1H), 7.95 (d, *J* = 2.3 Hz, 1H), 6.35 (d, *J* = 5.1 Hz, 1H), 4.92 (t, *J* =

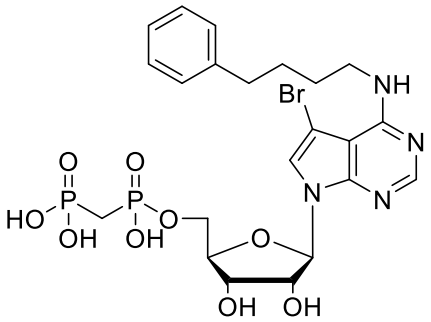
5.1 Hz, 1H), 4.85 – 4.79 (m, 2H), 4.79 – 4.72 (m, 3H), 4.60 (t, *J* = 4.7 Hz, 1H), 4.43 (q, *J* = 3.5 Hz, 1H), 4.30 – 4.18 (m, 2H), 2.42 – 2.23 (m, 2H). ^{13}C NMR (151 MHz, D_2O) *δ* 146.60, 145.90, 140.67, 139.80, 124.77, 121.57, 117.20, 91.51, 87.08, 77.34, 73.04, 66.57, 29.24. ^{31}P NMR (243 MHz, D_2O) *δ* 19.57 (d, *J* = 11.1 Hz), 16.66 (d, *J* = 11.7 Hz). **LC-MS** (*m/z*): 450.20 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 98.8%.

7-Bromo-7-deaza-N^6,N^6-dibutyl-AMPCP (65a, Bcy-353)



Compound **60b** (60 mg, 0.13 mmol), $PO(OCH_3)_3$ (6 mL) and methylenediphosphonic dichloride (162 mg, 0.65 mmol) were used. **Appearance**: white powder; **mp**: 86-88 ℃. **Yield**: 8 mg, 10%. ^{1}H NMR (600 MHz, D_2O) *δ* 8.28 (s, 1H), 7.93 (s, 1H), 6.30 (d, *J* = 5.9 Hz, 1H), 4.81 (s, 2H), 4.77 (s, 2H), 4.59 (t, *J* = 5.5 Hz, 1H), 4.47 (dd, *J* = 5.1, 3.6 Hz, 1H), 4.34 (d, *J* = 3.3 Hz, 1H), 4.21 – 4.14 (m, 2H), 3.91 – 3.79 (m, 4H), 3.68 (s, 1H), 2.41 (d, *J* = 6.2 Hz, 2H), 1.70 (p, *J* = 7.3 Hz, 4H), 1.30 (h, *J* = 7.3 Hz, 4H), 0.85 (t, *J* = 7.3 Hz, 6H). ^{13}C NMR (151 MHz, D_2O) *δ* 154.33, 151.76, 145.73, 128.82, 106.95, 94.53, 89.90, 86.75, 77.26, 73.18, 72.44, 55.30, 31.62, 30.18, 22.00, 15.79. ^{31}P NMR is uncertain. **LC-MS** (*m/z*): 615.20 $[M - H]^-$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 97.0%.

7-Bromo-7-deaza-N^6-(4-phenylbutyl)-AMPCP (65b, Bcy-359)



Compound **60c** (60 mg, 0.13 mmol), $PO(OCH_3)_3$ (6 mL) and methylenediphosphonic dichloride (162 mg, 0.65 mmol) were used. **Appearance**: white powder; **mp**: 184-186 °C. **Yield**: 5 mg, 6%. ^{1}H NMR (600 MHz, D_2O) δ 8.18 (s, 1H), 7.87 (s, 1H), 7.32 – 7.16 (m, 5H), 7.07 (t, *J* = 7.4 Hz, 1H), 6.28 (d, *J* = 6.1 Hz, 1H), (2 O*H*s are overlaid by D_2O), 4.72 – 4.63 (m, 2H), 4.59 – 4.47 (m, 1H), 4.36 (d, *J* = 3.9 Hz, 1H), 4.16 (t, *J* = 4.2 Hz, 2H), 3.70 (s, 2H), 3.57 (d, *J* = 6.7 Hz, 2H), 2.67 (s, 2H), 2.24 (t, *J* = 19.8 Hz, 2H), 1.78 (s, 4H). ^{13}C NMR (151 MHz, D_2O) δ 152.52, 148.83, 145.99, 145.33, 131.45, 131.25, 128.63, 127.01, 93.22, 89.68, 87.03, 77.29, 73.39, 72.45, 66.62, 44.65, 42.20, 37.30, 29.90, 28.95. ^{31}P NMR (243 MHz, D_2O) δ 18.10, 16.25. **LC-MS** (*m/z*): 636.40 $[M + H]^+$. Purity by **HPLC-UV** (254 nm)-ESI-MS: 94.5%.

8.5 Biology

8.5.1 Enzyme preparation and expression

8.5.1.1 Preparations of human umbilical cords and NTPDase1, -2, -3 and -8

The preparations of different enzymes (human umbilical cords and NTPDase1, -2, -3 and -8) were performed by Dr. Julie Pelletier and Prof. Dr. Jean Sévigny.

Human umbilical cords were obtained under approved institutional review board protocol (*Comité d'Éthique de la Recherche du CHU de Québec – Université Laval*) following written consent. They were minced and homogenized with a polytron in 95 mM NaCl, 0.1 mM phenylmethylsulfonyl fluoride and 45 mM tris(hydroxymethyl)aminomethane (TRIS) solution, pH 7.6. The homogenates were then filtered through a cheesecloth, centrifuged for 15 min at 600 g and the supernatants were centrifuged for 1 h at 100 000 g. The pellets were resuspended in 5 mM TRIS solution, pH 8.0 and 10% glycerol. All the purification steps were performed at 4 °C. The prepared proteins were kept at -80 °C.

The NTPDase1, -2, -3 and -8 isoenzymes were obtained by expression in COS-7 cells. The respective recombinant enzymes expression vectors containing the cDNA and Lipofectamine were added to the cells (human CD39 (GenBank accession no.

U87967)[191], human NTPDase2 (NM_203468)[192], human NTPDase3 (AF034840)[193], human NTPDase8 (AY430414)[194]). Membrane preparations containing the transmembrane proteins were prepared according to established protocols.[77,195] The aliquoted protein samples were stored at -80 ℃ until used in activity assays.

8.5.1.2 Preparations of soluble human CD73

The preparations and expression of soluble human CD73 enzyme were performed by Riham Idris and Tobias Claft.

Spodoptera frugiperda (Sf9) insect cells were used for recombinant expression of soluble human CD73. A 10 × His-tag was fused to the N-terminus of the cDNA encoding for the soluble human CD73-residues 27 to 549 (based on Genbank accession no. NM_002526 corresponding to the natural variant T376A P21589/VAR_022091/UniProtKB/Swiss-Prot). The generated construct was then ligated into the pFastBack1 vector between EcoRI/HindIII restriction sites. A transfection mix of 5 µL of recombinant bacmid (2000 ng/µL), 3 µL of X-tremeGENE HP DNA transfection reagent and 100 µL of transfection medium was used to transfect Sf9 cells grown at 1 × 10^6 cell/mL, then incubated at 27 ℃ for 96 h. After virus titer amplification and protein expression, the produced soluble enzyme was concentrated using Amicon® Ultra-15, 10 kDa cut-off (Merck Millipore, MA, USA), then subjected to high-capacity nickel-IMAC purification using HisPur™ Ni-NTA Spin Columns (#: 88226, Thermo Fisher Scientific, MA, USA). The purified soluble enzyme was subsequently aliquoted and stored at -80 ℃ until further use.

8.5.1.3 Preparations and expression of soluble human enzymes CD38, CD39, NPP1, -3, -4 and -5

The preparations and expression of various soluble human enzymes CD38, CD39, NPP1, -3, -4 and -5 were performed by and Salahuddin Mirza and Vittoria Lopez.

Proteins were recombinantly expressed as a soluble variant in Sf9 insect cells using ProEasy™ linearized baculovirus genomic DNA as previously reported.[67,196-197] Details of cloning tools are given in **Table 8.1** (cDNA of human NPP1 (NM_006258), NPP3, (NM_005021), NPP4 (NM_014936), NPP5 (NM_021572) and CD38 (NM_001775)). Briefly, the sequence for the transmembrane domain of each enzyme is trimmed and the genes of interest (GOI) were subcloned in the expression vector between restriction sites. The right plasmids were selected through gene sequencing performed by Eurofins Genomics. For transfection, 10% Cellfectin™, 1 µg of plasmid DNA containing GOI, and 2.5 µL of ProEasy™ DNA were dissolved in 100 µL of culture media and were allowed to incubate for 20 min at rt. The resulting DNA-cellfectin emulsion was used to infect semi-confluent Sf9 adherent cells culture for 96 h at 27 °C to amplify virus titer and protein expression. Medium-containing soluble enzymes were concentrated approximately up to 70-80% with Amicon® Ultra 15 mL centrifugal filters with variable cut-off limits (10-50 kDa, 2500 × G). Subsequently, His-tagged proteins were purified by HisPur™ Ni^{2+}-NTA spin columns by increasing the concentration of imidazole from 10 mM to 250 mM (*Manufacturer specs)*. The eluted enzymes were filtered and concentrated through buffer exchange to get rid of excess imidazole (10 mM HEPES, pH 7.4, 25 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ in 10% glycerol). Protein concentration was determined by the Lowry method based on the oxidation of aromatic amino acids (tryptophan and tyrosine) with the folin-ciocalteu reagent.[198]

Table 8.1. Cloning tools for the expression of soluble human enzymes

Enzyme	A. A.[a]	Exp. Vec.[b]	R. S.[c]	Primers[d]
CD38	43-300	*p-ACGP67-A*	XbaI/NotI	*f*′-5′-*GATC*-TCTAGA-**A**-GTCCCGAGGTGGCGCC-3′ *r*′-5′-*GATC*-GCGGCCGC**TTA**GTGGTGGT-GGTGGTGATGGTGGTGGTGGATCTCA-GATGTGCAAGATGAATCC-3′
CD39	38-478	*p-ACGP67-B*	BamHI/XbaI	*f*′-5′-*GATC*-GGATCCCCACC**ATG**GA-CCCAGAACAAAGCATTGCCA-3′ *r*′-5′-*GATC*-TCTAGA**TTA**ATGGTGGTGGTG-ATGATGGACATAGGTGGAGTGGGAGA-3′
NPP1	98-925	-	BamHI/NotI	*f*′-5′-*GATC*-GGATCCGCCACGAA-AGAAGTTAAAAGTTGCAAAGG-3′ *r*′-5′-*GATC*-GCGGCCGCTTAG-TCTTCTTGGCTAAAGGTTG-3′
NPP3	31-875	-	BamHI/NotI	*f*′-5′-*GATC*-GGATCCGCCACCATG-TCACTTGGATTAGGCCTGG-3′ *r*′-5′-*GATC*-GCGGCCGC**TTA**GTGGTGGTGG-TGGTGATGGTGGTGGTGAATAGTGGTTTC-AAATGTTGG-3′
NPP4	16-407	*p-ACGP67-A*	XbaI/NotI	*f*′-5′-*GATC*-TCTAGA-ATTTA-GAAGTGACTCTTCCTCTA-3′ *r*′-5′-*GATC*-GCGGCCGC-TTA-GTGGT-GGTGGTGGTGATGGTGGTGGTG-GGCTTCTGGGAGATTAATGC-3′
NPP5	25-430	*p-ACGP67-A*	XbaI/NotI	*f*′-5′-*GATC*-TCTAGA-**A**-CACCACCACCATCACCACCACCA-CCACCCAGACCAGCAAAAGGTTC-3′ *r*′-5′-*GATC*-GCGGCCGC**TTA**TGA-CCCCTCTTGGTCATATTC-3′

[a]Amino acids.

[b]Expression vector.

[c]Restriction site.

[d]Frame adjustment was made in *p-ACGP67-A* is denoted by red color. Irrelevant base pairs are shown in *italic*, underlined sequence represents restriction sites and bold base pairs represent the stop codon.

8.5.2 Malachite green assay for human CD39, NTPDases2, -3 and -8

The enzyme activity experiments were performed by Laura Schäkel and Areso Ahmadsay as previously described with a few adaptations.[199]

In general, the assay was conducted in transparent 96-well half area plates in a final volume of 50 µL. CD39 enzyme preparations were preincubated with or without compounds for 5 min at 37 °C in reaction buffer containing a final DMSO concentration of 2%. ATP substrate (50 µM, K_m = 17 µM)[195] was added to initiate the enzymatic dephosphorylation. The reaction was terminated after 15 min by adding the detection reagents (20 µL malachite green solution (0.6 mM) and 30 µL ammonium molybdate solution (20 mM) in sulfuric acid (1.5 M)). The released (inorganic) phosphate was quantified after 20 min at 25 °C by measuring the absorption of the malachite green-phosphomolybdate complex at 600 nm using a BMG PheraStar FS plate reader (BMG Labtech GmbH, Ortenberg, Germany). The phosphate concentration was calculated by subtracting the absorption of the negative control samples, which were incubated with denatured enzyme (90 °C, 15 min), and the inhibition was calculated as follows:

$$\%\ Inhibition = \frac{(B - T)}{B} * 100\ \%$$

where B is the average absorption of the positive control without inhibitor and T is the absorption in the presence of the test compound. Full concentration-inhibition curves were obtained for selected, potent compounds with inhibitor concentrations ranging from 0.1 to 300 µM. Three independent experiments (n = 3) were performed, and the data was analyzed with the GraphPad Prism 8 software (GraphPad software, San Diego, CA, USA). The K_i values of competitive inhibitors were calculated using the Cheng-Prusoff equation: $K_i = \frac{IC_{50}}{1+\frac{[S]}{K_m}}$.

For ticlopidine derivatives and analogs, the reaction buffer contained 80 mM TRIS-HCl, 5 mM $CaCl_2$, pH 7.4. Human umbilical cord membrane preparations containing membrane-bound CD39 were used for initial testing of ticlopidine derivatives and analogs at a concentration of 100 µM. To determine the inhibition type of compound **8k**, the concentration-inhibition curves and inhibition type experiments were performed using 50 ng membrane preparations of COS-7 cells containing recombinant CD39. The

substrate ATP (10-250 µM) in the absence and presence of various concentrations of inhibitor (10, 30 and 100 µM for **8k**). Experiments at recombinant human membrane-bound NTPDase2, -3 or -8 were performed with 100 µM ATP substrate (K_m (NTPDase2) = 70 µM; K_m (NTPDase3) = 75 µM; K_m (NTPDase8) = 46 µM)[195] and compound concentrations of 300 µM.

For 8-BuS-AMP and AMPCP derivatives and analogs, the reaction buffer contained 10 mM HEPES, 2 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4. Human umbilical cord membrane preparations (250 ng) expressing high amounts of CD39 or the respective recombinant COS-7 cell membrane preparations expressing the appropriate NTPDase isoenzyme (CD39, NTPDase2, -3 and -8, about 100 ng of protein depending on enzyme activity, adjusted to ensure 10-20% of substrate conversion)[77,200] with or without 50 µM inhibitor. To determine the inhibition type of compound 8-BuS-AMP (**1i**), the concentration-inhibition curves and inhibition type experiments were performed using 50 ng membrane preparations of COS-7 cells containing recombinant CD39.[77,200] The substrate ATP (10-250 µM) in the absence and presence of various concentrations of inhibitor (2, 4 and 8 µM for 8-BuS-AMP).

8.5.3 Capillary electrophoresis assay for soluble human CD39

The enzyme activity experiments were performed by Salahuddin Mirza as previously described.[45] The test compounds were initially investigated at a concentration of 50 µM (n = 3), 150 µM ATP (K_m = 67.9 µM), and 150 ng human recombinant soluble CD39 were added to initiate the reaction. The reaction buffer contained 10 mM HEPES, 2 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4 in a final volume of 100 µL. Incubation at 37 °C for 30 min, followed by termination of the enzymatic reaction by heating at 90 °C for 5 min. The samples were then diluted 1:20 with reaction buffer to perform separation of nucleotides by capillary electrophoresis. Reaction with soluble CD39 was conducted using DAD-detector with an absorbance maximum of 254 nm. Concentration-inhibition curves were generated at concentrations ranging from 0.01 to 300 µM (n = 3), plotted

with GraphPad Prism 7 software and the K_i value was calculated using the Cheng-Prusoff equation for competitive inhibitors.

Analysis was carried out using a P/ACE MDQ capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA) using a polyacrylamide-coated capillary [30 cm (10 cm effective length) × 50 µm (id), × 360 µm (od)]. Before each run, the capillary was rinsed with the background electrolyte (50 mM phosphate buffer, pH 6.5) for 1 min at 30 psi. Electrokinetic injection of samples by applying a voltage of -6 kV for 30 s at the capillary outlet, and separation of the fluorescent nucleotide derivatives by voltage application of -15 kV. Detection was performed at an excitation wavelength of 488 nm and an emission wavelength of 520 nm. Data collection and peak area analysis were performed by the P/ACE MDQ software 32 KARAT obtained from Beckman Coulter (Fullerton, CA, USA).

8.5.4 Radiometric assay for soluble human CD73

The experiments were performed by Katharina Sylvester, Riham Idris and Patrick Riziki as previously described.[45] Briefly, the respective test compound was incubated in the assay buffer consisting of 25 mM TRIS buffer, 140 mM NaCl and 25 mM NaH_2PO_4, pH 7.4 with 10 µL of each of the soluble human recombinant CD73 (0.09 µg/mL final concentration) and the substrate [2,8-^{3}H]AMP (specific activity 7.4 × 108 Bq/mmol, 20 mCi/mmol at 5 µM final concentration) in a 100 µL total volume. The enzymatic reaction was performed at 37 °C for 25 min in a shaking water bath. The reaction was quenched by addition of 500 µL of ice-cold precipitation buffer (100 mM $LaCl_3$, 100 mM sodium acetate, pH 4.0) then incubated on ice for at least 30 min. The unhydrolyzed substrate was then separated from the product adenosine through GF/B glass fiber filters using Brandel cell harvester (M-48, Brandel, MD, USA). After three washing steps with 400 µL ice-cold demineralized water, the filtrate was collected in scintillation vials, 5 mL scintillation cocktail (ULTIMA Gold XR9) was added and the radioactivity was measured using TRICARB 2900 TR, Packard/PerkinElmer counter. Background was corrected using negative control without the enzyme and

normalization was performed with positive control contains enzyme but not the test compound. Data was analyzed with GraphPad Prism software version 8 (GraphPad Software, La Jolla, USA) using nonlinear regression fit with variable slope. K_i value was calculated from IC_{50} value using Cheng-Prusoff equation. Three independent experiments (n = 3) were conducted, each in duplicates.

8.5.5 Selectivity studies on human NPP1

For ticlopidine derivatives and analogs, the experiments for the selectivity studies at human NPP1 were performed by Salahuddin Mirza as previously described.[201] *p*NP-TMP was used as an artificial substrate which results in the formation of the *p*-nitrophenolate anion with an absorption maximum of 400 nm. Crude soluble NPP1 (3.5 µg, expressed in insect cells) was mixed with test compound (20 µM final concentration for initial screening, 0.1-200 µM for determining concentration-dependent inhibition curves), 2% DMSO and 400 µM of *p*NP-TMP as a substrate in a final volume of 100 µL. The mixture was incubated for 30 min at 37 °C with gentle shaking, and the enzyme reaction was terminated by the addition of 20 µL of 1 M aqueous NaOH. The absorption was measured at 405 nm using a BMG PheraStar FS plate reader (BMG Labtech GmbH, Ortenberg, Germany). Three independent experiments (n = 3) were performed.

For 8-BuS-AMP derivatives and analogs, the experiments for the selectivity studies at human NPP1 were performed by Vittoria Lopez. The test compounds were investigated at a concentration of 50 µM (n = 6), 300 µM ATP and 800 ng human recombinant soluble NPP1 were added to initiate the reaction. The reaction buffer contained 10 mM CHES, 2 mM $CaCl_2$, 1 mM $MgCl_2$, pH 9.0 in a final volume of 100 µL. Incubation at 37 °C for 30 min, followed by termination of the enzymatic reaction by heating at 90 °C for 5 min. Analysis was carried out using a P/ACE MDQ capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA) using a polyacrylamide-coated capillary [30 cm (10 cm effective length) × 50 µm (id), × 360 µm (od)]. Before each run, the capillary was rinsed with the background electrolyte (50 mM phosphate buffer, pH 6.5) for 1 min at 30 psi. Electrokinetic injection of samples by applying a voltage

of -6 kV for 30 s at the capillary outlet, and separation of the fluorescent nucleotide derivatives by voltage application of -90 µA according to a published procedure.[202] Data collection and peak area analysis were performed by the P/ACE MDQ software 32 KARAT obtained from Beckman Coulter (Fullerton, CA, USA).

8.5.6 Selectivity studies on human NPP4

The experiments for the selectivity studies at human NPP4 were performed by Vittoria Lopez using two enzymatic assay system. The substrate employed was Ap4A (diadenosine tetraphosphate) with two detection method, bioluminescence detection (luciferase-based assay) and CE-based assay for analysis and quantification of the products. 8-BuS-AMP (**1i**) was tested at several concentration for a complete IC_{50} curve with AP_4A employed as a substrate which is cleaved by NPP4 to ATP and AMP. The reaction product ATP was quantified by luciferin-luciferase reaction.[197] A mixture of 1.4 µg of NPP4 (soluble form expressed in insect cells and purified),[197] test compound at different concentration ranging from 0.1 to 200 µM in 2% DMSO was incubated with 20 µM of AP_4A as a substrate for 60 min at 37 °C with gentle shaking. The reaction was terminated by heating at 90 °C for 5 min, and after cooling down on ice, 50 µL of *D*-luciferin dissolved in buffer (300 mM Tris-HCl, 15 mM $MgCl_2$, 100 ng *D*-luciferin, pH 7.8) and 50 µL luciferase (50 ng dissolved in H_2O) were added. The firefly luciferase reacts with *D*-luciferin in the presence of ATP produced by NPP4. The resulting luminescence was measured between 10-14 min at 560 nm using a BMG PheraStar FS plate reader. Three independent experiments, each in triplicate, were performed (n = 9). Compounds **28j**, **28r**, **30a**, **38h** and **48b** were tested at a final concentration of 50 µM, employing 300 µM of Ap_4A as substrate which was incubated with 1.2 µg of soluble human NPP4 for 90 min at 37 °C, followed by 5 min of enzyme deactivation at 95 °C and cooling on ice. For analysis and quantification of the products from the enzymatic reaction, a CE-based assay was used. In particular 30 cm capillary, 50 mM phosphate buffer pH 6.5, -90 µA, according to the published procedure.[202] Data collection and peak area analysis were performed by the P/ACE MDQ software 32

KARAT obtained from Beckman Coulter (Fullerton, CA, USA). Negative and positive controls, together with standards were used in parallel. Experiments performed in triplicate in two independent repetitions.

8.5.7 Selectivity studies on human NPP3, NPP5 and CD38

The experiments for the selectivity studies at human NPP3, NPP5, and CD38 were performed by Salahuddin Mirza in analogy to published procedures.[203] The enzymatic activity of the enzymes (soluble forms expressed in insect cells and purified) was measured using 1,N^6-etheno-nicotinamide adenine dinucleotide (ε-NAD^+) as a substrate, which is hydrolyzed to fluorescent 1,N^6-etheno-AMP (for NPP3 and NPP5) and 1,N^6-etheno-ADPR (for CD38). The enzymatic reactions were performed in the reaction buffer. For NPP3 and NPP5: 10 mM HEPES (pH 7.4), 500 µM $CaCl_2$, and 10 µM $ZnCl_2$; for CD38: 10 mM HEPES reaction buffer, pH 7.2. Purified NPP3 (90 ng), NPP5 (400 ng), CD38 (8 ng) were mixed with 20 µM of ε-NAD^+ and 50 µM of the test compounds and incubated at 37 °C for 30 min. The relative fluorescence at 270 nm excitation and 420 nm emission was detected by a fluorescence microplate reader (Flexstation, Medical Devices LLC. USA, Softmax Pro Software). Three independent experiments (n = 3) were performed.

9 Abbreviations

8-BuS-AMP	8-Butylthio-AMP
8-BuS-ADP	8-Butylthio-ADP
8-BuS-ATP	8-Butylthio-ATP
ε-NAD$^+$	1,N^6-Etheno-nicotinamide adenine dinucleotide
aa	Amino acid
AB680	[[(2*R*,3*S*,4*R*,5*R*)-5-[6-Chloro-4-[[(1*S*)-1-(2-fluorophenyl)-ethyl]amino]pyrazolo[3,4-*b*]pyridin-1-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid
ACR	Apyrase conserved region
ACS	Acute coronary syndromes
ADO	Adenosine
ADP	Adenosine diphosphate
ADPR	Adenosine diphosphate ribose
AIDS	Acquired immune deficiency syndrome
AMP	Adenosine monophosphate
AMPCP	Adenosine-5′-*O*-[(phosphonomethyl)phosphonic acid]
AP$_4$A	Diadenosine tetraphosphate
APs	Alkaline phosphatases
aq.	Aqueous
ARL 67156	N^6-Diethyl-*D*-β,γ-dibromo-methylene-ATP
ATP	Adenosine triphosphate
BSA	*N*,*O*-Bis(trimethylsilyl)acetamide
BTEA-Br	Benzyltriethylammonium bromide

cADPR	Cyclic adenosine diphosphate ribose
CD38	Cyclic ADP ribose hydrolase
CD39	NTPDase1
CD73	Ecto-5′-nucleotidase
cDNA	Complementary DNA
cGAMP	Cyclic guanosine monophosphate-adenosine monophosphate
CL_{int}	Internal clearance
CMP	Cytidine monophosphate
Compd.	Compound
COVID-19	Coronavirus disease 2019
CTLA-4	Cytotoxic T lymphocyte–associated protein-4
DCA	Dichloroacetic acid
DCM	Dichloromethane
dCMP	2'-Deoxycytidine 5'-monophosphate
DMAP	4-Dimethylaminopyridine
DMEA	*N*,*N*-Dimethylethylamine
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DTBP	Di-*tert*-butyl peroxide
EB virus	Epstein-Barr virus
eq.	Equivalent
ESI	Electrospray ionization
FDA	U.S. Food and Drug Administration

FL-ATP	N^6-(6-Fluoresceincarbamoyl)hexyl-ATP
GCAP	Germ cell alkaline phosphatase
GMP	Guanosine monophosphate
GOI	Genes of interest
GPCRs	G protein-coupled receptors
GPI	Glycosylphosphatidylinositol
HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
HMDS	Hexamethyldisilazane
HPLC	High performance liquid chromatography
HUVEC	Human umbilical vein endothelial cells
IAP	Intestinal alkaline phosphatase
$\boldsymbol{IC_{50}}$	Half maximal inhibitory concentration
IMP	Inosine monophosphate
$\boldsymbol{K_i}$	Inhibitory constant
Km	Michaelis-Menten constant
LC-MS	Liquid chromatography–mass spectrometry
LGIC	Ligand-gated ion channel
LHQW	Lianhuaqingwen
lit.	Literature
***Lp*CD39**	*Legionella pneumophila* CD39
mp	Melting point
MPI	Myocardial perfusion imaging
MRGPRs	Mas-related G protein-coupled receptors
$\mathbf{n_D^{20}}$	Refractive index (20 °C)

NAADP	Nicotinic acid adenine dinucleotide phosphate
NAD	Nicotinamide adenine dinucleotide
NADH	$NAD^+ + H$
NK cells	Natural killer cells
NMP	*N*-Methyl-2-pyrrolidone
NMR	Nuclear magnetic resonance
NPP	Ecto-nucleotide pyrophosphatase/phosphodiesterase
NTPDases	Nucleoside triphosphate diphosphohydrolases
P0	Purinergic-0
P1	Purinergic-1
P2	Purinergic-2
PD-1	Programmed cell death protein 1
PE	Petroleum ether
PLAP	Placental alkaline phosphatase
***p*NP-TMP**	*p*-Nitrophenyl thymidine 5′-monophosphate
proton sponge	1,8-Bis(dimethylamino)naphthalene
PSB	Pharmaceutical Sciences Bonn
PSVT	Paroxysmal supraventricular tachycardia
RNA	Ribonucleic acid
rt	Room temperature
SARs	Structure-activity relationships
satd.	Saturated
SD	Standard deviation
SEM	Standard error of the mean

Sf9	*Spodoptera frugiperda*
STING	Stimulator of interferon genes
$t_{1/2}$	Half-life
TCM	Traditional Chinese medicine
TEAC	Triethylammonium hydrogencarbonate buffer
TFA	Trifluoroacetic acid
***Tg*CD39**	*Toxoplasma gondii* CD39
THF	Tetrahydrofuran
Tim-3	T cell immunoglobulin and mucin domain containing-3
TLC	Thin layer chromatography
TMDs	Transmembrane domains
TMSBr	Bromotrimethylsilane
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TNAP	Tissue-nonspecific alkaline phosphatase
TRIS	Tris(hydroxymethyl)aminomethane
UDP	Uridine diphosphate
UMP	Uridine monophosphate
UTP	Uridine triphosphate
UV	Ultra-violet

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Publications

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