Synthesis and biological evaluation of novel ligands for the cannabinoid-like orphan G protein-coupled receptors GPR18 and GPR55

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1 Introduction

Right at this moment as we are reading these words, light is being absorbed by the retinal layer inside our eyes and converted by the photoreceptors on the surface of sensory cells into signals, which are rapidly processed by the brain to induce visual perception. Cell-surface receptors are protein structures anchored in the lipid bilayer of the cellular membranes. These machineries can recognize a wide array of stimuli from the surrounding environment like sound waves, temperature, pressure, chemicals from small molecules to peptides and even macromolecules like proteins. Upon binding to an activator, the receptor will initiate a chain of reactions, and thereby transmit external signals to a variety of intracellular sites. The final destination of intracellular signaling is usually the nucleus, where the activation of transcription factors turns on new gene expression and cell division can be induced, helping cells to respond to changes in the extracellular space and to communicate between each other so that biological processes of the whole organism can be regulated.

Based on membrane topology, a transmembrane receptor can be classified by how many times its peptide chain passes the lipid bilayer. Receptors that cross the cell membrane seven times are called seven-transmembrane receptors or 7-TM receptors. The common core structure of 7-TM receptors is composed of an extracellular N-terminus, followed by a bundle of seven α-helices embedded in the cell membrane connected by three extracellular and three intracellular loops, and finally an intracellular C-terminus (Figure 1.1).[1]

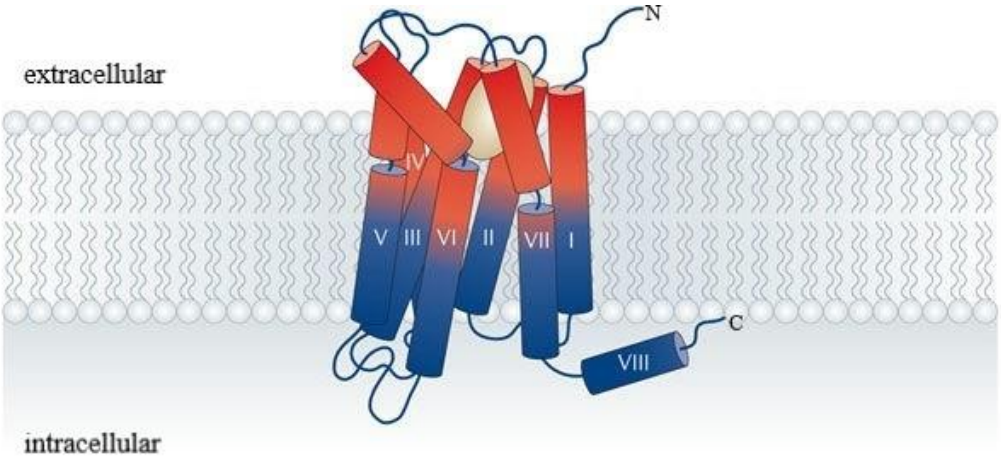


Figure 1.1: Typical structure of 7-TM receptors with seven transmembrane-crossing domains composed of extracellular modules (red) and intracellular modules (blue) (edited from Stevens *et al.*).[1]

Many members of this receptor superfamily are able to couple with heterotrimeric guanine nucleotide-binding proteins (G protein), and by this means signals can be forwarded. For this reason, 7-TM receptors are also called G protein-coupled receptors.

1.1 G protein-coupled receptors

Rhodopsin, one of the photoreceptors which helped us reading thus far, has been studied intensively. It is a remarkably stable protein, highly enriched in the bovine retina, and can therefore be obtained in large quantities for research purposes. At the time when the structure of rhodopsin was elucidated,[2] it was believed that the protein is supposed to be homologous to bacteriorhodopsin,[3] the light-sensitive proton pump from archaebacteria, because of the common seven transmembrane domains. Scientist at that time concluded that 7-TM core structure has to be a unique feature of all light-sensitive proteins. In fact, rhodopsin is able to link with G proteins, but bacteriorhodopsin does not. Only after the β_2-adrenergic receptor had been cloned, researchers acknowledged that seven membrane spans are indeed a signature feature of G protein-coupled receptors (abbr. GPCRs).[4] One could say that all GPCRs are 7-TM receptors but not vice versa.

For a long time, it was difficult to study the structure–function relationships of GPCRs due to their low expression levels in native tissue as well as their inadequate thermo- and detergent-stability. Bovine rhodopsin, with the advantages discussed above, was the first GPCR, of which a three-dimensional structure from the inactive, 11-*cis*-retinal bound conformation with a relative low resolution of 2.8 Å was obtained (Figure 1.2 A).[5] Until 2008, as the crystal structures of opsin in both ligand-free and G protein-interacting conformations were revealed,[6,7] rhodopsin had served as the only model to study GPCRs. Advances in crystallography as well as in protein engineering have led to acquisition of further GPCR structures including β_1[8]- and β_2[9]-receptors, dopamine D_3-receptor[10], histamine H_1-receptor[11], muscarinic M_2[12]- and M_3[13]-receptors, etc. Recently, the active, agonist-bound as well as the inactive, antagonist-bound crystal structures of the human A_{2A} adenosine receptor (A_{2A}AR) have been illustrated (Figure 1.2 B and C).[14]

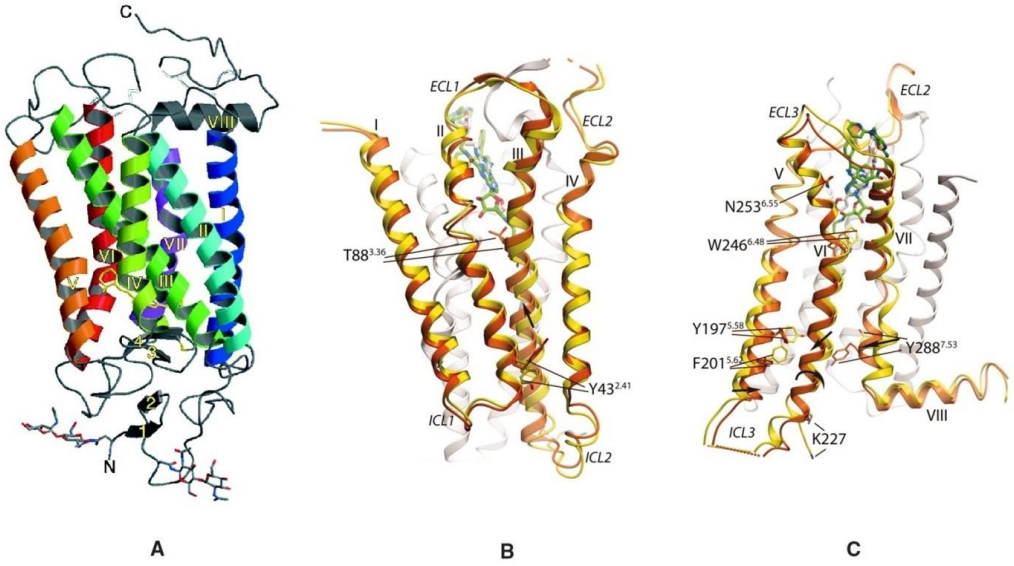


Figure 1.2: The first GPCR crystal structure: rhodopsin (A)[5] and a structural comparison between an agonist-bound conformation (A_{2A}AR/UK-432097 complex, orange) and an antagonist-bound conformation (A_{2A}AR/ZM241385 complex, yellow) of the human A_{2A} adenosine receptor (B: side view of helices I-IV and C: side view of helices V-VIII).[14]

An important consequence of revealing the GPCR crystal structures is the insight into the mechanism of activation through the conformational changes of the receptors from inactive to active state. In the resting state, the GPCR is coupled to its inactive G protein, which is composed of an α-, β- and γ-subunit. In this state, guanosine diphosphate (GDP) binds to the G_{α}-subunit (Figure 1.3, Stage 1). The activation of a GPCR by an agonistic ligand leads to a conformational change in the receptor which catalyzes the release of GDP from the G_{α}-subunit to promote the binding of guanosine triphosphate (GTP) (Figure 1.3, Stage 2). The G_{α}- and $G_{\beta\gamma}$-subunits dissociate subsequently from each other and independently interact with several downstream effector proteins, such as adenylyl cyclase, phospholipase C and ion channels (Figure 1.3, Stage 3). With its own intrinsic GTPase activity, the G_{α}-subunit can hydrolyze the attached GTP to GDP allowing it to re-associate with the $G_{\beta\gamma}$-subunit (Figure 1.3, Stage 4). During this stage the presence of a protein structural domain named regulator of G protein signaling (RGS) can drastically increase the rate of GTP hydrolysis via GTPase activation. The G protein heterotrimer now couples again to the receptor and the resting state (Stage 1) is restored.[15]

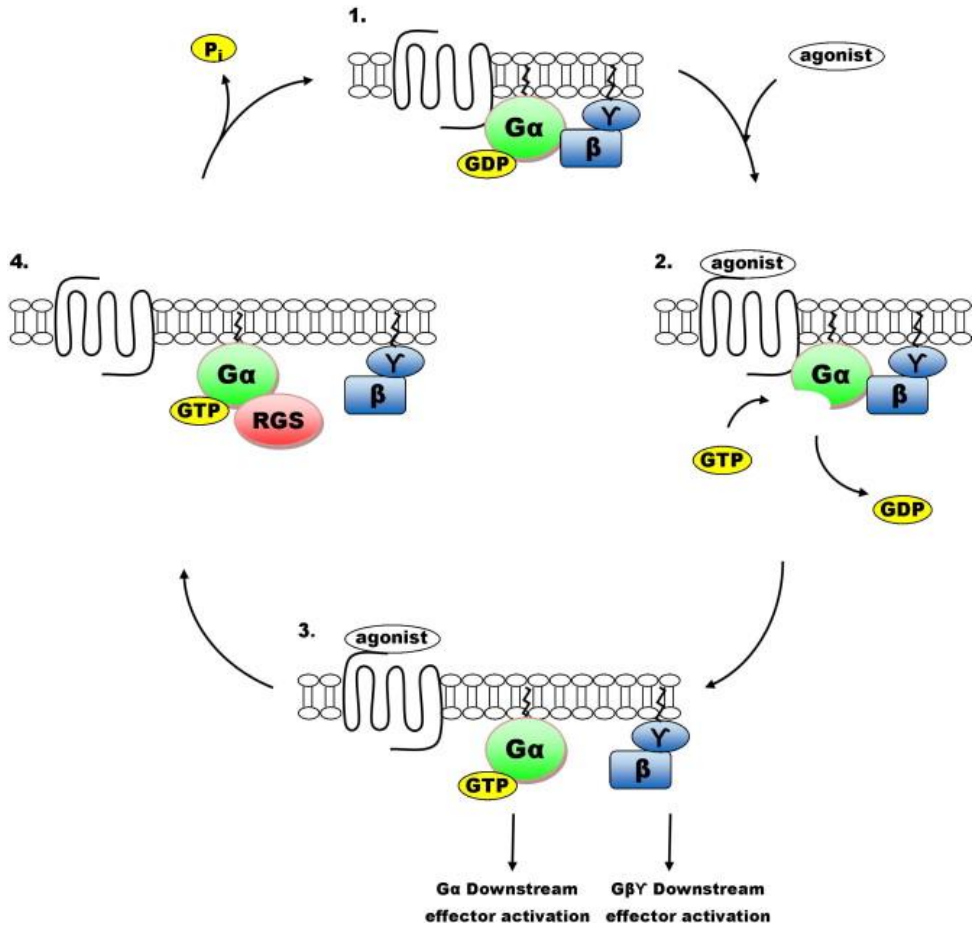


Figure 1.3: Activation cycle of a G protein-coupled receptor.[15]

According to the primary signal transduction pathway of the G_α-subunits, the G_α proteins can be divided into four groups. The G_s subfamily stimulates adenylyl cyclase thus facilitating the synthesis of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP), while the $G_{i/o}$ subfamily inhibits this enzyme resulting in a decrease of intracellular cAMP concentration. The second messenger cAMP acts as an activator of protein kinase A in the cytosol, which triggers further cellular effects through phosphorylation of other proteins. $G_{q/11}$-coupled receptors interact with phospholipase C that cleaves phosphatidylinositol-4,5-bisphosphate (PIP_2) into two second messengers: inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). The binding of IP_3 to its receptor induces the release of calcium from the endoplasmic reticulum into the cytoplasm, whereas DAG activates protein kinase C. The effectors of the $G_{12/13}$ pathway are Rho GTPases, whose function is to control the actin structures of the cytoskeleton.[16,17] Independently of heterotrimeric G proteins, agonist-occupied GPCRs are also able to recruit ß-arrestins from the cytoplasm after being phosphorylated by GPCR kinases (GRKs). The GPCR/β-arrestin complexes can subsequently affect the activity of mitogen-activated protein kinases (MAPKs), which regulate several cellular processes including proliferation, differentiation and apoptosis.[18]

The diversity of G protein-coupled receptors is enormous. With almost 800 members identified in human[19], they represent the largest class of membrane proteins capable of mediating most cellular responses as well as being responsible for vision, olfaction and taste. Based on sequence and structural similarity they are classified into six families, A-F.[20] Class A includes around 700 rhodopsin-like receptors representing the largest family; class B encloses 15 secretin receptor family members; class C consists of 15 metabotropic glutamate receptors. The classes D and E represent pheromone and cAMP receptors of fungi and slime molds. Finally, 24 frizzled receptors comprise class F. More recently, an alternative classification (GRAFS) was suggested[21], in which GPCRs are clustered into five families: **G**lutamate, **R**hodopsin, **A**dhesion, **F**rizzled/Taste2 and **S**ecretin. The largest family of rhodopsin-like receptors is divided further into four branches α, β, γ, δ according to phylogenetic similarity. Besides about 460 olfactory receptors, the rhodopsin family also contains most of the important GPCRs for pharmacology and drug development, for example opioid, purinergic, cannabinoid, muscarinic acetylcholine and several monoamine-activated receptors like adrenergic, histamine, dopamine, serotonin receptors etc. However, not all native transmitters for GPCRs have been found yet, there are still nearly 100 orphan receptors, for which the natural primary messengers remain undiscovered.[22]

1.2 Cannabinoid receptors

The plant *Cannabis sativa* has been used since ancient times for medicine, recreation, religious ceremonies as well as the source of hemp fiber, seed oils and livestock feed. Throughout history, the flowers and resin of cannabis have been prepared to treat an array of illnesses from menstrual disorders, gout, rheumatism, constipation, nausea and vomiting to fever, inflammation, pain, glaucoma, insomnia, epilepsy and anxiety.[23]

In 1964, Δ^9-tetrahydrocannabinol (Δ^9-THC or THC, **1**, Figure 1.4), the main psychoactive ingredient in cannabis was successfully isolated and characterized.[24] And after almost 3 decades, the first THC-stimulated receptor (aka. cannabinoid receptor 1 or CB_1) was cloned as an orphan receptor from a rat brain cDNA library.[25] Within a few years, the second subtype (CB_2) of cannabinoid receptors was found in a human leukemic cell line.[26] During this period, anandamide (**2**, Figure 1.4)[27] and 2-arachidonoylglycerol (2-AG, **3**, Figure 1.4)[28,29] were the first natural ligands of cannabinoid receptors, the so-called endocannabinoids, to be identified. These lipids are synthesized on-demand from cell membrane arachidonic acid derivatives. Anandamide functions as a partial agonist at cannabinoid receptors with a higher preference for the CB_1 than the CB_2 subtype. The partial agonistic nature as well as the affinity for the CB_1

receptor of anandamide is similar to that of THC. 2-AG, on the other hand, is a full agonist at cannabinoid receptors.[30] Later on, some other anandamide analogs and *N*-acyldopamines have also been found to act on cannabinoid receptors.[31] The discovery of THC along with many other cannabinoids produced by cannabis stimulated the synthesis of numerous analogs that included not only compounds structurally similar to phytocannabinoids but also analogs with different chemical structures, for example the potent CB agonist CP55,940 (**4**, Figure 1.4) and the CB_1 antagonist SR141716A (**5**, Figure 1.4).[32]

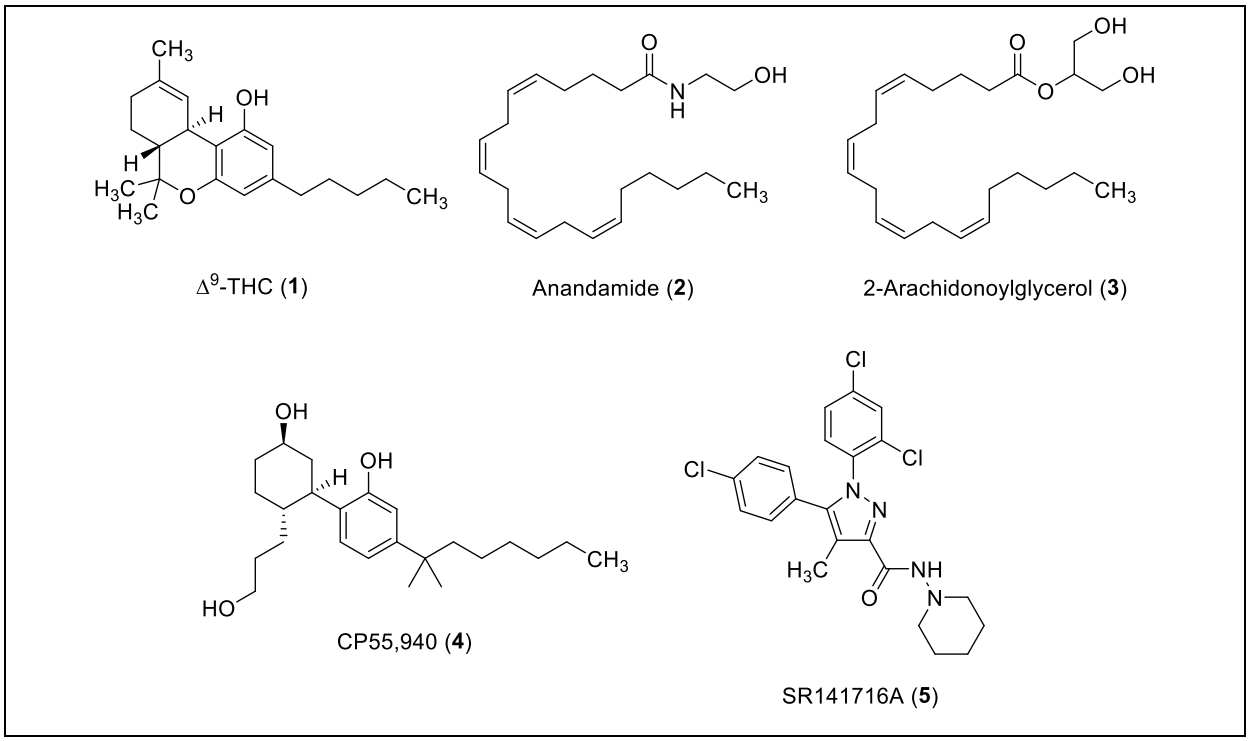


Figure 1.4: Structures of some prominent cannabinoids.

Cannabinoid receptor subtypes 1 and 2 are cell surface receptors possessing the typical seven transmembrane domains of GPCRs and belong to the branch α of class A (rhodopsin-like) GPCRs. Both receptors couple to $G_{i/o}$ proteins and are able to inhibit adenylate cyclase thus activate MAP kinase.[32] The CB_1 receptor is the major cannabinoid receptor in the central nervous system (CNS), highly expressed in cerebral cortex and hippocampus, regions that are responsible for behavioral effects of cannabinoids. Lower levels of CB_1 expression were also found in the peripheral nervous system as well as in many other tissues, such as bone marrow, heart, lung, prostate, testis, etc.[33] In addition to the $G_{i/o}$ pathway, CB_1 receptors can also signal through G_s proteins leading to cAMP accumulation, which could be in association with a co-expressed dopamine receptor.[34] The CB_2 receptor has 44 % amino acid identity with its CB_1 subtype. Predominant expression of CB_2 has been detected in cells of the immune system, especially in peripheral blood cells including B lymphocytes, natural killer cells and monocytes.

By activating the CB_2 receptors, immune cell migration and cytokine release can be regulated.[32] There are evidences that some CB_1 receptors are also expressed on non-neuronal cells, including immune cells[33] and CB_2 can be detected on some neurons, both central and peripheral ones.[35]

Although compounds that act on cannabinoid receptors possess potential therapeutic benefits, their applications are currently limited mainly owing to psychoactive side-effects. Only a few pharmaceuticals are on the market, these are Nabilone, a structural analog of THC, and Dronabinol or synthetic THC, which were approved to treat chemotherapy-induced nausea and vomiting[36] as well as to stimulate appetite in AIDS patients.[37] The CB_1 antagonist SR141716A (Rimonabant) had been approved as an antiobesity drug but was withdrawn later due to the serious depressive adverse effects leading to suicide.[38] Another cause for the moderate development of cannabinoid therapeutics is the fact that interactions of cannabinoids, both endogenous, phyto and synthetic, are not restricted to the CB_1 and CB_2 receptors, but also extend to other targets, for example, transient receptor potential cation channel vanilloid (TRPV) receptors,[39] peroxisome proliferator-activated receptors (PPARs)[40] and some orphan GPCRs like GPR18 and GPR55. As the main subjects of this thesis, GPR18 and GPR55 will be further described in the next chapters.

1.3 G protein-coupled receptor 18

Recently, an abnormal-cannabidiol-sensitive receptor has been identified as the orphan GPR18,[41] which mediates mesenteric vasodilation[42] in mice and microglial migration.[43] The activating effects induced by anandamide and abnormal cannabidiol (abn-CBD, **7**, Figure 1.5) on this receptor can also be mimicked by THC[44] and blocked by SR141716A.[42] It was also reported that *N*-arachidonylglycine (NAGly, **8**, Figure 1.5), an endogenous metabolite of ananamide, stimulated Ca^{2+}-influx and inihibited forskolin-induced cAMP production in GPR18-transfected Chinese hamster ovary (CHO) cells. The authors proposed that NAGly is an endogenous ligand of the $G_{\alpha i/o}$ protein-coupled receptor 18.[45] However, in a study examining the pharmacological interactions of different lipids with various GPCRs, NAGly did not activate GPR18.[46] A more recent study showed no inhibition of calcium release after application of NAGly on GPR18-expressing neurons.[47] In our work-group, established ß-arrestin assays of GPR18 could not confirm the above-mentioned proposal of NAGly being a GPR18 agonist.[48] Latest investigations point to the existence of another endogenous GPR18 activator: the polyunsaturated fatty acid metabolite Resolvin D2 (RvD2, **9**, Figure 1.5), which

is mainly involved in inflammatory processes,[49] but its confirmation as a GPR18 agonist is still awaited.

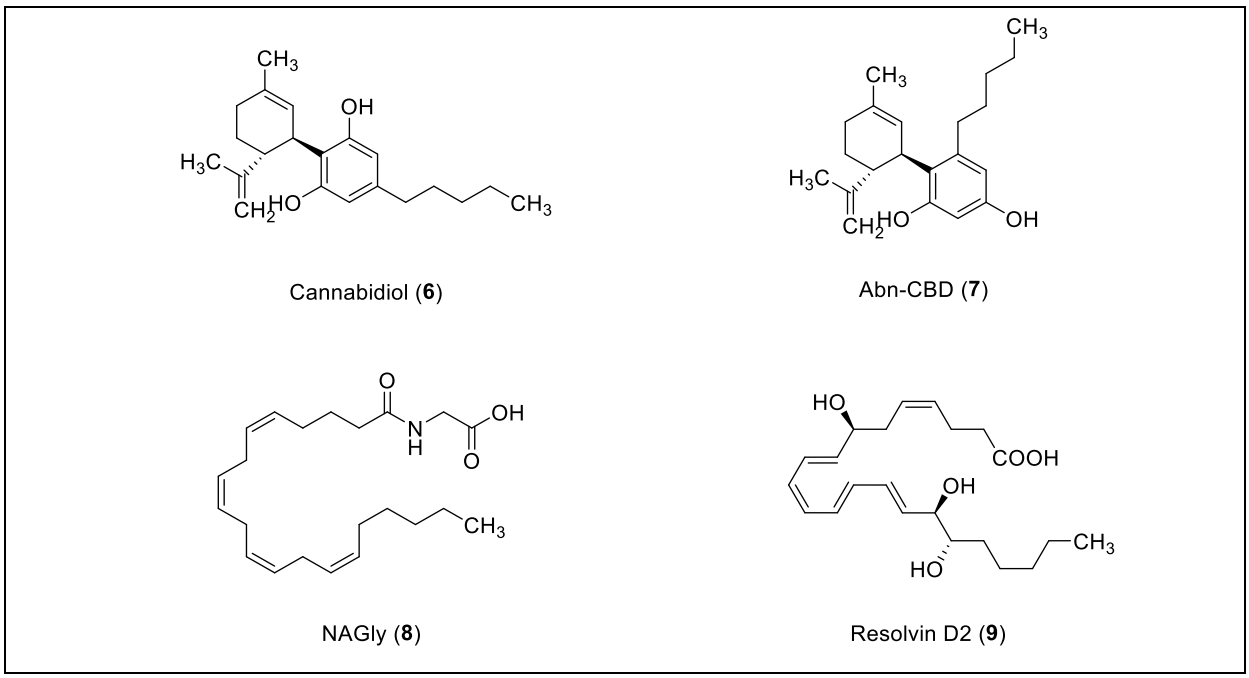


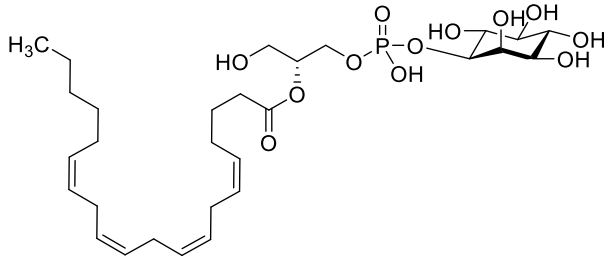
Figure 1.5: Cannabidiol, a major phytocannabinoid and its synthetic analog abnormal cannabidiol (abn-CBD) as well as the proposed endogenous GPR18 ligands *N*-arachidonoylglycine (NAGly) and Resolvin D2.

Despite sharing several ligands with cannabinoid receptors, GPR18 belongs to a different branch (branch δ) of class A, rhodopsin-type receptor family and displays low sequence identity with CB_1 and CB_2 (13% and 8%, respectively).[32] The 331-amino acid GPCR is mainly expressed in organs of the immune system, such as spleen and thymus. Accordingly, it was suggested to be involved in the regulation of the immune system.[45] Moderate GPR18 expression can be found in brain, testis, ovary, and lung.[50] More recently, the receptor was also detected in metastatic melanomas [51] and human spermatozoa. [52] Moreover, GPR18 was proposed to participate in normal T cell homeostasis in the small intestine.[53]

1.4 G protein-coupled receptor 55

The orphan G protein-coupled receptor 55 (GPR55) had been proposed as a candidate for a new cannabinoid receptor subtype. However, studies of cannabinoids on GPR55 delivered mixed results. In particular, activation of this receptor by THC[54], anandamide and CP55,940[55] has been reported earlier, but a more recent study could not demonstrate activation of GPR55 by THC and anandamide in ß-arrestin assays using human embryonic kidney cell line (HEK293 cells). In the same study, the cannabinoid receptor agonist CP55,940 has been found to

antagonize GPR55 and the CB_1 antagonist SR141716A was able to activate the receptor.[56] In an effort to examine the effects of different phospholipids on GPR55, Oka *et al.* observed that a mixture of lysophosphatidylinositols (LPIs) derived from soybean was capable of inducing phosphorylation of MAPKs and transient increase of intracellular calcium level in GPR55-expressing HEK293 cells.[57] In a further study, a particular species of LPI, 2-arachidonoyl-*sn*-glycero-3-phosphoinositol (2-AGPI, **10**, Figure 1.6) found in rat brain has been shown to be more potent than soybean LPI.[58]



2-AGPI (**10**)

Figure 1.6: Molecular structure of the proposed endogenous GPR55 activator: 1-LPI.

Similar to GPR18, GPR55 is also a member of branch δ of the rhodopsin-like (class A) receptors, thus exhibits low sequence identity to both CB_1 (13 %) and CB_2 (14 %) subtypes. The orphan receptor has also been found to couple with G_q, G_{12}, or G_{13} for signal transduction, which results in downstream activation of RhoA and phospholipase C (PLC).[54, 59] High expression of GPR55 can be detected in some parts of the mammalian brain such as caudate putamen, frontal cortex, striatum, hypothalamus, as well as in glial cells and large dorsal root ganglia neurons. mRNA of this receptor is also expressed in different cell types including lymphocytes, endothelial cells, prostate and ovarian cells, as well as in several organs like spleen, stomach and intestine.[59] A study has also determined a correlation of the GPR55/LPI system with the proliferation rate and aggressiveness of cancer cells.[60] Moreover, GPR55 could play a role in cardiovascular functions, nociception, inflammation and bone metabolism.[61] A very recent research report has discovered that GPR55 may be associated with obesity in humans.[62]

1.5 Synthetic ligands for GPR18 and GPR55

1.5.1 Atypical cannabinoids

Atypical cannabinoids are synthetic compounds derived from the natural cannabinoids in cannabis, for example, the above-mentioned abn-CBD. Both abn-CBD analogs: O-1602 (**11**) and O-1918 (**12**) do not bind with high affinity to either the cannabinoid CB_1 or CB_2 receptor

but exhibit totally different activities at GPR18 and GPR55. O-1602 is an agonist at both GPR18[44] and GPR55[55] while O-1918 exerts antagonistic activity at these receptors.[63,64]

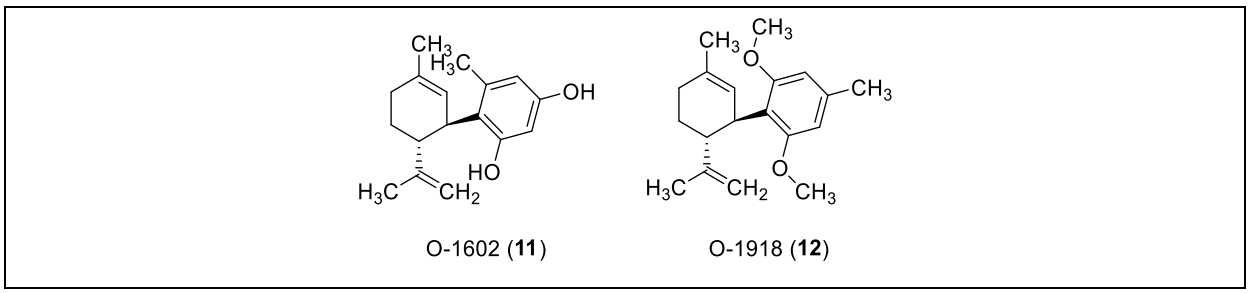


Figure 1.7: Molecular structures of two abn-CBD analogs: O-1602 and O-1918.

1.5.2 Coumarin derivatives

Several compounds with a coumarin scaffold have been shown to be a novel class of GPR55 antagonists. They exert from weak antagonistic properties to no activity at GPR18. Analysis of their structure-activity relationships indicates that the residue in position 7 of the coumarin scaffold is important for interaction with GPR55 and CB receptors. A long and bulky lipophilic residue at position 7 will lead to high inhibitory potencies of the compounds at GPR55. Thus, PSB-SB-487 (**13**) has been shown to be the most potent antagonist at GPR55 in this coumarin compound class possessing at the same time high affinities for the CB receptors. The absence of a substituent at position 7 led to an increased selectivity toward GPR55 and eradicated CB receptor binding but lowered the antagonistic activity of the coumarin derivative, e.g. PSB-SB-489 (**14**).[65]

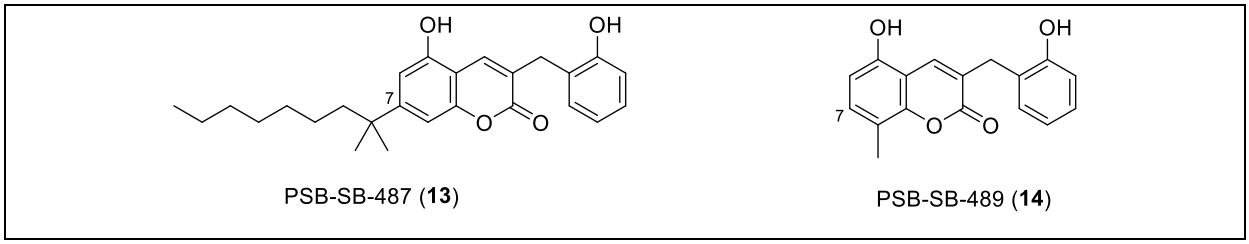


Figure 1.8: Coumarin derivatives as potent (selective) antagonists of GPR55.

1.5.3 Bicyclic imidazole-4-one derivatives

The imidazo[2,1-*b*]thiazin-3-one derivative **15** has been discovered as the most potent GPR18 antagonist among all bicyclic imidazole-4-one derivatives showing weak binding affinity to CB receptors and no interaction with GPR55 receptors. In contrast, imidazo[2,1-*b*]thiazin-3-one derivatives bearing benzylidene residues without bulky substituents such as **16** have been shown to be better tolerated by GPR55 while exhibiting no affinity to CB as well as GPR18 receptors.[48]

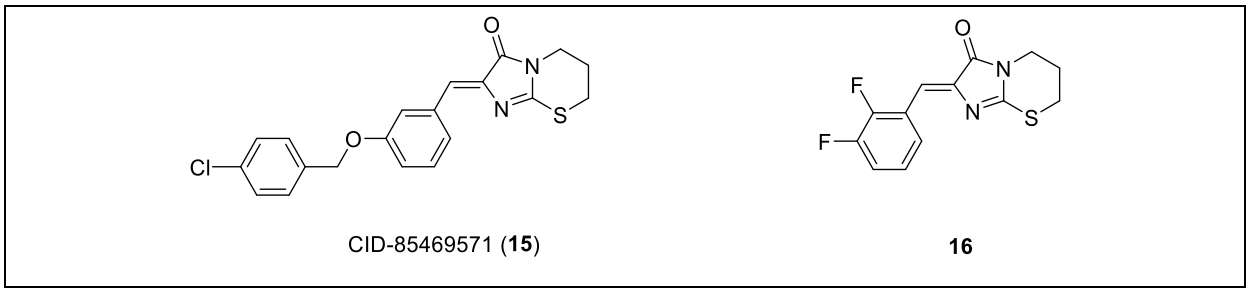


Fig. 1.5: Imidazo[2,1-*b*]thiazine derivatives as potent, selective antagonists of GPR18 and GPR55.

1.5.4 Magnolol derivatives

Most of the synthetic compounds based on the natural product magnolol are highly selective CB receptor ligands showing no or moderate inhibition of GPR18 and GPR55. None of them were able to activate these orphan receptors. However, some small modifications such as the methylation of a phenolic hydroxyl group could increase inhibitory potency at GPR55. Compound **17** is the most potent GPR55 antagonist among the magnolol series, but it is even more potent at the CB receptors and thus not selective.[66]

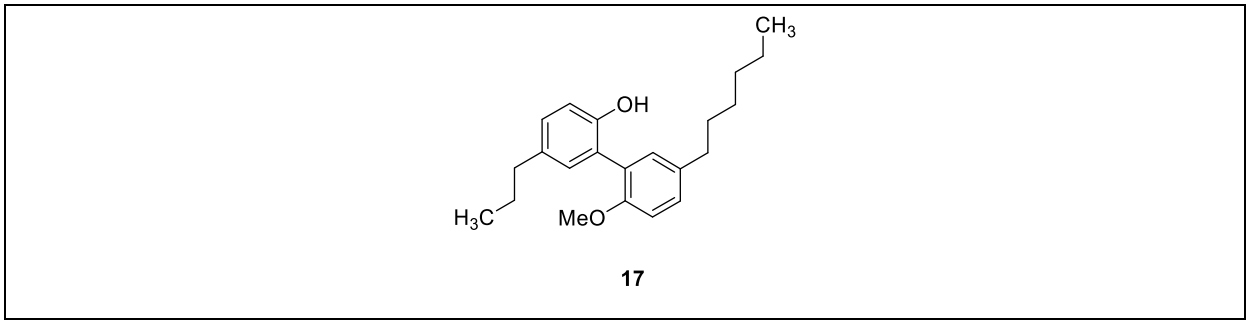


Fig. 1.6: A magnolol derivative which is a potent GPR55 antagonist, but also binds to CB receptors.

Summarized in Table 1.1 are the activities of some natural as well as synthetic ligands at GPR18 and GPR55.

Table 1.1: EC_{50}/IC_{50} values of selected ligands for GPR18 and GPR55 determined in different assays.

Compound	hGPR18		hGPR55	
	Activity	EC_{50} or IC_{50} (µM)	Activity	EC_{50} or IC_{50} (µM)
1 Δ^9-THC[65]	Agonist	4.61[a]	Antagonist	14.2[a]
10 1-LPI[48]	Antagonist	>10[a]	Agonist	1.00[a]
11 O-1602[44,55]	Agonist	0.065[b]	Agonist	0.013[c]
13 PSB-SB-487[65]	Antagonist	12.5[a]	Antagonist (CB affinity)	0.113[a]
14 PSB-SB-489[65]	Antagonist	>10[a]	Antagonist	1.77[a]
15 CID-85469571[65]	Antagonist	0.279[a]	Antagonist	>10[a]
16[48]	Antagonist	>10[a]	Antagonist	3.15[a]
17[66]	Antagonist	>10[a]	Antagonist (CB affinity)	3.25[a]

[a]EC_{50}/IC_{50} values obtained from ß-arrestin recruitment assays. [b]EC_{50}/IC_{50} values obtained from In-Cell Western assay to quantify MAPK phosphorylation. [c]EC_{50}/IC_{50} values obtained from [^{35}S]GTPγS binding assay.

2 Aims of the thesis

With around 800 members, G protein-coupled receptors comprise the largest and most important family of transmembrane receptors.[19] Signal transduction by GPCRs is involved in most physiological processes, from vision, smell and taste to neurological, cardiovascular, endocrine, and also reproductive functions, thus, making the GPCR superfamily a crucial target for therapeutic intervention. In fact, from all currently marketed drugs, more than 30 % are targeting GPCRs.[67] Despite continuous progresses in GPCR research, there is still a large number of orphan receptors. Deorphanizing receptors by revealing their natural transmitters can lead to solve their (patho)physiological roles.

Two orphan receptors are GPR18 and GPR55, which possibly have essential roles in the immune system, metabolism and cancer, and thus emerge as potential targets for new drugs. In this study, compounds of both natural and synthetic origin will be designed and subsequently evaluated in pharmacological assays at GPR18 and GPR55, and additionally for selectivity versus CB_1 and CB_2 receptors. The obtained data will provide information helping us to potentially discover the endogenous ligands of the receptors as well as to study structure-activity relationships, which serve as a basis to develop more potent and selective agonists, antagonists and modulators as pharmacological tools for research studies on these receptors.

3 Results and discussion

3.1 Synthesis of phenothiazine derivatives

3.1.1 Introduction

The discovery of the heterocyclic drug phenothiazine dates back to 1876 when Heinrich Caro investigated aniline-based dyes and synthesized a pigment known as methylene blue.[68] Initially it was used by Paul Ehrlich in his cell staining experiments. During these experiments he observed that methylene blue can also stain bacteria and parasites such as malaria. The compound was subsequently tested clinically and by the end of 19th century, methylene blue was being widely employed as the first synthetic drug for the treatment of malaria and later also livestock parasites. Further researches in the late 1940s led to the preparation of promethazine, which was the most powerful sedative at that time, and chlorpromazine, a low-potent but pioneer drug for the treatment of schizophrenia (Figure 3.1).

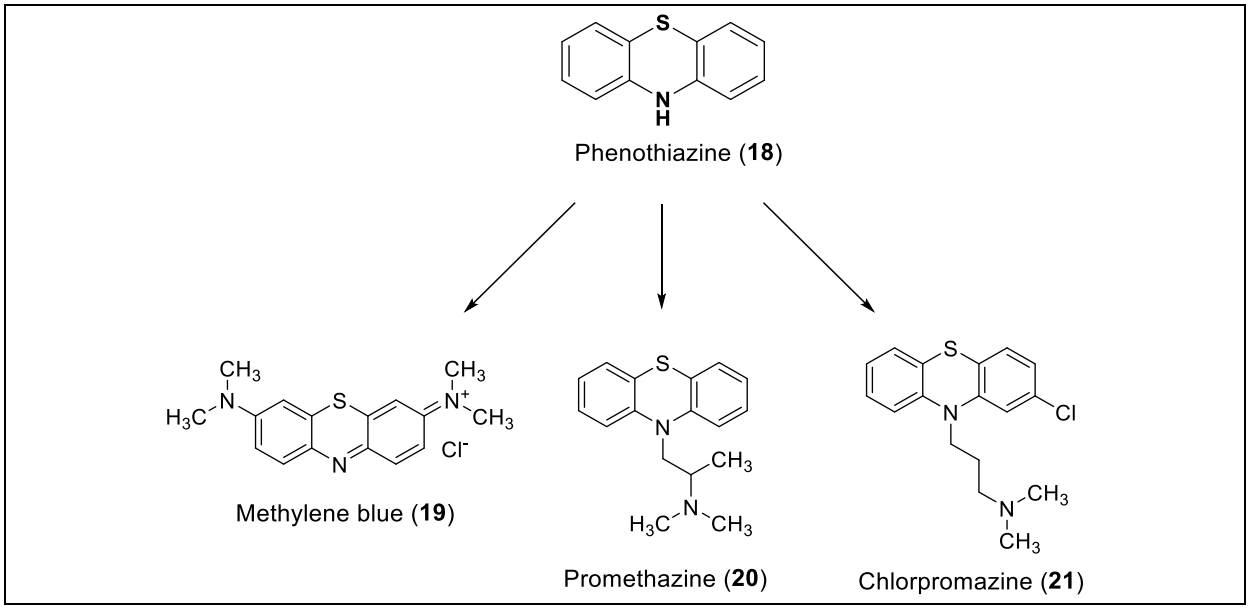


Figure 3.1: The phenothiazine parent compound and its early developed drugs.

Possessing a relative high lipophilicity, phenothiazine drugs can easily cross the blood-brain barrier and interact with a variety of receptors in the central nervous system including dopaminergic, serotonin, histamine, GABA, muscarinic and also α-adrenergic receptors. The effectiveness of phenothiazine-derived antipsychotics rely mainly on their ability to block the dopamine receptor subtype 2 (D_2 receptor) in the mesolimbic pathway of the brain.[69] However binding of these agents at D_2 receptors in other parts of the dopaminergic pathway as well as at different receptors cause mild to severe side effects like sedation, dry mouth, low blood

pressure, fast heart rate, weight gain and some drug-induced movement disorders, the so-called extrapyramidal symptoms. Phenothiazine antipsychotics with different types of side chains at the nitrogen atom usually have different potencies and side effect profiles. Aliphatic phenothiazines like chlorpromazine are weakly potent, and tend to have more anticholinergic than neurologic adverse effects, while piperazine-bearing analogs like perphenazine (**22**) and fluphenazine (**23**) are more potent but possess higher risk of extrapyramidal symptoms.[70]

In our workgroup, more than 400 drugs of a proprietary collection were screened using the ß-arrrestin assay at GPR18 to find new lead structures. As a result, chlorpromazine, perphenazine, fluphenazine, and fluphenazine sulfoxide (**24**) are the phenothiazine antipsychotics that appeared to be capable of inhibiting THC-induced GPR18 activation at low micromolar concentrations (Figure 3.2).

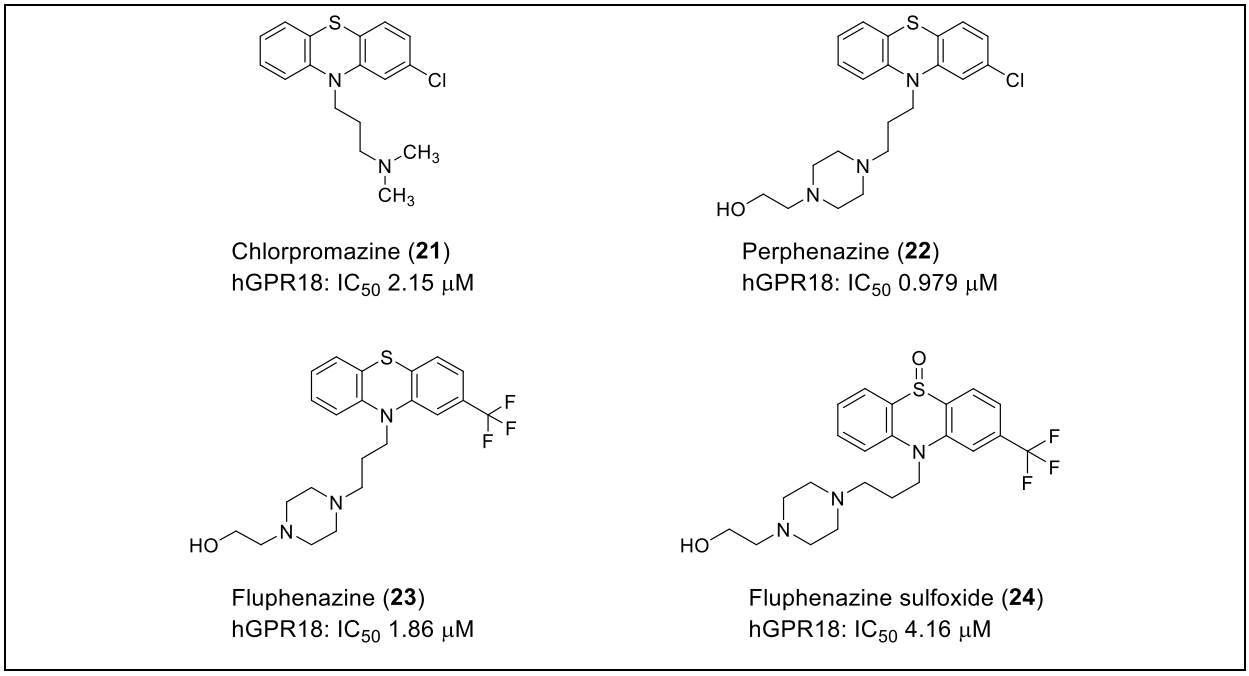


Figure 3.2: Some phenothiazine antipsychotics that act as GPR18 antagonists.

In the absence of the agonist THC, these drugs were even able to reduce the assay signal potentially indicating an inverse agonistic mode of action (Figure 3.3 A and B). In a later experiment, perphenazine in different concentrations was added to the activation of GPR18 by THC resulting in a suppression of the activation curves; the response was still increased but the maximal effect of THC could not be reached (Figure 3.3 C). This likely represents a non-competitive inhibition, in which the antagonist does not bind to the same binding site of the agonist, but induces a conformational change of the receptor, so that the affinity of the agonist is decreased.

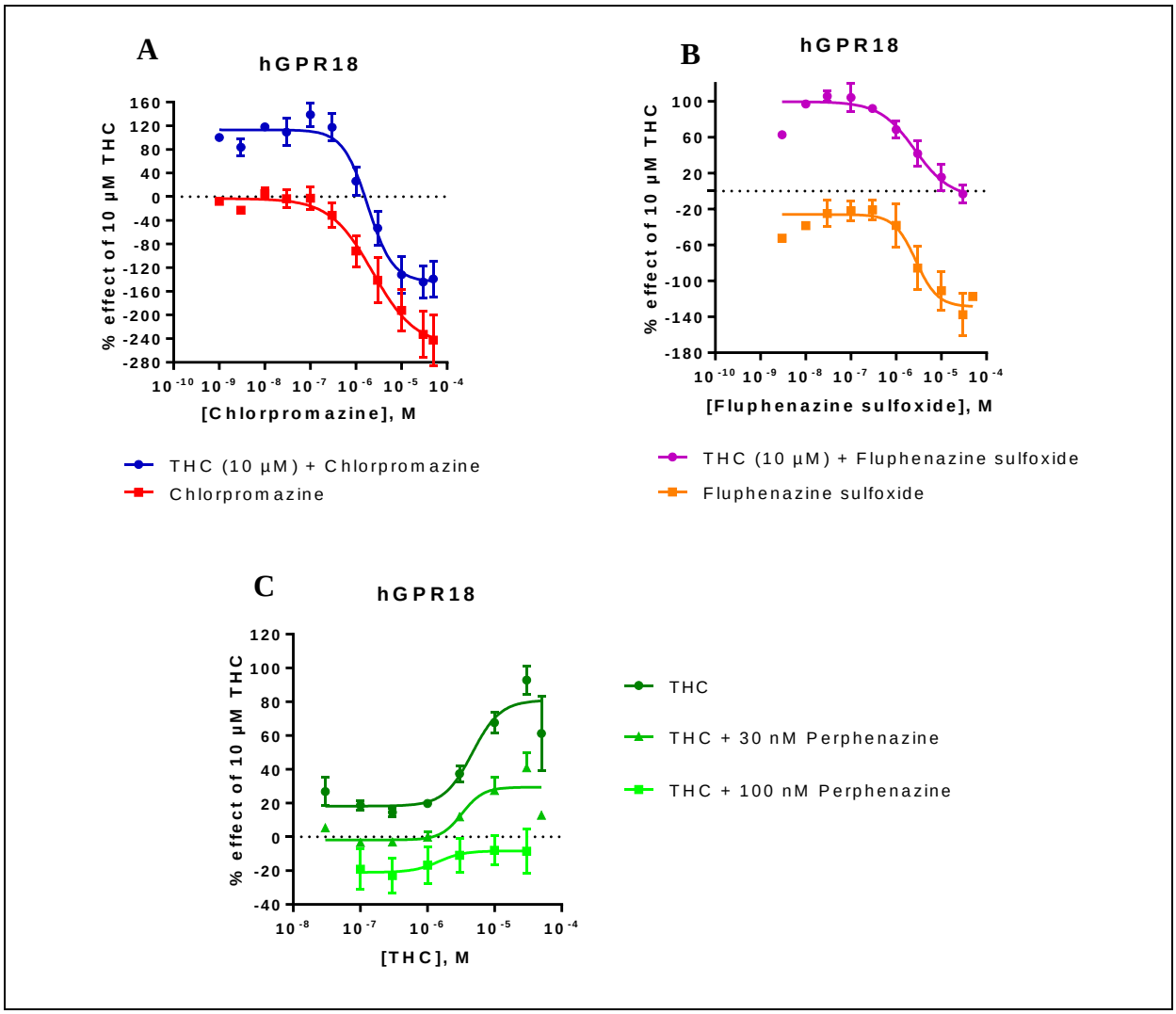


Figure 3.3: Effect of phenothiazine drugs at GPR18. **(**A) Inhibition curve of chlorpromazine in the presence of 10 µM THC (blue) and dose-response curve of chlorpromazine alone (red). **(**B): Inhibition curve of fluphenazine sulfoxide in the presence of 10 µM THC (green) and dose-response curve of fluphenazine sulfoxide alone (orange). (C) Activation curve of THC alone and in the presence of perphenazine in different concentrations.

A comparison between the potencies of the mentioned phenothiazine drugs at GPR18 revealed that a chlorine substitution at position 2 of the heterocyclic core is more beneficial than a trifluoromethyl group. And furthermore an unoxidized thioether was clearly more preferred than a sulfoxide residue. The propylpiperazinylethanol substituent at position N-10 remains unchanged in all these drugs; it was probably optimized for the activity at dopamine receptors. But for the investigation of GPR18 and GPR55, this residue can be modified to obtain more potent and selective agonists and antagonists. Figure 3.3 illustrates 2-chlorophenothiazine (**25**) as the main scaffold for the synthesis of compounds described in the following section.

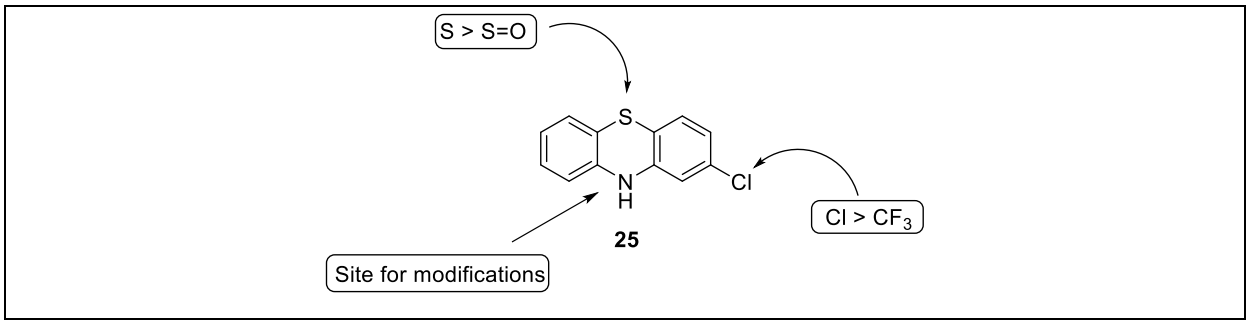


Figure 3.4: The core structure 2-chlorophenothiazine.

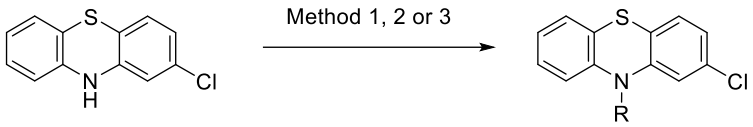
3.1.2 Alkylation at the N-10 position of 2-chlorophenothiazine

In most of the reactions, primary alkyl halides were used as versatile and readily available alkylating reagents. Since primary alkyl halides prefer an S_N2 mechanism, the reaction rate depends heavily on the strength of the nucleophile. However, 2-chlorophenothiazine possesses a low basicity and thus a weak nucleophilicity due to delocalization of the nitrogen non-bonding electron pair into the aromatic ring. *N*-Alkylation of such amines usually requires a powerful but non-nucleophilic base to deprotonate the amino group whereupon a negatively charged nitrogen with increased electron density and nucleophilicity will be formed. The impact of solvents on the nucleophile is not negligible. Polar protic solvents like alcohols, acetic acid, water etc. are able to create a shell around the nucleophilic anion through hydrogen bonding making it less reactive. On the other hand, polar aprotic solvents such as *N*,*N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), acetone, acetonitrile, do not form hydrogen bonds with the bare nucleophile leaving it more reactive.

Generally the alkylations of 2-chlorophenothiazine (**25**) using method 1 (Table 3.1) afforded moderate yields (32 - 52 %). In method 1, NaH was added as a single portion into a solution of 2-chlorophenothiazine at 0 °C and stirred for 10 minutes prior to treatment with the alkyl halide. However, the reaction of 1-bromo-3-chloropropane and 2-chlorophenothiazine under this conditions did not deliver the desired product **42**. Consequently, an alternative method described by Du *et al.*[71](method 2) was attempted and the compound could be obtained at 54 % yield. The key differences in the latter method are the reduction of solvent volume, a drastic increase of alkyl halide equivalent and an additional portion of NaH after a period of time. Consequently, method 2 was applied to a series of longer chain alkylations (compounds **30** – **34**) as well as for the preparation of **38** and **39** resulting in moderate to high yields. In cases of **35** and **37**, the combination of NaOH as a strong base and DMSO as s solvent also worked well. It is noteworthy that all those GPR18-inhibiting antipsychotics illustrated in Figure 3.2 have an aminopropyl residue at the N-10 position. Hence, some derivatives with a propyl linker (**40** –

43) were synthesized to investigate the importance of the substitution attached to this linker for the interaction with our biological targets.

Table 3.1: Methods for the synthesis of *N*-Alkylated phenothiazine derivatives and their isolated yields.



25 **26** - **44**, 12 - 87 %

Method 1: DMF, NaH (1.0 eq.), 10 min, R-X (1.2 eq.), rt, 4h
Method 2: R-X (3 eq.), DMF, NaH (2 x 1.1 eq.), rt, 1.5 h
Method 3: DMSO, NaOH (1.3 eq.), R-X (1.2 eq.), rt, overnight

Comp.	R	Method	Yield (%)[c]
26	methyl	**1**	50
27	ethyl	**1**	52
28	propyl	**1**	44
29	isopropyl	**1**	38
30	butyl	**2**	75
31	pentyl	**2**	82
32	hexyl	**2**	84
33	heptyl	**2**	81
34	octyl	**2**	87
35	cyclohexylmethyl	**3**	32
36	cyclohexylethyl	**1**[a]	34
37	benzyl	**3**	50
38	(3-methoxyphenyl)methyl	**2**	83
39	(2-cyanophenyl)methyl	**2**	28
40	3-phenylpropyl	**1**[b]	41
41	3-aminopropyl	**2**	58
42	3-chloropropyl	**2**	54
43	3-hydroxypropyl	**2**	12
44	indol-3-yl-ethyl	**2**	16

[a] reaction conducted at 60 °C for 4 h. [b] reaction conducted at 60 °C for 24 h. [c] isolated yield.

The most direct approach to prepare the indole-bearing derivative **44** would be an alkylation of 2-chlorophenothiazine **25** with the already available 3-(2-bromoethyl)indole **45**. The standard

strategy of employing NaH and DMF only gave a low yield of the main product along with several unknown impurities (Figure 3.5). A possible reason for this phenomenon could have been the presence of the unprotected indole NH-group, which can be deprotonated by NaH and subsequently undergo side reactions with one or more electrophiles in the reaction mixture. However, an attempted alkylation of 2-chlorophenothiazine with a Boc-protected 3-(2-bromoethyl)indole was not successful. In another effort using potassium *tert*-butoxide (KOtBu) and tetrahydofuran (THF), the product **44** could not be detected in the reaction mixture. Hence the synthesis of **44** certainly requires optimization.

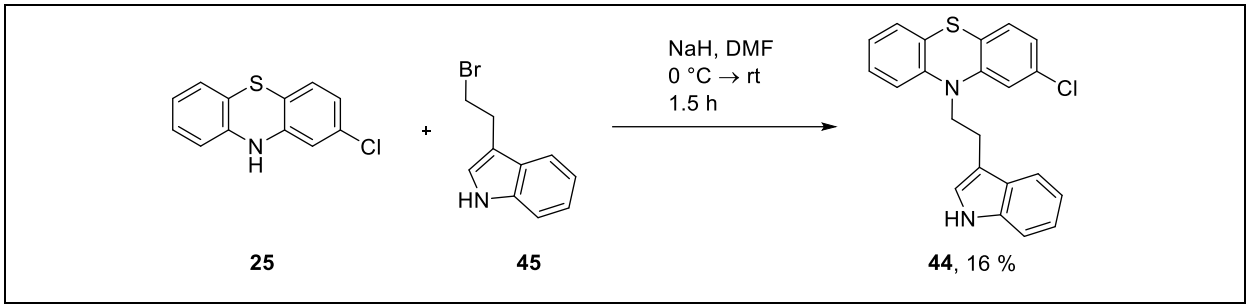


Figure 3.5: Synthesis of the phenothiazine-indole hybrid via alkylation.

There are a few cases where the yields were also low (**43** and **39**) probably due to the unprotected hydroxyl group or unexpected reduction of the cyano group by NaH.

3.1.3 Synthesis of 2-(1*H*-indol-3-yl)-1-(2-chloro-10*H*-phenothiazin-10-yl)ethanone (46)

In this part of the study, an amide derivative of **44** was prepared. This compound (**46**) has an additional carbonyl group at the first carbon of the ethyl linker. The synthesis starts with a temporary conversion of indole-3-acetic acid (**47**) to its acyl chloride. Thionyl chloride was the reagent of choice, superior to oxalyl chloride and phosphorus pentachloride because of its higher activity and easily removable gaseous by-products. The *N*-acylation between the resulting acyl chloride and 2-chlorophenothiazine was carried out twice under reflux conditions in two different solvents: toluene and *o*-xylene, which have relative high boiling points (110 and 144 °C, respectively) (Figure 3.6). The latter was found to be better since toluene gave a lower yield (13 %). Perhaps this type of reaction needs a high temperature to accelerate the reaction.

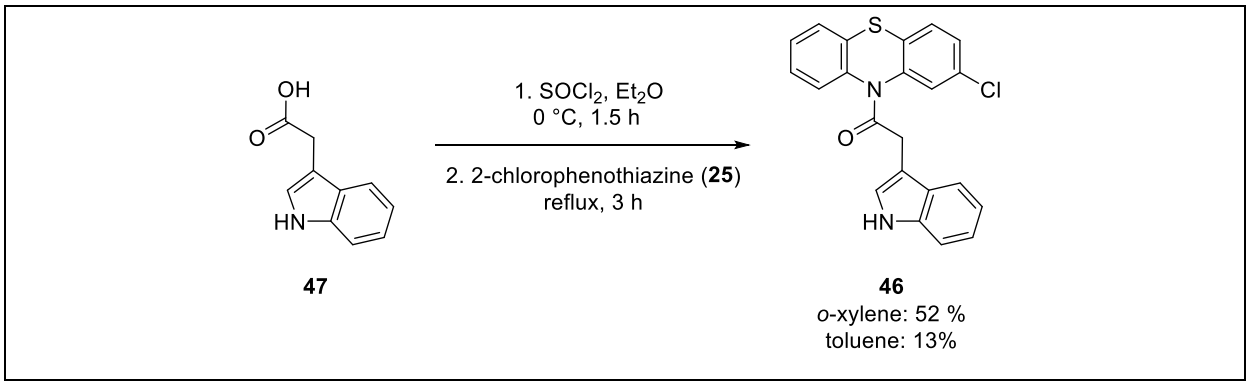


Figure 3.6: Two-step synthesis of compound **46** via acyl chloride conversion and subsequent acylation.

Due to the low yield in the direct synthesis of compound **44** via alkylation, an alternative route was probed, namely the amide group of compound **46** was left to react with three different reducing reagents: $LiAlH_4$, $NaBH_4$ and BH_3-THF complex, which have been reported to successfully reduce similar compounds as our starting material.[72] However, several experiments with $LiAlH_4$ always led to a cleavage of the amide bond giving 2-chlorophenothiazine and some unknown byproducts. Meanwhile in the reaction with $NaBH_4$ only a minor peak with the mass of the product was detected by LC-MS. Unfortunately, the first application of BF_3-THF did not deliver the desired product (Figure 3.7).

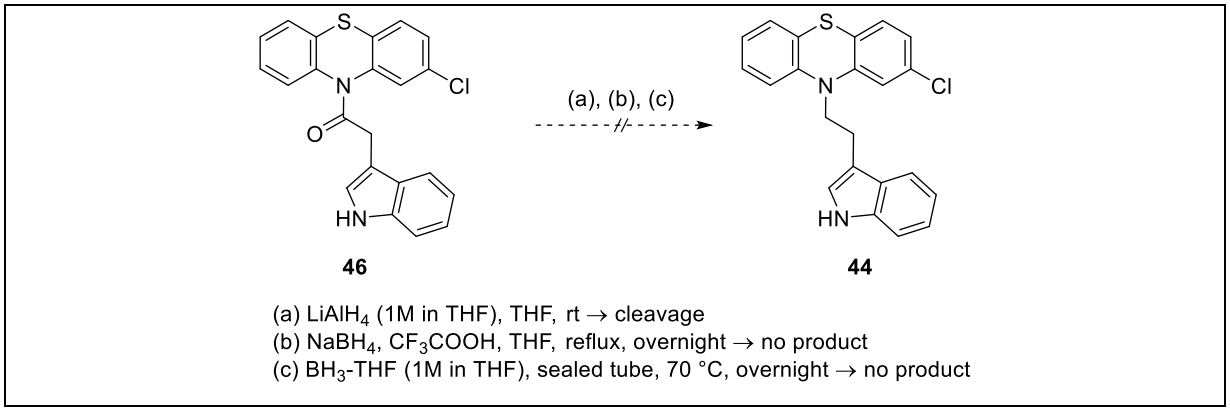


Figure 3.7: Attempts to synthesize compound **44** via amide reduction of **44**.

3.1.4 Synthesis of sulfonamide-linked phenothiazine analogs

It is well-known that the basic amino group in dopamine forms a salt bridge with the highly conserved Asp-114 residue in the binding site of dopamine receptors.[73] This feature is responsible for the activity of antipsychotics at D_2 receptors. It is possible to avoid this interaction just by abolishing the amino group. But if the presence of a hydrogen bond donor or acceptor is still required at this position, it is better to convert the amino group into a neutral polar substituent (sulfonamide, amide, urea etc.).

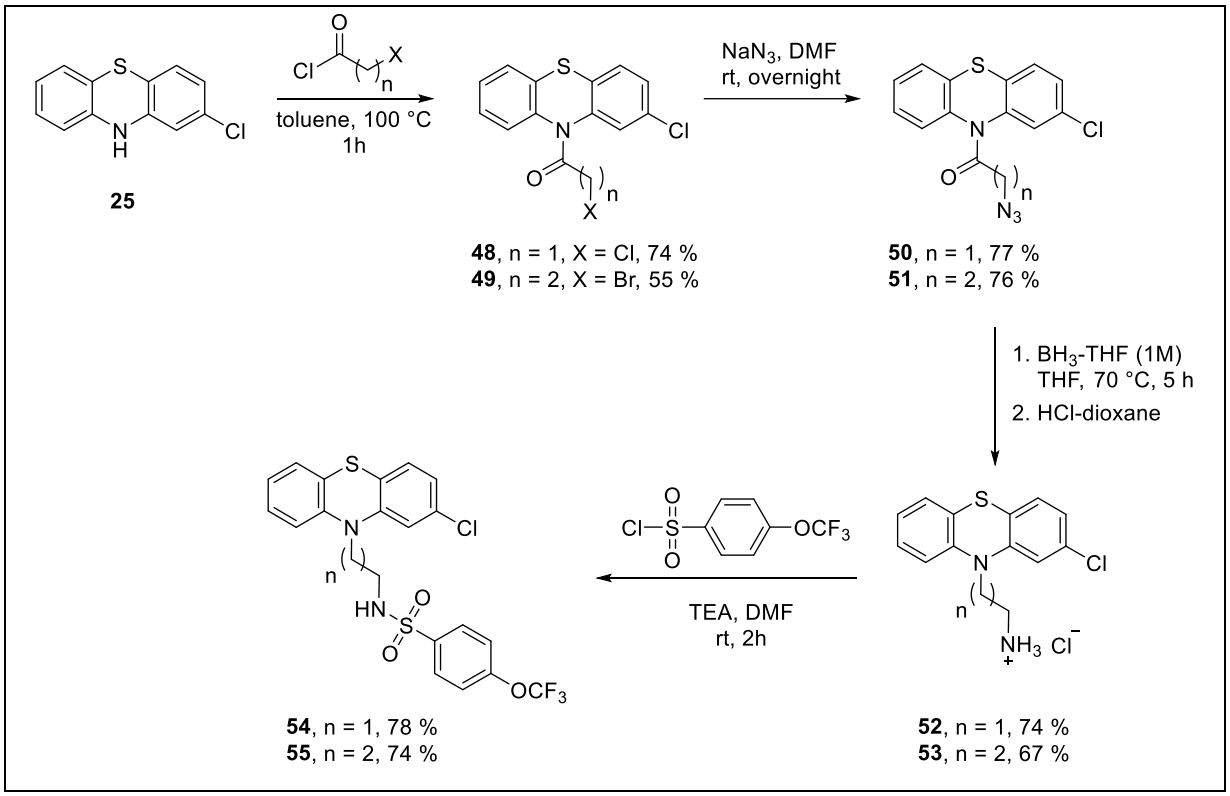


Figure 3.8: Multistep synthesis of sulfonamide-bearing phenothiazine derivatives

The synthesis of sulfonamide-linked phenothiazine analogs followed a multistep approach reported by Kastrinsky *et al.*[74] beginning with the acylation of 2-chlorophenothiazine by chloroacetyl chloride and 3-bromoproprionyl chloride, respectively. After the conversion of the halides (**48**, **49**) to the nitriles (**50**, **51**) using sodium azide, and combined reduction to the amine precursors (**52**, **53**) by BH_3-THF complex. These precursors were finally derivatized with 4-trifluoromethoxybenzenesulfonyl chloride to afford the products **54** and **55** (Figure 3.8). The obtained yields were sufficient and also comparable to those of literature.

3.2 Pharmacological assays of phenothiazine derivatives

3.2.1 Introduction

The antagonistic activity of some phenothiazine-derived drugs at GPR18 has just been discovered via ß-arrestin assays performed in our workgroup (see 3.1.1). The small ß-arrestin proteins are ubiquitously expressed and play an important role in the function of GPCRs. Upon activation, serine and threonine residues in the intracellular loops and the C-terminal tail of the agonist-bound receptor are phosphorylated by GPCR kinases (GRKs). These phosphorylated residues can be detected and bound by a ß-arrestin blocking recoupling of G protein subunits to the receptor/ß-arrestin complex, thus preventing further receptor stimulation.[75] This process is called desensitization.

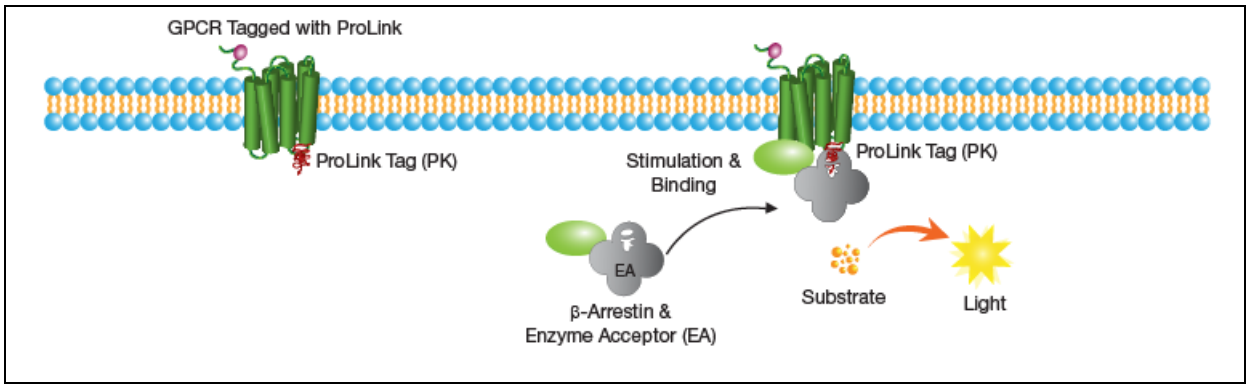


Figure 3.9: The principle of the PathHunter® ß-arrestin assay.

Based on this mechanism of ß-arrestin, a modified β-galactosidase complementation assay, referred to as PathHunter® technology, has been developed by DiscoverX (Fremont, CA, U.S.). The assay makes use of a small enzyme donor fragment ProLink™ (PK), which is tethered to the C-terminus of the target GPCR. This tagged receptor is co-expressed in cells producing a fusion protein of a β-arrestin with the larger, N-terminal deletion mutant of β-galactosidase (called enzyme acceptor or EA). Upon recruitment of β-arrestin to the PK-tagged GPCR, the two enzyme fragments merge together resulting in the formation of an active β-galactosidase enzyme. The increase in enzyme activity can be quantified with the aid of chemiluminescent detection reagents (Figure 3.9).[76] The ß-arrestin assay is not depending on G protein signaling and suitable for ligand screening and deorphanizing of GPCRs.

In this study, ß-arrestin recruitment assays using Chinese hamster ovary (CHO) cells stably expressing the target receptors were performed by Dr. Clara Schoeder. Agonistic activities of test compounds were compared to the effect of the GPR18 agonist THC (EC_{50} 4.61 µM) at 10 µM or GPR55 agonist LPI (EC_{50} 1 µM) at 1 µM. Whenever a compound shows an activation higher than 50 % (compared to the known agonist), full activation curves will be determined in order to calculate EC_{50} values. For antagonistic activity, if a compound exerts an inhibition of agonist-induced receptor stimulation higher than 50 %, full concentration-inhibition curves will be determined in order to calculate IC_{50} values. Curves were obtained in three separate experiments, each in duplicates.

3.2.2 Structure-activity relationships of phenothiazine derivatives

There are in total eighteen out of twenty five phenothiazine derivatives tested in ß-arrestin assays so far (Figure 3.10). None of them could activate the human GPR18 or GPR55 compared to the stimulation level of the known agonists. In the first set of compounds with different alkyl moieties substituted at the N-10 position. Derivatives carrying short alkyl side chain of up to 5 carbons (**26** – **31**) were able to inhibit GPR55 activation at low micromolar concentrations (IC_{50}

6.20 – 10.1 µM). In contrast, alkyl substituents with the length from 6 to 8 carbons (compounds **32 - 34**) were not tolerated. The binding pocket of GPR55 for the phenothiazine scaffold may not have enough depth for the binding of those compounds.

Table 3.2: Results from ß-arrestin assays of phenothiazine analogs at GPR18 and GPR55.

Comp.	**R**	**hGPR18**[a]		**hGPR55**[a]	
		EC_{50} ± SEM (µM) (% activation)[b]	IC_{50} ± SEM (µM) (% inhibition)[c]	EC_{50} ± SEM (µM) (% activation)[d]	IC_{50} ± SEM (µM) (% inhibition)[e]
26	methyl	>10 (-12 ± 16)	>10 (19 ± 9)	>10 (7 ± 5)	**10.1** ± 0.2
27	ethyl	>10 (12 ± 11)	>10 (5 ± 15)	>10 (-10 ± 13)	**6.70** ± 2.08
28	propyl	>10 (25 ± 3)	>10 (16 ± 12)	>10 (-2 ± 8)	**8.16** ± 1.57
29	isopropyl	>10 (39 ± 6)	>10 (1 ± 9)	>10 (6 ± 9)	**6.27** ± 1.74
30	butyl	>10 (-19 ± 12)	>10 (-36 ± 11)	>10 (-15 ± 12)	**7.22** ± 1.59
31	pentyl	>10 (-5 ± 12)	>10 (-47 ± 10)	>10 (9 ± 14)	**6.20** ± 1.24
32	hexyl	>10 (-3 ± 11)	>10 (16 ± 6)	>10 (28 ± 8)	>10 (28 ± 16)
33	heptyl	>10 (8 ± 6)	>10 (22 ± 7)	>10 (29 ± 9)	>10 (39 ± 11)
34	octyl	>10 (26 ± 8)	>10 (2 ± 10)	>10 (17 ± 12)	>10 (32 ± 16)
35	cyclohexylmethyl	>10 (17 ± 5)	>10 (-18 ± 13)	>10 (3 ± 11)	**8.41** ± 0.73
36	cyclohexylethyl	>10 (36 ± 11)	>10 (2 ± 10)	>10 (15 ± 11)	>10 (37 ± 14)
37	benzyl	>10 (18 ± 5)	>10 (7 ± 5)	>10 (30± 9)	**7.28** ± 1.41
38	(3-methoxyphenyl)methyl	>10 (8 ± 3)	>10 (31 ± 9)	>10 (0 ± 6)	(**58** ± 5)
39	(2-cyanophenyl)methyl	>10 (14 ± 9)	>10 (44 ± 15)	>10 (-6 ± 7)	(**53** ± 23)
40	3-phenylpropyl	>10 (39 ±9)	>10 (3 ± 16)	>10 (3 ± 11)	**5.29** ± 1.87
41	3-aminopropyl	>10 (-98 ± 15)	**4.89** ± 0.96	>10 (3 ± 16)	>10 (16 ± 14)
43	3-hydroxypropyl	n.d.	n.d.	>10 (-11 ± 11)	(**89** ± 16)
46	2-(indol-3-yl)-1-ethanoyl	n.d.	n.d.	>10 (-15 ± 10)	**7.48** ± 0.48

[a]receptors were expressed on CHO cells. [b]% activation in comparison to 10 µM THC induced luminescence signal. [c]% inhibition of 10 µM THC induced luminescence signal. [d]% inhibition of 1 µM LPI induced luminescence signal. [e]% activation in comparison to 1 µM LPI induced luminescence signal. All data with SEM resulted from three independent experiments, performed in duplicate.

In the series of compounds with cyclic substituents, compound **40** containing a phenyl ring separated by three carbons from the ring system was the most potent GPR55 antagonist possessing an IC_{50} value of 5.29 µM (Figure 3.10 B). By shortening the linker from three to one carbon, the potency was lowered to 7.28 µM as in the *N*-benzylated derivative **37**. A comparable potency could also be obtained from a phenothiazine analog bearing an indole residue **46** (IC_{50} 7.48 µM). Compounds linked with a substituted benzyl group (**38** and **39**) also exerted some inhibitory effects at GPR55, however more data are required to completely calculate the IC_{50} values. Through a comparison between the two related compounds **35** and **36**, it was shown that the extension of the linker at the cyclohexyl residue from one to two carbons clearly eliminated the antagonistic activity at GPR55.

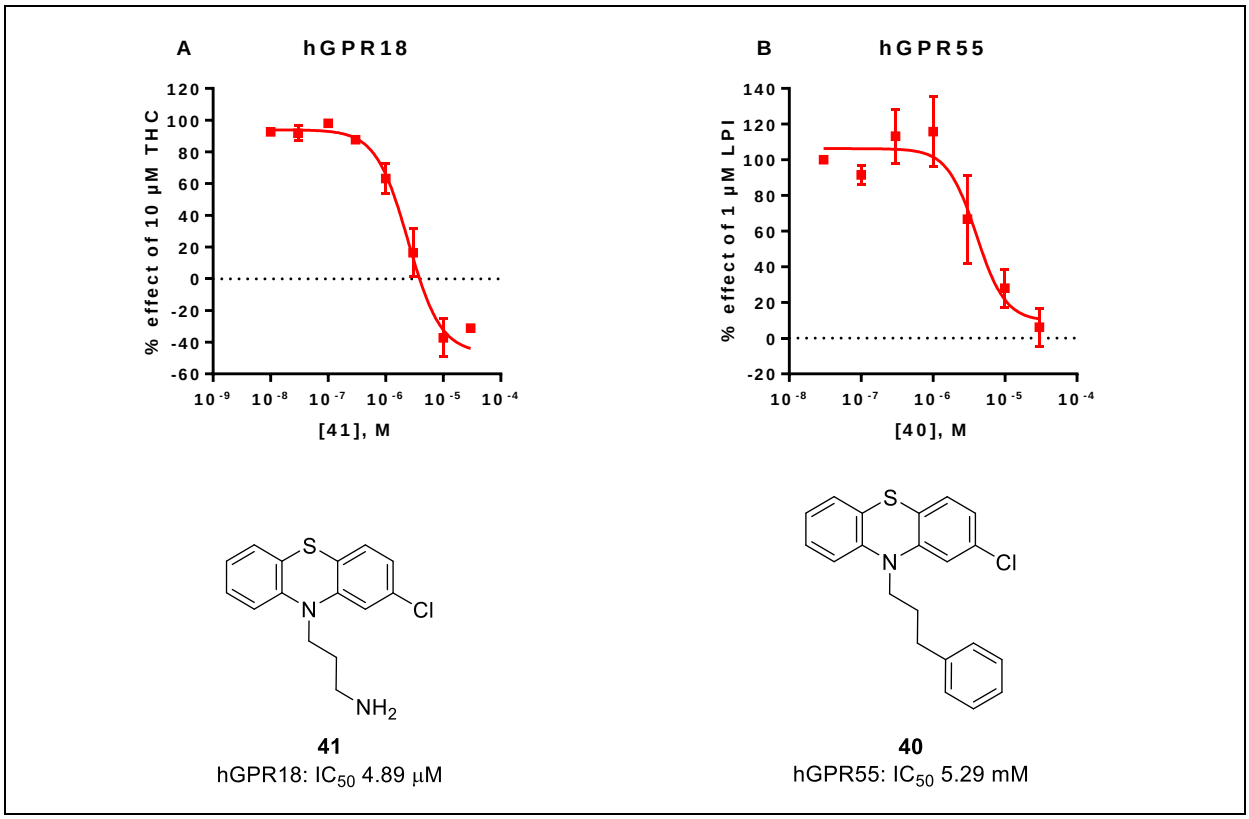


Figure 3.10: Inhibition curves and potencies of the most potent GPR18- and GPR55 antagonists from the phenothiazine series. (A) 2-Chloro-10-(3-aminopropyl)-10*H*-phenothiazine (**41**). (B) 2-Chloro-10-(3-phenylpropyl)-10*H*-phenothiazine (**40**).

As the only phenothiazine derivative containing a primary amino group in this series, compound **41** could inhibit THC-induced GPR18 activation with a moderate potency of 4.89 µM (Figure 3.10 A). It actually serves as the common core structure of some antipsychotics including chlorpromazine, perphenazine, fluphenazine and fluphenazine sulfoxide. The basic nitrogen atom might play an important role in the activity of those compounds at GPR18. The amino group in **41** was later exchanged for a hydroxyl group forming **43**, which in turn showed

GPR55-inhibiting properties in initial screening tests. However, an effect of **43** at GPR18 cannot be excluded, since assay data for this compound at the receptor are still missing.

3.3 Synthesis of *N*-acylamino acids

3.3.1 Introduction

N-Acylamino acids comprise a class of lipids, in which amino acids are linked covalently to long-chain fatty acids by an amide bond (Figure 3.11). To date, about 70 naturally occurring members as well as several synthetic analogs of the *N*-acylamino acid family have been identified.[77] However, for most of these molecules, their biological functions remain undiscovered.

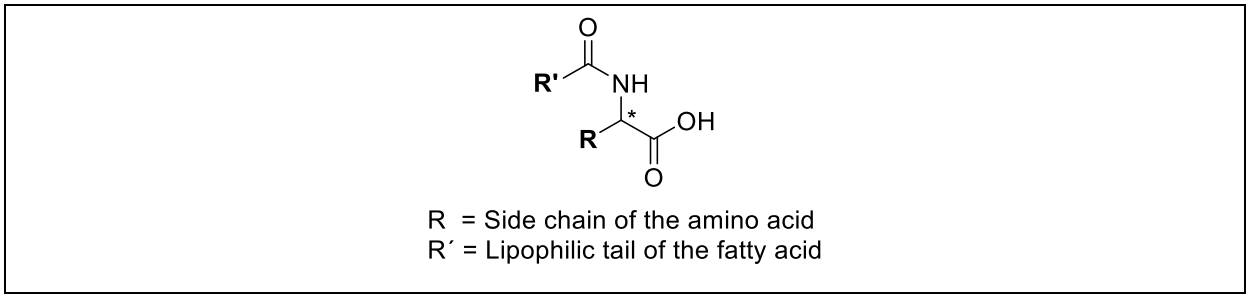


Figure 3.11: General chemical structure of *N*-acylamino acids.

N-Arachidonoylglycine (NAGly) was one of the first *N*-acylglycines to be identified *in vivo* in rat brain, spinal cord, small intestine, kidney and skin, at concentrations of approx. 50 - 140 pmol/g of dry tissue weight.[78] NAGly has been reported to be the physiological agonist of GPR18,[45] although this discovery could not be validated by some research groups regardless of test system.[46,47] A recent study concerning the presence of *N*-acylamino acids in rat brain has shown that conjugates of serine are the most abundant with 58.6 pmol/g wet brain for the palmitoyl derivative (**56**) and 35.2 pmol/g for the stearoyl analog (**57**).[79] Furthermore, *N*-palmitoylserine and *N*-arachidonoylserine (**58**) have been shown to exert neuroprotective effects in traumatic brain injury models.[80] Similar to fatty acyl serines, concentrations of palmitoyl (**59**) and stearoyl (**60**) glutamic acids also appeared to be high in rat brain (26.6 and 36.5 pmol/g wet brain, respectively).[79] These evidences, together with the fact that the eicosanoid lipids anandamide (**2**) and 2-arachidonoylglycerol (**3**) are natural transmitters of the endocannabinoid system, led us to assume that *N*-acylamino acids may serve as a new class of signaling molecules.

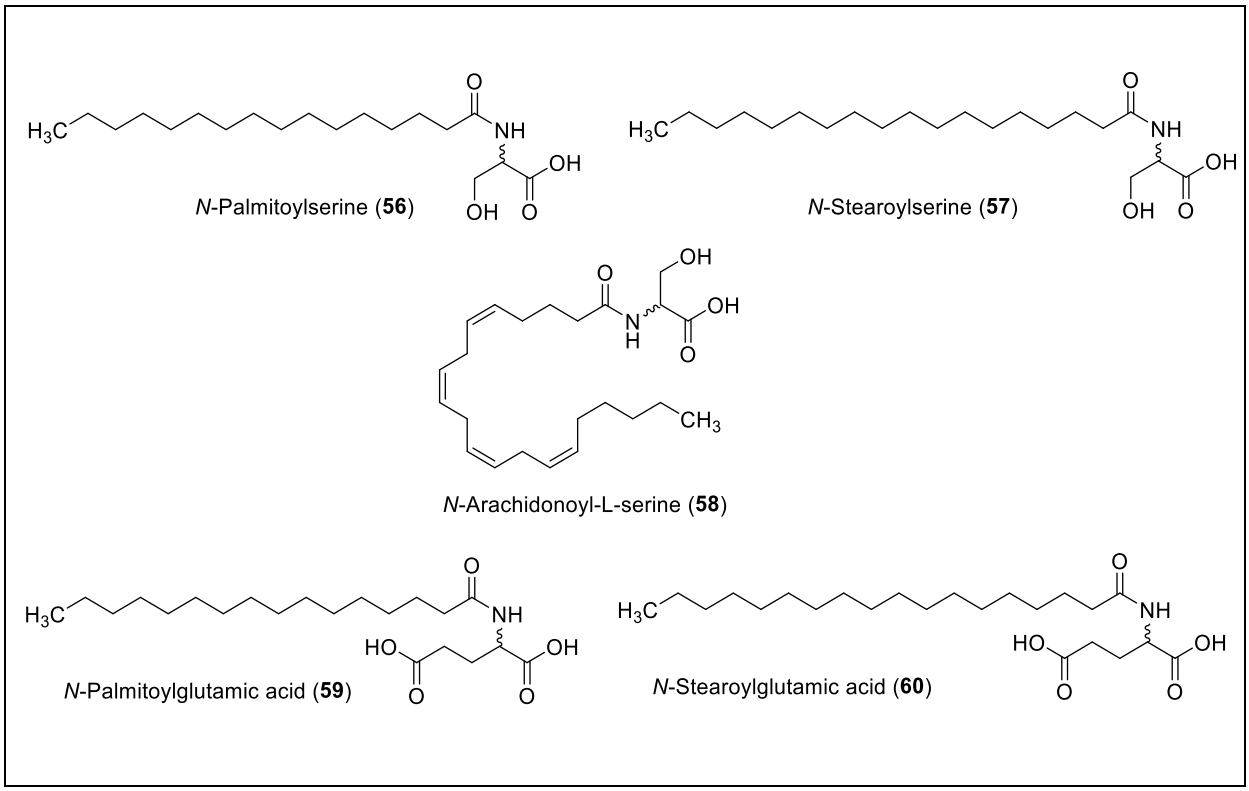


Figure 3.12: Some bioactive *N*-acylamino acids.

The formation of an amide bond at the N-terminus of amino acids can be performed in many ways. The main requirement is the conversion of carboxyl groups in fatty acids into other activated derivatives such as acyl halides, acyl azides, anhydrides, esters, etc. which provide good leaving groups after the attack of the nucleophilic amino function. This conversion can be conducted prior to the amide bond formation or *in situ* in a one-pot reaction. In this study we employed several methods to prepare *N*-acylamino acids.

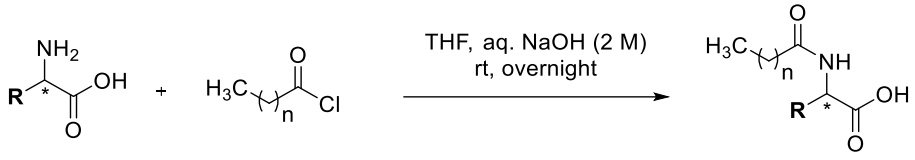
3.3.2 *N*-Acylation of amino acids using fatty acyl chlorides

A method for preparation of *N*-acylamino acids from fatty acyl chlorides has been described earlier.[81] This is a straightforward method since fatty acyl chlorides are readily available and inexpensive. The procedure required a single step, in which a solution of acyl chloride in THF was injected into the solution of the corresponding amino acid in aqueous NaOH. In contrast to the work-up protocol provided in the literature, a smaller amount of water was used to dilute the reaction mixture with the aim to decrease the amount of polar products dissolved in water and to thus increase the extraction yields.

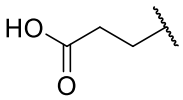
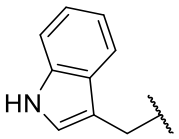
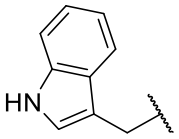
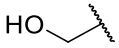
Some initial reactions were stirred for a maximal duration of 3 days and thin layer chromatography (TLC) of the reaction mixtures was performed frequently to check the progress of reaction. We could not reach completion of these reactions after 3 days but have found out that the most beneficial reaction duration was around 16 – 18 h (overnight stirring), since

durations of 2 or 3 days did not deliver any significant improvement in yields as shown by TLC analyses. In order to remove the remaining free fatty acids, the crude products were stirred in heptane for 30 min. In case of L-serine derivatives petroleum ether (bp. 40 – 60 °C) was used to avoid the formation of a gel-like structure.[82]

Table 3.3: Results from the *N*-acylations of four amino acids, L-glutamic acid, L- and D-tryptophan as well as L-serine, with different saturated fatty acyl chlorides.



Amino acid **61 - 64** | Fatty acyl chloride | **56**, **57**, **59**, **60**, **65 - 90** 17 - 89 %

Amino acid	R	Config. (*)[a]	Compound	n	Yield (%)[b]
L-Glutamic acid (61)		S	**65**	0	(3)
			66	1	(9)
			67	2	18
			68	4	17
			69	6	29
			70	8	69
			71	10	62
			72	12	36
			59	14	37
			60	16	36
L-Tryptophan (62)		S	**73**	0	82
			74	1	86
			75	2	32
			76	4	89
			77	6	87
			78	8	37
			79	10	73
			80	12	63
			81	14	67
			82	16	78
D-Tryptophan (63)		R	**83**	8	19
			84	10	74
			85	12	63
			86	14	63
			87	16	76
L-Serine (64)		S	**88**	8	27
			89	10	37
			90	12	53
			56	14	23
			57	16	53

[a] absolute configuration of the C_α of the amino acids. [b] isolated yield.

In general, reactions of tryptophan, regardless of its configuration, resulted in better yields than those of L-glutamic acid and L-serine probably because of the higher lipophilicity and thus better solubility of tryptophan in organic solvents. L-Glutamic acid derivatives with very short

acyl chain (acetyl **65**, propionyl **66**) are very polar and require individual optimization in extractive work-up. In some cases of tryptophan conjugates, the low yields are a result of loss during filtration.

3.3.3 Amide coupling of amino acids with free fatty acids

In contrast to the shorter fatty acids (C4 – C18), whose acyl chlorides were employed in the previous chapter, arachidic (C20) and arachidonic acids (C20:4) were only available as free fatty acids. Besides the option to activate the carboxyl group separately prior to the addition of the amino acid reaction partner, one can conduct a one-pot amide formation using a coupling reagent. In the latter case, the carboxyl residues in amino acids must be protected at first in order to prevent their interference in the subsequent coupling reaction. However, the prominent advantage of this one-pot method lies in the possibility to avoid material loss during isolation of fatty acyl chlorides. On a basis of an already published method by Dinda *et al.*,[83] we were able to synthesize several *N*-arachidoyl- and *N*-arachidonoylamino acids. In the first step, L-glutamic acid, L- and D-tryptophan were temporary converted to their corresponding acid chloride forms using thionyl chloride ($SOCl_2$). The chlorine was then replaced immediately by a methoxy group in the present of methanol acting as both reagent and solvent. A further neutralization step afforded the methyl esters **91** and **92** as oily compounds while methyl ester **93** did not undergo neutralization, and thus remained as a hydrochloride salt for better handling and storage. Subsequently, the three methyl esters were subjected to a reaction with either arachidic or arachidonoic acid in the presence of *N*,*N*'-dicyclohexylcarbodiimide (DCC) and 1-hydroxy-7-azabenzotriazole (HOAt) to form the acyl amino acid methyl esters **94** - **99**. The application of hydrochloride salt **93** turned out to work fine when the solution of this compound was treated with a portion of *N*,*N*-diisopropylethylamine (DIPEA) before being added to the other reagents. Finally, the ester functional group was cleaved under basic conditions giving the sodium salts of the *N*-acylamino acids, which were converted into the free lipoamino acids **100** – **105** upon neutralization with HCl (Figure 3.13).

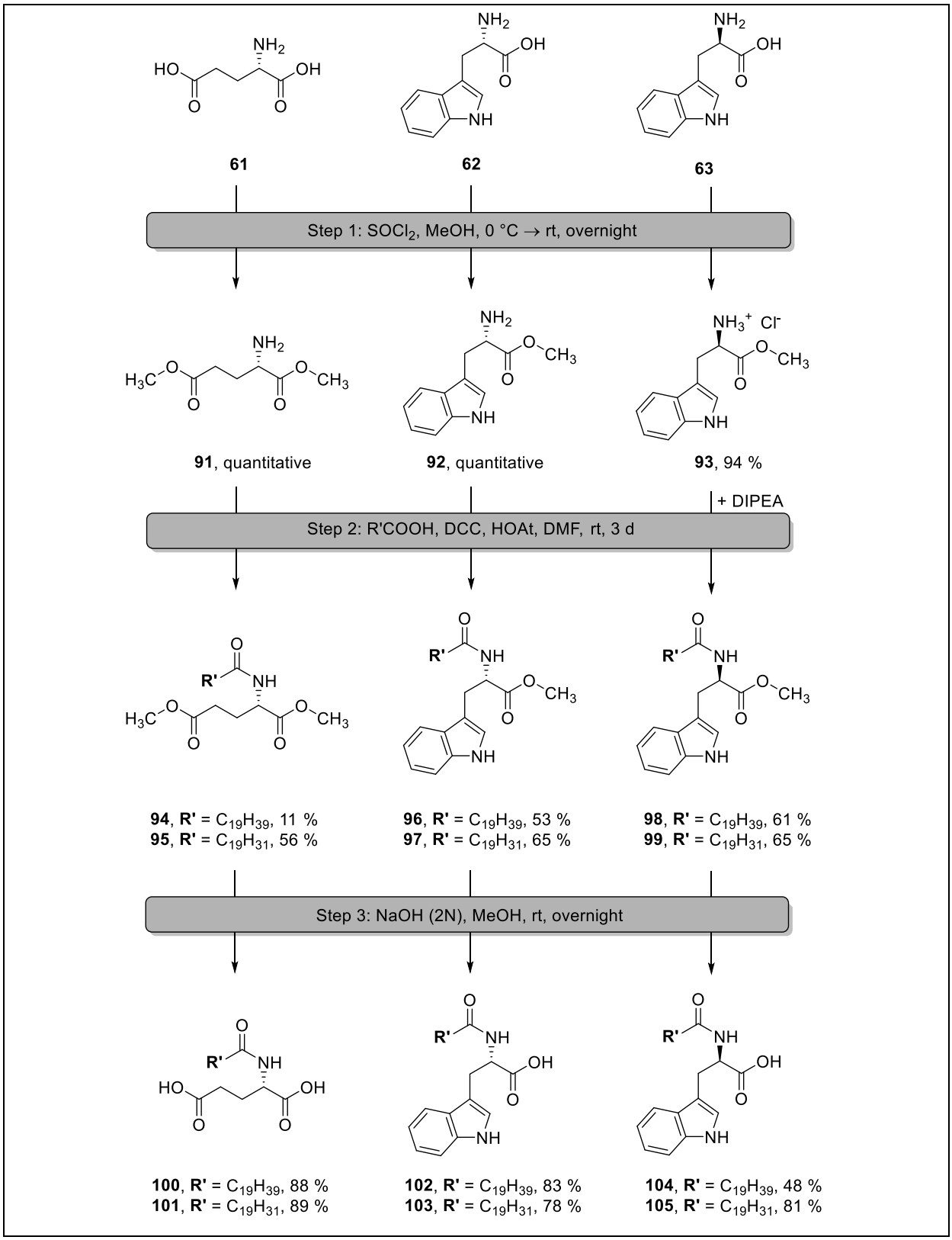


Figure 3.13: Preparation of *N*-acylamino acids from free fatty acids.

DCC is one of the classic coupling reagents, which is being widely used in peptide synthesis. The mechanism of DCC is illustrated in Figure 3.14 starting with a reaction of this coupling reagent with a carboxylic acid to form an ester *O*-acylisourea while the additive HOAt

suppresses the formation of the *N*-acylurea by-product and transfers the acyl group to the amino group to produce the desired amide and DCU, which is insoluble in most organic solvent and can be easily removed by filtration.

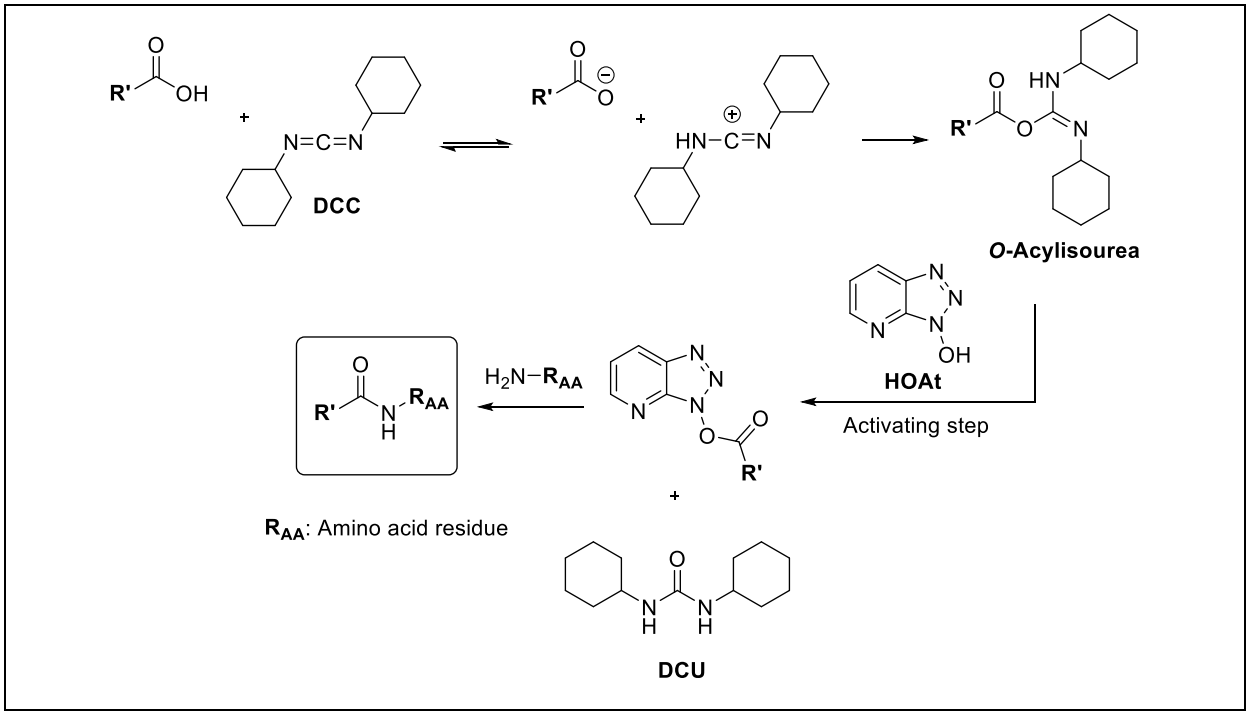


Figure 3.14: Mechanism of the amide coupling reaction with DCC and HOAt.

3.3.4 Esterification of *N*-acyl-L-tryptophan derivatives

The methyl esters **94** – **99** mentioned in the previous chapter were initially prepared as precursors for the synthesis of the free *N*-acylamino acid final products **100** – **105**. They were also tested in ß-arrestin assays at GPR18 and GPR55 to examine whether the carboxylic group is essential for the compounds' activities. Later on, as it was confirmed that our biological targets also tolerated the ester moiety, some active *N*-acyl-L-tryptophan derivatives were selected to undergo esterification with different alcohols including ethanol, propanol, etc. Quantitative yields were obtained when thionyl chloride had been added dropwise to an alcoholic solution of *N*-acyl-L-tryptophan at 0 °C before the reaction mixture was warmed to room temperature and stirred overnight (Figure 3.15).

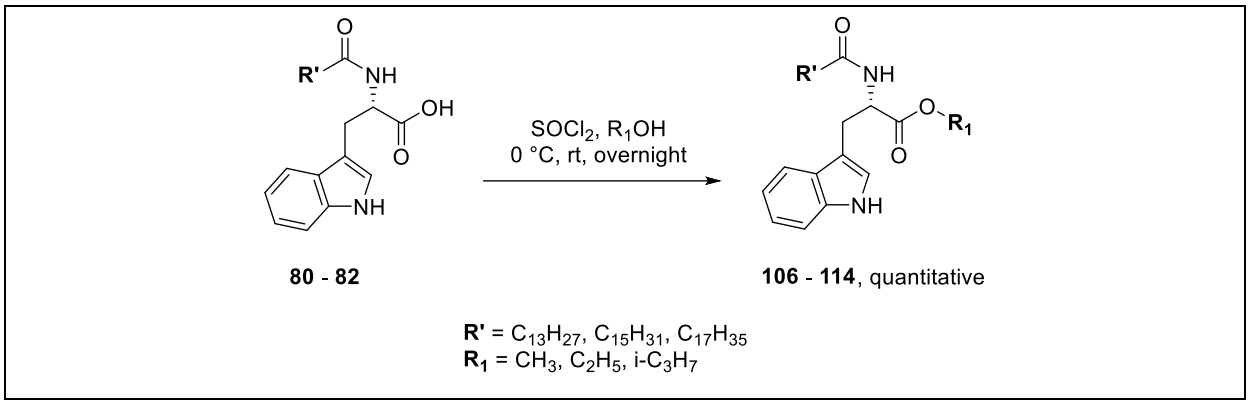


Figure 3.15: Esterified derivatives of *N*-acyl-L-tryptophans with different alcohols.

3.3.5 Racemization of *N*-acylamino acids

It is well known that amino acids can undergo racemization under basic condition.[84] The proton bound to the C_α atom can be abstracted by a base (e.g. hydroxide anion) resulting in a planar enolate intermediate. In a subsequent protonation, a hydrogen atom can be attached to both sides of the planar intermediate yielding both, L- and D-isomers (Figure 3.16). After a certain time, the reaction reaches an equilibrium and the mixture of the two isomers will become racemic.

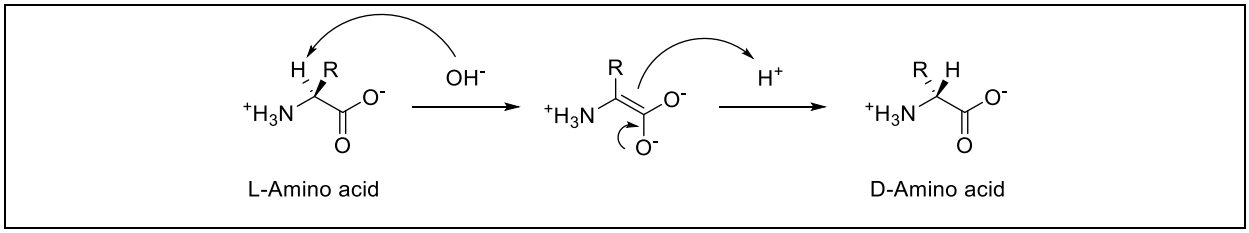


Figure 3.16: Mechanism of amino acid racemization in basic condition.

In order to examine, whether some reaction conditions involved 2N NaOH in this study could induce racemization, some *N*-acylamino acids were reprepared in a higher scale and their optical rotations were measured so that the specific rotations could be calculated using the equation 1.

$$[\alpha]_\lambda^T = \frac{\alpha(\text{obs})}{l \times c}$$

Equation 1: The specific rotation $[\alpha]_\lambda^T$ is calculated from the observed optical rotation α(obs) in degrees, l being the path length in decimeters, and c the concentration in g/mL, T is the temperature at which the measurement was taken (in degrees Celsius), and λ is the wavelength in nanometers.

By testing the solubility of *N*-dodecanoylamino acids in organic solvents, acetone was found to be able to dissolve large amounts of *N*-dodecanoylated glutamic acid (**71**) and tryptophan (**79**, **84**) derivatives. In case of the serine analog **89**, methanol was used. Compound **79** synthesized from L-tryptophan gave a positive rotation while **84** originated from the D-isomer showed equal and opposite rotation (Table 3.4). This led to the assumption that the two compounds are a pair of enantiomers and a racemization of tryptophan derivatives under the acylation conditions had not occurred. Results from the measurements of glutamic acid and serine derivatives also indicated non-racemic mixtures.

Table 3.4: Observed optical rotations as well as specific rotations of the four amino acid educts and some *N*-acylamino acids at given temperatures using a sodium lamp (λ = 589 nm). The path length was 1 dm, the concentration was 0.1 g/mL.

Comp.	Solvent	Temp. (°C)	α(obs) (°)	$[\alpha]_D^T$ (°)
L-Glutamic acid	0.5 N NaOH	25.2	-0.13	-1.3
L-Tryptophan	0.5 N NaOH	23.9	+0.48	+4.8
D-Tryptophan	0.5 N NaOH	24.7	-0.41	-4.1
L-Serine	0.5 N NaOH	25.7	-0.42	-4.2
71	acetone	20.0	-0.64	-6.4
79	acetone	25.2	+1.44	+14.4
84	acetone	21.3	-1.44	-14.4
89	methanol	20.0	+0.69	+6.9

3.4 Pharmacological assays of *N*-acylamino acids

3.4.1 ß-Arrestin assays at human GPR18 and GPR55

For the investigation of *N*-acylamino acids, ß-arrrestin assays at human GPR18 and GPR55 were performed by Dr. Clara Schoeder, Tabea Wiedenhöft and Andhika Mahardhika. In contrast to the assays of phenothiazine derivatives mentioned in 3.2.1, a novel GPR18 agonist was utilized instead of THC. PSB-KK-1415 is an indole-bearing compound, which was developed by our workgroup. It has an EC_{50} value of 19 nM at human GPR18.

So far, the major part of the *N*-acyl-L-tryptophan segment as well as some compounds from the L-glutamic acid and D-tryptophan series were tested. L-Glutamic acid derivatives were completely inactive at both GPR18 and GPR55; and no agonistic activity at the two orphan receptors could be detected for all species.

However, some GPR18-inhibiting activities of fatty *N*-acyl-L-tryptophans derivatives could be observed when the acyl side chain started to reach 12 carbons and was abolished at 20 carbons.

IC_{50} values of those compounds ranged from 6.32 µM (**81**, palmitoyl) to 9.08 µM (**79**, lauroyl). Interestingly the antagonistic effect was restored when L-tryptophan was coupled with arachidonic acid. The free acid form (**103**, IC_{50} 9.05 µM) is as potent as *N*-lauroyl-L-tryptophan (**79**), while its methyl ester derivative (**97**) possessed a higher potency (IC_{50} 5.27 µM, Figure 3.17 A). Esterification at the carboxyl group of L-tryptophan seems to be beneficial in terms of antagonistic activity. Regarding the inactivity of the *N*-eicosanoyl-L-tryptophan derivatives (**102** and **96**), it can be assumed that there is not enough space inside the binding pocket of GPR18 for this linear straight-chain alkyl residue. In contrast, the unsaturated arachidonoyl side chain, although having the same number of carbons, is able to fold up and would fit into the binding pocket.

Table 3.5: Results from ß-arrestin assays of *N*-acylamino acids at GPR18 and GPR55.

Comp.	**Amino acid**	**Fatty acid**	**hGPR18**[a]		**hGPR55**[a]	
			EC_{50} ± SEM (µM) (% activation)[b]	IC_{50} ± SEM (µM) (% inhibition)[c]	EC_{50} ± SEM (µM) (% activation)[d]	IC_{50} ± SEM (µM) (% inhibition)[e]
67	L-Glu	C4:0	>10 (8 ± 5)	>10 (-3 ± 10)	>10 (6 ± 6)	>10 (0 ± 8)
70		C10:0	>10 (0 ± 7)	>10 (-12 ± 8)	>10 (12 ± 10)	>10 (-2 ± 19)
71		C12:0	>10 (-11 ± 8)	>10 (7 ± 7)	>10 (24 ± 9)	>10 (-27 ± 21)
72		C14:0	>10 (16 ± 7)	>10 (-6 ± 10)	n.d.	n.d.
59		C16:0	>10 (32 ± 10)	>10 (22 ± 5)	n.d.	n.d.
60		C18:0	>10 (36 ± 9)	>10 (26 ± 6)	n.d.	n.d.
75	L-Trp	C4:0	>10 (6 ± 3)	>10 (3 ± 10)	n.d.	n.d.
76		C6:0	>10 (-11 ± 9)	>10 (-10 ± 9)	n.d.	n.d.
77		C8:0	>10 (12 ± 4)	>10 (-3 ± 10)	n.d.	n.d.
78		C10:0	>10 (1 ± 15)	>10 (-4 ± 7)	n.d.	n.d.
79		C12:0	>10 (-4 ± 10)	**9.08** ± 1.04	n.d.	n.d.
80		C14:0	>10 (-2 ± 9)	**6.51** ± 1.14	n.d.	n.d.
81		C16:0	>10 (-10 ± 7)	**6.32** ± 1.71	n.d.	n.d.
82		C18:0	>10 (-2 ± 10)	**7.48** ± 1.90	n.d.	n.d.
102		C20:0	>10 (-4 ± 4)	>10 (36 ± 11)	n.d.	n.d.
103		C20:4	>10 (-1 ± 5)	**9.05** ± 1.09	n.d.	n.d.

Table 3.5 continued

Comp.	Amino acid	Fatty acid	hGPR18[a]		hGPR55[a]	
			EC_{50} ± SEM (µM) (% activation)[b]	IC_{50} ± SEM (µM) (% inhibition)[c]	EC_{50} ± SEM (µM) (% activation)[d]	IC_{50} ± SEM (µM) (% inhibition)[e]
106	L-Trp-OMe	C14:0	>10 (-5 ± 2)	**10.2** ± 1.12	>10 (26 ± 9)	>10 (47 ± 9)
107		C16:0	>10 (-7 ± 3)	>10 (44 ± 7)	>10 (6 ± 30)	>10 (32 ± 15)
108		C18:0	>10 (2 ± 4)	>10 (17 ± 10)	>10 (19 ± 12)	>10 (-16 ± 8)
96		C20:0	>10 (-5 ± 7)	>10 (-5 ± 9)	n.d.	n.d.
97		C20:4	>10 (-3 ± 9)	**5.27** ± 2.65	n.d.	n.d.
109	L-Trp-OEt	C14:0	>10 (-14 ± 4)	**10.5** ± 1.02	>10 (21 ± 16)	>10 (41 ± 10)
110		C16:0	>10 (2 ± 4)	>10 (6 ± 4)	>10 (24 ± 13)	>10 (-10 ± 17)
111		C18:0	>10 (-9 ± 5)	>10 (21 ± 3)	>10 (-11 ± 9)	>10 (0 ± 14)
104	D-Trp	C20:0	>10 (-3 ± 7)	**9.02** ± 0.945	>10 (13 ± 11)	>10 (-95 ± 28)
105		C20:4	>10 (1 ± 17)	**6.65** ± 1.07	>10 (4 ± 10)	>10 (1 ± 9)
98	D-Trp-OMe	C20:0	>10 (-4 ± 3)	>10 (12 ± 5)	>10 (-10 ± 11)	>10 (-6 ± 14)
99		C20:4	>10 (-31 ± 7)	**6.94** ± 0.405	>10 (-9 ± 19)	**3.67** ± 1.36

[a]Receptors were expressed on CHO cells. [b]% activation in comparison to 0.1 µM PSB-KK-1415 induced luminescence signal. [c]% inhibition of 0.1 µM PSB-KK-1415 induced luminescence signal. [d]% inhibition of 1 µM LPI induced luminescence signal. [e]% activation in comparison to 1 µM LPI induced luminescence signal. All data with SEM resulted from three independent experiments, performed in duplicate.

After an increase in the inhibitory effect was observed in an esterified derivative, more methyl and ethyl esters of *N*-acyl-L-tryptophan (**106** – **114**) were introduced. However, compounds of this category were almost inactive. The boundary species are the two derivatives having the myristoyl side chain (**106** and **109**), both exhibiting very similar GPR18-inhibiting behaviour (IC_{50} 10.2 µM and 10.5 µM, respectively).

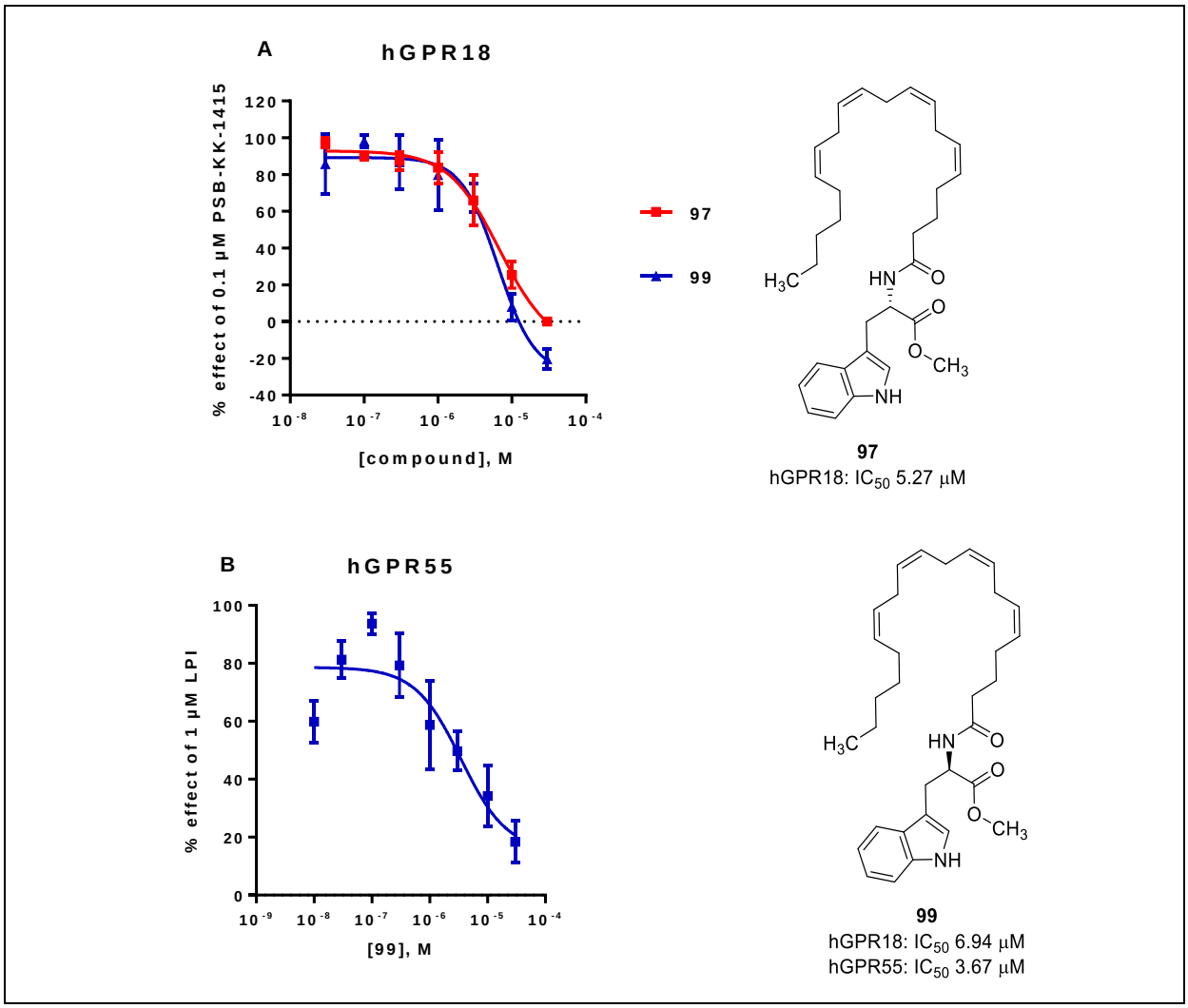


Figure 3.17: (A) *N*-Arachidonoyl-L-tryptophan methyl ester (**97**) and its D-isomer (**99**) showed comparable antagonistic activities at GPR18. (B) The D-isomer also acts as a GPR55 antagonist.

In the series of D-tryptophan derivatives, a different trend was observed. At first, a low activity (IC_{50} 9.02 µM) was detected with *N*-eicosanoyl-D-tryptophan (**104**), unlike its inactive L enantiomer **102**. Moreover, no increase in activity took place after a methyl esterification of *N*-arachidonoyl-D-tryptophan since both free acid (**105**) and methyl ester (**99**) derivatives acted as GPR18 antagonists with similar potencies (IC_{50} 6.65 µM and 6.94 µM, respectively). Additionally, the methyl ester **99** is one of the few tested *N*-acylamino acids, which could inhibit LPI-induced GPR55 activation (IC_{50} 3.67 µM, Figure 3.17 B).

3.4.2 Radioligand binding assays

Although the orphan GPR18 and GPR55 are structurally not related to the cannabinoid receptors CB_1 and CB_2, they are still able to interact with their natural and some synthetic ligands. In order to examine, whether the newly synthesized GPR18 and GPR55 antagonists

can bind to cannabinoid receptors, radioligand binding assays were performed. The binding affinity of a test compound for a receptor can be calculated indirectly by measuring its ability to compete with the binding of a known radioactive ligand to this receptor. The unlabeled test compound in increasing concentrations will compete with the radioligand in a fixed concentration and displace it from the binding site, leading to a decrease in the concentration of radioligand receptor complex and accordingly the measured radioactivity. The experiment is thus also known as competition binding assay. From the resulting data, the concentration of the unlabeled ligand that inhibits the binding of the radioactive ligand by 50 % (IC_{50} value) is obtained. The dissociation constant (K_i) of the unlabeled compound representing its affinity to the examined receptor can be calculated from the IC_{50} value using following Cheng-Prusoff equation. If the K_i value is low, the affinity of the unlabeled ligand for the receptor will be high.

$$K_i = \frac{IC_{50}}{1 + \frac{[L]}{K_D}}$$

Equation 2: Cheng-Prusoff equation. [L] is the concentration of the radioactive ligand used in the assay and K_D is the affinity of the radioligand for the receptor.

Radioligand binding assays of *N*-acylamino acids in this study were performed by Dr. Clara Schoeder and Andhika Mahardhika. The cannabinoid receptor agonist radioligand [^{3}H](-)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol ([^{3}H]CP55,940) with K_D values of 2.4 nM (at human CB_1) and 0.7 nM (at human CB_2), was used.

Table 3.6: Results from competition binding assays of *N*-acylamino acids at human CB_1 and CB_2 receptors.

Comp.	Amino acid	Fatty acid	**hCB$_1$**[a] K_i (µM) (% inhibition of specific binding)[b]	**hCB$_2$**[a] K_i (µM) (% inhibition of specific binding)[b]
67	L-Glu	C4:0	>10 (7 ± 3)	>10 (3 ± 7)
70		C10:0	>10 (25 ± 11)	>10 (15 ± 8)
71		C12:0	>10 (32 ± 10)	>10 (-1 ± 2)
72		C14:0	>10 (14 ± 4)	>10 (10 ± 5)
59		C16:0	>10 (37 ± 10)	>10 (25 ± 14)
60		C18:0	>10 (28 ± 5)	>10 (9 ± 3)

Table 3.6 continued

Comp.	Amino acid	Fatty acid	hCB_1[a] K_i (μM) (% inhibition of specific binding)[b]	hCB_2[a] K_i (μM) (% inhibition of specific binding)[b]
75	L-Trp	C4:0	>10 (18 ± 5)	>10 (1 ± 2)
76		C6:0	>10 (14 ± 10)	>10 (-3 ± 2)
77		C8:0	>10 (34 ± 11)	>10 (26 ± 11)
78		C10:0	>10 (2 ± 13)	>10 (9 ± 3)
79		C12:0	>10 (8 ± 3)	>10 (12 ± 3)
80		C14:0	>10 (18 ± 6)	>10 (30 ± 5)
81		C16:0	>10 (22 ± 3)	>10 (13 ± 3)
82		C18:0	>10 (16 ± 4)	>10 (12 ± 4)
102		C20:0	>10 (2 ± 9)	>10 (-10 ± 6)
103		C20:4	>10 (21 ± 8)	>10 (33 ± 5)
106	L-Trp-OMe	C14:0	>10 (45 ± 20)	>10 (39 ± 13)
107		C16:0	>10 (50 ± 11)	>10 (41 ± 11)
108		C18:0	>10 (-7 ± 8)	>10 (9 ± 7)
96		C20:0	>10 (30 ± 13)	>10 (11 ± 4)
97		C20:4	(**52** ± 6)	(**78** ± 8)
109	L-Trp-OEt	C14:0	**1.70** ± 0.08	**2.50** ± 0.05
110		C16:0	**0.27** ± 0.012	>10 (36 ± 13)
111		C18:0	>10 (5 ± 11)	>10 (5 ± 12)
104	D-Trp	C20:0	>10 (9 ± 12)	>10 (8 ± 7)
105		C20:4	**0.98** ± 0.31	>10 (31 ± 5)
98	D-Trp-OMe	C20:0	>10 (-3 ± 14)	>10 (-4 ± 3)
99		C20:4	**1.89** ± 0.52	**2.01** ± 0.29

[a]Receptors were expressed on CHO cells. [b]% inhibition of binding of 0.1 nM [^{3}H]CP55,940. All data with SEM resulted from three independent experiments, performed in duplicate.

Binding of *N*-acyltryptophan to CB_1 and CB_2 receptors occurred mostly when an arachidonoyl residue was present (Table 3.6, compounds **97**, **99**). In case of **103**, only CB_1 binding was observed (K_i 980 nM). This finding can be expected since the endogenous ligand anandamide (**2**) of cannabinoid receptors also possesses this residue, which may contribute greatly to the binding of this ligand to its biological targets. Nonetheless, *N*-myristoyl-L-tryptophan ethyl ester (**109**) has been found to have affinity to both CB_1 and CB_2 subtypes. By extending the fatty acyl side chain of this type of compound to palmitoyl (**110**), binding became selective toward CB_1 with a significant increase in affinity (K_i 207 nM), however, with a stearoyl residue (**111**), binding to cannabinoid receptors was completely eliminated.

3.5 Enzymatic hydrolysis of bovine phosphatidylinositol

Inspired by the study showing a considerable higher potency of 2-AGPI (**10**) as compare to soybean LPI, the intention of this experiment was to enzymatically prepare a mixture of 1-lysophosphatidylinositols (1-LPIs) from commercially available bovine liver PI, which consists of PIs with different fatty acyl residues at the *sn*-2 position (mainly C18:1, C18:2, C20:3 and C20:4). Under appropriate conditions, phospholipase A_1 will cleave the ester bond at *sn*-1 position of PI giving 1-LPIs and a free fatty acid (Figure 3.18).

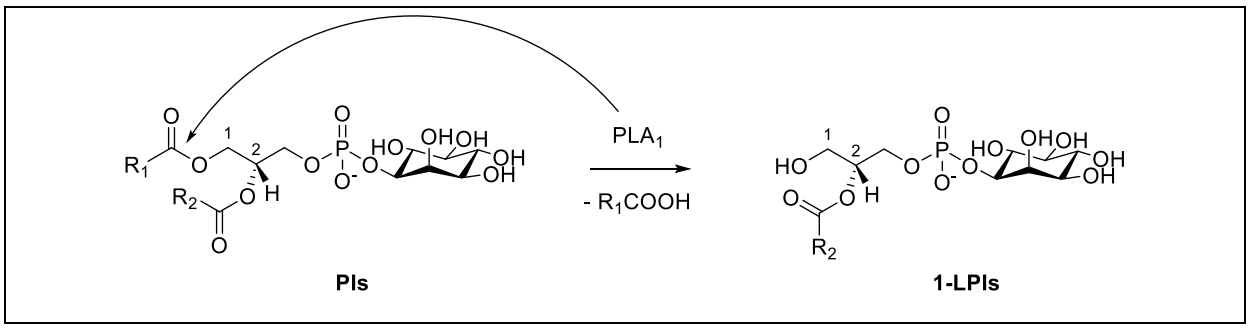


Figure 3.18: Hydrolysis of PIs using phospholipase A_1

The reaction protocol has been partly adopted from Oka *et al.* [58] Since the *Rhizopus delemar* lipase used by Oka was not available for us, a phospholipase A_1 from *Thermomyces lanuginosus* (10 kilo lipase unit per gram (KLU/g), purchased from Sigma) was used instead. In the first attempt, the amount of PLA_1 was calculated so that the enzyme can hydrolyze 5 equivalents of substrate. After the termination of the reaction and liquid-liquid extraction as described in the literature, the methanol-water layer was poured into a solid-phase extraction (SPE) tube. Aminopropyl-modified silica sorbent was chosen due to its usefulness in the separation of free fatty acids produced during the hydrolysis reaction.[85] The solvents in the sample were led to flow through the tube leaving compounds binding to the solid phase, which was then washed with a mixture of diethylether/acetic acid to elute all fatty acids (Fraction 2). Finally, the ionic

strength of the solid phase was reduced by adding methanol/ammonia, thus the stronger-retained phospholipids can be eluted (Fraction 3, Figure 3.19). All 3 fractions were examined via LC-MS but could not find any lysophosphatidylinositol (molecular weights from 591 – 623 were scanned).

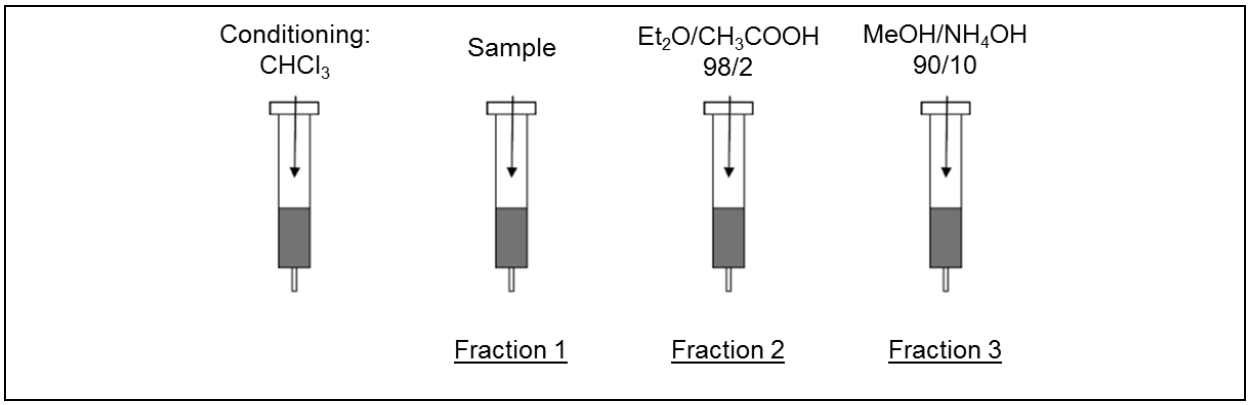
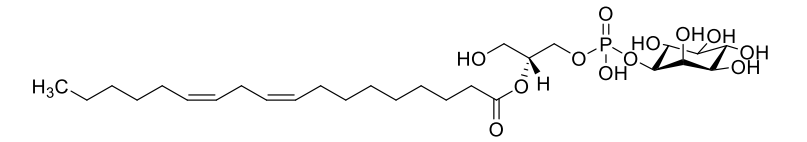


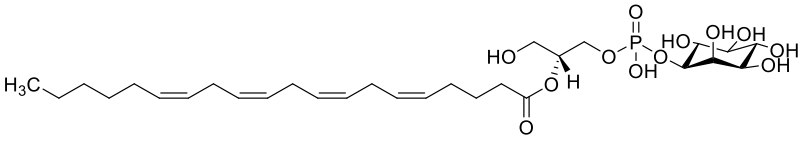
Figure 3.19: Purification of phospholipids employing aminopropyl SPE tube.

In a more recent attempt to hydrolyze 5 mg of bovine PI using phospholipase A_1 from *Thermomyces lanuginosus*, DMSO was added to the reaction mixture in order to increase the solubility of PI in acetate buffer. The reaction condition and purification protocol remained unchanged. The m/z values revealed in the LC-MS positive mode (Figure 3.20) of the third SPE fraction corresponded to few 1-LPI species varied in the unsaturated *sn*-2 acyl groups, namely linoleic acid (C18:2, **115**), arachidonic acid (C20:4, or 2-AGPI, **10**) and dihomo-γ-linoleic acid (C20:3, **116**) (Table 3.7).

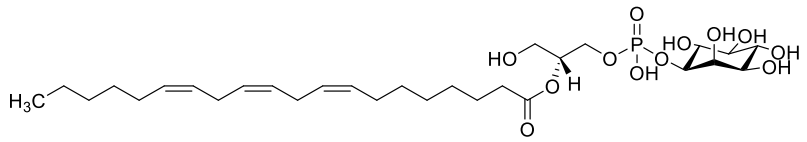
Table 3.7: Structures and m/z values of possible 1-LPIs identified in LC-MS spectra.



1-LPI/C18:2 (**115**) - Exact Mass: 596.30



1-LPI/C20:4 or 2-AGPI (**10**) - Exact Mass: 620.30



1-LPI/C20:3 (**116**) - Exact Mass: 622.31

1-LPI	Acyl residue	$[M+H]^+$	$[M+NH_4]^+$
115	C18:2	597	-
10	C20:4	621	638
116	C20:3	623	640

Moreover, the presence of these fatty acyl groups also complies with the fatty acid distribution in commercial bovine liver PI provided by Avanti Lipid. Besides $[M+H]^+$ ions, $[M+NH_4]^+$ adducts were also observed, probably due to the use of ammonia in SPE purification. In addition, there were some m/z values, which cannot be assigned to any compound yet.

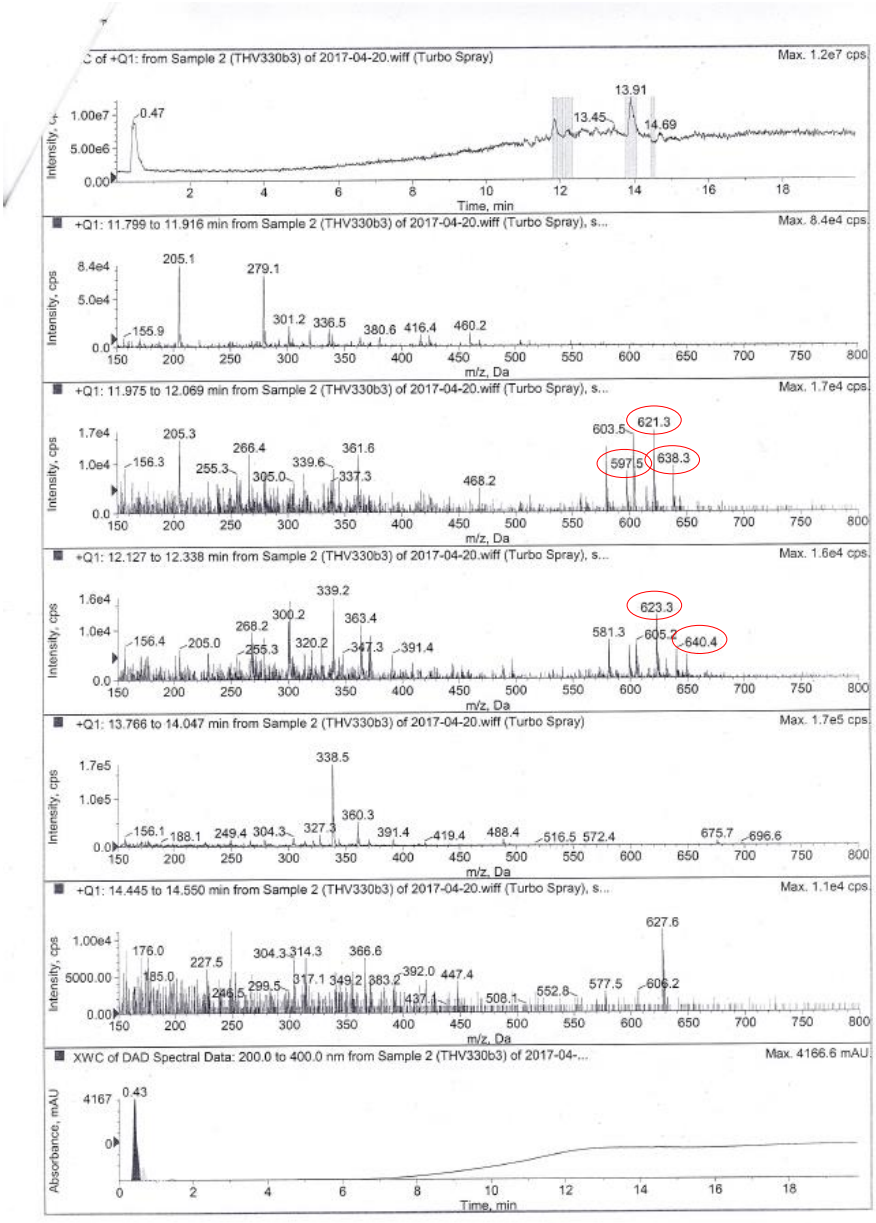


Figure 3.20: LC-MS spectra of the product from PI hydrolysis.

The addition of DMSO to the reaction medium has showed some positive effects on the phospholipase A_1-catalyzed hydrolysis of PI, as a few 1-LPI species were detected in the final SPE fraction by LC-MS analysis. The PLA_1 from *Thermomyces lanuginosus*, also known as Lecitase Ultra, has been reported to possess a single active site with both lipase and phospholipase activity, which can be enhanced by Ca^{2+} and Mg^{2+} ions.[86] However the pH-optimum for this enzyme is controversial, hitherto two values (8.5[86] and 5.0[87]) have been proposed. Furthermore, a complete hydrolysis of phosphatidylcholine (PC) to L-α-glycerylphosphorylcholine (L-α-GPC) catalyzed by Lecitase Ultra in *n*-hexane–water biphasic media has been described.[88] Hence, optimizations for both reaction condition as well as purification method are required.

4 Conclusions and outlook

The orphan G protein-coupled receptors GPR18 and GPR55 interact with cannabinoids, although they are only distantly related to the classical cannabinoid receptors CB_1 and CB_2. There is preliminary evidence for the involvement of GPR18 and GPR55 in various pathophysiological processes, and both receptors may have potential as future drug targets. Potent and selective pharmacological tool compounds, agonists and antagonists, are urgently required for target validation studies. Moreover, it is of great interest to identify physiological agonists of both orphan receptors. Physiological lipids have been postulated to activate the receptors, *N*-arachidonoylglycine (NAGly) for GPR18, and 1-lysophosphatidylinositol (1-LPI) for GPR55, but these have not been unambigously confirmed so far.

In the here presented study, synthetic and natural products including many new, not previously described compounds have been prepared and investigated as new ligands for these receptors.

Phenothiazine derivatives

Compounds of the first series were derived from the 2-chlorophenothiazine scaffold. This compound came into focus after the discovery of antagonistic activities of perphenazine and related antipsychotics at GPR18 observed in a screening approach of a library of approved drugs. At first, the basic propylamino group, a typical feature of these drugs, was replaced by several alkyl, cycloalkyl and aryl substituents via alkylation at the *N*-10 position of 2-chlorophenothiazine. The purpose of these modifications was to develop GPR18 antagonists without inhibitory activity at dopamine or other neurotransmitter receptors.

Appropriate conditions for preparing *N*-alkylated phenothiazine derivatives using NaH and DMF were investigated and optimized in this study. As a result, several alkyl- and aralkyl-substituted 2-chlorophenothiazines could be obtained in high yields (75–87%). Interestingly, these compounds lost their effect at GPR18 but were active as inhibitors of GPR55. The most potent phenothiazine-derived GPR55 antagonist was the 3-phenylpropyl-substituted derivative **40** displaying an IC_{50} value of 5.29 µM at GPR55 (see Fig. 4.1). Moreover, *N*-alkylated phenothiazine derivatives with side chains of up to five carbon atoms behaved as moderately potent antagonists at GPR55 (IC_{50} 6.20 – 10.1 µM). However, by exchange of the benzene ring in **40** for a primary amino group, the selectivity was shifted towards GPR18. Indeed, compound **41** was the only derivative in this series capable of inhibiting the activation of GPR18 (IC_{50} 4.89 µM) while being inactive at GPR55 (Figure 4.1). Therefore, it could be assumed that an

amino group was required for GPR18 antagonism. In future experiments, the amino group in **41** could be further modified, e.g. replaced by other non-basic functional groups such as amide or urea functions to study the role of the propylamino moiety for GPR18 inhibition. Further modifications may also be carried out at the phenyl group of **40** to develop more potent GPR55 antagonists.

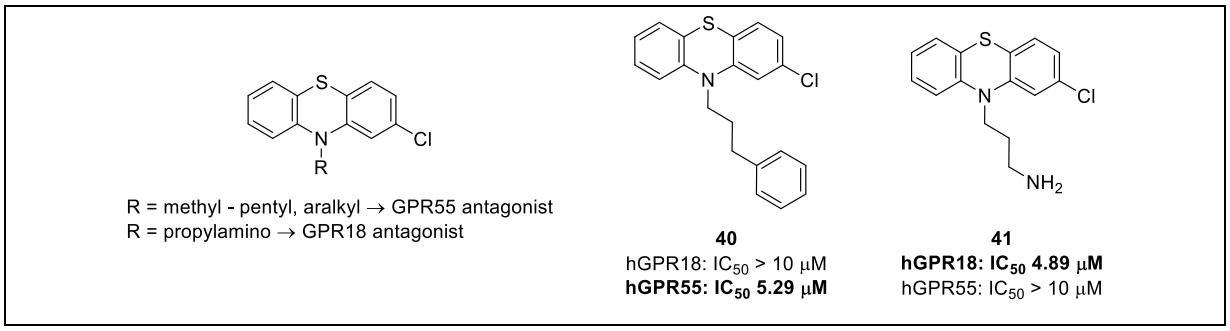


Figure 4.1: Novel phenothiazine-derived GPR18- and GPR55 antagonists.

N-Acylamino acids

Several different *N*-acylamino acids have been found in mammalian brains as well as the periphery, and a few have been shown to possess biological effects e.g., modulating pain, inflammation, and cardiovascular functions. Via acylation and amide coupling methods, various conjugates of amino acids and natural fatty acids were prepared. Among the four amino acids employed in the present study, tryptophan as a constituent of the prepared *N*-acylamino acids appeared to be an important moiety for inhibiting GPR18 activation. Particularly, amides of L-tryptophan and fatty acids with a side chain preferably between 12 and 18 carbon atoms antagonized GPR18 at low micromolar concentrations (IC_{50} 6.32 – 9.08 µM).

Furthermore, arachidonic acid also appeared to be a well-tolerated residue by GPR18 with the L-tryptophan methyl ester **97** being the most potent conjugate (IC_{50} 5.27 µM). However, this compound also showed binding affinities to both CB receptor subtypes in competition binding assays. Hydrolysis of the ester group of **97** eliminated binding to CB_1 and CB_2, and slightly decreased the potency at GPR18 (**103**, IC_{50} 9.05 µM). In case of D-tryptophan conjugates, the trend was different. Ester **99** possessed moderate inhibiting potencies at both GPR18 (IC_{50} 6.94 µM) and GPR55 (IC_{50} 3.67 µM) as well as binding affinities to both cannabinoid receptors. After hydrolysis, the activity of the resulting compound **105** at GPR18 remained almost unchanged (IC_{50} 6.65 µM) while the affinities towards GPR55 and CB_2 receptor were abolished (Figure 4.2). Thus, it was assumed that the carboxyl group plays an important role in the selectivity of *N*-acyltryptophan derivatives at cannabinoid-like receptors.

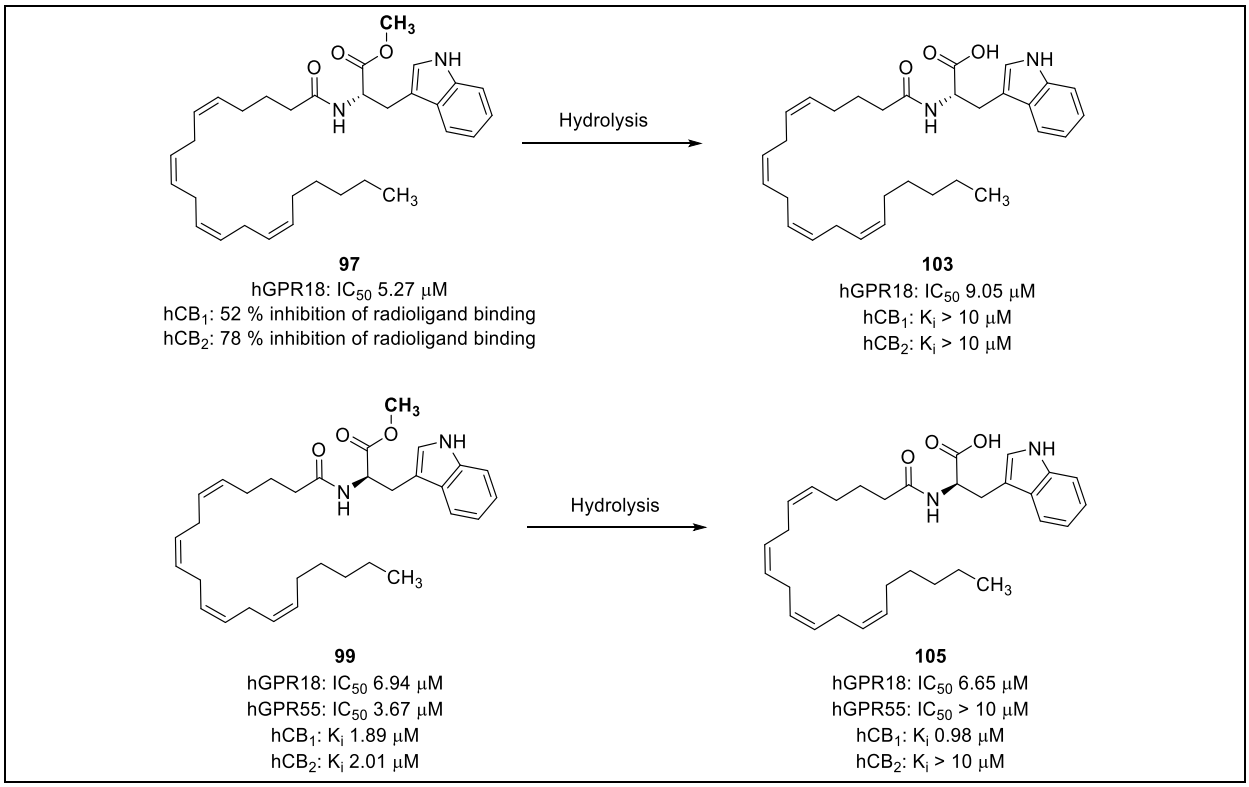


Figure 4.2: Enantiomers of *N*-arachidonoyltryptophan and their inhibitory potencies at GPR18, GPR55, CB_1 and CB_2 receptors.

Due to the preference of GPR18 for tryptophan as an amino acid residue in the *N*-acylamino acids, structural modifications at the indole ring system could be considered as a meaningful next step to identify more potent ligands.

In conclusion, in the present study, phenothiazine and *N*-acylamino acids have been identified as novel classes of ligands for the orphan receptors GPR18 and GPR55. The results provided herein will serve as a basis for further development of more potent and selective antagonists, which will be useful as pharmacological tools for elucidating the pathophysiological roles of these two orphan GPCRs and to validate them as future drug targets.

5 Experimental

5.1 Material for synthesis

5.1.1 Chemicals and solvents

Commercially available chemicals for synthesis were purchased from following producers: Sigma-Aldrich (Steinheim), Alfa Aesar (Karlsruhe), Acros Organics (Geel, Belgium), VWR (Siegburg), Merck (Darmstadt), Fluorochem (Hadfield, England) or TCI (Eschborn) and used without further purification. Solvents were obtained from various commercial sources. Dichloromethane was distilled over calcium hydride and other solvents were used without further purification.

5.1.2 Instruments and equipment

Thin layer chromatography (TLC)

Analytical thin layer chromatography (TLC) was performed on silica-coated aluminum plates containing a fluorescent indicator (Merck silica gel 60 F_{254}, Darmstadt, Germany).

Column chromatography

For column chromatography silica gel 60 (0.063-0.200 mm) from Merck (Darmstadt, Germany) was used.

Lyophilization

Lyophilization of compounds was performed with an Alpha 1-4 LSC freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany).

High performance liquid chromatography - mass spectrometry (HPLC-MS)

Low-resolution mass spectra were obtained on an API 2000 mass spectrometer (electrospray ionization, Applied Biosystems, Darmstadt, Germany) coupled to an HPLC system (Agilent 1100) using the following procedure: compounds were dissolved in methanol (1 mg/mL). A 10 µL sample of this solution was injected into the HPLC system containing a Phenomenex Luna C18 column (50 mm x 2.00 mm, particle size 3 µm). It was chromatographed using a gradient of water:methanol (containing 2 mM ammonium acetate, if not stated otherwise) from 90:10 to 0:100 within 30 min. The gradient was started after 10 min, the flow rate was 250 µL/min. UV absorption was detected using a diode array detector (from 190 to 900 nm) and purity was determined at 254 nm.

Nuclear magnetic resonance spectroscopy (NMR)

^{1}H-, ^{13}C- and ^{31}P-NMR spectra were recorded on a Bruker Avance 500 MHz or a Bruker Ascend 600 MHz spectrometer. Spectra were recorded at room temperature in DMSO-d_6, $CDCl_3$, MeOD or D_2O as indicated. Chemical shifts are given in parts per million (ppm) relative to the remaining protons of the deuterated solvents used as internal standard. Coupling constants are given in Hertz (Hz). Spin multiplicities are abbreviated as s (singlet), d (doublet), t (triplet), m (multiplet), br (broad). Alpha, beta and gamma carbons of amino acids are labelled as C_α, $C_ß$ and C_γ, respectively.

Melting point apparatus

For the determination of melting points a Büchi Melting Point apparatus B-560 was used, and melting points were not corrected.

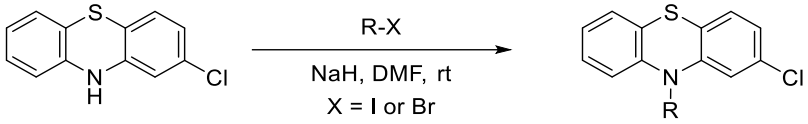
Optical rotation determination

The observed optical rotation was determined using a Bellingham + Stanley polarimeter with a sodium lamp (λ = 589 nm). The cell has a length of 1 dm. The concentration was 1 g/10 mL solvent.

5.2 Procedures for the synthesis of phenothiazine derivatives

5.2.1 General procedures for *N*-alkylation of 2-chlorophenothiazine

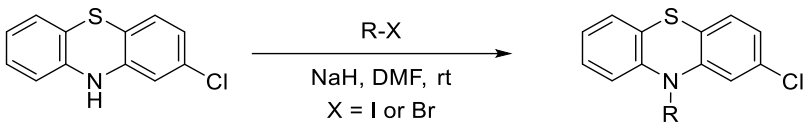
5.2.1.1 Method 1



25 **26 - 29, 36, 40**

2-Chlorophenothiazine (**25**, 200 mg, 0.86 mmol, 1 eq.) was dissolved in dimethylformamide (DMF, 20 mL). NaH (60% in mineral oil, 1 - 2 eq.) was added in small fractions to the reaction mixture at 0°C. After stirring for 10 min at 0°C, alkyl iodide or alkyl bromide (1.2 – 2.2 eq.) was added. The resulting solution was stirred at r. t. or 60 °C for 4 – 24 h, before being treated with water (40 mL). Dichloromethane (60 mL) was added to the solution and the organic phase was washed with a saturated solution of $NaHCO_3$ (40 mL), followed by an aqueous solution composed of 2 g of LiCl in 40 mL of water, and finally 3 times with water (40 mL). The organic phase was then dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel to afford the title compounds.

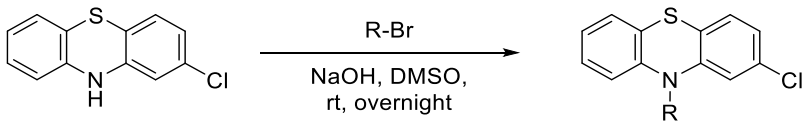
5.2.1.2 Method 2



25 **30 - 34**, **38**, **39**, **41 - 44**

To a mixture of 2-chlorophenothiazine (**25**, 233 mg, 1.00 mmol) and alkyl bromide or alkyl iodide (3 - 5 eq.) in dimethylformamide (DMF, 5 mL), NaH (60% in mineral oil, 48 mg, 1.2 mmol) was added in an ice-bath. The reaction mixture was stirred at r. t. for 1 h, and an additional portion of NaH (60% in mineral oil, 48 mg) was added upon cooling in an ice-bath. The reaction mixture was further stirred at the r. t. for 0.5 h before being treated with water (50 mL) and extracted with dichloromethane (3 x 30 mL). The combined organic phase was washed with an aqueous solution composed of 2 g of LiCl in 40 mL of water, and finally with water (3 x 50 mL). The organic phase was then dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by a column chromatography on silica gel and if necessary recrystallized to afford the title compounds.

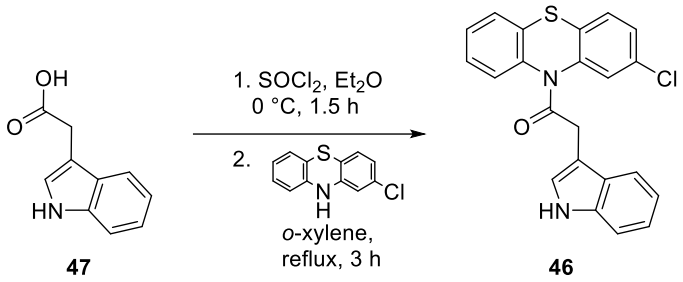
5.2.1.3 Method 3



25 **35**, **37**

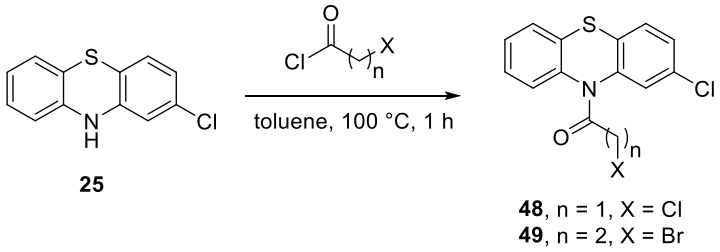
2-Chlorophenothiazine (**25**, 400 mg, 1.72 mmol, 1 eq.), NaOH (89 mg, 2.2 mmol, 1.3 eq.) and alkyl bromide (1.2 eq.) were dissolved in dimethyl sulfoxide (DMSO, 20 mL) and the mixture was stirred overnight at r. t. The reaction mixture was then poured into water and extracted with dichloromethane. The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel to afford the title compounds.

5.2.2 Procedure for the synthesis of 2-(1*H*-indol-3-yl)-1-(2-chloro-10*H*-phenothiazin-10-yl)ethanone (46)



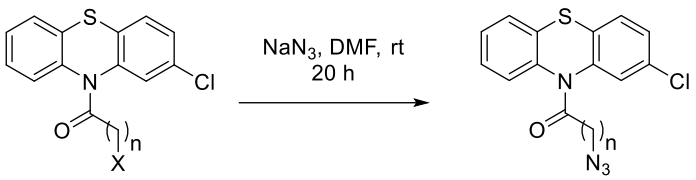
A solution of thionyl chloride ($SOCl_2$, 160 µL, 2.2 mmol) in diethyl ether (Et_2O, 5 mL) was added over a period of 5 min to a stirring suspension of indolyl-3-acetic acid (350 mg, 2.0 mmol) in diethyl ether (10 mL, dried over $MgSO_4$) at 0 °C. The reaction mixture was stirred at 0 °C for 1.5 h followed by removal of the solvent under vacuum affording a dark brown solid. Then a solution of 2-chlorophenothiazine (233 mg, 1.0 mmol) in *o*-xylene (20 mL) was added and the reaction mixture was refluxed for 3 h. The crude mixture was concentrated under vacuum then purified by column chromatography on silica gel (petroleum ether (b.p. 40 – 60 °C):ethyl acetate = 8:2) yielding a white solid.

5.2.3 General procedure for the acylation of 2-chlorophenothiazine



A solution of 2-chlorophenothiazine (**25**, 4.00 g, 17.1 mmol) in toluene (20.0 mL) was treated with chloroacetyl chloride (1.43 mL, 18.0 mmol) or 3-bromopropionyl chloride (1.82 mL, 18.0 mmol) and heated to 100 °C for 1 h. The mixture was cooled to r. t., concentrated under vacuum, taken up in a minimal amount of toluene and purified by column chromatography on silica gel (petroleum ether (b.p. 40 – 60 °C):ethyl acetate = 8:2) to afford the title compounds.

5.2.4 General procedure for the synthesis of azide derivatives

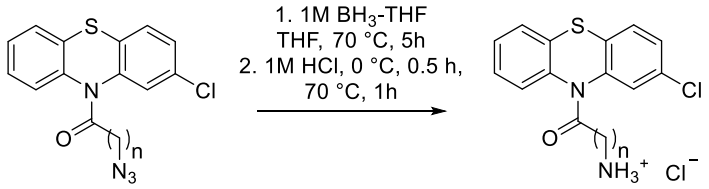


48, n = 1, X = Cl
49, n = 2, X = Br

50, n = 1
51, n = 2

A solution of a substituted alkyl halogenide (2.1 - 3.2 mmol) in DMF (10 mL) was treated with sodium azide (NaN_3, 3 eq.) and stirred at r. t. for 20 h. The solution was diluted with dichloromethane (100 mL) and the organic layer was washed with water (2 x 100 mL), followed by a saturated aqueous NaCl solution (100 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was taken up in a minimal amount of dichloromethane and purified by column chromatography on silica gel (petroleum ether (b.p. 40 – 60 °C):ethyl acetate = 9:1) to afford the title compounds.

5.2.5 General procedure for the reduction of azide derivatives to amines

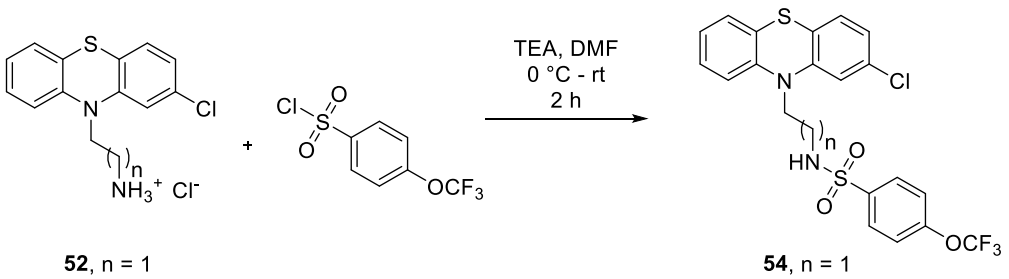


50, n = 1
51, n = 2

52, n = 1
53, n = 2

A solution of an azide derivative (4 – 6.3 mmol) in tetrahydrofuran (THF, 5 mL) was cooled to 0 °C and treated with a borane-tetrahydrofuran complex solution (BH_3-THF, 1 M in THF, 4 eq.). The flask was sealed, heated to 70 °C for 5 h, and then cooled to 0 °C. The solution was treated slowly and dropwise with a solution of aqueous 1 M HCl (8 eq.), stirred for an additional 0.5 h, and then heated to 70 °C for 1 h. The mixture was cooled to r. t., treated with aqueous 4 M NaOH until pH > 8, and then extracted with ethyl acetate (3 x 70 mL). The combined extracts were washed with saturated aqueous NaCl solution (100 mL), dried over $MgSO_4$, and concentrated under vacuum. The residue was dissolved in a minimal amount of dichloromethane and purified by column chromatography on silica gel (petroleum ether (b.p. 40 – 60 °C):ethyl acetate = 8:2 to remove nonpolar impurities followed by dichloromethane:methanol:NH_4OH : 17:2:1 to elute the product). The combined fractions of pure product were concentrated, dried azeotropically with toluene, and then suspended in diethyl ether (50 mL) and treated dropwise with a 4 N HCl-dioxane solution (1 mL). The solid that had precipitated was collected by filtration affording the title compounds.

5.2.6 General procedure for sulfonamide formation



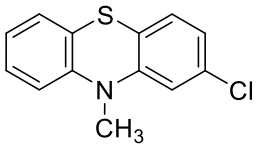
52, n = 1
53, n = 2

54, n = 1
55, n = 2

A solution of a substituted alkyl ammonium chloride (1 mmol) in DMF (5 mL) was cooled to 0 °C, treated with triethylamine (TEA, 293 µL, 2.1 mmol), and 4-trifluoromethoxybenzenesulfonyl chloride (187 µL, 1.1 mmol). The mixture was warmed to r. t. and stirred for 2 h. It was then poured into a saturated aqueous NaCl solution (50 mL), and extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were washed with saturated aqueous NaCl solution (2 x 50 mL), dried over $MgSO_4$, and concentrated under vacuum. The residue was dissolved in a minimal amount of dichloromethane, purified by column chromatography on silica gel (petroleum ether (b.p. 40 – 60 °C):ethyl acetate = 8:2) and recrystallized from ethanol affording the title compounds.

5.2.7 Analytical data of synthesized compounds

2-Chloro-10-methyl-10H-phenothiazine (26)[89]



The compound was prepared from methyl iodide (1.2 eq.), NaH (1 eq.), at r.t., in 4 h according to procedure 5.2.1.1. Chromatographic purification: petroleum ether (b.p. 40 – 60 °C):ethyl acetate = 95:5 as eluent.

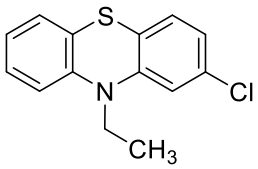
Appearance: white solid. **Yield:** 106 mg, 50 %. **Melting point:** 91 – 93 °C (lit. m.p. 81 – 83 °C).[1] **Purity by HPLC-UV (254 nm)-ESI-MS:** 98.6 %.

^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.16 – 7.09 (m, 2H, $H_{arom.}$), 7.01 (d, *J* = 8.1 Hz, 1H, $H_{arom.}$), 6.85 (m, 2H, $H_{arom.}$), 6.81 – 6.72 (m, 2H, $H_{arom.}$), 3.74 (s, 3H, CH_3).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 146.6 (2 x $C_{arom.}$, $C_{quat.}$), 133.4 ($C_{arom.}$, $C_{quat.}$), 127.6 ($C_{arom.}$, CH), 127.4 ($C_{arom.}$, CH), 127.0 ($C_{arom.}$, CH), 123.0 (2 x $C_{arom.}$, $C_{quat.}$), 122.3 (2 x $C_{arom.}$, CH), 114.5 ($C_{arom.}$, CH), 114.3 ($C_{arom.}$, CH), 35.2 (CH_3).

LC-ESI-MS (m/z): 248 $[M+H]^+$.

2-Chloro-10-ethyl-10H-phenothiazine (27)[90]



The compound was prepared from ethyl iodide (1.2 eq.), NaH (1 eq.), at r.t., in 5 h according to procedure 5.2.1.1. Chromatographic purification: petroleum ether (b.p. 40 – 60 °C):ethyl acetate = 95:5 as eluent.

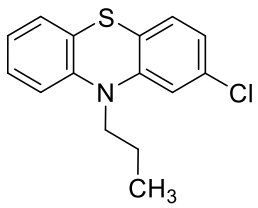
Appearance: white solid. **Yield:** 118 mg, 52 %. **Melting point:** 130 – 132 °C (lit. m.p. 120 °C).[2] **Purity by HPLC-UV (254 nm)-ESI-MS:** 98.2 %.

^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.16 – 7.05 (m, 2H, $H_{arom.}$), 6.99 (d, *J* = 8.1 Hz, 1H, $H_{arom.}$), 6.93 – 6.75 (m, 4H, $H_{arom.}$), 3.91 (s (br), 2H, CH_2), 1.39 (t, *J* = 6.9 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 145.8 (2 x $C_{arom.}$, $C_{quat.}$), 133.2 ($C_{arom.}$, $C_{quat.}$), 127.6 ($C_{arom.}$, CH), 127.4 (2 x $C_{arom.}$, CH), 122.8 (2 x $C_{arom.}$, $C_{quat.}$), 122.3 (2 x $C_{arom.}$, CH), 115.5 (2 x $C_{arom.}$, CH), 42.0 (CH_2), 12.8 (CH_3).

LC-ESI-MS (m/z): 262 $[M+H]^+$.

2-Chloro-10-propyl-10H-phenothiazine (28)[74]



The compound was prepared from *n*-propyl iodide (1.2 eq.), NaH (1 eq.), at r.t., in 24 h according to procedure 5.2.1.1. Chromatographic purification: petroleum ether (b.p. 40 – 60 °C):ethyl acetate = 95:5 as eluent.

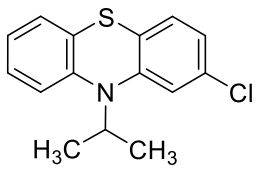
Appearance: pink-colored solid. **Yield:** 105 mg, 44 %. **Melting point:** 60 – 62 °C. **Purity by HPLC-UV (254 nm)-ESI-MS:** 98.7 %.

^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.17 – 7.07 (m, 2H, $H_{arom.}$), 7.00 (d, *J* = 8.1 Hz, 1H, $H_{arom.}$), 6.89 – 6.77 (m, 4H, $H_{arom.}$), 3.78 (s (br), 2H, N-C$\underline{H}_2$), 1.80 (sext, *J* = 7.3 Hz, 2H, C$\underline{H}_2$$CH_3$), 0.99 (t, *J* = 7.4 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 146.5 ($C_{arom.}$, $C_{quat.}$), 144.5 ($C_{arom.}$, $C_{quat.}$), 133.1 ($C_{arom.}$, $C_{quat.}$), 127.8 ($C_{arom.}$, CH), 127.4 ($C_{arom.}$, CH), 127.3 ($C_{arom.}$, CH), 124.7 ($C_{arom.}$, $C_{quat.}$), 123.4 ($C_{arom.}$, $C_{quat.}$), 122.8 ($C_{arom.}$, CH), 122.1 ($C_{arom.}$, CH), 115.7 (2 x $C_{arom.}$, CH), 49.2 (N-$\underline{C}H_2$), 20.0 (C$\underline{H}_2$$CH_3$), 11.3 ($CH_3$).

LC-ESI-MS (m/z): 276 $[M+H]^+$.

2-Chloro-10-(1-methylethyl)-10H-phenothiazine (29)



The compound was prepared from isopropyl iodide (2 eq.), NaH (2 eq.), at r.t., in 4 h according to procedure 5.2.1.1. Chromatographic purification: petroleum ether (b.p. 40 – 60 °C):ethyl acetate = 95:5 as eluent.

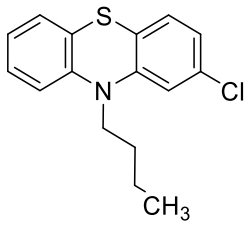
Appearance: off-white solid. **Yield:** 90 mg, 38 %. **Melting point:** 55 – 57 °C. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.6 %.

^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.15 – 7.08 (m, 4H, $H_{arom.}$), 7.00 (d, *J* = 8.2 Hz, 1H, $H_{arom.}$), 6.97 – 6.92 (m, 1H, $H_{arom.}$), 6.89 (dd, *J* = 8.2, 2.1 Hz, 1H, $H_{arom.}$), 4.25 (sept, *J* = 6.9 Hz, 1H, N-C<u>H</u>), 1.60 (d, *J* = 6.9 Hz, 6H, 2 x CH_3).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 145.9 ($C_{arom.}$, $C_{quat.}$), 143.9 ($C_{arom.}$, $C_{quat.}$), 132.8 ($C_{arom.}$, $C_{quat.}$), 127.7 ($C_{arom.}$, CH), 127.4 ($C_{arom.}$, CH), 127.3 ($C_{arom.}$, CH), 126.5 ($C_{arom.}$, $C_{quat.}$), 125.5 ($C_{arom.}$, $C_{quat.}$), 123.4 ($C_{arom.}$, CH), 122.8 ($C_{arom.}$, CH), 118.7 ($C_{arom.}$, CH), 118.5 ($C_{arom.}$, CH), 54.0 (N-<u>C</u>H), 22.0 (2 x CH_3).

LC-ESI-MS (m/z): 276 $[M+H]^+$.

***2-Chloro-10-butyl-10H-phenothiazine (30)*[91]**



The compound was prepared from iodobutane (5 eq.) according to procedure 5.2.1.2. Chromatographic purification: petroleum ether (b.p. 40 – 60 °C):ethyl acetate = 95:5 as eluent.

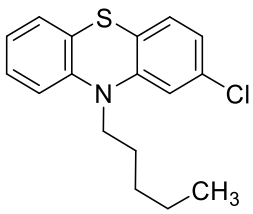
Appearance: colorless oil. **Yield:** 233 mg, 75 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 96.7 %.

^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.15 – 7.07 (m, 2H, $H_{arom.}$), 7.00 - 6.97 (m, 1H, $H_{arom.}$), 6.92 – 6.78 (m, 4H, $H_{arom.}$), 3.80 (s, 2H, N-CH_2), 1.74 (m, 2H, CH_2), 1.42 (m, 2H, CH_2), 0.92 (m, 3H, CH_3).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 146.6 ($C_{arom.}$, $C_{quat.}$), 144.6 ($C_{arom.}$, $C_{quat.}$), 133.2 ($C_{arom.}$, $C_{quat.}$), 127.8 ($C_{arom.}$, CH), 127.5 ($C_{arom.}$, CH), 127.4 ($C_{arom.}$, CH), 124.7 ($C_{arom.}$, $C_{quat.}$), 123.5 ($C_{arom.}$, $C_{quat.}$), 122.8 ($C_{arom.}$, CH), 122.2 ($C_{arom.}$, CH), 115.7 ($C_{arom.}$, CH), 114.9 ($C_{arom.}$, CH), 47.1 (CH_2), 28.9 (CH_2), 20.1 (CH_2), 13.8 (CH_3).

LC-ESI-MS (m/z): 290 $[M+H]^+$.

2-Chloro-10-pentyl-10H-phenothiazine (31)[92]



The compound was prepared from 1-bromopentane (5 eq.) according to procedure 5.2.1.2. Chromatographic purification: petroleum ether (b.p. 40 – 60 °C):ethyl acetate = 98:2 as eluent.

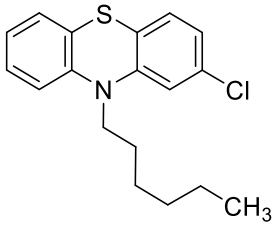
Appearance: colorless oil. **Yield:** 265 mg, 82 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.9 %.

^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.14 – 7.09 (m, 2H, $H_{arom.}$), 6.99 (d, *J* = 8.2 Hz, 1H, $H_{arom.}$), 6.90 – 6.79 (m, 4H, $H_{arom.}$), 3.80 (s, 2H, N-CH_2), 1.77 (quintet, *J* = 7.3 Hz, 2H, CH_2), 1.41 – 1.29 (m, 4H, 2 x CH_2), 0.88 (t, *J* = 7.2 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 146.5 ($C_{arom.}$, $C_{quat.}$), 144.5 ($C_{arom.}$, $C_{quat.}$), 133.1 ($C_{arom.}$, $C_{quat.}$), 127.8 ($C_{arom.}$, CH), 127.4 ($C_{arom.}$, CH), 127.3 ($C_{arom.}$, CH), 124.7 ($C_{arom.}$, $C_{quat.}$), 123.4 ($C_{arom.}$, $C_{quat.}$), 122.8 ($C_{arom.}$, CH), 122.1 ($C_{arom.}$, CH), 115.7 (2 x $C_{arom.}$, CH), 47.4 (CH_2), 29.1 (CH_2), 26.5 (CH_2), 22.3 (CH_2), 14.0 (CH_3).

LC-ESI-MS (m/z): 304 $[M+H]^+$.

2-Chloro-10-hexyl-10H-phenothiazine (32)



The compound was prepared from 1-bromohexane (5 eq.) according to procedure 5.2.1.2. Chromatographic purification: petroleum ether (b.p. 40 – 60 °C) as eluent.

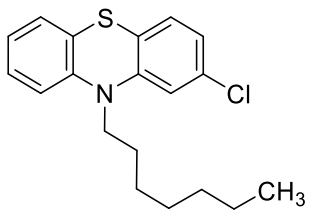
Appearance: colorless oil. **Yield:** 287 mg, 84 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.1 %.

^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.14 – 7.09 (m, 2H, $H_{arom.}$), 6.99 (d, *J* = 8.6 Hz, 1H, $H_{arom.}$), 6.94 – 6.79 (m, 4H, $H_{arom.}$), 3.80 (s, 2H, N-CH_2), 1.76 (quintet, *J* = 8.1 Hz, 2H, CH_2), 1.41 (s, 2H, CH_2), 1.29 - 1.27 (m, 4H, 2 x CH_2), 0.86 (t, *J* = 6.5 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 146.5 ($C_{arom.}$, $C_{quat.}$), 144.5 ($C_{arom.}$, $C_{quat.}$), 133.1 ($C_{arom.}$, $C_{quat.}$), 127.8 ($C_{arom.}$, CH), 127.4 ($C_{arom.}$, CH), 127.3 ($C_{arom.}$, CH), 124.7 ($C_{arom.}$, $C_{quat.}$), 123.5 ($C_{arom.}$, $C_{quat.}$), 122.8 ($C_{arom.}$, CH), 122.2 ($C_{arom.}$, CH), 115.7 (2 x $C_{arom.}$, CH), 47.5 (CH_2), 31.4 (CH_2), 26.8 (CH_2), 26.6 (CH_2), 22.6 (CH_2), 13.9 (CH_3).

LC-ESI-MS (m/z): 318 $[M+H]^+$.

2-Chloro-10-heptyl-10H-phenothiazine (33)



The compound was prepared from 1-bromoheptane (5 eq.) according to procedure 5.2.1.2. Chromatographic purification: petroleum ether (b.p. 40 – 60 °C) as eluent.

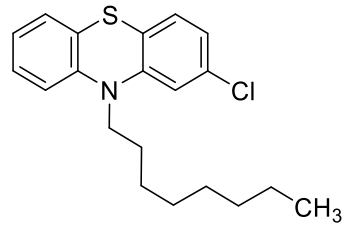
Appearance: light yellow oil. **Yield:** 288 mg, 81 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.4 %.

^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.12 – 7.09 (m, 2H, $H_{arom.}$), 6.99 (d, *J* = 8.1 Hz, 1H, $H_{arom.}$), 6.90 – 6.78 (m, 4H, $H_{arom.}$), 3.80 (s, 2H, N-CH_2), 1.76 (quintet, *J* = 7.6 Hz, 2H, CH_2), 1.42 - 1.37 (m, 2H, CH_2), 1.32 - 1.21 (m, 6H, 3 x CH_2), 0.84 (t, *J* = 6.8 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 146.6 ($C_{arom.}$, $C_{quat.}$), 144.5 ($C_{arom.}$, $C_{quat.}$), 133.2 ($C_{arom.}$, $C_{quat.}$), 127.8 ($C_{arom.}$, CH), 127.4 ($C_{arom.}$, CH), 127.3 ($C_{arom.}$, CH), 124.7 ($C_{arom.}$, $C_{quat.}$), 123.5 ($C_{arom.}$, $C_{quat.}$), 122.8 ($C_{arom.}$, CH), 122.2 ($C_{arom.}$, CH), 115.7 (2 x $C_{arom.}$, CH), 47.4 (CH_2), 31.7 (CH_2), 28.8 (CH_2), 26.8 (2 x CH_2), 22.5 (CH_2), 13.9 (CH_3).

LC-ESI-MS (m/z): 332 $[M+H]^+$.

2-Chloro-10-octyl-10H-phenothiazine (34)



The compound was prepared from 1-bromooctane (5 eq.) according to procedure 5.2.1.2. Chromatographic purification: petroleum ether (b.p. 40 – 60 °C) as eluent.

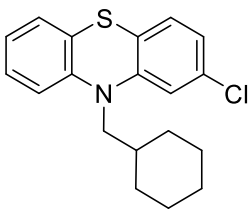
Appearance: light yellow oil. **Yield:** 322 mg, 87 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 98.2 %.

^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.13 – 7.09 (m, 2H, $H_{arom.}$), 6.99 (d, *J* = 8.0 Hz, 1H, $H_{arom.}$), 6.87 – 6.78 (m, 4H, $H_{arom.}$), 3.84 (s, 2H, N-CH_2), 1.76 (quintet, *J* = 7.3 Hz, 2H, CH_2), 1.40 (quintet, *J* = 7.1 Hz, 2H, CH_2), 1.31 - 1.19 (m, 8H, 4 x CH_2), 0.85 (t, *J* = 6.7 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 133.2 ($C_{arom.}$, $C_{quat.}$), 127.7 ($C_{arom.}$, CH), 127.4 (2 x $C_{arom.}$, CH), 124.8 ($C_{arom.}$, $C_{quat.}$), 123.5 ($C_{arom.}$, $C_{quat.}$), 122.9 ($C_{arom.}$, CH), 122.3 ($C_{arom.}$, CH), 115.7 (2 x $C_{arom.}$, CH), 47.3 (CH_2), 34.1 (CH_2), 32.8 (CH_2), 31.7 (CH_2), 29.1 (CH_2), 26.8 (CH_2), 22.6 (CH_2), 14.1 (CH_3), (2 x $C_{quat.}$ signals not visible).

LC-ESI-MS (m/z): 346 $[M+H]^+$.

2-Chloro-10-(cyclohexylmethyl)-10H-phenothiazine (35)



The compound was prepared from cyclohexylmethyl bromide (1.2 eq.) according to procedure 5.2.1.3. Chromatographic purification: petroleum ether (b.p. 40 – 60 °C) as eluent.

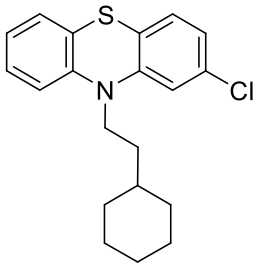
Appearance: colorless oil. **Yield:** 180 mg, 32 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 95.8 %.

^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.17 – 7.06 (m, 2H, $H_{arom.}$), 7.02 (d, *J* = 8.1 Hz, 1H, $H_{arom.}$), 6.96 – 6.77 (m, 4H, $H_{arom.}$), 3.65 (s (br), 2H, N-C$\underline{H}_2$), 1.93 – 1.78 (m, 3H, $H_{cyclohexyl}$), 1.72 – 1.69 (m, 2H, $H_{cyclohexyl}$), 1.54 (s, 1H, $H_{cyclohexyl}$), 1.27 – 1.05 (m, 3H, $H_{cyclohexyl}$), 0.98 - 0.77 (m, 2H, $H_{cyclohexyl}$).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 147.1 ($C_{arom.}$, $C_{quat.}$), 144.9 ($C_{arom.}$, $C_{quat.}$), 133.1 ($C_{arom.}$, $C_{quat.}$), 128.0 ($C_{arom.}$, CH), 127.6 ($C_{arom.}$, CH), 127.3 ($C_{arom.}$, CH), 125.4 ($C_{arom.}$, $C_{quat.}$), 124.1 ($C_{arom.}$, $C_{quat.}$), 122.8 ($C_{arom.}$, CH), 122.2 ($C_{arom.}$, CH), 116.1 ($C_{arom.}$, 2 x CH), 53.8 (N-$\underline{C}H_2$), 34.6 ($CH_{cyclohexyl}$), 30.9 (2 x $CH_{2,cyclohexyl}$), 26.6 (2 x $CH_{2,cyclohexyl}$), 25.7 ($CH_{2,cyclohexyl}$).

LC-ESI-MS (m/z): 330 $[M+H]^+$.

2-Chloro-10-(2-cyclohexylethyl)-10H-phenothiazine (36)



The compound was prepared from 2-cyclohexylethyl bromide (1.2 eq.), NaH (1 eq.), at 60 °C, in 4 h according to procedure 5.2.1.1. Chromatographic purification: petroleum ether (b.p. 40 – 60 °C) as eluent.

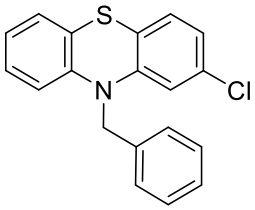
Appearance: colorless oil. **Yield:** 203 mg, 34 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 99.6 %.

^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.17 – 7.07 (m, 2H, $H_{arom.}$), 7.00 (d, *J* = 8.1 Hz, 1H, $H_{arom.}$), 6.91 – 6.73 (m, 4H, $H_{arom.}$), 3.78 (s (br), 2H, N-C$\underline{H}_2$), 1.75 – 1.60 (m, 6H, N-CH_2-C$\underline{H}_2$ and $H_{cyclohexyl}$), 1.46 – 1.37 (m, 1H, $H_{cyclohexyl}$), 1.28 – 1.09 (m, 4H, $H_{cyclohexyl}$), 1.00 - 0.92 (m, 2H, $H_{cyclohexyl}$).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 146.9 ($C_{arom.}$, $C_{quat.}$), 144.8 ($C_{arom.}$, $C_{quat.}$), 133.1 ($C_{arom.}$, $C_{quat.}$), 127.3 ($C_{arom.}$, 3 x CH), 125.7 ($C_{arom.}$, $C_{quat.}$), 124.7 ($C_{arom.}$, $C_{quat.}$), 122.7 ($C_{arom.}$, CH), 121.9 ($C_{arom.}$, CH), 115.7 ($C_{arom.}$, 2 x CH), 53.4 (N-$\underline{C}H_2$), 45.3 (N-CH_2-$\underline{C}H_2$), 35.8 ($CH_{cyclohexyl}$), 33.2 (2 x $CH_{2,cyclohexyl}$), 26.4 (2 x $CH_{2,cyclohexyl}$), 26.2 ($CH_{2,cyclohexyl}$).

LC-ESI-MS (m/z): 344 $[M+H]^+$.

2-Chloro-10-(phenylmethyl)-10H-phenothiazine (37)[93]



The compound was prepared from benzyl bromide (1.2 eq.) according to procedure 5.2.1.3. Chromatographic purification: petroleum ether (b.p. 40 – 60 °C):ethyl acetate = 98:2 as eluent.

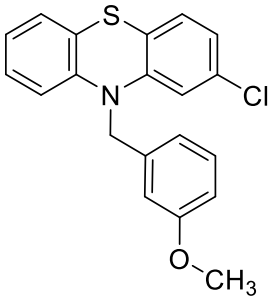
Appearance: colorless oil. **Yield:** 279 mg, 50 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 98.2 %.

^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.35 – 7.29 (m, 2H, $H_{arom.}$), 7.29 – 7.23 (m, 3H, $H_{arom.}$), 7.05 (dd, *J* = 7.6, 1.5 Hz, 1H, $H_{arom.}$), 6.98 – 6.92 (m, 2H, $H_{arom.}$), 6.87 – 6.78 (m, 2H, $H_{arom.}$), 6.62 (d, *J* = 8.1 Hz, 1H, $H_{arom.}$), 6.59 (d, *J* = 2.0 Hz, 1H, $H_{arom.}$), 5.06 (s, 2H, CH_2).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 143.8 ($C_{arom.}$, $C_{quat.}$), 136.0 ($C_{arom.}$, $C_{quat.}$), 133.1 ($C_{arom.}$, 2 x $C_{quat.}$), 128.9 ($C_{arom.}$, 2 x CH), 127.4 ($C_{arom.}$, CH), 127.3 ($C_{arom.}$, 2 x CH), 126.9 ($C_{arom.}$, CH), 126.5 ($C_{arom.}$, 2 x CH), 123.0 ($C_{arom.}$, $C_{quat.}$), 122.4 ($C_{arom.}$, $C_{quat.}$), 115.8 ($C_{arom.}$, 2 x CH), 115.6 ($C_{arom.}$, 2 x CH), 52.7 (CH_2).

LC-ESI-MS (m/z): 324 $[M+H]^+$.

2-Chloro-10-[(3-methoxyphenyl)methyl]-10H-phenothiazine (38)



The compound was prepared from 3-methoxybenzyl bromide (3 eq.) according to procedure 5.2.1.2. Chromatographic purification: petroleum ether (b.p. 40 – 60 °C):ethyl acetate = 98:2 as eluent.

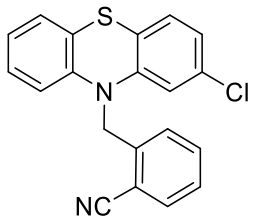
Appearance: colorless oil. **Yield:** 295 mg, 83 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.4 %.

^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.23 (t, *J* = 7.9, 1H, $H_{arom.}$), 7.05 (dd, *J* = 7.6, 1.5 Hz, 1H, $H_{arom.}$), 6.96 – 6.94 (m, 2H, $H_{arom.}$), 6.86 – 6.77 (m, 5H, $H_{arom.}$), 6.63 (d, *J* = 8.1, 1H, $H_{arom.}$), 6.60 (d, *J* = 2.0, 1H, $H_{arom.}$), 5.04 (s, 2H, CH_2), 3.75 (s, 3H, CH_3).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 160.1 ($C_{arom.}$, $C_{quat.}$), 145.7 ($C_{arom.}$, $C_{quat.}$), 143.7 ($C_{arom.}$, $C_{quat.}$), 133.1 ($C_{arom.}$, $C_{quat.}$), 129.9 ($C_{arom.}$, CH), 127.4 ($C_{arom.}$, CH), 127.2 ($C_{arom.}$, CH), 126.8 ($C_{arom.}$, CH), 123.1 ($C_{arom.}$, CH), 122.5 ($C_{arom.}$, CH), 118.8 ($C_{arom.}$, CH), 115.8 ($C_{arom.}$, CH), 115.6 ($C_{arom.}$, CH), 112.6 ($C_{arom.}$, CH), 112.1 ($C_{arom.}$, CH), 65.8 (CH_2), 55.2 (OCH_3), (3 x $C_{quat.}$ signals not visible).

LC-ESI-MS (m/z): 354 $[M+H]^+$.

2-Chloro-10-[(2-cyanophenyl)methyl]-10H-phenothiazine (39)



The compound was prepared from 2-(bromomethyl)benzonitrile (3 eq.) according to procedure 5.2.1.2. Chromatographic purification: petroleum ether (b.p. 40 – 60 °C):ethyl acetate = 95:5 as eluent.

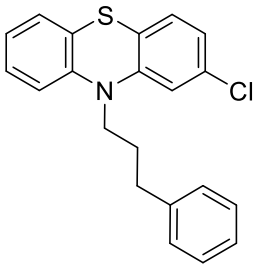
Appearance: pink-colored solid. **Melting point:** 88 – 90 °C. **Yield:** 98 mg, 28 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 94.2 %.

^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.73 (dd, *J* = 7.7, 1.3 Hz, 1H, $H_{arom.}$), 7.48 (td, *J* = 7.7, 7.6, 1.3 Hz, 1H, $H_{arom.}$), 7.41 (d, *J* = 7.9 Hz, 1H, $H_{arom.}$), 7.36 (td, *J* = 7.6, 7.6, 1.2 Hz, 1H, $H_{arom.}$), 7.10 (dd, *J* = 7.6, 1.5 Hz, 1H, $H_{arom.}$), 7.00 (td, *J* = 8.0, 7.9, 1.9 Hz, 2H, $H_{arom.}$), 6.88 (m, 2H, $H_{arom.}$), 6.60 – 6.55 (m, 2H, $H_{arom.}$), 5.23 (s, 2H, CH_2).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 145.4 ($C_{arom.}$, $C_{quat.}$), 143.3 ($C_{arom.}$, $C_{quat.}$), 140.0 ($C_{arom.}$, $C_{quat.}$), 133.4 ($C_{arom.}$, CH), 133.3 ($C_{arom.}$, $C_{quat.}$), 133.2 ($C_{arom.}$, CH), 127.9 ($C_{arom.}$, CH), 127.8 ($C_{arom.}$, CH), 127.7 ($C_{arom.}$, CH), 127.5 ($C_{arom.}$, CH), 127.3 ($C_{arom.}$, CH), 123.9 ($C_{arom.}$, $C_{quat.}$), 123.5 ($C_{arom.}$, CH), 122.9 ($C_{arom.}$, CH), 122.7 ($C_{arom.}$, $C_{quat.}$), 116.9 ($C_{arom.}$, $C_{quat.}$), 115.6 ($C_{arom.}$, CH), 115.5 ($C_{arom.}$, CH), 110.9 ($C_{arom.}$, CN), 50.7 (CH_2).

LC-ESI-MS (m/z): 349 $[M+H]^+$.

2-Chloro-10-(3-phenylpropyl)-10H-phenothiazine (40)



The compound was prepared from 3-phenylpropyl bromide (1.2 eq.), NaH (1 eq.), at 60 °C, in 24 h according to procedure 5.2.1.1. Chromatographic purification: petroleum ether (b.p. 40 – 60 °C):ethyl acetate = 95:5 as eluent.

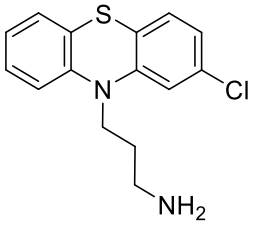
Appearance: pink-colored oil. **Yield:** 250 mg, 41 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.1 %.

^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.28 – 7.21 (m, 2H, $H_{arom.}$), 7.18 – 7.14 (m, 1H, $H_{arom.}$), 7.14 – 7.10 (m, 4H, $H_{arom.}$), 7.02 (d, *J* = 8.1 Hz, 1H, $H_{arom.}$), 6.91 – 6.73 (m, 4H, $H_{arom.}$), 3.82 (s (br), 2H, N-$C\underline{H}_2$), 2.73 (t, *J* = 7.5 Hz, 2H, $C\underline{H}_2$-C_6H_5), 2.16 – 2.06 (m, 1H, CH_2-$C\underline{H}_2$-CH_2).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 146.5 ($C_{arom.}$, $C_{quat.}$), 144.6 ($C_{arom.}$, $C_{quat.}$), 141.1 ($C_{arom.}$, $C_{quat.}$), 133.1 ($C_{arom.}$, $C_{quat.}$), 128.5 ($C_{arom.}$, 2 x CH), 128.4 ($C_{arom.}$, 2 x CH), 127.3 ($C_{arom.}$, 2 x CH), 125.9 ($C_{arom.}$, 2 x CH), 122.9 ($C_{arom.}$, $C_{quat.}$), 122.3 ($C_{arom.}$, $C_{quat.}$), 115.9 ($C_{arom.}$, 2 x CH), 115.8 ($C_{arom.}$, 2 x CH), 32.6 (3 x $CH_{2,propyl}$).

LC-ESI-MS (m/z): 352 $[M+H]^+$.

2-Chloro-10-(3-aminopropyl)-10H-phenothiazine (41)[94]



The compound was prepared from 3-bromopropylamine hydrobromide (5 eq.) according to procedure 5.2.1.2. Chromatographic purification: dichloromethane/methanol/aq. NH_3: 90/9/1 as eluent.

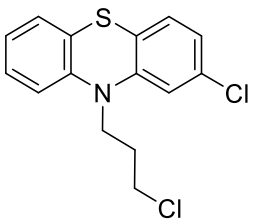
Appearance: dark green oil. **Yield:** 180 mg, 58 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 99.2 %.

^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.16 – 7.10 (m, 2H, $H_{arom.}$), 7.00 (d, *J* = 8.1 Hz, 1H, $H_{arom.}$), 6.91 (t, *J* = 7.5 Hz, 1H, $H_{arom.}$), 6.89 – 6.84 (m, 3H, $H_{arom.}$), 3.91 (t, *J* = 6.7 Hz, 2H, N-CH_2), 2.82 (t, *J* = 6.8 Hz, 2H, CH_2), 1.91 (m, 4H, CH_2, NH_2).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 146.5 ($C_{arom.}$, $C_{quat.}$), 144.5 ($C_{arom.}$, $C_{quat.}$), 133.2 ($C_{arom.}$, $C_{quat.}$), 127.9 ($C_{arom.}$, CH), 127.6 ($C_{arom.}$, CH), 127.4 ($C_{arom.}$, CH), 125.1 ($C_{arom.}$, $C_{quat.}$), 123.8 ($C_{arom.}$, $C_{quat.}$), 122.9 ($C_{arom.}$, CH), 122.3 ($C_{arom.}$, CH), 115.9 (2 x $C_{arom.}$, CH), 44.9 (CH_2), 39.5 (CH_2), 29.9 (CH_2).

LC-ESI-MS (m/z): 291 $[M+H]^+$.

2-Chloro-10-(3-chloropropyl)-10H-phenothiazine (42)[95]



The compound was prepared from 1-bromo-3-chloropropane (5 eq.) according to procedure 5.2.1.2. Chromatographic purification: petroleum ether (b.p. 40 – 60 °C):ethyl acetate = 95:5 as eluent.

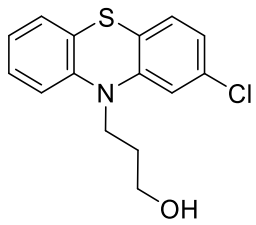
Appearance: light yellow oil. **Yield:** 180 mg, 54 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.6 %.

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 7.23 (ddd, *J* = 8.2, 7.3, 1.6 Hz, 1H, $H_{arom.}$), 7.19 – 7.15 (m, 2H, $H_{arom.}$), 7.12 (d, *J* = 2.1 Hz, 1H, $H_{arom.}$), 7.09 (dd, *J* = 8.2, 1.1 Hz, 1H, $H_{arom.}$), 7.03 – 6.95 (m, 2H, $H_{arom.}$), 4.04 (t, *J* = 6.7 Hz, 2H, CH_2-Cl), 3.72 (t, *J* = 6.3 Hz, 2H, N-CH_2), 2.10 (quint, 2H, CH_2-C$\underline{H}_2$-CH_2).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 146.3 ($C_{arom.}$, $C_{quat.}$), 143.9 ($C_{arom.}$, $C_{quat.}$), 132.7 ($C_{arom.}$, $C_{quat.}$), 128.3 ($C_{arom.}$, CH), 127.9 ($C_{arom.}$, CH), 127.5 ($C_{arom.}$, CH), 123.8 ($C_{arom.}$, $C_{quat.}$), 123.3 ($C_{arom.}$, CH), 123.2 ($C_{arom.}$, $C_{quat.}$), 122.5 ($C_{arom.}$, CH), 116.5 ($C_{arom.}$, CH), 115.9 ($C_{arom.}$, CH), 43.7 (CH_2), 42.7 (CH_2), 29.3 (CH_2).

LC-ESI-MS (m/z): 310 $[M]^+$, 312 $[M+2]^+$, 314 $[M+4]^+$.

2-Chloro-10-(3-hydroxypropyl)-10H-phenothiazine (43)[96]



The compound was prepared from 3-iodo-1-propanol (3 eq.) according to procedure 5.2.1.2. Chromatographic purification: petroleum ether (b.p. 40 – 60 °C):ethyl acetate = 6:4 as eluent. Recrystallization: *n*-hexane/ethyl acetate.

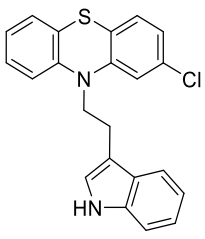
Appearance: pink-colored solid. **Melting point:** 132 – 133 °C. (lit. m.p. 118 - 119 °C).[96] **Yield:** 36 mg, 12 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.6 %.

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 7.20 (ddd, J = 8.2, 7.3, 1.6 Hz, 1H, $H_{arom.}$), 7.14 – 7.12 (m, 2H, $H_{arom.}$), 7.07 (d, J = 2.1 Hz, 1H, $H_{arom.}$), 7.05 (dd, J = 8.2, 1.2 Hz, 1H, $H_{arom.}$), 6.98 – 6.94 (m, 2H, $H_{arom.}$), 4.55 (t, J = 5.0, 1H, OH), 3.93 (t, J = 7.1, 2H, N-CH_2), 3.50 (td, J = 6.0, 6.0, 4.9 Hz, 2H, CH_2-OH), 1.86 – 1.77 (m, 2H, N-CH_2-CH_2).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 146.4 ($C_{arom.}$, $C_{quat.}$), 144.1 ($C_{arom.}$, $C_{quat.}$), 132.6 ($C_{arom.}$, $C_{quat.}$), 128.1 ($C_{arom.}$, CH), 127.9 ($C_{arom.}$, CH), 127.3 ($C_{arom.}$, CH), 123.3 ($C_{arom.}$, $C_{quat.}$), 123.0 ($C_{arom.}$, CH), 122.6 ($C_{arom.}$, $C_{quat.}$), 122.1 ($C_{arom.}$, CH), 116.3 ($C_{arom.}$, CH), 115.7 ($C_{arom.}$, CH), 58.0 (CH_2), 43.7 (CH_2), 29.6 (CH_2).

LC-ESI-MS (m/z): 292 $[M+H]^+$.

2-Chloro-10-[2-(1H-indol-3-yl)ethyl]-10H-phenothiazine (44)



The compound was prepared from 3-(2-bromoethyl)indole (3 eq.) according to procedure 5.2.1.2. Chromatographic purification: petroleum ether (b.p. 40 – 60 °C):ethyl acetate = 9:1 – 8:2 as eluent.

Appearance: off-white solid. **Melting point:** 142 – 144 °C. **Yield:** 75 mg, 16 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 95.9 %.

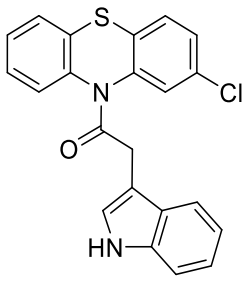
^{1}H-NMR (600 MHz, DMSO-d_6) δ: 10.84 (s, 1H, NH_{indole}), 7.51 (d, J = 7.9 Hz, 1H, $H_{arom.}$), 7.32 (d, J = 8.0 Hz, 1H, $H_{arom.}$), 7.27 – 7.21 (m, 2H, $H_{arom.}$), 7.19 – 7.11 (m, 4H, $H_{arom.}$), 7.06 (ddd, 8.0, 6.9, 1.1 Hz, 1H, $H_{arom.}$), 7.02 – 6.93 (m, 3H, $H_{arom.}$), 4.15 (t, J = 7.0 Hz, 2H, CH_2), 3.92 (t, J = 7.2 Hz, 2H, CH_2).

^{13}C-NMR (150 MHz, DMSO-d_6) δ: 146.4 ($C_{arom.}$, $C_{quat.}$), 144.0 ($C_{arom.}$, $C_{quat.}$), 136.2 ($C_{arom.}$, $C_{quat.}$), 132.6 ($C_{arom.}$, $C_{quat.}$), 128.2 ($C_{arom.}$, CH), 127.9 ($C_{arom.}$, CH), 127.4 ($C_{arom.}$, CH), 127.2 ($C_{arom.}$, $C_{quat.}$), 123.5 ($C_{arom.}$, $C_{quat.}$), 123.2 ($C_{arom.}$, CH), 123.0 ($C_{arom.}$, CH), 122.8 ($C_{arom.}$, $C_{quat.}$),

122.2 ($C_{arom.}$, CH), 121.1 ($C_{arom.}$, CH), 118.5 ($C_{arom.}$, CH), 118.2 ($C_{arom.}$, CH), 116.4 ($C_{arom.}$, CH), 115.9 ($C_{arom.}$, CH), 111.5 ($C_{arom.}$, CH), 111.3 ($C_{arom.}$, $C_{quat.}$), 47.7 (CH_2), 22.5 (CH_2).

LC-ESI-MS (m/z): 377 $[M+H]^+$.

2-(1H-Indol-3-yl)-1-(2-chloro-10H-phenothiazin-10-yl)ethanone (46)



The compound was prepared according to procedure 5.2.2.

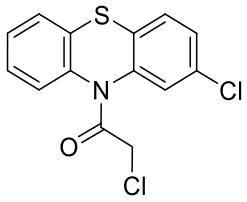
Appearance: white solid. **Melting point:** 210 - 212 °C. **Yield:** 224 mg, 52 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 95.6 %.

^{1}H-NMR (600 MHz, DMSO-d_6) δ: 10.82 (s, 1H, NH_{indole}), 7.79 (s, 1H, $H_{arom.}$), 7.70 (d, J = 7.9 Hz, 1H, $H_{arom.}$), 7.56 – 7.48 (m, 2H, $H_{arom.}$), 7.42 (td, J = 7.7, 1.4 Hz, 1H, $H_{arom.}$), 7.38 – 7.25 (m, 4H, $H_{arom.}$), 7.01 (t, 7.7 Hz, 1H, $H_{arom.}$), 6.92 – 6.86 (m, 2H, $H_{arom.}$), 3.92 (s, 2H, CH_2).

^{13}C-NMR (150 MHz, DMSO-d_6) δ: 170.0 (C=O), 140.2 ($C_{arom.}$, $C_{quat.}$), 138.7 ($C_{arom.}$, $C_{quat.}$), 136.4 ($C_{arom.}$, $C_{quat.}$), 132.5 ($C_{arom.}$, $C_{quat.}$), 131.9 ($C_{arom.}$, $C_{quat.}$), 131.8 ($C_{arom.}$, $C_{quat.}$), 129.4 ($C_{arom.}$, CH), 128.4 ($C_{arom.}$, CH), 128.0 ($C_{arom.}$, CH), 127.9 ($C_{arom.}$, CH), 127.8 ($C_{arom.}$, CH), 127.7 ($C_{arom.}$, CH), 127.4 ($C_{arom.}$, $C_{quat.}$), 127.3 ($C_{arom.}$, CH), 124.1 ($C_{arom.}$, CH), 121.4 ($C_{arom.}$, CH), 118.8 ($C_{arom.}$, CH), 118.7 ($C_{arom.}$, CH), 111.7 ($C_{arom.}$, CH), 107.5 ($C_{arom.}$, $C_{quat.}$), 31.4 (CH_2).

LC-ESI-MS (m/z): 391 $[M+H]^+$.

2-Chloro-1-(2-chloro-10H-phenothiazin-10-yl)ethanone (48)[74]



The compound was prepared from 2-chloroacetyl chloride (1.05 eq.) according to procedure 5.2.3.

Appearance: white solid. **Melting point:** 119 – 120 °C (lit. m.p. 115 - 118 °C).[97] **Yield:** 3.94 g, 74 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 95.3 %.

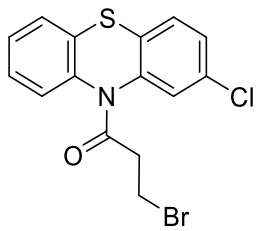
^{1}H-NMR (500 MHz, DMSO-d_6) δ: 7.79 (s, 1H, $H_{arom.}$), 7.68 (d, J = 7.7 Hz, 1H, $H_{arom.}$), 7.61 – 7.58 (m, 2H, $H_{arom.}$), 7.45 – 7.40 (m, 2H, $H_{arom.}$), 7.35 (td, J = 7.6, 1.4 Hz, 1H, $H_{arom.}$), 4.53 (s, 2H, CH_2).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 165.1 (C=O), 138.8 ($C_{arom.}$, $C_{quat.}$), 137.2 ($C_{arom.}$, $C_{quat.}$), 132.1 ($C_{arom.}$, $C_{quat.}$), 131.8 ($C_{arom.}$, $C_{quat.}$), 131.5 ($C_{arom.}$, $C_{quat.}$), 129.3 ($C_{arom.}$, CH), 128.3 ($C_{arom.}$,

CH), 127.9 ($C_{arom.}$, CH), 127.8 ($C_{arom.}$, CH), 127.5 ($C_{arom.}$, CH), 127.0 ($C_{arom.}$, CH), 126.9 ($C_{arom.}$, CH), 42.8 (CH_2).

LC-ESI-MS (m/z): 310 $[M+H]^+$.

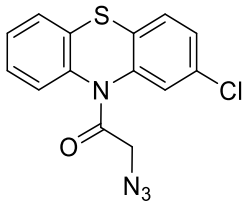
3-Bromo-1-(2-chloro-10H-phenothiazin-10-yl)propanone (49)[98]



The compound was prepared from 3-bromopropionyl chloride (1.05 eq.) according to procedure 5.2.3 and used directly in the next step without further analysis and purification

Appearance: white solid. **Yield:** 4.5 g, 55 %. **LC-ESI-MS (m/z):** 369 $[M+H]^+$.

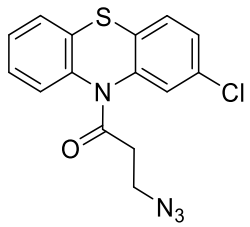
2-Azido-1-(2-chloro-10H-phenothiazin-10-yl)ethanone (50)[74]



The compound was prepared from **48** (3.2 mmol) according to procedure 5.2.4 and used directly in the next step without further analysis and purification.

Appearance: white solid. **Yield:** 792 mg, 77 %. **LC-ESI-MS (m/z):** 317 $[M+H]^+$.

3-Azido-1-(2-chloro-10H-phenothiazin-10-yl)propanone (51)



The compound was prepared from **49** (2.1 mmol) according to procedure 5.2.4.

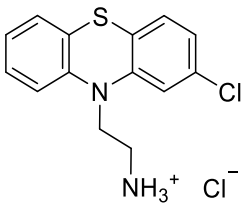
Appearance: white solid. **Melting point:** 102 – 103 °C. **Yield:** 526 mg, 76 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 98.0 %.

^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.55 (s, 1H, $H_{arom.}$), 7.48 – 7.42 (m, 2H, $H_{arom.}$), 7.36 – 7.31 (m, 2H, $H_{arom.}$), 7.28 – 7.19 (m, 2H, $H_{arom.}$), 3.67 – 3.50 (m, 2H, CH_2), 2.73 (s, 1H), 2.61 (s, 1H).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 169.1 (C=O), 139.3 ($C_{arom.}$, $C_{quat.}$), 137.8 ($C_{arom.}$, $C_{quat.}$), 133.2 ($C_{arom.}$, $C_{quat.}$), 132.9 ($C_{arom.}$, $C_{quat.}$), 131.6 ($C_{arom.}$, $C_{quat.}$), 128.6 ($C_{arom.}$, CH), 128.3 ($C_{arom.}$, CH), 127.6 ($C_{arom.}$, CH), 127.4 ($C_{arom.}$, 2 x CH), 127.3 ($C_{arom.}$, CH), 127.1 ($C_{arom.}$, CH), 47.2 (CH_2), 33.9 (CH_2).

LC-ESI-MS (m/z): 331 $[M+H]^+$.

2-(2-Chloro-10H-phenothiazin-10-yl)ethanamine hydrochloride (52)[74]



The compound was prepared from **50** (6.3 mmol) according to procedure 5.2.5.

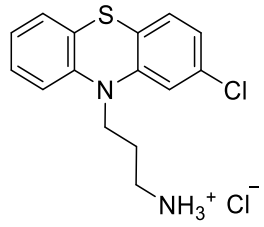
Appearance: white solid. **Melting point:** 283 – 284 °C. **Yield:** 1.45 g, 74 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 99.0 %.

^{1}H-NMR (600 MHz, MeOD) δ: 7.31 (dd, *J* = 7.8, 1.5 Hz, 1H, $H_{arom.}$), 7.26 (dd, *J* = 7.7, 1.5 Hz, 1H, $H_{arom.}$), 7.21 (d, *J* = 8.2 Hz, 1H, $H_{arom.}$), 7.16 – 7.11 (m, 2H, $H_{arom.}$), 7.08 (td, *J* = 7.5, 1.1 Hz, 1H, $H_{arom.}$), 7.06 (dd, *J* = 8.2, 2.1 Hz, 1H, $H_{arom.}$), 4.28 (t, *J* = 6.2 Hz, 2H, N-CH_2), 3.34 (t, 6.0 Hz, 2H, $C\underline{H}_2$-NH_3^+).

^{13}C-NMR (125 MHz, MeOD) δ: 146.2 ($C_{arom.}$, $C_{quat.}$), 143.8 ($C_{arom.}$, $C_{quat.}$), 133.3 ($C_{arom.}$, $C_{quat.}$), 128.1 ($C_{arom.}$, CH), 127.6 ($C_{arom.}$, CH), 127.5 ($C_{arom.}$, CH), 126.1 ($C_{arom.}$, $C_{quat.}$), 125.1 ($C_{arom.}$, $C_{quat.}$), 123.6 ($C_{arom.}$, CH), 122.9 ($C_{arom.}$, CH), 115.7 ($C_{arom.}$, CH), 115.6 ($C_{arom.}$, CH), 43.8 (CH_2), 36.1 (CH_2).

LC-ESI-MS (m/z): 277 $[M\text{-}Cl]^+$.

3-(2-Chloro-10H-phenothiazin-10-yl)propanamine hydrochloride (53)[74]



The compound was prepared from **51** (4.0 mmol) according to procedure 5.2.5.

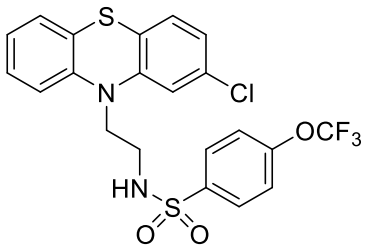
Appearance: white solid. **Melting point:** 241 – 243 °C (lit. m.p. 233 – 235 °C).[99] **Yield:** 874 mg, 67 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 100.0 %.

^{1}H-NMR (600 MHz, MeOD) δ: 7.32 – 7.25 (m, 1H, $H_{arom.}$), 7.22 (dd, *J* = 7.7, 1.5 Hz, 1H, $H_{arom.}$), 7.16 (d, *J* = 8.2 Hz, 1H, $H_{arom.}$), 7.13 – 7.08 (m, 2H, $H_{arom.}$), 7.04 (td, *J* = 7.5, 1.1 Hz, 1H, $H_{arom.}$), 7.02 (dd, *J* = 8.2, 2.1 Hz, 1H, $H_{arom.}$), 4.11 (t, *J* = 6.4 Hz, 2H, N-CH_2), 3.11 – 3.05 (m, 2H, $C\underline{H}_2$-NH_3^+), 2.21 – 2.11 (m, 2H, CH_2-$C\underline{H}_2$-CH_2).

^{13}C-NMR (125 MHz, MeOD) δ: 148.2 ($C_{arom.}$, $C_{quat.}$), 145.9 ($C_{arom.}$, $C_{quat.}$), 134.9 ($C_{arom.}$, $C_{quat.}$), 129.5 ($C_{arom.}$, CH), 129.2 ($C_{arom.}$, CH), 128.9 ($C_{arom.}$, CH), 127.3 ($C_{arom.}$, $C_{quat.}$), 126.3 ($C_{arom.}$, $C_{quat.}$), 124.8 ($C_{arom.}$, CH), 124.1 ($C_{arom.}$, CH), 117.8 ($C_{arom.}$, CH), 117.6 ($C_{arom.}$, CH), 45.5 (CH_2), 38.8 (CH_2), 26.3 (CH_2).

LC-ESI-MS (m/z): 291 $[M\text{-}Cl]^+$.

N-[2-(2-Chloro-10H-phenothiazin-10-yl)ethyl]-4-(trifluoromethoxy)benzenesulfonamide (54)[74]



The compound was prepared from **52** (1.0 mmol) according to procedure 5.2.6.

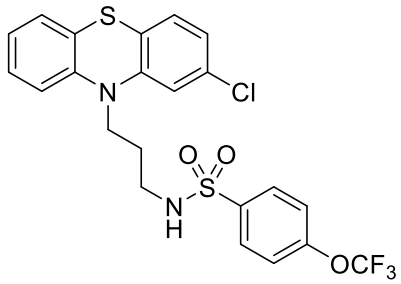
Appearance: white crystalline solid. **Melting point:** 100 – 102 °C. **Yield:** 392 mg, 78 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 95.0 %.

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 7.98 (s (br), 1H, NH), 7.88 (dt, *J* = 9.0, 2.9, 2.0 Hz, 2H, $H_{arom.}$), 7.51 – 7.46 (m, 2H, $H_{arom.}$), 7.18 (td, *J* = 7.8, 1.6 Hz, 1H, $H_{arom.}$), 7.15 – 7.11 (m, 2H, $H_{arom.}$), 7.00 – 6.92 (m, 4H, $H_{arom.}$), 3.95 (t, *J* = 6.6 Hz, 2H, N-CH_2), 3.09 (t, *J* = 6.6 Hz, 2H, $C\underline{H}_2$-NH).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 150.9 ($C_{arom.}$, $C_{quat.}$), 145.9 ($C_{arom.}$, $C_{quat.}$), 143.6 ($C_{arom.}$, $C_{quat.}$), 139.4 ($C_{arom.}$, $C_{quat.}$), 132.6 ($C_{arom.}$, $C_{quat.}$), 129.1 ($C_{arom.}$, 2 x CH), 128.3 ($C_{arom.}$, CH), 127.9 ($C_{arom.}$, CH), 127.4 ($C_{arom.}$, CH), 123.7 ($C_{arom.}$, $C_{quat.}$), 123.4 ($C_{arom.}$, CH), 122.9 ($C_{arom.}$, $C_{quat.}$), 122.5 ($C_{arom.}$, CH), 121.5 ($C_{arom.}$, 2 x CH), 120.0 (q, *J*(C,F) = 256 Hz, CF_3), 116.1 ($C_{arom.}$, CH), 115.7 ($C_{arom.}$, CH), 46.3 (CH_2), 39.4 (CH_2).

LC-ESI-MS (m/z): 501 $[M+H]^+$.

N-[3-(2-Chloro-10H-phenothiazin-10-yl)propyl]-4-(trifluoromethoxy)benzenesulfonamide (55)[74]



The compound was prepared from **53** (1.0 mmol) according to procedure 5.2.6.

Appearance: white crystalline solid. **Melting point:** 105 – 107 °C. **Yield:** 382 mg, 74 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 95.7 %.

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 7.83 (dt, *J* = 9.0, 3.0, 2.2 Hz, 2H, $H_{arom.}$), 7.78 (t, *J* = 5.6 Hz, 1H, NH), 7.52 – 7.47 (m, 2H, $H_{arom.}$), 7.19 (ddd, *J* = 8.2, 7.4, 1.5 Hz, 1H, $H_{arom.}$), 7.15 – 7.11 (m, 2H, $H_{arom.}$), 7.02 – 6.94 (m, 4H, $H_{arom.}$), 3.86 (t, *J* = 6.8 Hz, 2H, N-CH_2), 2.90 (td, *J* = 7.0, 5.5 Hz, 2H, $C\underline{H}_2$-NH), 1.75 (p, *J* = 6.9 Hz, 2H, N-CH_2-$C\underline{H}_2$).

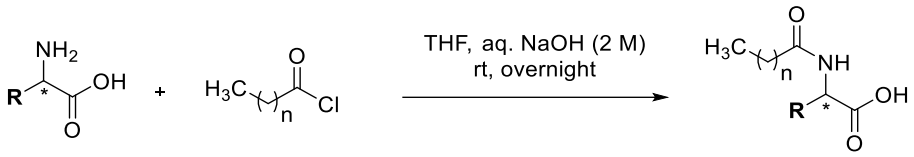
^{13}C-NMR (125 MHz, DMSO-d_6) δ: 150.8 ($C_{arom.}$, $C_{quat.}$), 146.4 ($C_{arom.}$, $C_{quat.}$), 144.0 ($C_{arom.}$, $C_{quat.}$), 139.5 ($C_{arom.}$, $C_{quat.}$), 132.6 ($C_{arom.}$, $C_{quat.}$), 129.1 ($C_{arom.}$, 2 x CH), 128.2 ($C_{arom.}$, CH),

127.8 ($C_{arom.}$, CH), 127.4 ($C_{arom.}$, CH), 123.8 ($C_{arom.}$, $C_{quat.}$), 123.2 ($C_{arom.}$, CH), 123.1 ($C_{arom.}$, $C_{quat.}$), 122.4 ($C_{arom.}$, CH), 121.5 ($C_{arom.}$, 2 x CH), 120.0 (q, J(C,F) = 256 Hz, CF_3), 116.5 ($C_{arom.}$, CH), 115.9 ($C_{arom.}$, CH), 43.9 (CH_2), 40.2 (CH_2), 26.5 (CH_2).

LC-ESI-MS (m/z): 515 $[M+H]^+$.

5.3 Procedures for the synthesis of *N*-acylamino acids

5.3.1 General procedure for the *N*-acylation of amino acids using fatty acid chlorides



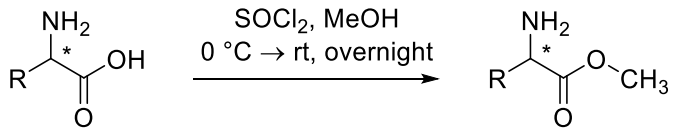
Amino acid
61 - 64

Fatty acyl chloride

56, **57**, **59**, **60**, **65 - 90**

A solution of fatty acid chloride (2.2 mmol) in THF (2 mL) was added dropwise to a stirred solution of the corresponding α-amino acid (2.0 mmol) in 2 N NaOH (5 mL) at 0 °C. The resulting mixture was stirred at 0°C for additional 5 min. After subsequent stirring overnight at r. t., the reaction mixture was diluted with 20 mL of water, cooled to 0 °C, and the pH was adjusted to ≤ 2 by addition of concentrated HCl (37 %). The mixture was diluted with water (30 mL) and poured into a separating funnel, then extracted with ethyl acetate (3 x 30 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude products were purified by stirring them in heptane (30 mL) for 30 min (or petroleum ether (b.p. 40 – 60 °C) in case of L-serine derivatives) then filtered to remove remaining free fatty acids.

5.3.2 General procedure for the esterification of amino acids



Amino acid
61 - 63

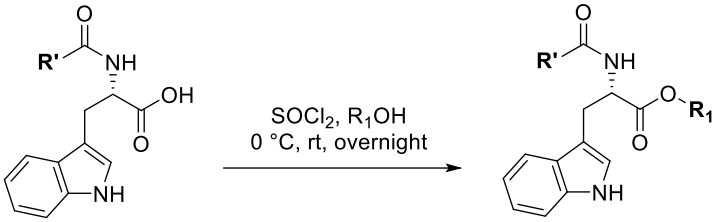
91 - 93

5.3.2.1 Method 1

Thionyl chloride ($SOCl_2$, 2.91 mL, 40 mmol) was added dropwise to a solution of amino acid (20 mmol) in methanol (20 – 50 mL, depending on solubility) at 0 °C, and the resulting mixture was warmed to r. t. and kept stirring overnight. The resulting solution was concentrated under

vacuum and dissolved in fresh methanol (50 mL). The solution was neutralized to pH 7.0 by adding solid $NaHCO_3$. After filtration of the precipitate, the solvent was evaporated to give the crude amino acid methyl ester as an oil. Dichloromethane (10 mL) was added to the resulting oil to precipitate salts such as NaCl and $NaHCO_3$. The solution was filtered and concentrated under vacuum to afford the desired neutral amino acid methyl ester.

5.3.2.2 Method 2 for *N*-acyl-L-tryptophan derivatives

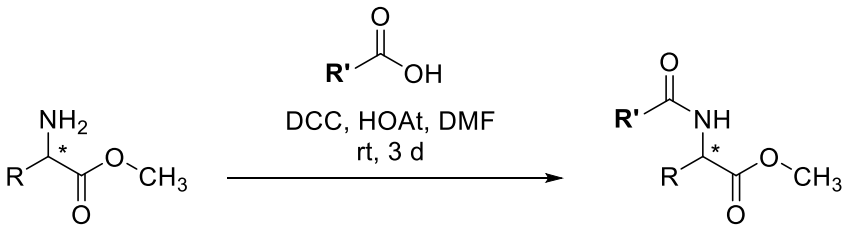


80 - 82 **106 - 114**

R' = $C_{13}H_{27}$, $C_{15}H_{31}$, $C_{17}H_{35}$
R_1 = CH_3, C_2H_5, i-C_3H_7

Thionyl chloride ($SOCl_2$, 72 µL, 1.0 mmol) was added dropwise to a solution of *N*-acyl-L-tryptophan (0.25 mmol) in alcohol (3 mL) at 0 °C, and the mixture was warmed to r. t. and kept stirring overnight. The resulting solution was concentrated under vacuum giving a crude solid mixture, which was then repeatedly dissolved in diethyl ether and concentrated under vacuum to afford the acylamino acid ester.

5.3.3 General procedure for amide coupling of amino acid methyl esters and fatty acids

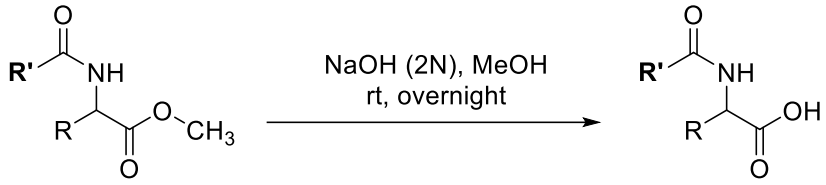


91 - 93 **94 - 99**

A solution of amino acid methyl ester (2.0 mmol) in DMF (3 mL) was added to a solution of a fatty acid (1.0 mmol) in DMF (2 mL) at 0 °C. Subsequently, *N*,*N*'-dicyclohexylcarbodiimide (DCC, 206 mg, 1.0 mmol) and 1-hydroxy-7-azabenzotriazole (HOAt, 136 mg, 1.0 mmol) were added consecutively to the reaction mixture and stirred for 3 days. The reaction mixture was then taken up in ethyl acetate (30 mL), and filtered through a sintered funnel to remove the formed dicyclohexylurea (DCU). The filtrate containing the product was washed with 2N HCl (3 × 20 ml), brine (1 × 20 ml), saturated $NaHCO_3$ (3 × 20 ml), brine (1 × 20 ml), and finally

dried over $MgSO_4$. The resulting solution was concentrated under vacuum and final purification was performed on a silica gel column using a mixture of petroleum ether (b.p. 40 – 60 °C) and ethyl acetate (1:1) as eluent.

5.3.4 General procedure for the hydrolysis of methyl esters



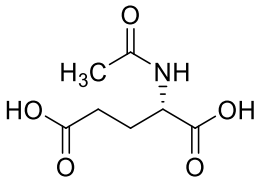
94 - 99 **100 - 105**

An aqueous NaOH solution (2 mL, 2 N) was added dropwise to a solution of a previously prepared *N*-acylamino acid methyl ester (0.2 mmol) in methanol (5 mL) at r. t. The reaction mixture was then stirred overnight and placed in a rotary evaporator to remove the methanol. The remaining aqueous solution containing the sodium salt of the amphiphilic product was mixed with 30 mL of water followed by washing with diethyl ether (2 × 20 mL) to remove any unreacted ester. Then the pH value of the aqueous layer was adjusted to 2 using 1 M HCl, and the organic compound was further extracted with ethyl acetate (3 × 20 mL). The extract was dried over $MgSO_4$ and finally concentrated under vacuum to afford the desired *N*-acylamino acid.

5.3.5 Analytical data of synthesized compounds

5.3.5.1 L-Glutamic acid derivatives

N-Acetyl-L-glutamic acid (65)[100]



The compound was prepared according to procedure 5.3.1.

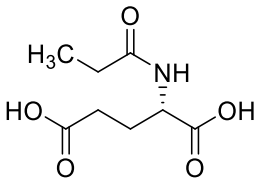
Appearance: white solid. **Melting point:** 188 - 190 °C (lit. m.p. 183 – 190 °C).[101] **Yield:** 36 mg, 3 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 96.8 %.

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 12.16 (s (br), 2H, 2 x COOH), 8.06 (d, J = 7.8 Hz, 1H, NH), 4.17 (ddd, J = 9.1, 7.8, 5.2 Hz, 1H, $C_\alpha H$), 2.30 – 2.21 (m, 2H, $C_\gamma H_2$), 1.97 – 1.88 (m, 1H, $C_\beta H$), 1.79 – 1.68 (m, 1H, $C_\beta H$), 1.83 (s, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 173.8 (C=O), 173.5 (C=O), 169.5 (C=O), 51.3 ($C_\alpha H$), 30.3 (CH_2), 26.6 (CH_2), 22.5 (CH_3).

LC-ESI-MS (m/z): 190 $[M+H]^+$.

N-Propionyl-L-glutamic acid (66)



The compound was prepared from L-glutamic acid and propionyl chloride according to procedure 5.3.1.

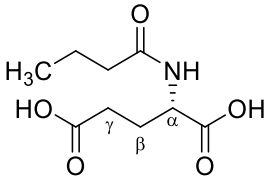
Appearance: white solid. **Melting point:** 143 – 144 °C. **Yield:** 37 mg, 9 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 100.0 %.

^{1}H-NMR (500 MHz, DMSO-*d*$_6$) δ: 12.29 (s (br), 2H, 2 x COOH), 7.97 (d, *J* = 7.8 Hz, 1H, NH), 4.18 (ddd, *J* = 9.1, 7.8, 5.1 Hz, 1H, $C_\alpha H$), 2.33 – 2.17 (m, 2H, $C_\gamma H_2$), 2.11 (qd, *J* = 7.5, 3.7 Hz, 2H, CH_2-CO_{acyl}), 1.99 – 1.88 (m, 1H, $C_\beta H$), 1.80 – 1.69 (m, 1H, $C_\beta H$), 0.98 (t, *J* = 7.6 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 173.8 (C=O), 173.6 (C=O), 173.3 (C=O), 51.2 ($C_\alpha H$), 30.3 (CH_2), 28.3 (CH_2), 26.5 (CH_2), 9.9 (CH_3).

LC-ESI-MS (m/z): 204 $[M+H]^+$.

N-Butyryl-L-glutamic acid (67)



The compound was prepared from L-glutamic acid and butyric acid chloride according to procedure 5.3.1.

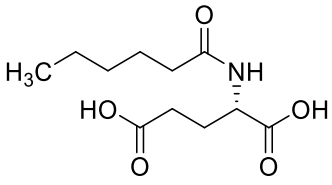
Appearance: white solid. **Melting point:** 101 – 102 °C. **Yield:** 96 mg, 18 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 100.0 %.

^{1}H-NMR (500 MHz, DMSO-*d*$_6$) δ: 12.29 (s (br), 2H, 2 x COOH), 7.99 (d, *J* = 7.9 Hz, 1H, NH), 4.23 – 4.15 (m, 1H, $C_\alpha H$), 2.32 – 2.18 (m, 2H, $C_\gamma H_2$), 2.08 (t, *J* = 7.2 Hz, 2H, CH_2-CO_{acyl}), 2.00 – 1.89 (m, 1H, $C_\beta H$), 1.78 – 1.70 (m, 1H, $C_\beta H$), 1.50 (sext, *J* = 7.4 Hz, 2H, $CH_{2\text{-}acyl}$), 0.84 (t, *J* = 7.4 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 173.8 (C=O), 173.6 (C=O), 172.4 (C=O), 51.2 ($C_\alpha H$), 37.1 (CH_2), 30.3 (CH_2), 26.5 (CH_2), 18.8 (CH_2), 13.6 (CH_3).

LC-ESI-MS (m/z): 218 $[M+H]^+$.

N-Hexanoyl-L-glutamic acid (68)[102]



The compound was prepared from L-glutamic acid and hexanoyl chloride according to procedure 5.3.1.

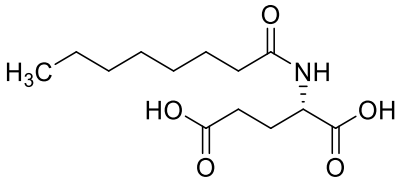
Appearance: white solid. **Melting point:** 92 - 93 °C. **Yield:** 101 mg, 17 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 100.0 %.

^{1}H-NMR (500 MHz, DMSO-*d*$_6$) δ: 12.26 (s (br), 2H, 2 x COOH), 7.99 (d, *J* = 7.9 Hz, 1H, NH), 4.21 – 4.16 (m, 1H, $C_\alpha H$), 2.28 – 2.21 (m, 2H, $C_\gamma H_2$), 2.09 (td, *J* = 7.2, 2.5 Hz, 2H, CH_2-CO_{acyl}), 1.98 – 1.90 (m, 1H, $C_\beta H$), 1.78 – 1.70 (m, 1H, $C_\beta H$), 1.47 (quintet, *J* = 7.4 Hz, 2H, $CH_{2\text{-acyl}}$), 1.30 - 1.18 (m, 4H, 2 x $CH_{2\text{-acyl}}$), 0.84 (t, *J* = 7.0 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 173.8 (C=O), 173.6 (C=O), 172.5 (C=O), 51.1 ($C_\alpha H$), 35.1 (CH_2), 30.9 (CH_2), 30.2 (CH_2), 26.5 (CH_2), 25.0 (CH_2), 21.9 (CH_2), 13.9 (CH_3).

LC-ESI-MS (m/z): 246 $[M+H]^+$.

N-Octanoyl-L-glutamic acid (69)[103]



The compound was prepared from L-glutamic acid and octanoyl chloride according to procedure 5.3.1.

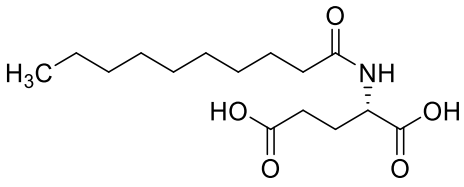
Appearance: white solid. **Melting point:** 100 - 102 °C (lit. m.p. 92 - 93 °C).[104] **Yield:** 188 mg, 29 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 100.0 %.

^{1}H-NMR (500 MHz, DMSO-*d*$_6$) δ: 12.28 (s (br), 2H, 2 x COOH), 7.98 (d, *J* = 7.9 Hz, 1H, NH), 4.21 – 4.16 (m, 1H, $C_\alpha H$), 2.28 – 2.21 (m, 2H, $C_\gamma H_2$), 2.09 (td, *J* = 7.2, 2.6 Hz, 2H, CH_2-CO_{acyl}), 1.97 – 1.90 (m, 1H, $C_\beta H$), 1.78 – 1.70 (m, 1H, $C_\beta H$), 1.47 (quintet, *J* = 6.9 Hz, 2H, $CH_{2\text{-acyl}}$), 1.28 - 1.19 (m, 8H, 4 x $CH_{2\text{-acyl}}$), 0.85 (t, *J* = 7.1 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 173.8 (C=O), 173.6 (C=O), 172.5 (C=O), 51.1 ($C_\alpha H$), 35.2 (CH_2), 31.3 (CH_2), 30.2 (CH_2), 28.6 (CH_2), 28.5 (CH_2), 26.5 (CH_2), 25.4 (CH_2), 22.2 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 274 $[M+H]^+$.

N-Decanoyl-L-glutamic acid (70)[103]



The compound was prepared from L-glutamic acid and decanoyl chloride according to procedure 5.3.1.

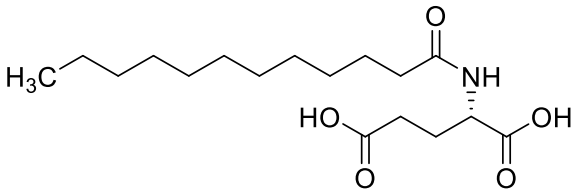
Appearance: white solid. **Melting point:** 76 °C. (lit. m.p. 85 - 87 °C).[105] **Yield:** 499 mg, 69 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 100.0 %.

^{1}H-NMR (500 MHz, DMSO-*d*$_6$) δ: 12.27 (s (br), 2H, 2 x COOH), 7.98 (d, *J* = 7.9 Hz, 1H, NH), 4.20 – 4.16 (m, 1H, $C_\alpha H$), 2.26 – 2.23 (m, 2H, $C_\gamma H_2$), 2.09 (td, *J* = 7.3, 2.7 Hz, 2H, CH_2-CO_{acyl}), 1.96 – 1.89 (m, 1H, $C_\beta H$), 1.78 – 1.71 (m, 1H, $C_\beta H$), 1.46 (quintet, *J* = 7.3 Hz, 2H, $CH_{2\text{-acyl}}$), 1.28 - 1.19 (m, 12H, 6 x $CH_{2\text{-acyl}}$), 0.85 (t, *J* = 6.9 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 173.8 (C=O), 173.5 (C=O), 172.5 (C=O), 51.1 ($C_\alpha H$), 35.2 (CH_2), 31.4 (CH_2), 30.2 (CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 26.5 (CH_2), 25.4 (CH_2), 22.2 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 302 $[M+H]^+$.

N-Dodecanoyl-L-glutamic acid (71)[103]



The compound was prepared from L-glutamic acid and dodecanoyl chloride according to procedure 5.3.1.

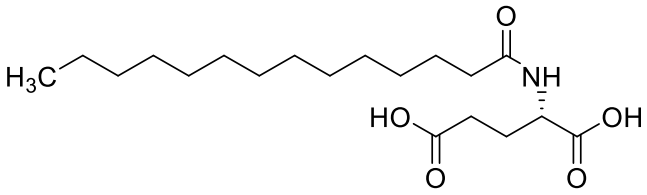
Appearance: white solid. **Melting point:** 111 – 113 °C. (lit. m.p. 96 - 99 °C).[105] **Yield:** 490 mg, 62 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 100.0 %. $[\alpha]^{25}_D$ = -6.4° (c = 0.1 g/mL, in acetone).

^{1}H-NMR (500 MHz, DMSO-*d*$_6$) δ: 12.27 (s (br), 2H, 2 x COOH), 7.98 (d, *J* = 7.9 Hz, 1H, NH), 4.21 – 4.16 (m, 1H, $C_\alpha H$), 2.27 – 2.23 (m, 2H, $C_\gamma H_2$), 2.09 (td, *J* = 7.3, 2.7 Hz, 2H, CH_2-CO_{acyl}), 1.98 – 1.90 (m, 1H, $C_\beta H$), 1.78 – 1.70 (m, 1H, $C_\beta H$), 1.47 (quintet, *J* = 6.8 Hz, 2H, $CH_{2\text{-acyl}}$), 1.28 - 1.19 (m, 16H, 8 x $CH_{2\text{-acyl}}$), 0.85 (t, *J* = 6.7 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 173.8 (C=O), 173.5 (C=O), 172.5 (C=O), 51.1 ($C_\alpha H$), 35.2 (CH_2), 31.4 (CH_2), 30.2 (CH_2), 29.1 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 26.5 (CH_2), 25.3 (CH_2), 22.2 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 330 $[M+H]^+$.

N-Tetradecanoyl-L-glutamic acid (72)[103]



The compound was prepared from L-glutamic acid and tetradecanoyl chloride according to procedure 5.3.1.

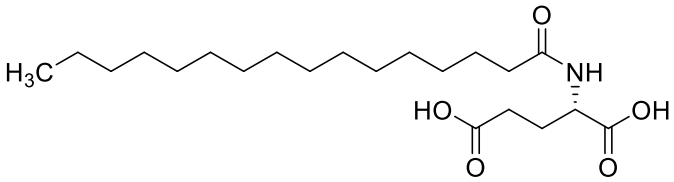
Appearance: white solid. **Melting point:** 113 – 114 °C. **Yield:** 258 mg, 36 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 95.5 %.

^{1}H-NMR (600 MHz, DMSO-*d*$_6$) δ: 12.29 (s (br), 2H, 2 x COOH), 7.99 (d, *J* = 7.8 Hz, 1H, NH), 4.18 (dt, *J* = 8.6, 5.1 Hz, 1H, $C_\alpha H$), 2.26 – 2.23 (m, 2H, $C_\gamma H_2$), 2.10 – 2.07 (m, 2H, CH_2-CO_{acyl}), 1.95 – 1.92 (m, 1H, $C_\beta H$), 1.75 – 1.73 (m, 1H, $C_\beta H$), 1.46 (p, *J* = 7.1 Hz, 2H, $CH_{2\text{-acyl}}$), 1.27 – 1.22 (m, 20H, 10 x $CH_{2\text{-acyl}}$), 0.84 (t, *J* = 6.9 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 173.8 (C=O), 173.6 (C=O), 172.5 (C=O), 51.2 ($C_\alpha H$), 35.2 (CH_2), 31.3 (CH_2), 30.2 (CH_2), 29.2 – 29.1 (5 x CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 26.5 (CH_2), 25.4 (CH_2), 22.2 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 358 $[M+H]^+$.

N-Hexadecanoyl-L-glutamic acid (59)[106]



The compound was prepared from L-glutamic acid and hexadecanoyl chloride according to procedure 5.3.1.

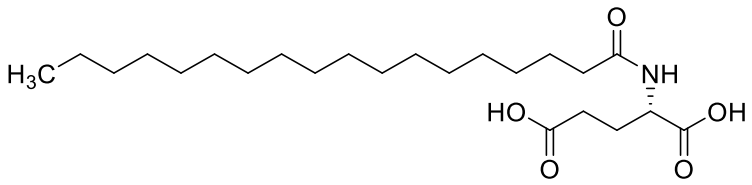
Appearance: white solid. **Melting point:** 114 – 117 °C. **Yield:** 285 mg, 37 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 99.7 %.

^{1}H-NMR (600 MHz, DMSO-*d*$_6$) δ: 12.29 (s (br), 2H, 2 x COOH), 7.99 (d, *J* = 7.7 Hz, 1H, NH), 4.17 (dt, *J* = 8.6, 5.1 Hz, 1H, $C_\alpha H$), 2.26 – 2.23 (m, 2H, $C_\gamma H_2$), 2.10 – 2.07 (m, 2H, CH_2-CO_{acyl}), 1.96 – 1.91 (m, 1H, $C_\beta H$), 1.76 – 1.70 (m, 1H, $C_\beta H$), 1.46 (p, *J* = 7.1 Hz, 2H, $CH_{2\text{-acyl}}$), 1.27 – 1.22 (m, 24H, 12 x $CH_{2\text{-acyl}}$), 0.84 (t, *J* = 6.9 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 173.8 (C=O), 173.5 (C=O), 172.5 (C=O), 51.2 ($C_\alpha H$), 35.2 (CH_2), 31.4 (CH_2), 30.2 (CH_2), 29.2 – 29.1 (7 x CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 26.5 (CH_2), 25.4 (CH_2), 22.2 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 386 $[M+H]^+$.

N-Octadecanoyl-L-glutamic acid (60)[106]



The compound was prepared from L-glutamic acid and octadecanoyl chloride according to procedure 5.3.1.

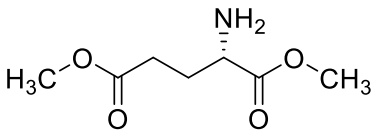
Appearance: white solid. **Melting point:** 107 – 110 °C (lit. m.p. 113 °C).[107] **Yield:** 296 mg, 36 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.1 %.

^{1}H-NMR (600 MHz, DMSO-*d*$_6$) δ: 12.22 (s (br), 2H, 2 x COOH), 7.99 (d, *J* = 7.8 Hz, 1H, NH), 4.18 (dt, *J* = 8.7, 5.2 Hz, 1H, $C_\alpha H$), 2.27 – 2.21 (m, 2H, $C_\gamma H_2$), 2.11 – 2.06 (m, 2H, CH_2-CO_{acyl}), 1.96 – 1.91 (m, 1H, $C_\beta H$), 1.76 – 1.70 (m, 1H, $C_\beta H$), 1.46 (p, *J* = 7.1 Hz, 2H, $CH_{2\text{-acyl}}$), 1.27 – 1.22 (m, 28H, 14 x $CH_{2\text{-acyl}}$), 0.84 (t, *J* = 6.9 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 173.8 (C=O), 173.6 (C=O), 172.5 (C=O), 51.2 ($C_\alpha H$), 35.2 (CH_2), 31.4 (CH_2), 30.2 (CH_2), 29.2 – 29.1 (5 x CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 28.7 (CH_2), 26.5 (CH_2), 25.4 (CH_2), 24.6 (CH_2), 22.2 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 414 $[M+H]^+$.

L-Glutamic acid dimethyl ester (91)[108]



The compound was prepared from L-glutamic acid according to procedure 5.3.2.1.

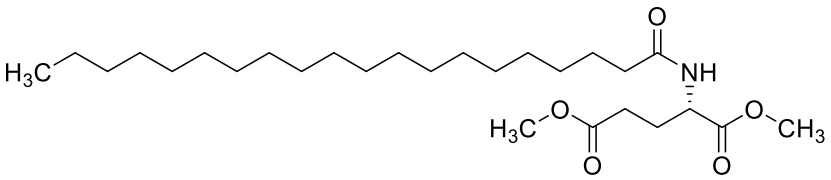
Appearance: colorless oil. **Yield:** quantitative. **Purity by HPLC-UV (254 nm)-ESI-MS:** 100.0 %.

^{1}H-NMR (600 MHz, DMSO-*d*$_6$) δ: 8.57 (s, 2H, NH_2), 4.00 (t, *J* = 6.7 Hz, 1H, $C_\alpha H$), 3.72 (s, 3H, OCH_3), 3.59 (s, 3H, OCH_3), 2.60 – 2.43 (m, 2H, $C_\gamma H_2$), 2.05 (td, *J* = 8.5, 6.6 Hz, 2H, $C_\beta H_2$).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 172.3 (C=O), 169.8 (C=O), 52.9 ($C_\alpha H$), 51.7 (CH_3), 51.4 (CH_3), 29.1 (CH_2), 25.4 (CH_2).

LC-ESI-MS (m/z): 176 $[M+H]^+$.

N-Eicosanoyl-L-glutamic acid dimethyl ester (94)



The compound was prepared from **91** and eicosanoic acid according to procedure 5.3.3.

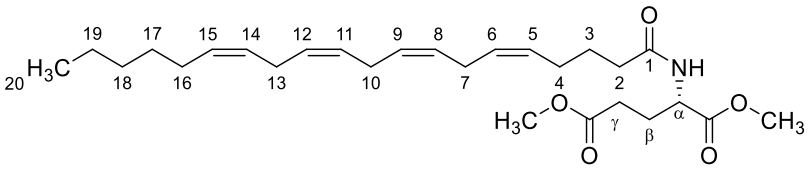
Appearance: white powder. **Melting point:** 88 – 90 °C. **Yield:** 52 mg, 11 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 100.0 %.

^{1}H-NMR (600 MHz, $CDCl_3$) δ: 6.12 (d, *J* = 7.6 Hz, 1H, NH), 4.61 (dt, *J* = 7.7, 4.8 Hz, 1H, $C_\alpha H$), 3.73 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 2.44 – 2.31 (m, 2H, $C_\gamma H_2$), 2.19 (t, *J* = 7.9 Hz, 2H, CH_2-CO_{acyl}), 2.00 – 1.95 (m, 2H, $C_\beta H_2$), 1.61 – 1.56 (m, 4H, 2 x $CH_{2\text{-acyl}}$), 1.28 – 1.23 (m, 30H, 15 x $CH_{2\text{-acyl}}$), 0.86 (t, *J* = 6.9 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 173.3 (C=O), 173.1 (C=O), 172.5 (C=O), 52.5 ($C_\alpha H$), 51.8 (OCH_3), 51.5 (OCH_3), 36.6 (CH_2), 31.9 (CH_2), 30.1 (CH_2), 29.7 – 29.6 (10 x CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 27.4 (CH_2), 25.5 (CH_2), 22.7 (CH_2), 14.1 ($CH_{3\text{-acyl}}$).

LC-ESI-MS (m/z): 470 $[M+H]^+$.

N-Arachidonoyl-L-glutamic acid dimethyl ester (95)



The compound was prepared from **91** and arachidonic acid according to procedure 5.3.3.

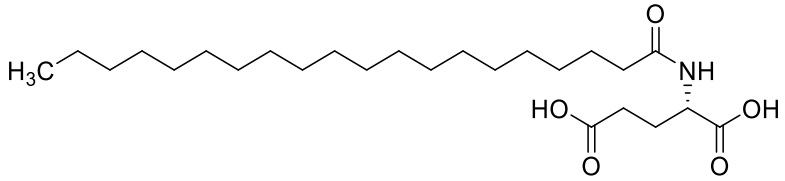
Appearance: light yellow oil. **Yield:** 145 mg, 56 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 100.0 %.

^{1}H-NMR (600 MHz, DMSO-d_6) δ: 8.17 (d, *J* = 7.5 Hz, 1H, NH), 5.39 – 5.26 (m, 8H, 8 x CH_{sp2} (C5, C6, C8, C9, C11, C12, C14, C16)), 4.24 (ddd, *J* = 9.1, 7.5, 5.3 Hz, 1H, $C_\alpha H$), 3.59 (s, 3H, OCH_3), 3.57 (s, 3H, OCH_3), 2.79 (dt, *J* = 19.5, 5.6 Hz, 6H, 3 x CH_2 (C7, C10, C13)), 2.40 – 2.32 (m, 2H, $C_\gamma H_2$), 2.11 (t, *J* = 7.4 Hz, 2H, CH_2 (C2)), 2.01 (q, *J* = 7.2 Hz, 4H, 2 x CH_2 (C4, C16)), 1.99 – 1.92 (m, 1H, $C_\beta H$), 1.84 – 1.76 (m, 1H, $C_\beta H$), 1.53 (p, *J* = 7.4 Hz, 2H, CH_2 (C3)), 1.35 – 1.19 (m, 6H, 3 x CH_2 (C17, C18, C19)), 0.84 (t, *J* = 7.0 Hz, 3H, CH_3 (C20)).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 172.6 (C=O), 172.4 (C=O), 172.3 (C=O), 130.1 (CH_{sp2}), 129.5 (CH_{sp2}), 128.3 (CH_{sp2}), 128.2 (CH_{sp2}), 128.1 (CH_{sp2}), 127.9 (CH_{sp2}), 127.8 (CH_{sp2}), 127.7 (CH_{sp2}), 51.9 ($C_\alpha H$), 51.5 (OCH_3), 51.2 (OCH_3), 34.5 (CH_2), 31.0 (CH_2), 29.8 (CH_2), 28.8 (CH_2), 26.7 (CH_2), 26.3 (CH_2), 26.2 (CH_2), 25.4 – 25.3 (4 x CH_2), 22.1 (CH_2), 14.1 ($CH_{3\text{-acyl}}$).

LC-ESI-MS (m/z): 462 $[M+H]^+$.

N-Eicosanoyl-L-glutamic acid (100)



The compound was prepared from **94** according to procedure 5.3.4.

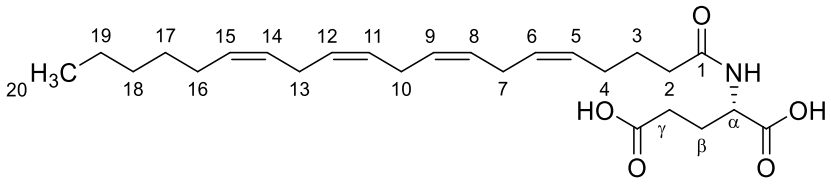
Appearance: white powder. **Melting point:** 118 – 120 °C. **Yield:** 30 mg, 88 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 100.0 %.

^{1}H-NMR (600 MHz, DMSO-d_6) δ: 12.29 (s (br), 2H, 2 x COOH), 7.99 (d, J = 7.9 Hz, 1H, NH), 4.18 (dt, J = 8.3, 4.2 Hz, 1H, $C_\alpha H$), 2.28 – 2.21 (m, 2H, $C_\gamma H_2$), 2.08 (td, J = 7.3, 3.9 Hz, 2H, CH_2-CO_{acyl}), 1.98 – 1.88 (m, 1H, $C_\beta H$), 1.78 – 1.69 (m, 1H, $C_\beta H$), 1.46 (p, J = 7.0 Hz, 2H, $CH_{2\text{-acyl}}$), 1.26 – 1.18 (m, 32H, 16 x $CH_{2\text{-acyl}}$), 0.84 (t, J = 6.8 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 173.8 (C=O), 172.6 (C=O), 172.5 (C=O), 51.2 ($C_\alpha H$), 35.2 (CH_2), 31.4 (CH_2), 30.3 (CH_2), 29.3 – 29.1 (10 x CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 26.5 (CH_2), 25.4 (CH_2), 24.6 (CH_2), 22.3 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 442 $[M+H]^+$.

N-Arachidonoyl-L-glutamic acid (101)



The compound was prepared from **95** according to procedure 5.3.4.

Appearance: light yellow oil. **Yield:** 85 mg, 89 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 100.0 %.

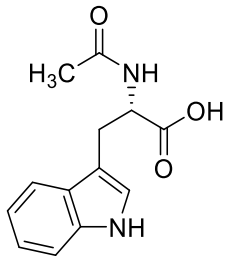
^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.07 (s (br), 2H, 2 x COOH), 6.40 (d, *J* = 7.2 Hz, 1H, NH), 5.40 – 5.30 (m, 8H, 8 x CH_{sp2} (C5, C6, C8, C9, C11, C12, C14, C16)), 4.64 (q, *J* = 6.6 Hz, 1H, $C_{\alpha}H$), 2.82 – 2.77 (m, 6H, 3 x CH_2 (C7, C10, C13)), 2.53 – 2.44 (m, 2H, $C_{\gamma}H_2$), 2.25 (t, *J* = 7.8 Hz, 2H, CH_2 (C2)), 2.22 – 2.08 (m, 4H, 2 x CH_2 (C4, C16)), 2.03 (q, *J* = 7.2 Hz, 2H, $C_{ß}H_2$), 1.69 (p, *J* = 7.4 Hz, 2H, CH_2 (C3)), 1.36 – 1.23 (m, 6H, 3 x CH_2 (C17, C18, C19)), 0.86 (t, *J* = 6.8 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 178.2 (C=O), 176.1 (C=O), 173.7 (C=O), 130.5 (CH_{sp2}), 128.9 (CH_{sp2}), 128.8 (CH_{sp2}), 128.6 (CH_{sp2}), 128.3 (CH_{sp2}), 128.1 (CH_{sp2}), 127.8 (CH_{sp2}), 127.5 (CH_{sp2}), 51.4 ($C_{\alpha}H$), 35.7 (CH_2), 31.5 (CH_2), 29.6 (CH_2), 29.3 (CH_2), 27.2 (CH_2), 26.7 (CH_2), 26.6 (CH_2), 25.6 (3 x CH_2), 25.3 (CH_2), 22.6 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 434 $[M+H]^+$.

5.3.5.2 L-Tryptophan derivatives

N-Acetyl-L-tryptophan (73)[109]



The compound was prepared from L-tryptophan and acetyl chloride according to procedure 5.3.1.

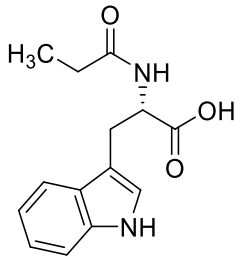
Appearance: white solid. **Melting point:** 189 – 190 °C (lit. m.p. 185 – 187 °C).[110] **Yield:** 402 mg, 82 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 99.4 %.

^{1}H-NMR (500 MHz, DMSO-*d*$_6$) δ: 12.54 (s (br), 1H, COOH), 10.79 (s, 1H, NH_{indole}), 8.09 (d, *J* = 7.8 Hz, 1H, NH_{amide}), 7.52 (d, *J* = 7.8 Hz, 1H, $H_{arom.}$), 7.32 (dt, *J* = 8.2, 0.9 Hz, 1H, $H_{arom.}$), 7.12 (d, *J* = 2.4 Hz, 1H, $H_{arom.}$), 7.05 (ddd, *J* = 8.1, 7.0, 1.2 Hz, 1H, $H_{arom.}$), 6.97 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H, $H_{arom.}$), 4.45 (ddd, *J* = 8.6, 7.8, 5.1 Hz, 1H, $C_{\alpha}H$), 3.15 (dd, *J* = 14.7, 5.1 Hz, 1H, $C_{\beta}H$), 2.98 (dd, *J* = 14.6, 8.6 Hz, 1H, $C_{\beta}H$), 1.79 (s, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 173.7 (C=O), 169.4 (C=O), 136.3 ($C_{arom.}$, $C_{quat.}$), 127.4 ($C_{arom.}$, $C_{quat.}$), 123.6 ($C_{arom.}$, CH), 121.1 ($C_{arom.}$, CH), 118.5 ($C_{arom.}$, CH), 118.3 ($C_{arom.}$, CH), 111.5 ($C_{arom.}$, CH), 110.1 ($C_{arom.}$, $C_{quat.}$), 53.1 ($C_{\alpha}H$), 27.3 (CH_2), 22.6 (CH_3).

LC-ESI-MS (m/z): 247 $[M+H]^+$.

N-Propionyl-L-tryptophan (74)



The compound was prepared from L-tryptophan and propionyl chloride according to procedure 5.3.1.

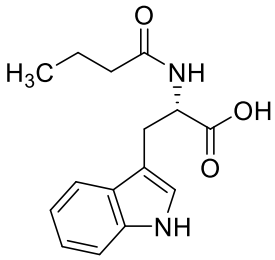
Appearance: white solid. **Melting point:** 178 – 179 °C (lit. m.p. 172 – 174 °C).[111] **Yield:** 446 mg, 86 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 98.9 %.

^{1}H-NMR (500 MHz, DMSO-*d*$_6$) δ: 10.78 (s, 1H, NH_{indole}), 7.97 (d, *J* = 7.9 Hz, 1H, NH_{amide}), 7.52 (d, *J* = 8.0 Hz, 1H, $H_{arom.}$), 7.32 (dt, *J* = 8.1, 0.9 Hz, 1H, $H_{arom.}$), 7.11 (d, *J* = 2.4 Hz, 1H, $H_{arom.}$), 7.05 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H, $H_{arom.}$), 6.97 (ddd, *J* = 7.9, 6.9, 1.0 Hz, 1H, $H_{arom.}$), 4.46 (td, *J* = 8.3, 5.1 Hz, 1H, $C_{\alpha}H$), 3.15 (dd, *J* = 14.7, 5.0 Hz, 1H, $C_{\beta}H$), 2.99 (dd, *J* = 14.6, 8.6 Hz, 1H, $C_{\beta}H$), 2.04 (qd, *J* = 7.5, 3.0 Hz, 2H, CH_2-CO_{acyl}), 0.92 (t, *J* = 7.6 Hz, 3H, CH_3), (COOH signal not visible).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 173.7 (C=O), 173.0 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.4 ($C_{arom.}$, $C_{quat.}$), 123.6 ($C_{arom.}$, CH), 121.0 ($C_{arom.}$, CH), 118.5 ($C_{arom.}$, CH), 118.3 ($C_{arom.}$, CH), 111.5 ($C_{arom.}$, CH), 110.2 ($C_{arom.}$, $C_{quat.}$), 53.1 ($C_{\alpha}H$), 28.4 (CH_2), 27.3 (CH_2), 9.8 (CH_3).

LC-ESI-MS (m/z): 261 $[M+H]^+$.

N-Butyryl-L-tryptophan (75)



The compound was prepared from L-tryptophan and butyric acid chloride according to procedure 5.3.1.

Appearance: yellow oil. **Yield:** 212 mg, 32 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 99.1 %.

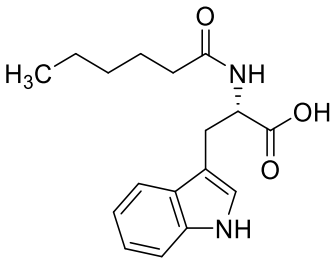
^{1}H-NMR (500 MHz, DMSO-*d*$_6$) δ: 12.27 (s (br), 1H, COOH), 10.78 (s, 1H, NH_{indole}), 7.99 (d, *J* = 7.9 Hz, 1H, NH_{amide}), 7.52 (d, *J* = 7.9 Hz, 1H, $H_{arom.}$), 7.32 (d, *J* = 8.1 Hz, 1H, $H_{arom.}$), 7.12 (d, *J* = 2.5 Hz, 1H, $H_{arom.}$), 7.09 – 7.01 (m, 1H, $H_{arom.}$), 7.00 – 6.93 (m, 1H, $H_{arom.}$), 4.49 – 4.45 (m, 1H, $C_{\alpha}H$), 3.16 (dd, *J* = 14.6, 5.1 Hz, 1H, $C_{\beta}H$), 2.99 (dd, *J* = 14.6, 8.9 Hz, 1H, $C_{\beta}H$), 2.04 (td, *J* = 7.2, 3.7 Hz, 2H, CH_2-CO_{acyl}), 1.44 (sext, *J* = 7.3 Hz, 2H, $CH_{2\text{-}acyl}$), 0.77 (t, *J* = 7.3 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 173.7 (C=O), 172.2 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.4 ($C_{arom.}$, $C_{quat.}$), 123.6 ($C_{arom.}$, CH), 121.0 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 118.3 ($C_{arom.}$, CH),

111.5 ($C_{arom.}$, CH), 110.2 ($C_{arom.}$, $C_{quat.}$), 52.9 ($C_{\alpha}H$), 37.2 (CH_2), 27.3 (CH_2), 18.7 (CH_2), 13.6 (CH_3).

LC-ESI-MS (m/z): 275 $[M+H]^+$.

N-Hexanoyl-L-tryptophan (76) [83]



The compound was prepared from L-tryptophan and hexanoyl chloride according to procedure 5.3.1.

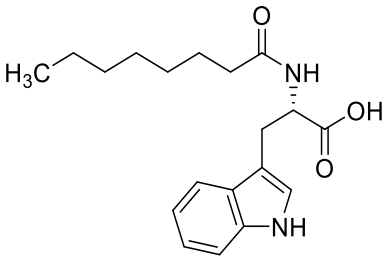
Appearance: white solid. **Melting point:** 106 – 108 °C (lit. m.p. 132 - 133 °C).[112] **Yield:** 648 mg, 89 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 98.3 %.

^{1}H-NMR (500 MHz, MeOD) δ: 7.60 (d, *J* = 8.0 Hz, 1H, $H_{arom.}$), 7.36 (d, *J* = 8.1 Hz, 1H, $H_{arom.}$), 7.13 – 7.10 (m, 2H, $H_{arom.}$), 7.05 – 7.02 (m, 1H, $H_{arom.}$), 4.78 (dd, *J* = 8.5, 4.9 Hz, 1H, $C_{\alpha}H$), 3.39 (ddd, *J* = 14.8, 5.0, 0.9 Hz, 1H, $C_{\beta}H$), 3.19 (ddd, *J* = 14.7, 8.5, 0.7 Hz, 1H, $C_{\beta}H$), 2.18 (td, *J* = 7.5, 1.4 Hz, 2H, CH_2-CO_{acyl}), 1.55 – 1.51 (m, 2H, $CH_{2\text{-}acyl}$), 1.32 – 1.25 (m, 2H, $CH_{2\text{-}acyl}$), 1.23 – 1.17 (m, 2H, $CH_{2\text{-}acyl}$), 0.89 (t, *J* = 7.3 Hz, 3H, CH_3), (OH, NH signals not visible).

^{13}C-NMR (125 MHz, MeOD) δ: 174.8 (C=O), 173.9 (C=O), 136.6 ($C_{arom.}$, $C_{quat.}$), 127.4 ($C_{arom.}$, $C_{quat.}$), 122.8 ($C_{arom.}$, CH), 120.9 ($C_{arom.}$, CH), 118.3 ($C_{arom.}$, CH), 117.8 ($C_{arom.}$, CH), 110.8 ($C_{arom.}$, CH), 109.7 ($C_{arom.}$, $C_{quat.}$), 53.1 ($C_{\alpha}H$), 35.4 (CH_2), 30.9 (CH_2), 27.1 (CH_2), 25.1 (CH_2), 21.9 (CH_2), 12.8 (CH_3).

LC-ESI-MS (m/z): 303 $[M+H]^+$.

N-Octanoyl-L-tryptophan (77) [83]



The compound was prepared from L-tryptophan and octanoyl chloride according to procedure 5.3.1.

Appearance: white solid. **Melting point:** 96 – 97 °C. **Yield:** 687 mg, 87 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.7 %.

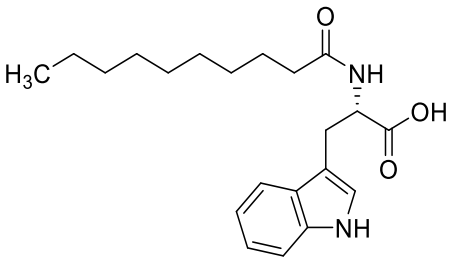
^{1}H-NMR (500 MHz, DMSO-*d*$_6$) δ: 12.37 (s (br), 1H, COOH), 10.78 (s, 1H, NH_{indole}), 7.98 (d, *J* = 7.9 Hz, 1H, NH_{amide}), 7.52 (d, *J* = 8.0 Hz, 1H, $H_{arom.}$), 7.31 (dt, *J* = 8.1, 0.9 Hz, 1H, $H_{arom.}$), 7.11 (d, *J* = 2.4 Hz, 1H, $H_{arom.}$), 7.05 (td, *J* = 8.1, 7.0, 1.2 Hz, 1H, $H_{arom.}$), 6.96 (td, *J* = 8.0, 7.0,

1.0 Hz, 1H, $H_{arom.}$), 4.46 (td, *J* = 8.8, 7.8, 5.0 Hz, 1H, $C_{\alpha}H$), 3.15 (dd, *J* = 14.7, 5.0 Hz, 1H, $C_{\beta}H$), 2.98 (dd, *J* = 14.7, 8.9 Hz, 1H, $C_{\beta}H$), 2.05 (td, *J* = 7.3, 2.4 Hz, 2H, CH_2-CO_{acyl}), 1.43 – 1.36 (m, 2H, $CH_{2\text{-acyl}}$), 1.25 – 1.11 (m, 8H, 4 x $CH_{2\text{-acyl}}$), 0.84 (t, *J* = 7.1 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 173.7 (C=O), 172.3 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.4 ($C_{arom.}$, $C_{quat.}$), 123.6 ($C_{arom.}$, CH), 120.9 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 118.3 ($C_{arom.}$, CH), 111.5 ($C_{arom.}$, CH), 110.2 ($C_{arom.}$, $C_{quat.}$), 52.9 ($C_{\alpha}H$), 35.2 (CH_2), 31.3 (CH_2), 28.6 (CH_2), 28.6 (CH_2), 27.3 (CH_2), 25.3 (CH_2), 22.2 (CH_2), 14.0 (CH_3).

LC-ESI-MS (m/z): 331 $[M+H]^+$.

***N-Decanoyl-L-tryptophan (78)*[83]**



The compound was prepared from L-tryptophan and decanoyl chloride according to procedure 5.3.1.

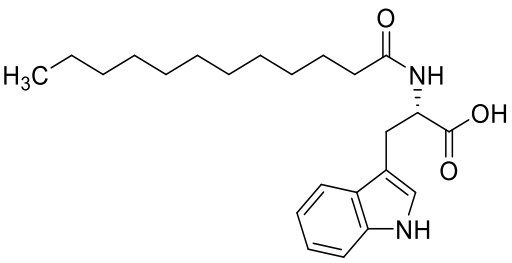
Appearance: white solid. **Melting point:** 85 – 87 °C. **Yield:** 322 mg, 37 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.3 %.

^{1}H-NMR (500 MHz, DMSO-*d*$_6$) δ: 12.47 (s (br), 1H, COOH), 10.78 (s, 1H, NH_{indole}), 7.98 (d, *J* = 7.9 Hz, 1H, NH_{amide}), 7.51 (d, *J* = 7.9 Hz, 1H, $H_{arom.}$), 7.31 (dt, *J* = 8.1, 0.9 Hz, 1H, $H_{arom.}$), 7.10 (d, *J* = 2.4 Hz, 1H, $H_{arom.}$), 7.04 (td, *J* = 8.1, 7.0, 1.2 Hz, 1H, $H_{arom.}$), 6.96 (td, *J* = 8.0, 6.9, 1.1 Hz, 1H, $H_{arom.}$), 4.46 (td, *J* = 8.4, 5.0 Hz, 1H, $C_{\alpha}H$), 3.14 (dd, *J* = 14.7, 5.1 Hz, 1H, $C_{\beta}H$), 2.98 (dd, *J* = 14.6, 8.8 Hz, 1H, $C_{\beta}H$), 2.04 (td, *J* = 7.3, 2.2 Hz, 2H, CH_2-CO_{acyl}), 1.42 – 1.36 (m, 2H, $CH_{2\text{-acyl}}$), 1.27 – 1.10 (m, 12H, 6 x $CH_{2\text{-acyl}}$), 0.85 (t, *J* = 6.9 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 173.7 (C=O), 172.3 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.4 ($C_{arom.}$, $C_{quat.}$), 123.6 ($C_{arom.}$, CH), 120.9 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 118.3 ($C_{arom.}$, CH), 111.4 ($C_{arom.}$, CH), 110.2 ($C_{arom.}$, $C_{quat.}$), 52.9 ($C_{\alpha}H$), 35.2 (CH_2), 31.4 (CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 27.3 (CH_2), 25.3 (CH_2), 22.2 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 359 $[M+H]^+$.

N-Dodecanoyl-L-tryptophan (79)[113]



The compound was prepared from L-tryptophan and dodecanoyl chloride according to procedure 5.3.1.

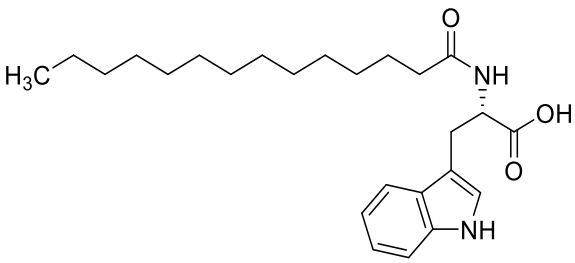
Appearance: white solid. **Melting point:** 113 – 115 °C. (lit. m.p. 107 - 109 °C).[114] **Yield:** 678 mg, 73 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.6 %. $[\alpha]^{25}_{D}$ = +14.4° (c = 0.1 g/mL, in acetone).

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 12.49 (s (br), 1H, COOH), 10.78 (s, 1H, NH_{indole}), 7.98 (d, *J* = 7.9 Hz, 1H, NH_{amide}), 7.52 (d, *J* = 7.9 Hz, 1H, $H_{arom.}$), 7.31 (dt, *J* = 8.1, 0.9 Hz, 1H, $H_{arom.}$), 7.11 (d, *J* = 2.4 Hz, 1H, $H_{arom.}$), 7.04 (td, *J* = 8.1, 7.0, 1.2 Hz, 1H, $H_{arom.}$), 6.96 (td, *J* = 7.9, 6.9, 1.0 Hz, 1H, $H_{arom.}$), 4.46 (td, *J* = 8.4, 5.1 Hz, 1H, $C_{\alpha}H$), 3.15 (dd, *J* = 14.6, 5.0 Hz, 1H, $C_{\beta}H$), 2.98 (dd, *J* = 14.6, 8.8 Hz, 1H, $C_{\beta}H$), 2.04 (td, *J* = 7.4, 1.9 Hz, 2H, CH_2-CO_{acyl}), 1.39 (quintet, *J* = 7.3 Hz, 2H, $CH_{2\text{-acyl}}$), 1.26 – 1.15 (m, 16H, 8 x $CH_{2\text{-acyl}}$), 0.84 (t, *J* = 6.8 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 173.6 (C=O), 172.3 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.4 ($C_{arom.}$, $C_{quat.}$), 123.6 ($C_{arom.}$, CH), 120.9 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 118.3 ($C_{arom.}$, CH), 111.4 ($C_{arom.}$, CH), 110.2 ($C_{arom.}$, $C_{quat.}$), 52.9 ($C_{\alpha}H$), 35.2 (CH_2), 31.4 (CH_2), 29.1 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 27.3 (CH_2), 25.3 (CH_2), 22.2 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 387 $[M+H]^+$.

N-Tetradecanoyl-L-tryptophan (80)[115]



The compound was prepared from L-tryptophan and tetradecanoyl chloride according to procedure 5.3.1.

Appearance: white solid. **Melting point:** 103 – 104 °C. **Yield:** 522 mg, 63 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.7 %.

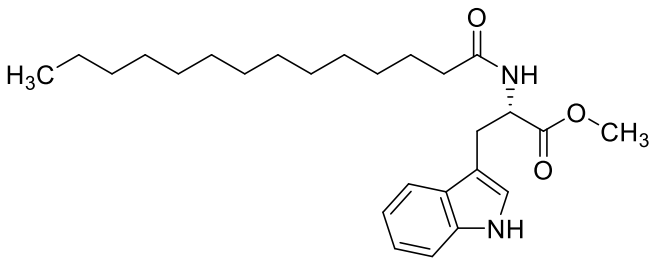
^{1}H-NMR (600 MHz, DMSO-d_6) δ: 12.52 (s (br), 1H, COOH), 10.79 (s, 1H, NH_{indole}), 7.99 (d, *J* = 7.9 Hz, 1H, NH_{amide}), 7.51 (d, *J* = 7.9 Hz, 1H, $H_{arom.}$), 7.31 (d, *J* = 8.1 Hz, 1H, $H_{arom.}$), 7.11 (d, *J* = 2.4 Hz, 1H, $H_{arom.}$), 7.04 (t, *J* = 7.5 Hz, 1H, $H_{arom.}$), 6.96 (t, *J* = 7.4 Hz, 1H, $H_{arom.}$), 4.46 (td, *J* = 8.4, 5.0 Hz, 1H, $C_{\alpha}H$), 3.14 (dd, *J* = 14.7, 5.1 Hz, 1H, $C_{\beta}H$), 2.98 (dd, *J* = 14.6, 8.8 Hz,

1H, $C_\beta H$), 2.04 (td, *J* = 7.4, 2.4 Hz, 2H, CH_2-CO_{acyl}), 1.39 (p, *J* = 7.2 Hz, 2H, $CH_{2\text{-}acyl}$), 1.27 – 1.12 (m, 20H, 10 x $CH_{2\text{-}acyl}$), 0.84 (t, *J* = 6.9 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 173.7 (C=O), 172.3 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.4 ($C_{arom.}$, $C_{quat.}$), 123.6 ($C_{arom.}$, CH), 120.9 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 118.3 ($C_{arom.}$, CH), 111.5 ($C_{arom.}$, CH), 110.2 ($C_{arom.}$, $C_{quat.}$), 52.9 ($C_\alpha H$), 35.3 (CH_2), 31.5 (CH_2), 29.2 (4 x CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.7 (CH_2), 27.3 (CH_2), 25.3 (CH_2), 22.3 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 415 $[M+H]^+$.

N-Tetradecanoyl-L-tryptophan methyl ester (106)[115]



The compound was prepared from **80** and methanol according to procedure 5.3.2.2.

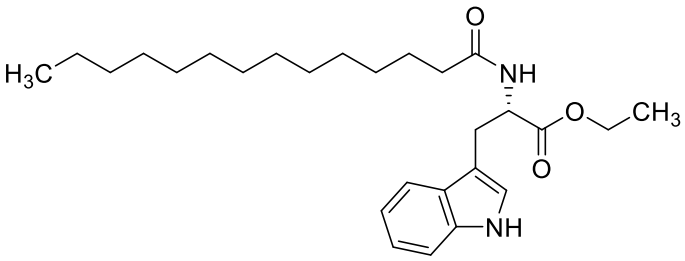
Appearance: off-white solid. **Melting point:** 55 – 57 °C. **Yield:** 110 mg, quantitative. **Purity by HPLC-UV (254 nm)-ESI-MS:** 96.3 %.

^{1}H-NMR (600 MHz, DMSO-*d*$_6$) δ: 10.82 (s, 1H, NH_{indole}), 8.17 (d, *J* = 7.5 Hz, 1H, NH_{amide}), 7.47 (d, *J* = 7.9 Hz, 1H, $H_{arom.}$), 7.32 (d, *J* = 8.1 Hz, 1H, $H_{arom.}$), 7.12 (d, *J* = 2.3 Hz, 1H, $H_{arom.}$), 7.05 (t, *J* = 7.5 Hz, 1H, $H_{arom.}$), 6.96 (t, *J* = 7.4 Hz, 1H, $H_{arom.}$), 4.49 (td, *J* = 8.1, 5.8 Hz, 1H, $C_\alpha H$), 3.56 (s, 3H, OCH_3), 3.12 (dd, *J* = 14.6, 5.7 Hz, 1H, $C_\beta H$), 3.00 (dd, *J* = 14.6, 8.6 Hz, 1H, $C_\beta H$), 2.05 (td, *J* = 7.2, 2.7 Hz, 2H, CH_2-CO_{acyl}), 1.39 (p, *J* = 7.3 Hz, 2H, $CH_{2\text{-}acyl}$), 1.29 – 1.10 (m, 20H, 10 x $CH_{2\text{-}acyl}$), 0.84 (t, *J* = 6.8 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 172.7 (C=O), 172.4 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.2 ($C_{arom.}$, $C_{quat.}$), 123.7 ($C_{arom.}$, CH), 121.1 ($C_{arom.}$, CH), 118.5 ($C_{arom.}$, CH), 118.1 ($C_{arom.}$, CH), 111.6 ($C_{arom.}$, CH), 109.7 ($C_{arom.}$, $C_{quat.}$), 53.1 ($C_\alpha H$), 51.9 (OCH_3), 35.1 (CH_2), 31.5 (CH_2), 29.2 (4 x CH_2), 29.1 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 27.2 (CH_2), 25.3 (CH_2), 22.3 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 429 $[M+H]^+$.

N-Tetradecanoyl-L-tryptophan ethyl ester (109)



The compound was prepared from **80** and ethanol according to procedure 5.3.2.2.

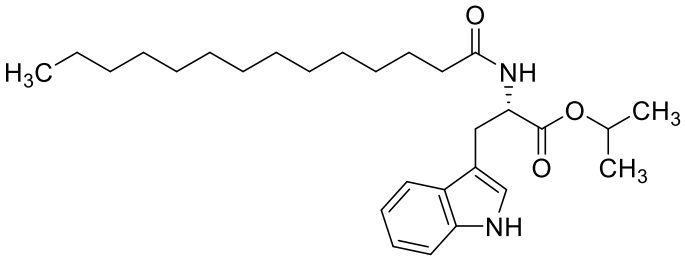
Appearance: pink-colored solid. **Melting point:** 60 – 62 °C. **Yield:** 115 mg, quantitative. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.5 %.

^{1}H-NMR (600 MHz, DMSO-d_6) δ: 10.80 (s, 1H, NH_{indole}), 8.13 (d, J = 7.6 Hz, 1H, NH_{amide}), 7.48 (d, J = 7.9 Hz, 1H, $H_{arom.}$), 7.32 (dt, J = 8.1, 0.9 Hz, 1H, $H_{arom.}$), 7.12 (d, J = 2.4 Hz, 1H, $H_{arom.}$), 7.05 (ddd, J = 8.1, 7.0, 1.2 Hz, 1H, $H_{arom.}$), 6.96 (ddd, J = 7.9, 7.0, 1.1 Hz, 1H, $H_{arom.}$), 4.47 (td, J = 8.1, 5.9 Hz, 1H, $C_{\alpha}H$), 4.00 (q, J = 7.1, 2H, $CH_{2\text{-ethyl}}$), 3.12 (dd, J = 14.7, 5.8 Hz, 1H, $C_{\beta}H$), 3.01 (dd, J = 14.5, 8.5 Hz, 1H, $C_{\beta}H$), 2.06 (td, J = 7.3, 3.8 Hz, 2H, CH_2-CO_{acyl}), 1.41 (p, J = 6.8 Hz, 2H, $CH_{2\text{-acyl}}$), 1.30 – 1.11 (m, 20H, 10 x $CH_{2\text{-acyl}}$), 1.07 (t, J = 7.1 Hz, 3H, $CH_{3\text{-ethyl}}$), 0.84 (t, J = 6.8 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 172.4 (C=O), 172.2 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.2 ($C_{arom.}$, $C_{quat.}$), 123.7 ($C_{arom.}$, CH), 121.0 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 118.1 ($C_{arom.}$, CH), 111.5 ($C_{arom.}$, CH), 109.7 ($C_{arom.}$, $C_{quat.}$), 60.4 ($CH_{2\text{-ethyl}}$), 53.2 ($C_{\alpha}H$), 35.1 (CH_2), 31.4 (CH_2), 29.2 (4 x CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.6 (CH_2), 27.2 (CH_2), 25.3 (CH_2), 22.2 (CH_2), 14.0 ($CH_{3\text{-ethyl}}$), 14.0 ($CH_{3\text{-acyl}}$).

LC-ESI-MS (m/z): 443 $[M+H]^+$.

N-Tetradecanoyl-L-tryptophan isopropyl ester (112)



The compound was prepared from **80** and isopropanol according to procedure 5.3.2.2.

Appearance: pink-colored sticky solid. **Melting point:** 44 – 45 °C. **Yield:** 121 mg, quantitative. **Purity by HPLC-UV (254 nm)-ESI-MS:** 95.9 %.

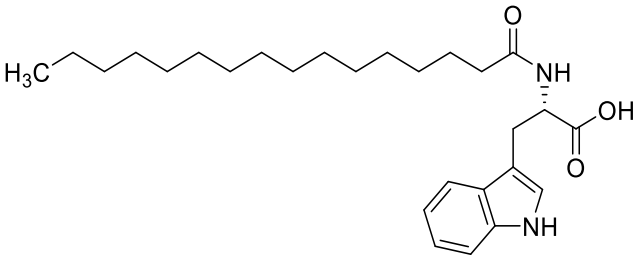
^{1}H-NMR (600 MHz, DMSO-d_6) δ: 10.80 (s, 1H, NH_{indole}), 8.11 (d, J = 7.5 Hz, 1H, NH_{amide}), 7.48 (d, J = 7.9 Hz, 1H, $H_{arom.}$), 7.31 (dt, J = 8.2, 1.0 Hz, 1H, $H_{arom.}$), 7.12 (d, J = 2.4 Hz, 1H, $H_{arom.}$), 7.05 (ddd, J = 8.1, 7.0, 1.2 Hz, 1H, $H_{arom.}$), 6.96 (ddd, J = 8.0, 7.0, 1.1 Hz, 1H, $H_{arom.}$),

4.80 (sept, *J* = 6.3 Hz, 1H, $CH_{isopropyl}$), 4.43 (td, *J* = 8.1, 6.1 Hz, 1H, $C_{\alpha}H$), 3.10 (dd, *J* = 14.5, 6.0 Hz, 1H, $C_{\beta}H$), 2.98 (dd, *J* = 14.5, 8.5 Hz, 1H, $C_{\beta}H$), 2.05 (m, 2H, CH_2-CO_{acyl}), 1.47 – 1.36 (m, 2H, $CH_{2\text{-}acyl}$), 1.30 – 1.14 (m, 20H, 10 x $CH_{2\text{-}acyl}$), 1.12 (d, *J* = 6.3 Hz, 3H, $CH_{3\text{-}isopropyl}$), 1.00 (d, *J* = 6.2 Hz, 3H, $CH_{3\text{-}isopropyl}$), 0.84 (t, *J* = 7.0 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 172.4 (C=O), 171.7 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.3 ($C_{arom.}$, $C_{quat.}$), 123.7 ($C_{arom.}$, CH), 121.0 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 118.2 ($C_{arom.}$, CH), 111.5 ($C_{arom.}$, CH), 109.8 ($C_{arom.}$, $C_{quat.}$), 67.8 ($CH_{isopropyl}$), 53.4 ($C_{\alpha}H$), 35.1 (CH_2), 31.4 (CH_2), 29.2 (4 x CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.6 (CH_2), 27.2 (CH_2), 25.3 (CH_2), 22.2 (CH_2), 21.6 ($CH_{3\text{-}isopropyl}$), 21.4 ($CH_{3\text{-}isopropyl}$), 14.1 ($CH_{3\text{-}acyl}$).

LC-ESI-MS (m/z): 457 $[M+H]^+$.

***N-Hexadecanoyl-L-tryptophan (81)*[116]**



The compound was prepared from L-tryptophan and hexadecanoyl chloride according to procedure 5.3.1.

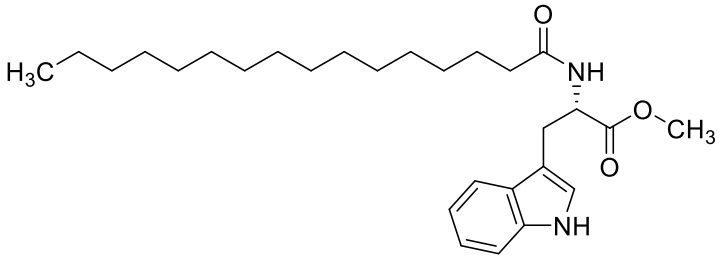
Appearance: white solid. **Melting point:** 96 – 98 °C. (lit. m.p. 95 – 97 °C).[114] **Yield:** 598 mg, 67 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.0 %.

^{1}H-NMR (600 MHz, DMSO-*d*$_6$) δ: 12.49 (s (br), 1H, COOH), 10.79 (s, 1H, NH_{indole}), 7.99 (d, *J* = 7.9 Hz, 1H, NH_{amide}), 7.51 (d, *J* = 7.9 Hz, 1H, $H_{arom.}$), 7.31 (d, *J* = 8.1 Hz, 1H, $H_{arom.}$), 7.11 (d, *J* = 2.3 Hz, 1H, $H_{arom.}$), 7.04 (t, *J* = 7.5 Hz, 1H, $H_{arom.}$), 6.96 (t, *J* = 7.4 Hz, 1H, $H_{arom.}$), 4.46 (td, *J* = 8.4, 5.0 Hz, 1H, $C_{\alpha}H$), 3.14 (dd, *J* = 14.7, 5.1 Hz, 1H, $C_{\beta}H$), 2.98 (dd, *J* = 14.7, 8.8 Hz, 1H, $C_{\beta}H$), 2.04 (td, *J* = 7.3, 2.3 Hz, 2H, CH_2-CO_{acyl}), 1.39 (p, *J* = 7.2 Hz, 2H, $CH_{2\text{-}acyl}$), 1.27 – 1.12 (m, 24H, 12 x $CH_{2\text{-}acyl}$), 0.84 (t, *J* = 6.9 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 173.7 (C=O), 172.3 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.4 ($C_{arom.}$, $C_{quat.}$), 123.6 ($C_{arom.}$, CH), 120.9 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 118.3 ($C_{arom.}$, CH), 111.5 ($C_{arom.}$, CH), 110.2 ($C_{arom.}$, $C_{quat.}$), 52.9 ($C_{\alpha}H$), 35.2 (CH_2), 31.4 (CH_2), 29.2 (6 x CH_2), 29.1 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 27.3 (CH_2), 25.3 (CH_2), 22.2 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 443 $[M+H]^+$.

N-Hexadecanoyl-L-tryptophan methyl ester (107)[116]



The compound was prepared from **81** and methanol according to procedure 5.3.2.2.

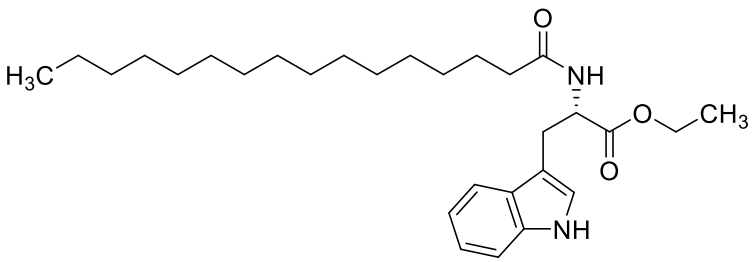
Appearance: off-white solid. **Melting point:** 66 – 68 °C. (lit. m.p. 69 – 70 °C).[114] **Yield:** 113 mg, quantitative. **Purity by HPLC-UV (254 nm)-ESI-MS:** 94.9 %.

^{1}H-NMR (600 MHz, DMSO-d_6) δ: 10.82 (s, 1H, NH_{indole}), 8.17 (d, J = 7.5 Hz, 1H, NH_{amide}), 7.47 (d, J = 7.9 Hz, 1H, $H_{arom.}$), 7.31 (d, J = 8.1 Hz, 1H, $H_{arom.}$), 7.11 (d, J = 2.3 Hz, 1H, $H_{arom.}$), 7.04 (t, J = 7.4 Hz, 1H, $H_{arom.}$), 6.96 (t, J = 7.4 Hz, 1H, $H_{arom.}$), 4.49 (td, J = 8.2, 5.7 Hz, 1H, $C_\alpha H$), 3.56 (s, 3H, OCH_3), 3.12 (dd, J = 14.6, 5.6 Hz, 1H, $C_\beta H$), 3.00 (dd, J = 14.6, 8.6 Hz, 1H, $C_\beta H$), 2.05 (td, J = 7.3, 2.7 Hz, 2H, CH_2-CO_{acyl}), 1.39 (p, J = 7.3 Hz, 2H, $CH_{2\text{-}acyl}$), 1.29 – 1.10 (m, 24H, 12 x $CH_{2\text{-}acyl}$), 0.84 (t, J = 6.9 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 172.7 (C=O), 172.4 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.2 ($C_{arom.}$, $C_{quat.}$), 123.7 ($C_{arom.}$, CH), 121.0 ($C_{arom.}$, CH), 118.5 ($C_{arom.}$, CH), 118.1 ($C_{arom.}$, CH), 111.5 ($C_{arom.}$, CH), 109.7 ($C_{arom.}$, $C_{quat.}$), 53.1 ($C_\alpha H$), 51.8 (OCH_3), 35.1 (CH_2), 31.4 (CH_2), 29.2 (6 x CH_2), 29.1 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 27.2 (CH_2), 25.3 (CH_2), 22.2 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 457 $[M+H]^+$.

N-Hexadecanoyl-L-tryptophan ethyl ester (110)[117]



The compound was prepared from **81** and ethanol according to procedure 5.3.2.2.

Appearance: pink-colored solid. **Melting point:** 67 – 69 °C. (lit. m.p. 56 – 58 °C).[117] **Yield:** 123 mg, quantitative. **Purity by HPLC-UV (254 nm)-ESI-MS:** 96.2%.

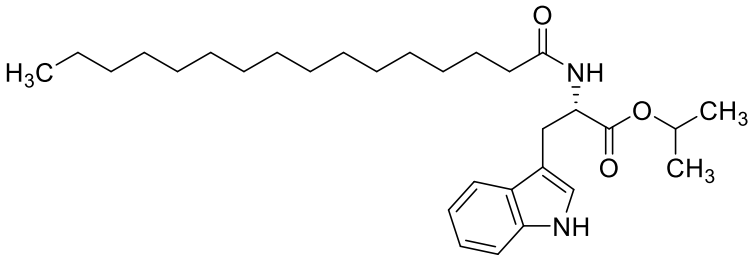
^{1}H-NMR (600 MHz, DMSO-d_6) δ: 10.80 (s, 1H, NH(indole)), 8.13 (d, J = 7.6 Hz, 1H, NH(amide)), 7.48 (d, J = 8.0 Hz, 1H, $H_{arom.}$), 7.32 (dt, J = 8.1, 1.0 Hz, 1H, $H_{arom.}$), 7.12 (d, J = 2.4 Hz, 1H, $H_{arom.}$), 7.05 (ddd, J = 8.1, 7.0, 1.2 Hz, 1H, $H_{arom.}$), 6.96 (ddd, J = 7.9, 7.0, 1.0 Hz,

1H, $H_{arom.}$), 4.47 (td, *J* = 8.1, 5.9 Hz, 1H, $C_{\alpha}H$), 4.00 (q, *J* = 7.1, 2H, $CH_{2\text{-ethyl}}$), 3.12 (dd, *J* = 14.6, 5.8 Hz, 1H, $C_{\beta}H$), 3.01 (dd, *J* = 14.4, 8.5 Hz, 1H, $C_{\beta}H$), 2.05 (td, *J* = 7.3, 3.7 Hz, 2H, CH_2-CO_{acyl}), 1.41 (p, *J* = 7.3 Hz, 2H, $CH_{2\text{-acyl}}$), 1.30 – 1.12 (m, 24H, 12 x $CH_{2\text{-acyl}}$), 1.07 (t, *J* = 7.1 Hz, 3H, $CH_{3\text{-ethyl}}$), 0.84 (t, *J* = 6.9 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 172.4 (C=O), 172.2 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.3 ($C_{arom.}$, $C_{quat.}$), 123.7 ($C_{arom.}$, CH), 121.0 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 118.1 ($C_{arom.}$, CH), 111.5 ($C_{arom.}$, CH), 109.8 ($C_{arom.}$, $C_{quat.}$), 60.4 ($CH_{2\text{-ethyl}}$), 53.2 ($C_{\alpha}H$), 35.1 (CH_2), 31.4 (CH_2), 29.2 (6 x CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.6 (CH_2), 27.2 (CH_2), 25.3 (CH_2), 22.2 (CH_2), 14.0 ($CH_{3\text{-ethyl}}$), 14.0 ($CH_{3\text{-acyl}}$).

LC-ESI-MS (m/z): 471 $[M+H]^+$.

N-Hexadecanoyl-L-tryptophan isopropyl ester (113)



The compound was prepared from **81** and isopropanol according to procedure 5.3.2.2.

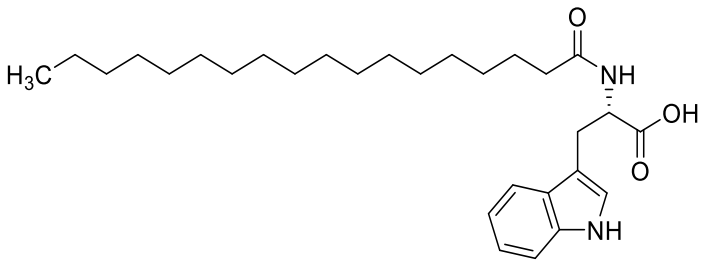
Appearance: off-white solid. **Melting point:** 65 – 66 °C. **Yield:** 240 mg, quantitative. **Purity by HPLC-UV (254 nm)-ESI-MS:** 95.6 %.

^{1}H-NMR (600 MHz, DMSO-*d*$_6$) δ: 10.80 (s, 1H, NH_{indole}), 8.11 (d, *J* = 7.6 Hz, 1H, NH_{amide}), 7.48 (d, *J* = 8.0 Hz, 1H, $H_{arom.}$), 7.31 (dt, *J* = 8.2, 0.9 Hz, 1H, $H_{arom.}$), 7.11 (d, *J* = 2.4 Hz, 1H, $H_{arom.}$), 7.04 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 1H, $H_{arom.}$), 6.96 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H, $H_{arom.}$), 4.80 (sept, *J* = 6.3 Hz, 1H, $CH_{isopropyl}$), 4.42 (ddd, *J* = 8.4, 7.5, 6.1 Hz, 1H, $C_{\alpha}H$), 3.10 (dd, *J* = 14.7, 6.2 Hz, 1H, $C_{\beta}H$), 2.99 (dd, *J* = 14.8, 8.5 Hz, 1H, $C_{\beta}H$), 2.05 (td, *J* = 7.3, 6.1 Hz, 2H, CH_2-CO_{acyl}), 1.45 – 1.36 (m, 2H, $CH_{2\text{-acyl}}$), 1.30 – 1.17 (m, 24H, 12 x $CH_{2\text{-acyl}}$), 1.12 (d, *J* = 6.3 Hz, 3H, $CH_{3\text{-isopropyl}}$), 1.00 (d, *J* = 6.3 Hz, 3H, $CH_{3\text{-isopropyl}}$), 0.84 (t, *J* = 7.2 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 172.4 (C=O), 171.7 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.3 ($C_{arom.}$, $C_{quat.}$), 123.7 ($C_{arom.}$, CH), 121.0 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 118.2 ($C_{arom.}$, CH), 111.5 ($C_{arom.}$, CH), 109.8 ($C_{arom.}$, $C_{quat.}$), 67.8 ($CH_{isopropyl}$), 53.4 ($C_{\alpha}H$), 35.1 (CH_2), 31.4 (CH_2), 29.2 (6 x CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.6 (CH_2), 27.2 (CH_2), 25.3 (CH_2), 22.2 (CH_2), 21.6 ($CH_{3\text{-isopropyl}}$), 21.4 ($CH_{3\text{-isopropyl}}$), 14.1 ($CH_{3\text{-acyl}}$).

LC-ESI-MS (m/z): 485 $[M+H]^+$.

N-Octadecanoyl-L-tryptophan (82)[118]



The compound was prepared from L-tryptophan and octadecanoyl chloride according to procedure 5.3.1.

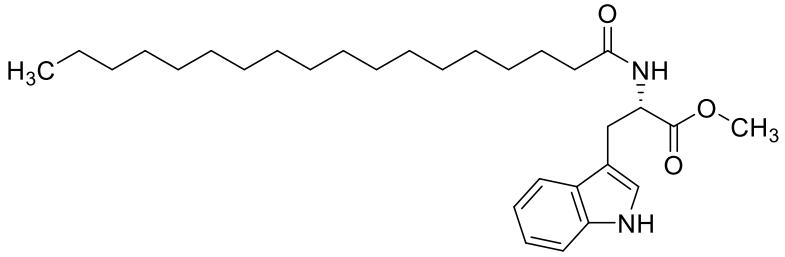
Appearance: white solid. **Melting point:** 93 – 95 °C. (lit. m.p. 95 – 97 °C).[114] **Yield:** 738 mg, 78 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 95.9 %.

^{1}H-NMR (600 MHz, DMSO-d_6) δ: 12.33 (s (br), 1H, COOH), 10.79 (s, 1H, NH_{indole}), 7.99 (d, J = 7.7 Hz, 1H, NH_{amide}), 7.51 (d, J = 7.8 Hz, 1H, $H_{arom.}$), 7.31 (d, J = 8.0 Hz, 1H, $H_{arom.}$), 7.10 (s, 1H, $H_{arom.}$), 7.04 (t, J = 7.1 Hz, 1H, $H_{arom.}$), 6.96 (t, J = 7.5 Hz, 1H, $H_{arom.}$), 4.45 (td, J = 8.4, 4.1 Hz, 1H, $C_{\alpha}H$), 3.13 (d, J = 4.9 Hz, 1H, $C_{\beta}H$), 2.98 (dd, J = 14.7, 8.9 Hz, 1H, $C_{\beta}H$), 2.04 (m, 2H, CH_2-CO_{acyl}), 1.39 (m, 2H, $CH_{2\text{-acyl}}$), 1.26 – 1.14 (m, 28H, 14 x $CH_{2\text{-acyl}}$), 0.84 (t, J = 7.1 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 174.0 (C=O), 172.6 (C=O), 136.5 ($C_{arom.}$, $C_{quat.}$), 127.7 ($C_{arom.}$, $C_{quat.}$), 123.9 ($C_{arom.}$, CH), 121.3 ($C_{arom.}$, CH), 118.7 ($C_{arom.}$, CH), 118.6 ($C_{arom.}$, CH), 111.8 ($C_{arom.}$, CH), 110.5 ($C_{arom.}$, $C_{quat.}$), 53.3 ($C_{\alpha}H$), 35.6 (CH_2), 31.7 (CH_2), 29.5 (8 x CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.0 (CH_2), 27.6 (CH_2), 25.6 (CH_2), 22.6 (CH_2), 14.4 (CH_3).

LC-ESI-MS (m/z): 471 $[M+H]^+$.

N-Octadecanoyl-L-tryptophan methyl ester (108)[118]



The compound was prepared from **82** and methanol according to procedure 5.3.2.2.

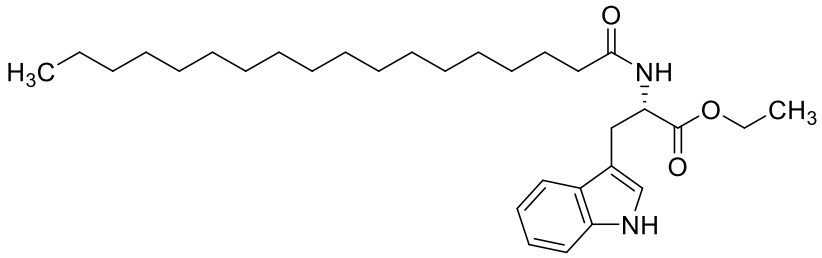
Appearance: off-white solid. **Melting point:** 73 – 75 °C. (lit. m.p. 71 – 73 °C).[114] **Yield:** 123 mg, quantitative. **Purity by HPLC-UV (254 nm)-ESI-MS:** 95.8 %.

^{1}H-NMR (600 MHz, DMSO-*d*$_6$) δ: 10.82 (s, 1H, NH_{indole}), 8.16 (d, *J* = 7.6 Hz, 1H, NH_{amide}), 7.47 (d, *J* = 7.9 Hz, 1H, $H_{arom.}$), 7.31 (d, *J* = 8.1 Hz, 1H, $H_{arom.}$), 7.11 (d, *J* = 2.4 Hz, 1H, $H_{arom.}$), 7.04 (t, *J* = 7.5 Hz, 1H, $H_{arom.}$), 6.96 (t, *J* = 7.3 Hz, 1H, $H_{arom.}$), 4.49 (td, *J* = 8.3, 5.7 Hz, 1H, $C_{\alpha}H$), 3.56 (s, 3H, OCH_3), 3.12 (dd, *J* = 14.6, 5.6 Hz, 1H, $C_{\beta}H$), 3.00 (dd, *J* = 14.6, 8.6 Hz, 1H, $C_{\beta}H$), 2.05 (td, *J* = 7.3, 2.7 Hz, 2H, CH_2-CO_{acyl}), 1.39 (p, *J* = 7.3 Hz, 2H, $CH_{2\text{-}acyl}$), 1.29 – 1.08 (m, 28H, 14 x $CH_{2\text{-}acyl}$), 0.84 (t, *J* = 6.9 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 172.7 (C=O), 172.4 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.2 ($C_{arom.}$, $C_{quat.}$), 123.7 ($C_{arom.}$, CH), 121.0 ($C_{arom.}$, CH), 118.5 ($C_{arom.}$, CH), 118.1 ($C_{arom.}$, CH), 111.5 ($C_{arom.}$, CH), 109.8 ($C_{arom.}$, $C_{quat.}$), 53.2 ($C_{\alpha}H$), 51.9 (OCH_3), 35.1 (CH_2), 31.4 (CH_2), 29.2 (8 x CH_2), 29.1 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 27.2 (CH_2), 25.3 (CH_2), 22.2 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 485 $[M+H]^+$.

N-Octadecanoyl-L-tryptophan ethyl ester (111)



The compound was prepared from **82** and ethanol according to procedure 5.3.2.2.

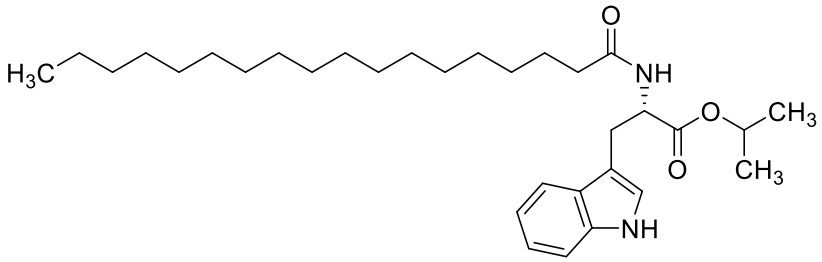
Appearance: pink-colored solid. **Melting point:** 73 – 74 °C. **Yield:** 126 mg, quantitative. **Purity by HPLC-UV (254 nm)-ESI-MS:** 94.9 %.

^{1}H-NMR (500 MHz, DMSO-*d*$_6$) δ: 10.80 (s, 1H, NH_{indole}), 8.13 (d, *J* = 7.6 Hz, 1H, NH_{amide}), 7.48 (d, *J* = 8.0 Hz, 1H, $H_{arom.}$), 7.32 (dt, *J* = 8.1, 0.9 Hz, 1H, $H_{arom.}$), 7.11 (d, *J* = 2.4 Hz, 1H, $H_{arom.}$), 7.04 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H, $H_{arom.}$), 6.96 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H, $H_{arom.}$), 4.47 (td, *J* = 8.3, 6.1 Hz, 1H, $C_{\alpha}H$), 4.00 (q, *J* = 7.1, 2H, $CH_{2\text{-}ethyl}$), 3.12 (dd, *J* = 14.7, 5.8 Hz, 1H, $C_{\beta}H$), 3.01 (dd, *J* = 14.6, 8.3 Hz, 1H, $C_{\beta}H$), 2.05 (td, *J* = 7.3, 3.7 Hz, 2H, CH_2-CO_{acyl}), 1.41 (p, *J* = 7.4 Hz, 2H, $CH_{2\text{-}acyl}$), 1.30 – 1.12 (m, 28H, 14 x $CH_{2\text{-}acyl}$), 1.07 (t, *J* = 7.1 Hz, 3H, $CH_{3\text{-}ethyl}$), 0.84 (t, *J* = 6.8 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 172.4 (C=O), 172.2 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.3 ($C_{arom.}$, $C_{quat.}$), 123.7 ($C_{arom.}$, CH), 121.0 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 118.1 ($C_{arom.}$, CH), 111.5 ($C_{arom.}$, CH), 109.7 ($C_{arom.}$, $C_{quat.}$), 60.4 ($CH_{2\text{-ethyl}}$), 53.2 ($C_{\alpha}H$), 35.1 (CH_2), 31.4 (CH_2), 29.2 (8 x CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.6 (CH_2), 27.2 (CH_2), 25.2 (CH_2), 22.2 (CH_2), 14.0 ($CH_{3\text{-ethyl}}$), 14.0 (CH_3).

LC-ESI-MS (m/z): 499 $[M+H]^+$.

N-Octadecanoyl-L-tryptophan isopropyl ester (114)



The compound was prepared from **82** and isopropanol according to procedure 5.3.2.2.

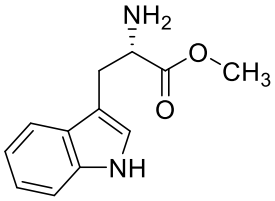
Appearance: brown solid. **Melting point:** 63 – 64 °C. **Yield:** 202 mg, quantitative. **Purity by HPLC-UV (254 nm)-ESI-MS:** 91.0 %.

^{1}H-NMR (600 MHz, DMSO-d_6) δ: 10.80 (s, 1H, NH_{indole}), 8.11 (d, J = 7.5 Hz, 1H, NH_{amide}), 7.48 (d, J = 7.8 Hz, 1H, $H_{arom.}$), 7.31 (dt, J = 8.1, 0.9 Hz, 1H, $H_{arom.}$), 7.11 (d, J = 2.4 Hz, 1H, $H_{arom.}$), 7.04 (ddd, J = 8.1, 6.9, 1.1 Hz, 1H, $H_{arom.}$), 6.96 (ddd, J = 7.9, 6.9, 1.0 Hz, 1H, $H_{arom.}$), 4.80 (sept, J = 6.3 Hz, 1H, $CH_{isopropyl}$), 4.43 (td, J = 8.1, 6.2 Hz, 1H, $C_{\alpha}H$), 3.10 (dd, J = 14.6, 6.1 Hz, 1H, $C_{\beta}H$), 2.99 (dd, J = 14.5, 8.4 Hz, 1H, $C_{\beta}H$), 2.11 – 2.00 (m, 2H, CH_2-CO_{acyl}), 1.45 – 1.36 (m, 2H, $CH_{2\text{-acyl}}$), 1.29 – 1.17 (m, 28H, 14 x $CH_{2\text{-acyl}}$), 1.12 (d, J = 6.3 Hz, 3H, $CH_{3\text{-isopropyl}}$), 1.00 (d, J = 6.2 Hz, 3H, $CH_{3\text{-isopropyl}}$), 0.84 (t, J = 6.8 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 172.4 (C=O), 171.7 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.3 ($C_{arom.}$, $C_{quat.}$), 123.7 ($C_{arom.}$, CH), 121.0 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 118.2 ($C_{arom.}$, CH), 111.5 ($C_{arom.}$, CH), 109.8 ($C_{arom.}$, $C_{quat.}$), 67.8 ($CH_{isopropyl}$), 53.4 ($C_{\alpha}H$), 35.1 (CH_2), 31.4 (CH_2), 29.2 (8 x CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.6 (CH_2), 27.2 (CH_2), 25.3 (CH_2), 22.2 (CH_2), 21.6 ($CH_{3\text{-isopropyl}}$), 21.4 ($CH_{3\text{-isopropyl}}$), 14.1 ($CH_{3\text{-acyl}}$).

LC-ESI-MS (m/z): 513 $[M+H]^+$.

L-Tryptophan methyl ester (92)[119]



The compound was prepared from L-tryptophan according to procedure 5.3.2.1.

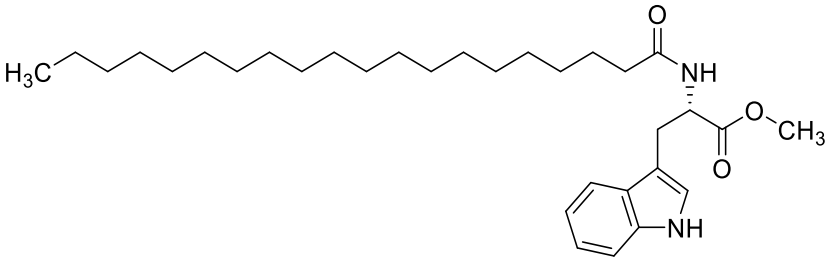
Appearance: light yellow oil. **Yield:** 4.38 g, quantitative. **Purity by HPLC-UV (254 nm)-ESI-MS:** 98.9 %.

^{1}H-NMR (500 MHz, DMSO-*d*$_6$) δ: 10.82 (s, 1H, NH_{indole}), 7.47 (d, *J* = 8.0 Hz, 1H, $H_{arom.}$), 7.32 (dt, *J* = 8.1, 0.9 Hz, 1H, $H_{arom.}$), 7.11 (d, *J* = 2.3 Hz, 1H, $H_{arom.}$), 7.05 (ddd, *J* = 8.1, 7.0, 1.2 Hz, 1H, $H_{arom.}$), 6.96 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 1H, $H_{arom.}$), 3.62 (t, *J* = 6.3 Hz, 1H, $C_\alpha H$), 3.54 (s, 3H, OCH_3), 3.01 (ddd, *J* = 14.2, 6.2, 0.8 Hz, 1H, $C_\beta H$), 2.92 (ddd, *J* = 14.2, 6.6, 0.8 Hz, 1H, $C_\beta H$), (NH_2 signal not visible).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 175.8 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.5 ($C_{arom.}$, $C_{quat.}$), 123.7 ($C_{arom.}$, CH), 120.9 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 118.3 ($C_{arom.}$, CH), 111.5 ($C_{arom.}$, CH), 110.1 ($C_{arom.}$, $C_{quat.}$), 55.3 ($C_\alpha H$), 51.4 (OCH_3), 30.9 (CH_2).

LC-ESI-MS (m/z): 219 $[M+H]^+$.

N-Eicosanoyl-L-tryptophan methyl ester (96)



The compound was prepared from **92** and eicosanoic acid according to procedure 5.3.3.

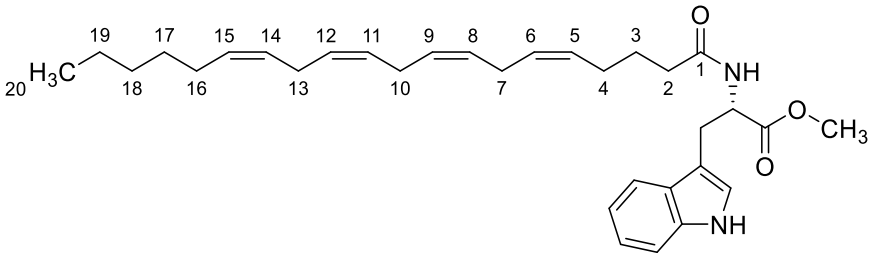
Appearance: white solid. **Melting point:** 93 – 96 °C. **Yield:** 270 mg, 53 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.9 %.

^{1}H-NMR (600 MHz, DMSO-*d*$_6$) δ: 10.80 (s, 1H, NH_{indole}), 8.14 (d, *J* = 8.3 Hz, 1H, NH_{amide}), 7.47 (d, *J* = 8.0 Hz, 1H, $H_{arom.}$), 7.31 (d, *J* = 8.5 Hz, 1H, $H_{arom.}$), 7.11 (d, *J* = 2.6 Hz, 1H, $H_{arom.}$), 7.05 (t, *J* = 7.4 Hz, 1H, $H_{arom.}$), 6.96 (t, *J* = 7.4 Hz, 1H, $H_{arom.}$), 4.61 – 4.35 (m, 1H, $C_\alpha H$), 3.56 (s, 3H, OCH_3), 3.14 (dd, *J* = 14.6, 6.0 Hz, 1H, $C_\beta H$), 3.00 (dd, *J* = 14.7, 8.8 Hz, 1H, $C_\beta H$), 2.06 – 2.03 (m, 2H, CH_2-CO_{acyl}), 1.39 (p, *J* = 7.2 Hz, 2H, $CH_{2\text{-}acyl}$), 1.26 – 1.15 (m, 32H, 16 x $CH_{2\text{-}acyl}$), 0.84 (t, *J* = 7.4, 3H, $CH_{3\text{-}acyl}$).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 172.7 (C=O), 172.4 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.2 ($C_{arom.}$, $C_{quat.}$), 123.7 ($C_{arom.}$, CH), 121.0 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 118.1 ($C_{arom.}$, CH), 111.5 ($C_{arom.}$, CH), 109.7 ($C_{arom.}$, $C_{quat.}$), 53.1 ($C_{\alpha}H$), 51.8 (OCH_3), 35.1 (CH_2), 31.4 (CH_2), 29.1 (10 x CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.6 (CH_2), 27.2 (CH_2), 25.2 (CH_2), 22.2 (CH_2), 14.0 ($CH_{3\text{-acyl}}$).

LC-ESI-MS (m/z): 513 $[M+H]^+$.

N-Arachidonoyl-L-tryptophan methyl ester (97)



The compound was prepared from **92** and arachidonic acid according to procedure 5.3.3.

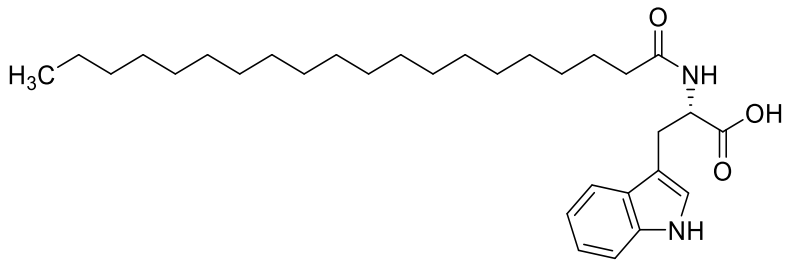
Appearance: yellow oil. **Yield:** 370 mg, 65 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 99.5 %.

^{1}H-NMR (600 MHz, DMSO-d_6) δ: 10.82 (s, 1H, NH_{indole}), 8.19 (d, J = 7.6 Hz, 1H, NH_{amide}), 7.47 (d, J = 7.9 Hz, 1H, $H_{arom.}$), 7.32 (d, J = 8.1 Hz, 1H, $H_{arom.}$), 7.11 (d, J = 2.3 Hz, 1H, $H_{arom.}$), 7.05 (t, J = 7.2 Hz, 1H, $H_{arom.}$), 6.96 (t, J = 7.6 Hz, 1H, $H_{arom.}$), 5.37 – 5.28 (m, 8H, 8 x CH_{sp2} (C5, C6, C8, C9, C11, C12, C14, C16)), 4.49 (td, J = 8.0, 5.8 Hz, 1H, $C_{\alpha}H$), 3.55 (s, 3H, OCH_3), 3.12 (dd, J = 14.5, 5.8 Hz, 1H, $C_{\beta}H$), 3.01 (dd, J = 14.6, 8.5 Hz, 1H, $C_{\beta}H$), 2.82 – 2.74 (m, 6H, 3 x CH_2 (C7, C10, C13)), 2.14 – 2.03 (m, 2H, CH_2 (C2)), 2.02 – 1.95 (m, 4H, 2 x CH_2 (C4, C16)), 1.48 (p, J = 7.4 Hz, 2H, CH_2 (C3)), 1.32 – 1.21 (m, 6H, 3 x CH_2 (C17, C18, C19)), 0.84 (t, J = 6.9, Hz, 3H, CH_3 (C20)).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 172.9 (C=O), 172.5 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 130.4 (CH_{sp2}), 129.9 (CH_{sp2}), 128.6 (CH_{sp2}), 128.5 (CH_{sp2}), 128.4 (CH_{sp2}), 128.2 (CH_{sp2}), 128.1 (CH_{sp2}), 127.9 (CH_{sp2}), 127.5 ($C_{arom.}$, $C_{quat.}$), 124.0 ($C_{arom.}$, CH), 121.4 ($C_{arom.}$, CH), 118.8 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 111.9 ($C_{arom.}$, CH), 110.0 ($C_{arom.}$, $C_{quat.}$), 53.5 ($C_{\alpha}H$), 52.2 (OCH_3), 34.9 (CH_2), 31.3 (CH_2), 29.2 (CH_2), 27.6 (CH_2), 27.1 (CH_2), 26.6 (CH_2), 25.7 – 25.6 (4 x CH_2), 22.4 (CH_2), 14.4 ($CH_{3\text{-acyl}}$).

LC-ESI-MS (m/z): 505 $[M+H]^+$.

N-Eicosanoyl-L-tryptophan (102)



The compound was prepared from **96** according to procedure 5.3.4.

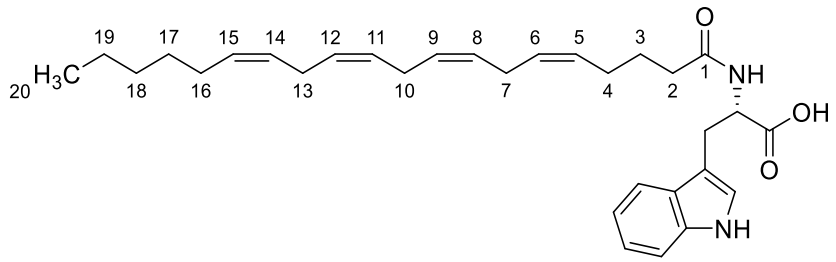
Appearance: white solid. **Melting point:** 95 – 98 °C. **Yield:** 83 mg, 83 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 96.3 %.

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 12.49 (s (br), 1H, COOH), 10.78 (s, 1H, NH_{indole}), 7.97 (d, J = 7.9 Hz, 1H, NH_{amide}), 7.51 (d, J = 7.8 Hz, 1H, $H_{arom.}$), 7.31 (d, J = 8.1 Hz, 1H, $H_{arom.}$), 7.10 (d, J = 2.3 Hz, 1H, $H_{arom.}$), 7.04 (ddd, J = 8.0, 6.9, 1.1 Hz, 1H, $H_{arom.}$), 6.98 – 6.92 (m, 1H, $H_{arom.}$), 4.46 (td, J = 8.4, 5.1 Hz, 1H, $C_{\alpha}H$), 3.14 (dd, J = 14.7, 5.1 Hz, 1H, $C_{\beta}H$), 2.98 (dd, J = 14.6, 8.8 Hz, 1H, $C_{\beta}H$), 2.04 (td, J = 7.3, 1.4 Hz, 2H, CH_2-CO_{acyl}), 1.39 (p, J = 7.3 Hz, 2H, $CH_{2\text{-}acyl}$), 1.30 – 1.10 (m, 32H, 16 x $CH_{2\text{-}acyl}$), 0.84 (t, J = 6.5, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 173.7 (C=O), 172.3 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.4 ($C_{arom.}$, $C_{quat.}$), 123.6 ($C_{arom.}$, CH), 120.9 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 118.3 ($C_{arom.}$, CH), 111.4 ($C_{arom.}$, CH), 110.2 ($C_{arom.}$, $C_{quat.}$), 52.9 ($C_{\alpha}H$), 35.2 (CH_2), 31.4 (CH_2), 29.2 – 29.1 (10 x CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 27.3 (CH_2), 25.3 (CH_2), 22.3 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 499 $[M+H]^+$.

N-Arachidonoyl-L-tryptophan (103)



THV328

The compound was prepared from **97** according to procedure 5.3.4.

Appearance: yellow oil. **Yield:** 77 mg, 78 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 99.1 %.

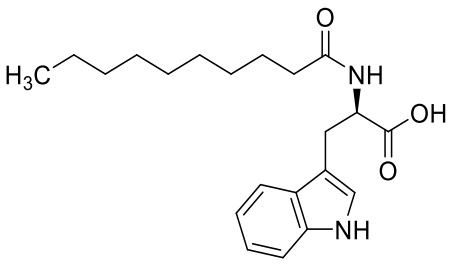
^{1}H-NMR (600 MHz, DMSO-d_6) δ: 12.48 (s (br), 1H, COOH), 10.79 (s, 1H, NH_{indole}), 8.02 (d, J = 7.8 Hz, 1H, NH_{amide}), 7.51 (d, J = 7.8 Hz, 1H, $H_{arom.}$), 7.31 (d, J = 8.1 Hz, 1H, $H_{arom.}$), 7.10 (d, J = 2.3 Hz, 1H, $H_{arom.}$), 7.04 (t, J = 7.2 Hz, 1H, $H_{arom.}$), 6.96 (t, J = 7.4 Hz, 1H, $H_{arom.}$), 5.39 – 5.25 (m, 8H, 8 x CH_{sp2} (C5, C6, C8, C9, C11, C12, C14, C16)), 4.46 (td, J = 8.3, 5.2 Hz, 1H, $C_{\alpha}H$), 3.14 (dd, J = 14.6, 5.1 Hz, 1H, $C_{\beta}H$), 2.98 (dd, J = 14.6, 8.7 Hz, 1H, $C_{\beta}H$), 2.83 – 2.70 (m, 6H, 3 x CH_2 (C7, C10, C13)), 2.13 – 2.03 (m, 2H, CH_2 (C2)), 2.02 – 1.94 (m, 4H, 2 x CH_2 (C4, C16)), 1.47 (pd, J = 7.5, 1.9 Hz, 2H, CH_2 (C3)), 1.34 – 1.18 (m, 6H, 3 x CH_2 (C17, C18, C19)), 0.84 (t, J = 6.9, Hz, 3H, CH_3 (C20)).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 173.7 (C=O), 172.1 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 130.1 (CH_{sp2}), 129.6 (CH_{sp2}), 128.3 (CH_{sp2}), 128.2 (CH_{sp2}), 128.1 (CH_{sp2}), 127.9 (CH_{sp2}), 127.8 (CH_{sp2}), 127.7 (CH_{sp2}), 127.4 ($C_{arom.}$, $C_{quat.}$), 123.6 ($C_{arom.}$, CH), 120.1 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 118.3 ($C_{arom.}$, CH), 111.5 ($C_{arom.}$, CH), 110.2 ($C_{arom.}$, $C_{quat.}$), 53.0 ($C_{\alpha}H$), 34.8 (CH_2), 30.9 (CH_2), 28.9 (CH_2), 27.3 (CH_2), 26.8 (CH_2), 26.4 (CH_2), 25.4 – 25.3 (4 x CH_2), 22.1 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 491 $[M+H]^+$.

5.3.5.3 D-Tryptophan derivatives

N-Decanoyl-D-tryptophan (83)



The compound was prepared from D-tryptophan and decanoyl chloride according to procedure 5.3.1.

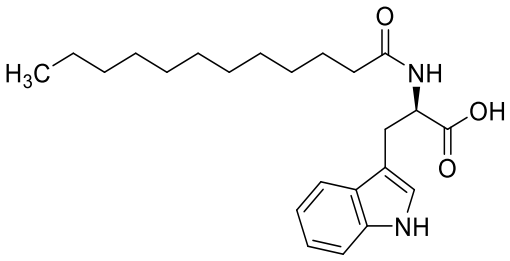
Appearance: white solid. **Melting point:** 92 – 95 °C. (lit. m.p. 93 – 95 °C).[120] **Yield:** 134 mg, 19 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 98.1 %.

^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.31 (s (br), 1H, NH_{indole}), 7.54 (d, J = 7.9 Hz, 1H, $H_{arom.}$), 7.32 (d, J = 8.1 Hz, 1H, $H_{arom.}$), 7.17 (t, J = 7.5 Hz, 1H, $H_{arom.}$), 7.09 (t, J = 7.5 Hz, 1H, $H_{arom.}$), 6.98 (s, 1H, $H_{arom.}$), 6.12 (d, J = 7.5 Hz, 1H, NH_{amide}), 4.91 (q, J = 5.6 Hz, 1H, $C_{\alpha}H$), 3.32 (qd, J = 14.9, 5.4 Hz, 2H, $C_{\beta}H_2$), 2.08 (t, J = 7.7 Hz, 2H, CH_2-CO_{acyl}), 1.48 (p, J = 7.0 Hz, 2H, $CH_{2\text{-}acyl}$), 1.30 – 1.08 (m, 12H, 6 x $CH_{2\text{-}acyl}$), 0.85 (t, J = 7.1, 3H, CH_3), (OH signal not visible).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 175.0 (C=O), 174.3 (C=O), 136.1 ($C_{arom.}$, $C_{quat.}$), 127.7 ($C_{arom.}$, $C_{quat.}$), 123.2 ($C_{arom.}$, CH), 122.2 ($C_{arom.}$, CH), 119.7 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 111.4 ($C_{arom.}$, CH), 109.5 ($C_{arom.}$, $C_{quat.}$), 53.3 ($C_\alpha H$), 36.4 (CH_2), 31.8 (CH_2), 29.4 (CH_2), 29.3 (2 x CH_2), 29.1 (CH_2), 27.0 (CH_2), 25.4 (CH_2), 22.6 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 359 $[M+H]^+$.

N-Dodecanoyl-D-tryptophan (84)



The compound was prepared from D-tryptophan and dodecanoyl chloride according to procedure 5.3.1.

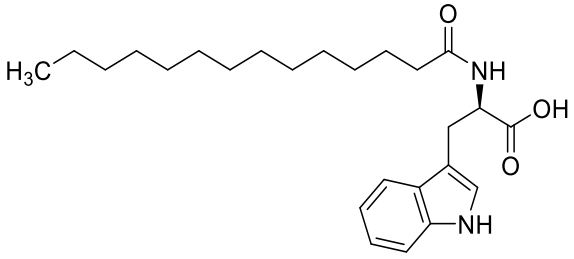
Appearance: white solid. **Melting point:** 106 - 108 °C. **Yield:** 568 mg, 74 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.1 %. $[\alpha]^{21}_D$ = -14.4° (c = 0.1 g/mL, in acetone).

^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.28 (s (br), 1H, NH_{indole}), 7.54 (d, *J* = 7.9 Hz, 1H, $H_{arom.}$), 7.33 (d, *J* = 8.1 Hz, 1H, $H_{arom.}$), 7.17 (t, *J* = 7.5 Hz, 1H, $H_{arom.}$), 7.09 (t, *J* = 7.4 Hz, 1H, $H_{arom.}$), 6.99 (s, 1H, $H_{arom.}$), 6.07 (d, *J* = 7.4 Hz, 1H, NH_{amide}), 4.91 (q, *J* = 5.7 Hz, 1H, $C_\alpha H$), 3.33 (qd, *J* = 15.0, 5.4 Hz, 2H, $C_\beta H_2$), 2.08 (t, *J* = 7.6 Hz, 2H, CH_2-CO_{acyl}), 1.49 (p, *J* = 7.2 Hz, 2H, $CH_{2\text{-}acyl}$), 1.31 – 1.16 (m, 16H, 8 x $CH_{2\text{-}acyl}$), 0.86 (t, *J* = 7.0, 3H, CH_3), (OH signal not visible).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 175.0 (C=O), 174.3 (C=O), 136.1 ($C_{arom.}$, $C_{quat.}$), 127.7 ($C_{arom.}$, $C_{quat.}$), 123.2 ($C_{arom.}$, CH), 122.2 ($C_{arom.}$, CH), 119.7 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 111.4 ($C_{arom.}$, CH), 109.6 ($C_{arom.}$, $C_{quat.}$), 53.3 ($C_\alpha H$), 36.4 (CH_2), 31.9 (CH_2), 29.6 (2 x CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 27.0 (CH_2), 25.4 (CH_2), 22.7 (CH_2), 14.2 (CH_3).

LC-ESI-MS (m/z): 387 $[M+H]^+$.

N-Tetradecanoyl-D-tryptophan (85)



The compound was prepared from D-tryptophan and tetradecanoyl chloride according to procedure 5.3.1.

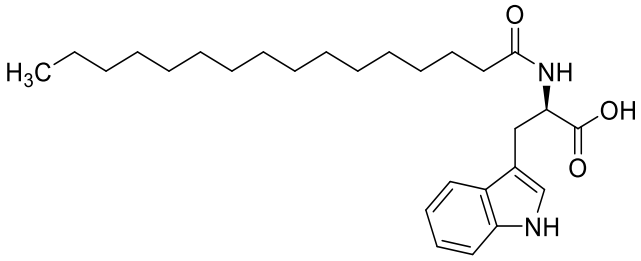
Appearance: off-white solid. **Melting point:** 100 - 102 °C. **Yield:** 523 mg, 63 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 98.8 %.

^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.29 (s (br), 1H, NH_{indole}), 7.54 (d, *J* = 7.9 Hz, 1H, $H_{arom.}$), 7.32 (d, *J* = 8.2 Hz, 1H, $H_{arom.}$), 7.17 (t, *J* = 7.5 Hz, 1H, $H_{arom.}$), 7.09 (t, *J* = 7.5 Hz, 1H, $H_{arom.}$), 6.98 (s, 1H, $H_{arom.}$), 6.08 (d, *J* = 7.5 Hz, 1H, NH_{amide}), 4.92 (q, *J* = 5.6 Hz, 1H, $C_{\alpha}H$), 3.33 (qd, *J* = 15.0, 5.3 Hz, 2H, $C_{\beta}H_2$), 2.08 (t, *J* = 7.8 Hz, 2H, CH_2-CO_{acyl}), 1.49 (p, *J* = 7.2 Hz, 2H, $CH_{2\text{-}acyl}$), 1.31 – 1.13 (m, 20H, 10 x $CH_{2\text{-}acyl}$), 0.85 (t, *J* = 6.9, 3H, CH_3), (OH signal not visible).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 175.1 (C=O), 174.2 (C=O), 136.0 ($C_{arom.}$, $C_{quat.}$), 127.7 ($C_{arom.}$, $C_{quat.}$), 123.1 ($C_{arom.}$, CH), 122.2 ($C_{arom.}$, CH), 119.7 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 111.4 ($C_{arom.}$, CH), 109.5 ($C_{arom.}$, $C_{quat.}$), 53.3 ($C_{\alpha}H$), 36.4 (CH_2), 31.9 (CH_2), 29.7 (CH_2), 29.6 (3 x CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 27.0 (CH_2), 25.4 (CH_2), 22.6 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 415 $[M+H]^+$.

N-Hexadecanoyl-D-tryptophan (86)



The compound was prepared from D-tryptophan and hexadecanoyl chloride according to procedure 5.3.1.

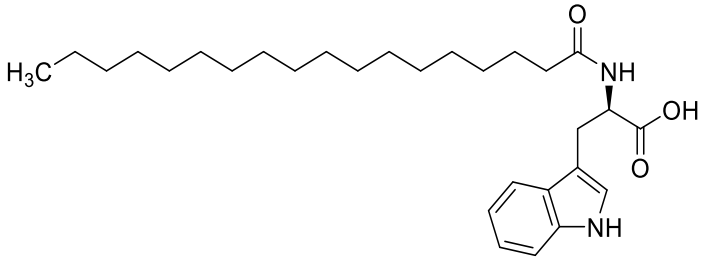
Appearance: white solid. **Melting point:** 95 - 97 °C. (lit. m.p. 98 – 100 °C).[121] **Yield:** 555 mg, 63 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 96.3 %.

^{1}H-NMR (600 MHz, DMSO-d_6) δ: 12.49 (s (br), 1H, COOH), 10.79 (s, 1H, NH_{indole}), 7.99 (d, *J* = 7.9 Hz, 1H, NH_{amide}), 7.51 (d, *J* = 7.9 Hz, 1H, $H_{arom.}$), 7.31 (d, *J* = 8.1 Hz, 1H, $H_{arom.}$), 7.10 (d, *J* = 2.3 Hz, 1H, $H_{arom.}$), 7.04 (t, *J* = 7.5 Hz, 1H, $H_{arom.}$), 6.96 (t, *J* = 7.4 Hz, 1H, $H_{arom.}$), 4.46 (td, *J* = 8.4, 5.0 Hz, 1H, $C_{\alpha}H$), 3.14 (dd, *J* = 14.6, 5.0 Hz, 1H, $C_{\beta}H$), 2.97 (dd, *J* = 14.6, 8.8 Hz, 1H, $C_{\beta}H$), 2.04 (td, *J* = 7.3, 2.3 Hz, 2H, CH_2-CO_{acyl}), 1.39 (p, *J* = 7.4 Hz, 2H, $CH_{2\text{-}acyl}$), 1.29 – 1.05 (m, 24H, 12 x $CH_{2\text{-}acyl}$), 0.84 (t, *J* = 6.8 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 173.7 (C=O), 172.3 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.4 ($C_{arom.}$, $C_{quat.}$), 123.6 ($C_{arom.}$, CH), 121.0 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 118.3 ($C_{arom.}$, CH), 111.5 ($C_{arom.}$, CH), 110.2 ($C_{arom.}$, $C_{quat.}$), 52.9 ($C_{\alpha}H$), 35.3 (CH_2), 31.5 (CH_2), 29.2 (6 x CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 27.3 (CH_2), 25.3 (CH_2), 22.3 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 443 $[M+H]^+$.

N-Octadecanoyl-D-tryptophan (87)



The compound was prepared from D-tryptophan and octadecanoyl chloride according to procedure 5.3.1.

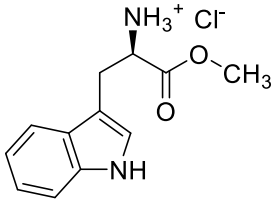
Appearance: white solid. **Melting point:** 97 - 99 °C. (lit. m.p. 96 – 98 °C).[120] **Yield:** 716 mg, 76 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 96.8 %.

^{1}H-NMR (600 MHz, DMSO-d_6) δ: 12.36 (s (br), 1H, OH), 10.79 (s, 1H, NH_{indole}), 7.99 (d, J = 7.9 Hz, 1H, NH_{amide}), 7.51 (d, J = 7.9 Hz, 1H, $H_{arom.}$), 7.31 (d, J = 8.0 Hz, 1H, $H_{arom.}$), 7.10 (d, J = 2.2 Hz, 1H, $H_{arom.}$), 7.04 (t, J = 7.5 Hz, 1H, $H_{arom.}$), 6.96 (t, J = 7.5 Hz, 1H, $H_{arom.}$), 4.46 (td, J = 8.3, 4.1 Hz, 1H, $C_\alpha H$), 3.14 (dd, J = 14.6, 5.0 Hz, 1H, $C_\beta H$), 2.97 (dd, J = 14.6, 8.9 Hz, 1H, $C_\beta H$), 2.04 (td, J = 7.3, 2.1 Hz, 2H, CH_2-CO_{acyl}), 1.39 (p, J = 7.4 Hz, 2H, $CH_{2\text{-}acyl}$), 1.28 – 1.11 (m, 28H, 14 x $CH_{2\text{-}acyl}$), 0.84 (t, J = 6.9 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 173.7 (C=O), 172.3 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.4 ($C_{arom.}$, $C_{quat.}$), 123.6 ($C_{arom.}$, CH), 121.0 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 118.3 ($C_{arom.}$, CH), 111.5 ($C_{arom.}$, CH), 110.2 ($C_{arom.}$, $C_{quat.}$), 53.0 ($C_\alpha H$), 35.3 (CH_2), 31.5 (CH_2), 29.2 (8 x CH_2), 29.1 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 27.3 (CH_2), 25.3 (CH_2), 22.3 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 471 $[M+H]^+$.

D-Tryptophan methyl ester (93)[122]



The compound was prepared as hydrochloride salt from D-tryptophan according to procedure 5.3.2.1 without neutralization and subsequent work-up.

Appearance: white solid. **Melting point:** 217 °C. **Yield:** 3.6 g, 94 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.2 %.

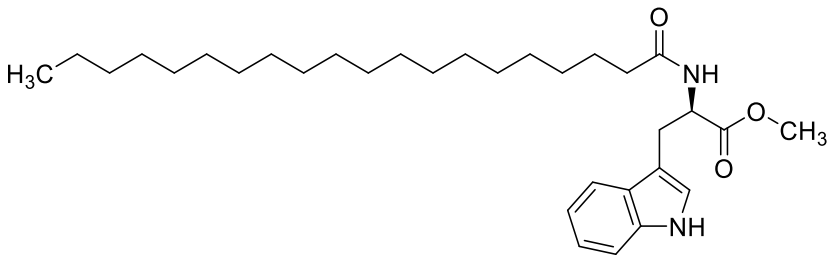
^{1}H-NMR (500 MHz, DMSO-d_6) δ: 11.09 (s, 1H, NH_{indole}), 8.60 (s, 3H, NH_3^+), 7.50 (d, J = 7.9 Hz, 1H, $H_{arom.}$), 7.36 (d, J = 8.1 Hz, 1H, $H_{arom.}$), 7.24 (d, J = 2.4 Hz, 1H, $H_{arom.}$), 7.08 (t, J = 7.3

Hz, 1H, $H_{arom.}$), 7.00 (t, *J* = 7.5 Hz, 1H, $H_{arom.}$), 4.20 (t, *J* = 6.2 Hz, 1H, $C_{\alpha}H$), 3.64 (s, 3H, OCH_3), 3.36 – 3.25 (m, 2H, $C_{\beta}H_2$).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 170.2 (C=O), 136.6 ($C_{arom.}$, $C_{quat.}$), 127.3 ($C_{arom.}$, $C_{quat.}$), 125.4 ($C_{arom.}$, CH), 121.6 ($C_{arom.}$, CH), 119.1 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 111.2 ($C_{arom.}$, CH), 106.8 ($C_{arom.}$, $C_{quat.}$), 53.1 ($C_{\alpha}H$), 53.1 (OCH_3), 26.6 (CH_2).

LC-ESI-MS (m/z): 219 $[M\text{-}Cl]^+$.

N-Eicosanoyl-D-tryptophan methyl ester (98)



The compound was prepared from **93** and eicosanoic acid according to procedure 5.3.3 with an additional portion of *N*,*N*-diisopropylethylamine (DIPEA, 1.1 eq.).

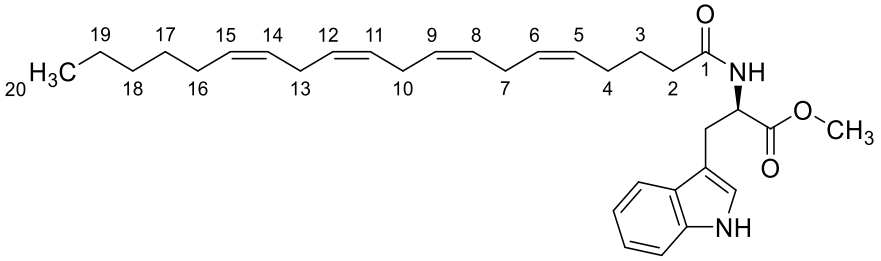
Appearance: white solid. **Melting point:** 88 - 89 °C. **Yield:** 314 mg, 61 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.9 %.

^{1}H-NMR (600 MHz, DMSO-*d*$_6$) δ: 10.82 (s, 1H, NH_{indole}), 8.16 (d, *J* = 7.6 Hz, 1H, NH_{amide}), 7.47 (d, *J* = 7.7 Hz, 1H, $H_{arom.}$), 7.31 (d, *J* = 8.1 Hz, 1H, $H_{arom.}$), 7.11 (d, *J* = 2.3 Hz, 1H, $H_{arom.}$), 7.04 (t, *J* = 7.5 Hz, 1H, $H_{arom.}$), 6.96 (t, *J* = 7.5 Hz, 1H, $H_{arom.}$), 4.49 (td, *J* = 8.3, 5.6 Hz, 1H, $C_{\alpha}H$), 3.56 (s, 3H, OCH_3), 3.12 (dd, *J* = 14.6, 5.6 Hz, 1H, $C_{\beta}H$), 3.00 (dd, *J* = 14.6, 8.6 Hz, 1H, $C_{\beta}H$), 2.05 (td, *J* = 7.3, 2.7 Hz, 2H, CH_2-CO_{acyl}), 1.39 (p, *J* = 7.6 Hz, 2H, $CH_{2\text{-}acyl}$), 1.28 – 1.11 (m, 32H, 16 x $CH_{2\text{-}acyl}$), 0.84 (t, *J* = 6.9 Hz, 3H, $CH_{3\text{-}acyl}$).

^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ: 173.0 (C=O), 172.7 (C=O), 136.5 ($C_{arom.}$, $C_{quat.}$), 127.5 ($C_{arom.}$, $C_{quat.}$), 124.0 ($C_{arom.}$, CH), 121.3 ($C_{arom.}$, CH), 118.8 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 111.8 ($C_{arom.}$, CH), 110.1 ($C_{arom.}$, $C_{quat.}$), 53.5 ($C_{\alpha}H$), 52.2 (OCH_3), 35.4 (CH_2), 31.7 (CH_2), 29.5 (10 x CH_2), 29.4 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 28.9 (CH_2), 27.5 (CH_2), 25.6 (CH_2), 22.5 (CH_2), 14.4 ($CH_{3\text{-}acyl}$).

LC-ESI-MS (m/z): 513 $[M+H]^+$.

N-Arachidonoyl-D-tryptophan methyl ester (99)



The compound was prepared from **93** and arachidonic acid according to procedure 5.3.3 with an additional portion of *N*,*N*-diisopropylethylamine (DIPEA, 1.1 eq.).

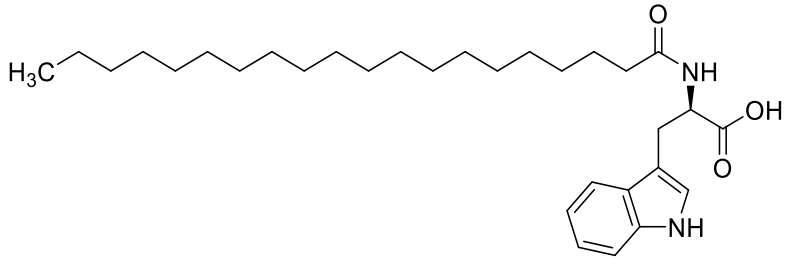
Appearance: light yellow oil. **Yield:** 370 mg, 65 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.6 %.

^{1}H-NMR (600 MHz, DMSO-d_6) δ: 10.82 (s, 1H, NH_{indole}), 8.20 (d, J = 7.5 Hz, 1H, NH_{amide}), 7.47 (d, J = 7.9 Hz, 1H, $H_{arom.}$), 7.32 (d, J = 8.1 Hz, 1H, $H_{arom.}$), 7.11 (d, J = 2.5 Hz, 1H, $H_{arom.}$), 7.05 (t, J = 7.5 Hz, 1H, $H_{arom.}$), 6.96 (t, J = 7.5 Hz, 1H, $H_{arom.}$), 5.39 – 5.26 (m, 8H, 8 x CH_{sp2} (C5, C6, C8, C9, C11, C12, C14, C15)), 4.49 (td, J = 8.1, 5.9 Hz, 1H, $C_{\alpha}H$), 3.55 (s, 3H, OCH_3), 3.12 (dd, J = 14.6, 5.8 Hz, 1H, $C_{\beta}H$), 3.01 (dd, J = 14.6, 8.5 Hz, 1H, $C_{\beta}H$), 2.82 – 2.71 (m, 6H, 3 x CH_2 (C7, C10, C13)), 2.14 – 2.03 (m, 2H, CH_2 (C2)), 2.03 – 1.93 (m, 4H, 2 x CH_2 (C4, C16)), 1.53 – 1.43 (m, 2H, CH_2 (C3)), 1.34 – 1.18 (m, 6H, 3 x CH_2 (C17, C18, C19)), 0.83 (t, J = 6.9 Hz, 3H, CH_3 (C20)).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 172.7 (C=O), 172.2 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 130.1 (CH_{sp2}), 129.6 (CH_{sp2}), 128.3 (CH_{sp2}), 128.2 (CH_{sp2}), 128.1 (CH_{sp2}), 127.9 (CH_{sp2}), 127.8 (CH_{sp2}), 127.7 (CH_{sp2}), 127.2 ($C_{arom.}$, $C_{quat.}$), 123.7 ($C_{arom.}$, CH), 121.1 ($C_{arom.}$, CH), 118.5 ($C_{arom.}$, CH), 118.1 ($C_{arom.}$, CH), 111.6 ($C_{arom.}$, CH), 109.7 ($C_{arom.}$, $C_{quat.}$), 53.2 ($C_{\alpha}H$), 51.8 (OCH_3), 34.6 (CH_2), 31.0 (CH_2), 28.9 (CH_2), 27.3 (CH_2), 26.7 (CH_2), 26.3 (CH_2), 25.4 – 25.3 (4 x CH_2), 22.1 (CH_2), 14.1 ($CH_{3\text{-acyl}}$).

LC-ESI-MS (m/z): 505 $[M+H]^+$.

N-Eicosanoyl-D-tryptophan (104)



The compound was prepared from **98** according to procedure 5.3.4.

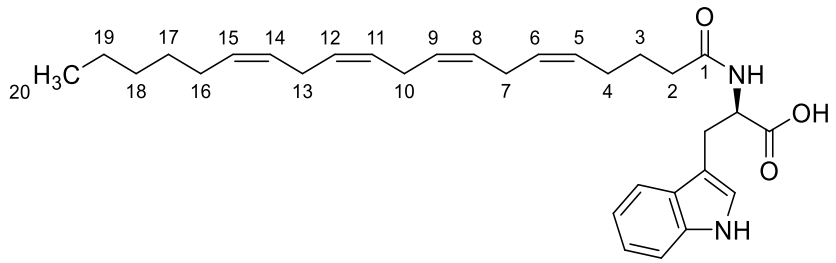
Appearance: white solid. **Melting point:** 83 - 84 °C. **Yield:** 48 mg, 48 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 98.1 %.

^{1}H-NMR (600 MHz, DMSO-d_6) δ: 12.43 (s (br), 1H, COOH), 10.79 (s, 1H, NH_{indole}), 7.99 (d, J = 7.9 Hz, 1H, NH_{amide}), 7.51 (d, J = 7.9 Hz, 1H, $H_{arom.}$), 7.31 (d, J = 8.1 Hz, 1H, $H_{arom.}$), 7.10 (d, J = 2.2 Hz, 1H, $H_{arom.}$), 7.04 (t, J = 7.5 Hz, 1H, $H_{arom.}$), 6.95 (t, J = 7.5 Hz, 1H, $H_{arom.}$), 4.45 (td, J = 8.3, 5.0 Hz, 1H, $C_{\alpha}H$), 3.14 (dd, J = 14.7, 5.1 Hz, 1H, $C_{\beta}H$), 2.98 (dd, J = 14.6, 8.9 Hz, 1H, $C_{\beta}H$), 2.04 (td, J = 7.3, 2.1 Hz, 2H, CH_2-CO_{acyl}), 1.39 (p, J = 7.3 Hz, 2H, $CH_{2\text{-acyl}}$), 1.29 – 1.08 (m, 32H, 16 x $CH_{2\text{-acyl}}$), 0.84 (t, J = 6.8 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 173.7 (C=O), 172.3 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 127.4 ($C_{arom.}$, $C_{quat.}$), 123.6 ($C_{arom.}$, CH), 120.9 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 118.3 ($C_{arom.}$, CH), 111.4 ($C_{arom.}$, CH), 110.2 ($C_{arom.}$, $C_{quat.}$), 53.0 ($C_{\alpha}H$), 35.3 (CH_2), 31.5 (CH_2), 29.2 (10 x CH_2), 29.1 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 27.3 (CH_2), 25.3 (CH_2), 22.3 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 499 $[M+H]^+$.

N-Arachidonoyl-D-tryptophan (105)



The compound was prepared from **99** according to procedure 5.3.4.

Appearance: brown oil. **Yield:** 79 mg, 81 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 97.9 %.

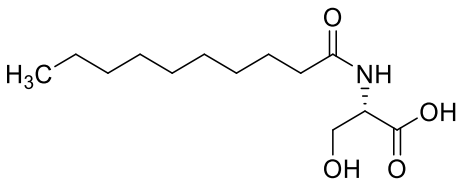
^{1}H-NMR (600 MHz, DMSO-d_6) δ: 12.50 (s (br), 1H, COOH), 10.79 (s, 1H, NH_{indole}), 8.01 (d, J = 7.8 Hz, 1H, NH_{amide}), 7.51 (d, J = 7.9 Hz, 1H, $H_{arom.}$), 7.31 (d, J = 8.1 Hz, 1H, $H_{arom.}$), 7.10 (d, J = 2.3 Hz, 1H, $H_{arom.}$), 7.04 (t, J = 7.6 Hz, 1H, $H_{arom.}$), 6.95 (t, J = 7.4 Hz, 1H, $H_{arom.}$), 5.37 – 5.26 (m, 8H, 8 x CH_{sp2} (C5, C6, C8, C9, C11, C12, C14, C15)), 4.46 (td, J = 8.3, 5.1 Hz, 1H, $C_{\alpha}H$), 3.14 (dd, J = 14.6, 5.8 Hz, 1H, $C_{\beta}H$), 2.98 (dd, J = 14.6, 8.7 Hz, 1H, $C_{\beta}H$), 2.82 – 2.70 (m, 6H, 3 x CH_2 (C7, C10, C13)), 2.12 – 2.03 (m, 2H, CH_2 (C2)), 2.03 – 1.93 (m, 4H, 2 x CH_2 (C4, C16)), 1.47 (pd, J = 7.6, 1.9 Hz, 2H, CH_2 (C3)), 1.34 – 1.18 (m, 6H, 3 x CH_2 (C17, C18, C19)), 0.83 (t, J = 6.9 Hz, 3H, CH_3 (C20)).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 173.7 (C=O), 172.1 (C=O), 136.2 ($C_{arom.}$, $C_{quat.}$), 130.1 (CH_{sp2}), 129.6 (CH_{sp2}), 128.3 (CH_{sp2}), 128.2 (CH_{sp2}), 128.1 (CH_{sp2}), 127.9 (CH_{sp2}), 127.8 (CH_{sp2}), 127.7 (CH_{sp2}), 127.4 ($C_{arom.}$, $C_{quat.}$), 123.6 ($C_{arom.}$, CH), 121.0 ($C_{arom.}$, CH), 118.4 ($C_{arom.}$, CH), 118.3 ($C_{arom.}$, CH), 111.4 ($C_{arom.}$, CH), 110.2 ($C_{arom.}$, $C_{quat.}$), 53.1 ($C_{\alpha}H$), 34.8 (CH_2), 31.0 (CH_2), 28.9 (CH_2), 27.3 (CH_2), 26.8 (CH_2), 26.4 (CH_2), 25.4 – 25.3 (4 x CH_2), 22.1 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 491 $[M+H]^+$.

5.3.5.4 L-Serine derivatives

N-Decanoyl-L-serine (88)[123]



The compound was prepared from L-serine and decanoyl chloride according to procedure 5.3.1.

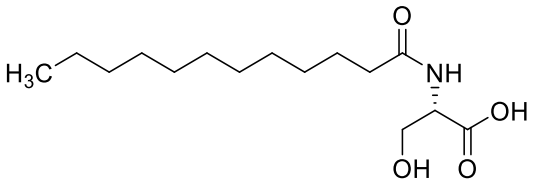
Appearance: white solid. **Melting point:** 79 - 80 °C. **Yield:** 139 mg, 27 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 100.0 %.

^{1}H-NMR (600 MHz, DMSO-d_6) δ: 12.37 (s (br), 1H, COOH), 7.86 (d, J = 7.9 Hz, 1H, NH), 4.25 (dt, J = 7.7, 4.7 Hz, 1H, $C_{\alpha}H$), 3.65 (dd, J = 10.9, 5.4 Hz, 1H, $C_{\beta}H$), 3.59 (dd, J = 10.9, 4.4 Hz, 1H, $C_{\beta}H$), 2.12 (t, J = 7.5 Hz, 2H, CH_2-CO_{acyl}), 1.49 – 1.30 (m, 2H, $CH_{2\text{-}acyl}$), 1.28 – 1.18 (m, 12H, 6 x $CH_{2\text{-}acyl}$), 0.85 (t, J = 6.8 Hz, 3H, CH_3), (CH_2-O<u>H</u> signal not visible).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 172.5 (C=O), 172.3 (C=O), 61.6 (CH_2), 54.7 ($C_{\alpha}H$), 35.2 (CH_2), 31.5 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.8 (2 x CH_2), 25.4 (CH_2), 22.3 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 260 $[M+H]^+$.

N-Dodecanoyl-L-serine (89)[123]



The compound was prepared from L-serine and dodecanoyl chloride according to procedure 5.3.1.

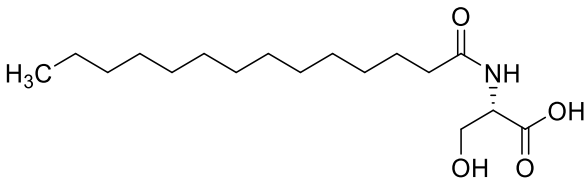
Appearance: white solid. **Melting point:** 83 - 85 °C. (lit. m.p. 103 °C).[124] **Yield:** 210 mg, 37 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 100.0 %. $[\alpha]^{20}_{D}$ = +6.9° (c = 0.1 g/mL, in methanol)

^{1}H-NMR (600 MHz, DMSO-*d_6*) δ: 12.45 (s (br), 1H, COOH), 7.86 (d, *J* = 7.9 Hz, 1H, NH), 4.89 (s (br), 1H, CH_2-O<u>H</u>), 4.25 (dt, *J* = 8.0, 4.9 Hz, 1H, C_αH), 3.65 (dd, *J* = 10.9, 5.4 Hz, 1H, C_βH), 3.59 (dd, *J* = 10.9, 4.4 Hz, 1H, C_βH), 2.11 (t, *J* = 7.5 Hz, 2H, CH_2-CO_{acyl}), 1.46 (p, *J* = 7.0 Hz, 2H, $CH_{2\text{-acyl}}$), 1.28 – 1.19 (m, 16H, 8 x $CH_{2\text{-acyl}}$), 0.84 (t, *J* = 6.9 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d_6*) δ: 172.5 (C=O), 172.3 (C=O), 61.6 (CH_2), 54.7 (C_αH), 35.2 (CH_2), 31.5 (CH_2), 29.2 (2 x CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 25.4 (CH_2), 22.2 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 288 $[M+H]^+$.

N-Tetradecanoyl-L-serine (90)[123]



The compound was prepared from L-serine and tetradecanoyl chloride according to procedure 5.3.1.

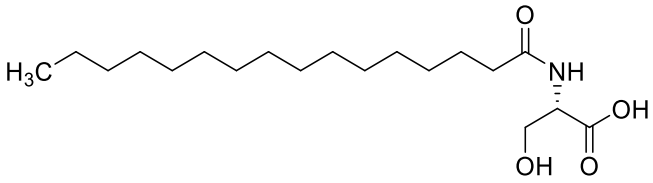
Appearance: white solid. **Melting point:** 73 - 76 °C. **Yield:** 333 mg, 53 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 100.0 %.

^{1}H-NMR (600 MHz, DMSO-*d_6*) δ: 12.19 (s (br), 1H, COOH), 7.86 (d, *J* = 7.9 Hz, 1H, NH), 4.89 (s (br), 1H, CH_2-O<u>H</u>), 4.25 (ddd, *J* = 7.9, 5.4, 4.3 Hz, 1H, C_αH), 3.65 (dd, *J* = 10.9, 5.4 Hz, 1H, C_βH), 3.59 (dd, *J* = 10.9, 4.4 Hz, 1H, C_βH), 2.12 (t, *J* = 7.4 Hz, 2H, CH_2-CO_{acyl}), 1.50 – 1.42 (m, 2H, $CH_{2\text{-acyl}}$), 1.29 – 1.19 (m, 20H, 10 x $CH_{2\text{-acyl}}$), 0.84 (t, *J* = 6.9 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-*d_6*) δ: 172.4 (C=O), 172.3 (C=O), 61.6 (CH_2), 54.7 (C_αH), 35.2 (CH_2), 33.8 (CH_2), 31.4 (CH_2), 29.3 – 28.6 (6 x CH_2), 25.4 (CH_2), 24.6 (CH_2), 22.2 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 316 $[M+H]^+$.

N-Hexadecanoyl-L-serine (56)[123]



The compound was prepared from L-serine and hexadecanoyl chloride according to procedure 5.3.1.

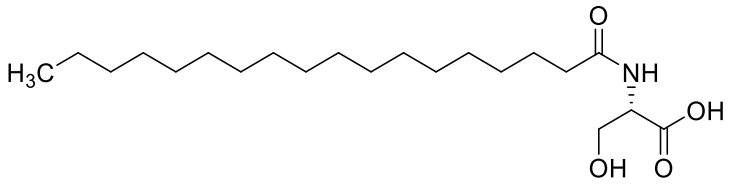
Appearance: white solid. **Melting point:** 90 - 93 °C. (lit. m.p. 96 – 99 °C).[125] **Yield:** 158 mg, 23 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 100.0 %.

^{1}H-NMR (600 MHz, DMSO-d_6) δ: 12.3 (s (br), 1H, COOH), 7.86 (d, *J* = 7.9 Hz, 1H, NH), 4.89 (s (br), 1H, CH_2-O<u>H</u>), 4.25 (dt, *J* = 7.9, 4.9 Hz, 1H, $C_\alpha H$), 3.65 (dd, *J* = 10.9, 5.4 Hz, 1H, $C_\beta H$), 3.59 (dd, *J* = 10.9, 4.4 Hz, 1H, $C_\beta H$), 2.12 (t, *J* = 7.5 Hz, 2H, CH_2-CO_{acyl}), 1.49 – 1.43 (m, 2H, $CH_{2\text{-acyl}}$), 1.27 – 1.19 (m, 24H, 12 x $CH_{2\text{-acyl}}$), 0.84 (t, *J* = 6.9 Hz, 3H, CH_3).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 172.5 (C=O), 172.3 (C=O), 61.6 (CH_2), 54.7 ($C_\alpha H$), 35.2 (CH_2), 31.4 (CH_2), 29.2 – 28.7 (10 x CH_2), 25.4 (CH_2), 22.3 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 344 $[M+H]^+$.

N-Octadecanoyl-L-serine (57)[126]



The compound was prepared from L-serine and octadecanoyl chloride according to procedure 5.3.1.

Appearance: white solid. **Melting point:** 77 - 79 °C. (lit. m.p. 103 °C).[127] **Yield:** 394 mg, 53 %. **Purity by HPLC-UV (254 nm)-ESI-MS:** 100.0 %.

^{1}H-NMR (600 MHz, DMSO-d_6) δ: 11.9 (s (br), 1H, COOH), 7.85 (d, *J* = 7.9 Hz, 1H, NH), 4.24 (dt, *J* = 7.8, 4.9 Hz, 1H, $C_\alpha H$), 3.65 (dd, *J* = 10.9, 5.4 Hz, 1H, $C_\beta H$), 3.58 (dd, *J* = 10.9, 4.4 Hz, 1H, $C_\beta H$), 2.17 (t, *J* = 7.4 Hz, 2H, CH_2-CO_{acyl}), 1.50 – 1.43 (m, 2H, $CH_{2\text{-acyl}}$), 1.28 – 1.18 (m, 28H, 14 x $CH_{2\text{-acyl}}$), 0.84 (t, *J* = 6.9 Hz, 3H, CH_3), (CH_2-O<u>H</u> signal not visible).

^{13}C-NMR (125 MHz, DMSO-d_6) δ: 172.4 (C=O), 172.3 (C=O), 61.6 (CH_2), 54.7 ($C_\alpha H$), 35.2 (CH_2), 33.8 (CH_2), 31.4 (CH_2), 29.2 – 28.7 (10 x CH_2), 25.4 (CH_2), 24.6 (CH_2), 22.2 (CH_2), 14.1 (CH_3).

LC-ESI-MS (m/z): 372 $[M+H]^+$.

5.4 Enzymatic hydrolysis of bovine liver phosphatidylinositol

Bovine liver PI (5 mg, average MW = 902, ~ 5.54 µmol, purchased from Avanti Lipid) was suspended in 2 mL of 50 mM acetate buffer (pH 5.7) containing 10 mM $CaCl_2$ and 100 mM NaCl. The solution was then treated with DMSO (200 µL) and Phospholipase A_1 from *Thermomyces lanuginosus* (2.31 µL, 10 kilo lipase unit per gram (KLU/g), density = 1.2 g/mL, 5 eq., purchased from Sigma) then stirred at r. t. for 1 h. The enzymatic reaction was terminated by adding 0.4 mL of 200 mM EDTA and 15 mL of chloroform:methanol (1:2, v/v). After the addition of chloroform (5 mL) and water (5.6 mL), the methanol-water layer was taken and subjected to a chloroform-conditioned solid phase extraction (SPE) tube (normal phase, aminopropyl-modified silica, 6 mL tube volume, 500 mg bed weight, purchased from Macherey-Nagel, Düren). After the application of the sample (fraction 1), the tube was washed with a mixture of diethyl ether:acetic acid (98:2) to remove the free fatty acids (fraction 2) and subsequently eluted with a mixture of methanol:NH_4OH (9:1) (fraction 3). All fractions were dried under a gentle stream of argon at r. t.

5.5 ß-Arrestin assays on GPR18

Recruitment of β-arrestin to GPR18 was investigated using a β-galactosidase enzyme fragment complementation assay (β-arrestin PathHunter™ assay, DiscoverX, Fremont, CA, U.S.). Chinese hamster ovary (CHO) cells stably expressing GPR18 were seeded in a volume of 90 µL into a white-bottomed 96-well plate and incubated at a density of 40.000 cells/well in assay medium (Opti-MEM, 2 % fetal calf serum (FCS), 100 U/mL penicillin, 100 µg/mL streptomycin, 800 µg/mL geneticin and 300 µg/mL hygromycin (P/S/G/H medium)) for 24 h at 37 °C, 5 % CO_2. The medium was then changed to 0 % FCS assay medium (Opti-MEM, 100 U/mL penicillin, 100 µg/mL streptomycin, 800 µg/mL geneticin and 300 µg/mL hygromycin (P/S/G/H medium)) prior to the assay. The test compounds and the agonist were separately diluted in phosphate-buffered saline (PBS buffer) containing 0.1 % bovine serum albumin (BSA) and 10 % DMSO.

For antagonistic activity, about 5 µL of antagonist solution were added to the cells followed by an incubation for 1 h at 37 °C, 5 % CO_2. After this first incubation, agonist was added in a volume of 5 µL followed by a second incubation for 1.5 hour at 37 °C, 5 % CO_2. The detection reagent was prepared by mixing the chemiluminescent substrate Galacton-Star®, Emerald-II™ and a lysis buffer A (150 mM NaCl, 5 mM K_2HPO_4 5 mM KH_2PO_4, 25 mM $Mg(CH_3COO)_2$, 2.5 % CHAPS) in a ratio of 1:5:19. The detection reagent was added in a volume of 50 µL per well and the measurement plate was incubated for 1 h at r.t. Luminescence was then measured in a Luminometer (TopCount NXT, Packard / Perkin-Elmer). The data were analyzed using GraphPad PRISM Version 6.01 (San Diego, CA, USA).

For the determination of potential agonistic properties of the test compounds, the assay was performed as described above for antagonist testing except that PBS buffer containing 0.1 % BSA and 10 % DMSO was added in place of potential antagonists and after a 60 min incubation the test compounds were added.

5.6 ß-Arrestin assays on GPR55

CHO cells stably expressing GPR55 were used (F-12 10 % FCS, 100 U/mL penicillin, 100 µg/mL streptomycin, 800 µg/mL geneticin und 300 µg/mL hygromycin (P/S/G/H medium). For receptor stimulation, the GPR55 agonist lysophosphatidylinositol (LPI) was employed (1 µM) and the test compounds were screened were screened at 10 µM for antagonistic activity.

The step procedures of the assay were similar to the recruitment of β-arrestin molecules on GPR18, except the detection reagent was varied and obtained by mixing the chemiluminescent substrate Galacton-Star (2 mM) with the luminescence enhancer Emerald-II and lysis buffer B (10 mM TRIS, 1 mM EDTA, 100 mM NaCl, 5 mM $MgCl_2$, 1% Triton-X; pH 8) in a ratio of 1:5:19.

5.7 Radioligand binding assays on cannabinoid receptors

Competition binding assays were performed versus the cannabinoid receptor agonist radioligand [^{3}H](-)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol ([^{3}H]CP55,940) in concentration of 0.1 nM. As a source for human CB1 and CB2 receptors, membrane preparations of Chinese hamster ovary (CHO) cells stably expressing the respective receptor subtype were used. The test compounds were diluted in DMSO (final concentration of 10 µM). In case inhibition of radioligand binding was 50 % or more, full concentration-inhibition curves were determined to calculate K_i values.

The binding assays were performed at r. t. in 96-well plates. Each well contained 15 µL of test compound, 465 µL of assay buffer (50 mM Tris-HCl, 3 mM $MgCl_2$, 0.1 % BSA, pH 7.4), 60 µL of radioligand solution and 60 µL of CB_1 membrane suspension (30 µg of protein per well) or CB_2 membrane suspension (16 µg protein per well). Total binding was determined in the presence of 2.5 % DMSO and nonspecific binding in the presence of 10 µM of unlabeled CP55,940. Three separate experiments were conducted, each in duplicates. The mixtures were incubated for 120 min at r. t. to reach equilibrium conditions. Incubation was terminated by rapid filtration using a 96-channel harvester through GF/C glass fiber filters that were previously presoaked in 0.3 % aqueous polyethyleneimine solution for 30 min. Filters were washed three times with ice-cold washing buffer (50 mM Tris-HCl 0.1 % BSA, pH 7.4). The glass fiber filters were then dried for 90 min at 50 °C. Radioactivity of the plates were measured in a Topcount Microplate Scintillation and Luminescence Counter after 10 h of pre-incubation with 50 µL of scintillation cocktail.

6 Abbreviations

2-AG	2-arachidonoylglycerol
2-AGPI	2-arachidonoyl-*sn*-glycero-3-phosphoinositol
A_{2A}AR	A_{2A} adenosine receptor
abn-CBD	abnormal cannabidiol
ADP	adenosine diphosphate
AIDS	acquired immunodeficiency syndrome
anandamide	*N*-arachidonoylethanolamine
ATP	adenosine triphosphate
b.p.	boiling point
br	broad
BSA	bovine serum albumin
cAMP	cyclic adenosine monophosphate
CHO	chinese hamster ovary
conc.	concentration
d	doublet
DAG	diacylglycerol
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DCM	dichloromethane
DIPEA	*N*,*N*-diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
EC50	Half maximal effective concentration
EDTA	ethylenediaminetetraacetic acid
eq.	equivalents
ESI	electrospray ionization
FCS	fetal calf serum
GABA	gamma-aminobutyric acid
GDP	guanosine diphosphate
GPCR	G protein-coupled receptor

GRK	GPCR kinase
GRK	G protein-coupled receptor kinase
GTP	guanosine triphosphate
h	human
HEK293	human embryonic kidney cell line
HOAt	1-hydroxy-7-azabenzotriazole
HPLC	high-performance liquid chromatography
IC_{50}	half maximal inhibitory concentration
IP_3	inositol-1,4,5-trisphosphate
K_D	equilibrium dissociation constant
K_i	equilibrium dissociation constant for an inhibitor
KLU	kilo lipase unit
KOtBu	potassium *tert*-butoxide
LC	liquid chromatography
LPI	lysophosphatidylinositol
L-α-GPC	L-α-glycerylphosphorylcholine
m	multiplet
m.p.	melting point
MAPK	mitogen-activated protein kinase
MeOH	methanol
MHz	megahertz
MS	mass spectrometry
NAGly	N-arachidonoylglycine
NMR	nuclear magnetic resonance spectroscopy
PBS	phosphate buffered saline
PC	phosphatidylcholine
PI	phosphatidylinositol
PIP_2	phosphatidylinositol-4,5-bisphosphate
PLA_1	phospholipase A_1
PLC	phospholipase C
PPAR	peroxisome proliferator-activated receptor

ppm	parts per million
q	quartet
quat.	quarternary
r.t.	room temperature
RGS	regulator of G protein signaling
RP	reversed phase
RvD2	Resolvin D2
s	singlet
SEM	standard error of the mean
S_N2	nucleophilic aromatic substitution
SPE	solid-phase extraction
t	triplet
TEA	triethylamine
THC	Δ^9-tetrahydrocannabinol
THF	tetrahydrofuran
TLC	thin layer chromatography
TM	transmembrane
TRPV	transient receptor potential cation channel vanilloid
UDP	uridine diphosphate
UTP	uridine triphosphate
UV	ultraviolet

7 References

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