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Novel Divalent and Irreversibly-Binding Ligands for the CB1 Cannabinoid GPCR

A thesis submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

By

Nils Kahlcke

School of Chemical Sciences
University of Auckland
May 2017
Abstract

The cannabinoid 1 (CB1) G-protein coupled receptor is potentially an important therapeutic target however clinically approved drugs have displayed undesirable side-effects. There is a need for pharmacological tools to more fully characterise receptor binding and downstream signalling processes. During this study, the synthetic preparation and pharmacological evaluation of multifunctional molecular probes based on the known high-affinity CB1 receptor ligand Rimonabant (14) were investigated.

Synthetic methods to install a linker attachment handle at the C3 position of the pyrazole core have been identified and used to develop CB1 ligands that retain high affinity upon linker attachment. A large library of test compounds possessing C3 linkers of varying lengths and chemical composition were prepared. Disappointingly, it appeared that the addition of linkers longer than six carbons appear to significantly attenuate ligand affinity for the CB1 receptor, either by reduced accessed to the orthosteric GPCR binding site or more general problems including non-specific cell membrane binding or very low solubility.

In contrast, a route was also developed for synthesising heterodivalent probes of varying length containing of the CB1 receptor ligand Rimonabant (14) and the known dopamine 2 (D2) receptor agonist PPHT-NH2 (NK242) to investigate literature reports of altered GPCR signalling upon co-activation of these receptors. Using the same library of linkers it was found that although CB1 receptor affinity again proved low, these divalent ligands retained extremely high affinity for the D2 receptor, in some cases even higher than the parent ligand.

Finally, based on results of molecular modelling studies which have been achieved in collaboration with other research groups, a library of several CB1R ligands has been developed and synthesised which are potentially able to irreversibly bind to the human CB1 receptor, as a foundation for ongoing work to identify useful pharmacological probes for the human CB1 receptor.
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<table>
<thead>
<tr>
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<th>Description</th>
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<tbody>
<tr>
<td>δ</td>
<td>delta, chemical shift</td>
</tr>
<tr>
<td>Alloc</td>
<td>allyloxycarbonyl</td>
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<tr>
<td>Asp</td>
<td>aspartic acid</td>
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<tr>
<td>aq.</td>
<td>aqueous</td>
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<td>Ac</td>
<td>acetate</td>
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<td>Ar</td>
<td>aryl</td>
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<tr>
<td>Boc</td>
<td>t-butyloxycarbonyl</td>
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<tr>
<td>bp</td>
<td>boiling point</td>
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<tr>
<td>Bu</td>
<td>butyl</td>
</tr>
<tr>
<td>tBu</td>
<td>tertiary butyl</td>
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<tr>
<td>cat.</td>
<td>catalytic</td>
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<tr>
<td>Cbz</td>
<td>Carboxybenzyl</td>
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<tr>
<td>cm⁻¹</td>
<td>wave number</td>
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<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>DCM</td>
<td>methylene chloride</td>
</tr>
<tr>
<td>DMAP</td>
<td>N,N-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DME</td>
<td>dimethoxyethane</td>
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<tr>
<td>DCM</td>
<td>dichloromethane</td>
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<tr>
<td>DBU</td>
<td>1,8-diazabicyclo[5.4.0]undec-7-ene</td>
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<tr>
<td>DCC</td>
<td>N,N'-dicyclohexylcarbodiimide</td>
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<tr>
<td>DIC</td>
<td>N,N'-diisopropylcarbodiimide</td>
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<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
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<td>DMSO</td>
<td>dimethylsulfoxide</td>
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<td>DIPEA</td>
<td>diisopropylethylamine</td>
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<td>Name</td>
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<tr>
<td>ECS</td>
<td>endocannabinoid system</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray ionization</td>
</tr>
<tr>
<td>EDCI</td>
<td>1-ethyl-3-(3-dimethylaminopropyl)carbodiimide</td>
</tr>
<tr>
<td>EtO</td>
<td>diethylether</td>
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<tr>
<td>eq.</td>
<td>equivalent</td>
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<td>ethyl</td>
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<tr>
<td>EtOAc</td>
<td>ethyl acetate</td>
</tr>
<tr>
<td>EtOH</td>
<td>ethanol</td>
</tr>
<tr>
<td>et al.</td>
<td>et alii (and others)</td>
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<tr>
<td>Fmoc</td>
<td>9-fluorenly methoxycarbonyl</td>
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<tr>
<td>GPCR</td>
<td>G protein-coupled receptor</td>
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<tr>
<td>g</td>
<td>gram(s)</td>
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<tr>
<td>g/mol</td>
<td>gram(s) per mole</td>
</tr>
<tr>
<td>h</td>
<td>hour(s)</td>
</tr>
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<td>HATU</td>
<td>O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate</td>
</tr>
<tr>
<td>HBTU</td>
<td>O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate</td>
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<tr>
<td>HCl</td>
<td>hydrochloric acid</td>
</tr>
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<td>HCTU</td>
<td>O-(6-chlorobenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate</td>
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<tr>
<td>HRMS</td>
<td>high resolution mass spectrometry</td>
</tr>
<tr>
<td>HSQC</td>
<td>heteronuclear single-quantum correlation spectroscopy</td>
</tr>
<tr>
<td>HOAT</td>
<td>1-hydroxy-7-azabenzotriazole</td>
</tr>
<tr>
<td>HOBT</td>
<td>1-hydroxy-1H-benzotriazole</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
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<tr>
<td>HRMS</td>
<td>high resolution mass spectrometry</td>
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<tr>
<td>Hz</td>
<td>hertz</td>
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<tr>
<td>IR</td>
<td>infrared</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>IBX</td>
<td>2-iodoxybenzoic acid</td>
</tr>
<tr>
<td>J</td>
<td>coupling constant</td>
</tr>
<tr>
<td>$K_i$</td>
<td>inhibitory constant</td>
</tr>
<tr>
<td>L</td>
<td>litre</td>
</tr>
<tr>
<td>LC-MS</td>
<td>liquid chromatography-mass spectrometry</td>
</tr>
<tr>
<td>LHMDS</td>
<td>lithium hexamethyldisilazane</td>
</tr>
<tr>
<td>Lys</td>
<td>lysine</td>
</tr>
<tr>
<td>M</td>
<td>molar</td>
</tr>
<tr>
<td>m/z</td>
<td>mass to charge ratio</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>mg</td>
<td>milligram(s)</td>
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<td>mole(s)</td>
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<tr>
<td>mp</td>
<td>melting point</td>
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<td>MS</td>
<td>mass spectroscopy</td>
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<td>acetonitrile</td>
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<tr>
<td>MeOH</td>
<td>methanol</td>
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<tr>
<td>MgSO$_4$</td>
<td>magnesium sulphate</td>
</tr>
<tr>
<td>Na$_2$CO$_3$</td>
<td>sodium carbonate</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
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<tr>
<td>NEt$_3$</td>
<td>triethylamine</td>
</tr>
<tr>
<td>PEG</td>
<td>polyethylene glycol</td>
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<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>Phe</td>
<td>phenylalanine</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<td>--------------</td>
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<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>R</td>
<td>unspecified group</td>
</tr>
<tr>
<td>RT</td>
<td>room temperature</td>
</tr>
<tr>
<td>SAR</td>
<td>structure-activity relationship</td>
</tr>
<tr>
<td>SPPS</td>
<td>solid-phase peptide synthesis</td>
</tr>
<tr>
<td>t</td>
<td>tert (tertiary)</td>
</tr>
<tr>
<td>THC</td>
<td>(-) trans-Δ9-tetrahydrocannabinol</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>Trp</td>
<td>tryptophan</td>
</tr>
<tr>
<td>Tyr</td>
<td>tyrosine</td>
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<tr>
<td>TIS</td>
<td>triisopropylsilane</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>TM</td>
<td>transmembrane</td>
</tr>
<tr>
<td>ULTRA</td>
<td>DCM : MeOH 3:1 (v/v) with 5% NH₃ (aq., 25%)</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>Val</td>
<td>Valine</td>
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Chapter 1 Introduction

1.1 G protein-coupled receptors

G protein-coupled receptors (GPCRs) comprise the largest protein superfamily of the human genome, with more than 800 identified members, and are generally found embedded in the cellular phospholipid membrane. Over 30% of currently used drugs are targeted at GPCRs, however, these drugs target only 50–60 GPCRs, leaving the majority of human GPCRs unexplored for drug discovery. Despite the very central role that the study of these receptors plays in pharmacological research today, it is only in the last thirty years that there has been any general acceptance they even exist. The award of the Nobel Prize in 2012 in chemistry to Kobilka and Lefkowitz for their outstanding work on GPCRs illustrates the importance of a better structure understanding on GPCR cell signalling nowadays.

GPCRs share a common structural signature of seven transmembrane (7TM) α-helices with an extracellular N-terminus and an intracellular C-terminus and are found in almost all mammals, fungi and yeast. The 7TM helices are connected via three intra- and three extra-cellular loops.

Figure 1: General model of a GPCR showing three extra- and intracellular loops. (Picture adapted from A/Prof Nader Moniri, Mercer University, USA)
GPCRs are classically clustered into five major classes (classes A – E) and numerous subfamilies based on their amino acid sequences. The members of these different subclasses have low sequence identity (SI) within the 7TM segments. For example 460 GPCRs are predicted to be olfactory receptors with a SI of ≤ 25%. Class A (Rhodopsin-like receptors) is the largest class, with more than 650 members.

![Diagram of GPCR Superfamily](image)

**Figure 2:** Classification of GPCRs.

### 1.1.1 Activation of G proteins

The main function of GPCRs is signal transduction after ligand binding. This generally occurs upon activation of a guanine nucleotide binding protein (G protein). These heterotrimeric G proteins consist of three subunits (α, β and γ) and are activated in a multi-step process, consisting of the ligand binding to the receptor, conformation change and binding of a G protein to a GPCR (Scheme 1):
Introduction

Initially, the receptor interacts intracellularly with a non-activated heterotrimeric G protein (1), after which a ligand binds extracellularly to the same receptor (2) which changes its conformation (3). The G protein is activated via phosphorylation of inactive guanosine diphosphate (GDP) to active guanosine triphosphate (GTP) at the α-unit (4), that subsequently splits off and activates other downstream proteins (signal transduction) (5). Finally the G-α-subunit is deactivated through GTPase-activating proteins (GAPs) in a complex process and recombines with the G-β,γ-subunit (6).

Numerous different G-subunits exist, with different cell types having different distributions. The effects of the GPCRs are related to which G proteins it couples and also which G proteins are available inside the cell. The CB1 receptors and D2 receptors are known to bind to Gᵢ and Gₛ-proteins. The general effect of activated Gᵢ-proteins is inducing a “chemical brake” to the cell via inhibition of adenylate cyclase. This enzyme converts adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) which is essential for the direct or indirect activation of several cellular proteins. Depending on the role and location of the cell, these effects may be inhibitory or excitatory. For example, adenosine A₂A-receptors (A₂AR), histamine H₂-receptors (H₂R) or β-adrenergic-receptors (βARs) bind to the Gₛ-protein subunit. Contrary to the Gᵢ-protein, the Gₛ-protein subunit activates adenylate cyclase and
Introduction

augments the concentration of cAMP thus initiating cell processes such as phosphorylation of proteins involved in cell metabolism.

GPCRs also recruit other proteins for signaling, most prominently β-arrestin-1 and -2. Arrestins serve multiple functions as regulators of G protein coupling and receptor localisation, and are essential elements of multiple GPCR signaling cascades involving kinases, phosphatases, and ubiquitin ligases. Arrestin signaling has been generally thought to occur in the absence of bound G protein, and although this has been recently challenged, arrestin-mediated signaling is likely to be mediated by a different ligand/receptor conformation from those involved in Gα interactions.

1.2 Distribution and structure of the CB1 receptor

The CB1 receptor is a typical GPCR with a 7 transmembrane structure. It is most densely expressed within the CNS and is comprised of 472 amino acids, while the CB2 receptor consists of 360 amino acids. Both receptors are activated within the endocannabinoid system upon ligand binding, naturally within the body by endocannabinoids or introduced into the body by consuming phytoprogenic or synthetic cannabinoids. The CB1 receptor is found predominantly in the brain and spinal cord, as well as some peripheral tissues e.g. gastrointestinal systems. Its central distribution is not homogenous and is most densely located in the basal ganglia, hippocampus and the cerebral cortex. The CB1 receptor and the CB2 receptor have a different distribution. The CB1R is found predominantly in the central nervous system (CNS) e.g. brain while the CB2R is mainly distributed in the immune system e.g. spleen and lymph glands, as well in some parts of the nervous system.
The classical role of a GPCR is to detect the presence of an extracellular ligand and to transmit the information through the plasma membrane. In case of agonist binding this results in the activation of a heterotrimeric G protein and so leads to the modulation of downstream effector proteins. The effect of a ligand on the structure and biophysical properties of a receptor and the ultimate biological response is called ligand efficacy. Naturally occurring or synthetic ligands can be clustered into four different efficacy classes:

1. *Full agonists* are enabled to generate the maximum receptor stimulation
2. *Partial agonists* are unable to attain full receptor activity even at saturating concentrations
3. *Neutral antagonists* do not have an effect on the signalling activity but they can prevent other ligands from binding to the receptor
4. *Inverse agonists* reduce the level of basal activity below that of the unliganded receptor
Introduction

Figure 4: Model to illustrate the concept of ligand efficacy.\textsuperscript{13}

Agonists and antagonists are known to bind in different ways to receptors. For example the binding of a full agonist to the CB1 receptor results in a conformational change of the receptor and stabilises its active state, leading to signal transduction. Some receptors are able to change their conformation from an inactive state to the active state even without ligand binding. The CB1 receptor is known to have ‘constitutive activity’ and thus it can spontaneously adopt an active conformational state in the absence of agonist binding.\textsuperscript{14} The two state-model of receptor activation can explain the influence of different ligands to the receptor.\textsuperscript{15}

Figure 5: Two state-model for illustrating the effects of ligand binding to receptors.\textsuperscript{14}

The inactive receptor state [R] and the activated receptor state [R*] are in equilibrium. Upon ligand binding one of the states is going to be stabilised. A full agonist will stabilise the active state [R*] while
an inverse agonist stabilises the inactive state \([R]\). A neutral antagonist will neither stabilise the active nor the inactive receptor state and both states will still coexist.\(^{14}\)

Superagonists are a type of agonists that are capable of producing a maximal response greater than the endogenous agonist for the target receptor, and thus has an efficacy of more than 100%.\(^{16}\) For example, goserelin is a superagonist of the gonadotropin-releasing hormone receptor.

allosteric ligands are substances which indirectly influence the effects of a primary ligand that directly activates or deactivates the function of a target protein.\(^{17}\) allosteric ligands bind to a site distinct from that of the orthosteric binding site. They stabilise a conformation of the protein structure that affects either the binding or the efficacy of the primary ligand.

### 1.3 Cannabinoids and the cannabinoid receptors

Cannabinoids are a diverse class of compounds having the affinity to bind to cannabinoid receptors. All natural occurring cannabinoids are constituents of the hemp plant *Cannabis sativa* that has been used as a recreational drug due to its psychoactive effects and also for medical purposes for over a thousand years.\(^{18}\) They have been shown to produce effects on the behaviour of humans and animals, such as: enhanced body awareness, un-coordination, sleepiness, cognitive impairments, mood alterations and many more.\(^{19}\) Consumers show dependence upon chronic administration and also withdrawal symptoms upon cessation, inducing nervousness, restlessness, anxiety and sleep disturbance.\(^{20}\) However, a clear-cut abstinence syndrome has been reported only rarely, supposedly because of the long biological half-life period in bodies.

Cook *et al.* reported withdrawal symptoms observed in CB1R agonist tolerant mice after injecting a CB1R antagonist\(^{21}\) and chronic exposure to cannabis is suspected to cause long-term impairment.\(^{22}\) Nevertheless cannabinoids have therapeutically useful effects, such as analgesia, appetite-stimulating, antiemetic, antispasmodic and sleep-inducing effects.\(^{18}\)

Before the discovery of cannabinoid receptors, all research into the activity of natural and synthetic cannabinoids involved use of *in vivo* animal models with all their associated ethical disadvantages. The identification of the cannabinoid CB1 receptor in 1988\(^{23}\) and the CB2 receptor in 1993\(^{24}\) were major breakthroughs in cannabinoid research. The sequence identity between both receptors within the transmembrane \(\alpha\)-helix is 68% and 44% throughout the whole protein\(^{24}\):
**Introduction**

![Figure 6: Model of the CB1 and CB2 receptor, demonstrating their different sequence identity.](image)

CB1 rat, mice and human receptors have been cloned and show an overall SI of 97-99% while cloned CB2 receptors only have an overall SI of 82%.\(^1\)

**Update:**

All of the references incorporated in the introduction have been published until June 2015. In 2016, 2 articles have been published disclosing the crystal structures for the CB1 receptor.\(^2\)

1.3.1 Cannabinoid receptor agonists

Unless stated otherwise, all reported all data and values refer to the human cannabinoid receptors.

There are over 85 terpenoid compounds known as natural cannabinoids and they are exclusively found in cannabis.\(^2\) The evolutionary reason for producing such an array is not clear but there are suggestions that these compounds act as antimicrobials and antioxidants. Apart from the cannabinoids, hemp is known to produce other natural products thought to be of biological and medicinal interest including flavonoids, carotenoids and terpenes. The identification of CB1 and CB2 receptors has come mainly from the development of potent agonists, which can be classified into four different groups according to their chemical structures.\(^3\)

**Group I cannabinoids,** also known as classical cannabinoids, are dibenzopyran congeners that are natural constituents of the hemp plant. Naturally occurring cannabinoids derived from hemp consist of 21 carbons and are all very lipophilic. To this group belongs the primary psychoactive constituent of cannabis, \((-\)\-)\textit{Δ}^\textit{9}-\textit{trans}-tetrahydrocannabinol (THC) \(^1\). THC is a CB1R agonist that mimics the effects of endogenous cannabinoids. It was isolated in 1964\(^2\) and its absolute stereochemistry was determined in by Mechoulam \textit{et al.} in 1967.\(^2\)}
Introduction

Other group I cannabinoids found in hemp are metabolites of THC, such as \((-\Delta^8\)-trans-Tetrahydrocannabinol\) 2 or cannabinol 3. HU-210⁹⁴⁴ 4, is a synthetic group I cannabinoid designed and synthesised by Mechoulam and co-workers that shows an 800 fold times higher efficacy than THC³⁰:

![Chemical structures of THC and its metabolites](image1)

**Figure 7:** Group I cannabinoids comprise a dibenzopyran moiety.

Group II cannabinoids, also known as nonclassical cannabinoids, were developed as bicyclic and tricyclic analogues of THC lacking the pyran ring.³¹ For example, the most studied cannabinoid receptor agonist CP 55,940 6 is a group II cannabinoid. 6 was synthesised by Pfizer in 1974 but was not released due to its strong side effects, such as vertigo, anxiety, paranoia and hallucinations. However, it has found widespread use in the study of the endocannabinoid system to identify possible new analgesics and anti-inflammatories. 6 was the first cannabinoid known to bind to both CB1R and CB2R with similar affinity and has affinity 45 times greater than THC.²³

Other group II cannabinoids include CP 47,497 5, a CB1 selective agonist and one of the psychoactive compounds within the ‘legal high’ Spice.³² CP 55,244 7 is another CB1 receptor agonist, featuring a tricyclic core.

![Chemical structures of group II cannabinoids](image2)

**Figure 8:** Group II cannabinoids containing a bi- or tricyclic core, based on THC lacking the pyran ring.
Introduction

**Group III cannabinoids** contain aminoalkylindoles, the most common example being WIN 55212-2 8, which is a non-selective potent CB receptor agonist, originally synthesised by Sterling Winthrop as cyclooxygenase inhibitor but found to inhibit electrically-stimulated contractions of mouse vas deferens and adenylyl cyclase in brain membranes.33 This insight led to the discovery that the compound acted through cannabinoid receptors. Other members of this subclass of cannabinoids act selectively, such as JWH-15 9 which was found to be a CB2R selective agonist,34 while AM-694 10, which is a halogenated group III cannabinoid, acts selectively as a CB1R agonist.35

![Figure 9: Group III cannabinoids comprise of agonists with an aminoalkylindole core structures.](image)

**Figure 9: Group III cannabinoids comprise of agonists with an aminoalkylindole core structures.**

**Group IV cannabinoids** are the endogenous mammalian cannabinoids. They are derivatives of arachidonic acid 11, which is a polyunsaturated omega-6 fatty acid. The first endogenous cannabinoid to be isolated was anandamide 12 from mammalian brain in 1992.36 12 is a non-selective cannabinoid receptor agonist. Another non-selective CB receptor agonist is 2-arachidonylglycerol 13, the ester of arachidonic acid and glycerol, which was found in mouse and rat brain.37 Both are synthesised on demand in response to elevations of intracellular calcium. Despite the significant structural differences compared to THC 1 Reggio et al. showed that they have a very similar three dimensional structure38 and share the feature of high lipophilicity.

![Figure 10: Group IV cannabinoids comprise the endogenous agonists that are congeners of arachidonic acid 11.](image)

**Figure 10: Group IV cannabinoids comprise the endogenous agonists that are congeners of arachidonic acid 11.**
Introduction

Besides the four groups of cannabinoids discussed in detail, there are a variety of other ligands based on different structural motifs that also bind to the cannabinoid receptors. A potential therapeutic uses of CB receptor agonists

Cannabis is one of the most traditional versatile herbal medicines with applications in pain-therapy, inflammation, anxiety, psychosis, epilepsy, muscle spasticity and many more claimed. One of the best characterised medical effects of cannabinoids is their capability to reduce pain transmission. They are found to be effective analgesics in both acute or chronic pain. The majority of these analgesic effects are mediated by CB1 receptors that are located in in the central and the peripheral nervous systems. Furthermore, cannabinoids are used for reduction of the nausea and vomiting in cancer chemotherapy, as anti-spasmodics in multiple sclerosis, as anti-diarrheals for decreased intestinal motility, as anti-glaucoma agents for reduction of intranocular pressure, as anti-proliferative agents of glioma growth and for treatment of diseases such as phobias and post-traumatic stress. Also the THC formulation Sativex® which is a mixture of THC and cannabidiol is used to alleviate neuropathic pain, spasticity, overactive bladder, and other symptoms of multiple sclerosis. Nevertheless negative side effects such as alterations in cognition and memory, dysphoria/euphoria and sedation restrict the therapeutic use of cannabinoid receptor agonists.

1.3.2 Cannabinoid receptor antagonists / inverse agonists

The first reported cannabinoid inverse agonist was SR141716A [(-)-cis-3-[2-hydroxy-4(1,1-dimethyl-heptyl)phenyl]-trans-4-(3-hydroxy-propyl)cyclohexanol] (Rimonabant) developed by Sanofi Recherche in 1994. The discovery of this highly potent inverse agonist intensified the interest in scientific research on cannabinoids by providing a valuable pharmacological tool for investigating the structure of the CB1 receptor and its role within the endocannabinoid system. This compound is based on a 1,5-diarylpyrazole structure and acts as a CB1R selective inverse agonist which blocks the actions of various cannabinoid receptor agonists in vivo. Rimonabant has been reported as a pure inverse agonist at low (nanomolar) concentrations with a higher selectivity for the CB1 receptor than the CB2 receptor, albeit it is not CB1 receptor specific as it blocks both receptors at adequately high doses:
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Figure 11: Examples of synthetic cannabinoid receptor antagonists.

Structure-activity relationship (SAR) studies of Rimonabant

Rimonabant 14 was found to behave as an inverse agonist, which means that not only does it block the CB1 receptor but produces effects that suppresses constitutive signalling of CB1 by stabilising the inactive receptor state (see Figure 4). Some authors have continued to use the term antagonist, and some authors have used the term antagonist/inverse agonist to describe Rimonabant 14 and Taranabant 20. It should be pointed out that the term antagonist emphasises its ability to antagonise the agonist effect, and the term inverse agonist emphasises its ability to inhibit constitutive CB1R activity in the absence of an agonist.

Molecular modelling studies of Rimonabant 14 revealed a hydrogen bond between the oxygen of the inverse agonist and lysine 192 (Lys192) residue of the CB1 receptor. McAllister and co-workers discovered in modelling studies that this hydrogen bond is capable of stabilising a salt bridge between Lys192 from the third helix and Asp366 of the sixth helix. This salt bridge is only present within the inactive state of the CB1 receptor and absent in the active state.47
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After the development of Rimonabant many studies to gain better understanding of CB1R antagonism were undertaken and various compounds have since been synthesised resulting in an increasing knowledge on cannabinoid receptor bindings. The majority of all reported CB1R antagonists\textsuperscript{48} so far have a similar structure to Rimonabant \textsuperscript{14} and the pharmacophore unveils five characteristics for CB antagonism:

A cyclic core [C], such as pyrazol in Rimonabant \textsuperscript{14} that is connected to two aromatic moieties [A+B]. Furthermore [C] needs to be connected to a hydrogen bond acceptor [D] that also connects [C] with a lipophilic moiety [E].

Structure-activity relationship studies on CB1 receptor antagonism showed that for optimum affinity that one aromatic moiety has to be favourably in the N-1 position of the pyrazole core and is most effective as a 2,4-dichloro-substituted phenyl ring. Halogens other than chlorine or additional halogens on this aromatic moiety were found to decrease both affinity and activity.\textsuperscript{49}

The second aromatic moiety needs to be connected in C-5 position of the pyrazole core and should ideally be \textit{para}-substituted with a halogen, although alkyl chains have also been shown to be tolerated.\textsuperscript{49} An aliphatic ring connected to the carboxamide, such as the piperidinyl rest in Rimonabant \textsuperscript{14}, showed to have the best activity, while other groups such as alkyl chains, alcohols, ethers or alkyl amides decreased affinity.

Muccioli and co-workers reported a change in activity from antagonism to agonism when the piperidine ring is substituted with a pentyl or heptyl chain in Rimonabant \textsuperscript{14}.\textsuperscript{50} The results suggests that the C-3 position of the pyrazole core is involved in agonism while position N-1 and C-5 are involved in antagonism. It has also been shown that the lack of a carboxamide oxygen next to the pyrazole core results in decreased binding affinity. Congeners missing an oxygen in this position were found to act as neutral antagonists which strenghens the hypothesis of an interaction between Lys192 of the CB1R and the oxygen of the pharmacophore as a hydrogen bond acceptor.\textsuperscript{51}

\textbf{Figure 12:} SAR studies of the CB1 antagonist Rimonabant proposed five characteristics for antagonism.
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Second generation cannabinoid receptor antagonists

SR147778 (Surinabant) 15 is a CB1R selective antagonist developed by Sanofi-Recherche in 2004. It was found to be 800 fold more selective for the CB1 receptor than for the CB2 receptor. Structurally, it resembles Rimonabant 14 with slight changes. It bears an ethyl group in the 4 position of the pyrazol ring instead of a methyl group and the phenyl group in 5 position is substituted with a bromine instead of a chlorine.

SR144528 18 is a highly selective CB2 receptor antagonist which was also developed by Sanofi Recherche in 1997. Its structure differs from Rimonabant in some points, as it contains a 4-methyl benzyl group connected to the 1-position of pyrazol instead of a 2,4-dichloro phenyl group in Rimonabant. Furthermore is the piperidine ring substituted by a norbornyl moiety.

In the last three decades Makriyannis and co-workers have developed various cannabinoid receptor ligands with some of them acting as antagonists. AM251 16 consists of a p-iodo phenyl group attached to C-5 of the pyrazol instead of a p-chloro phenyl group in Rimonabant 14. It exhibits a slightly better affinity to the CB1 receptor and is about two fold more selective for CB1 receptor than Rimonabant. AM281 17 is another antagonist based on the structure of Rimonabant that contains a morpholine ring instead of the piperidine ring in AM251 16. It was found to bind with a 340 fold selectivity to the CB1R. AM-630 19 has a aminoalkylindole structure similar to the group III agonists (see Figure 9) but was found to act as a cannabinoid selective inverse agonist with a 165-fold selectivity for CB2R compared to CB1R.

MK-0364 20 (Taranabant) is an acyclic antagonist that contains a 1,2-diaryl motif that is believed to mimic the 1,5 diaryl structure of Rimonabant 14. It was developed by Merck in 2006 and found to act as a potent CB1R selective antagonist with a similar affinity for the CB1 receptor than Rimonabant.

Potential uses of CB1 antagonists and inverse agonists

The steady increase in obesity worldwide over the past five decades is related with an increase in healthcare costs and even worse in co-morbidities, such as diabetes, hypertension, cancer, arthritis, cardiovascular disease. Obesity is now the most common nutritional disorder in Western industrialised countries. Defined as a body mass index (BMI, weight/height$^2$) of greater than 30, obesity arises from overconsumption of fatty and sugary foods. The prevalence of obese people in the USA and Europe has reached epidemic levels in recent years.

Cannabinoid receptor antagonists and inverse agonists are promising compounds for the treatment of obesity due to their anorectic effects. For example Rimonabant 14, an inverse agonist was found to be an effective drug helping to decrease the body mass index of overweight or obese patients. As it suppresses appetite, the opposite effect to cannabinoid receptor agonists such as THC 1 it is also known as ‘inverse marijuana’. More positive effects of Rimonabant from clinical studies are assisting people to quit smoking and the potential of diminishing the chance of resumption cocaine and opioid
addicted people from relapsing. There are also some indications for lowering the recidivism rate of alcohol addicted people.61 Rats were found to have an improved short term memory after ingestion of Rimonabant, again opposite to the effects of cannabinoid receptor agonists that usually impair this ability.62 Nevertheless Rimonabant was found to have negative side effects which forced Sanofi to stop the program in 2008, less than 2 years after the approval for the European market. Independent studies suggested more intense side effects than had been found by the manufacturer clinical studies. Reports of severe depression and suicidal thoughts were frequent. The drug was officially withdrawn from European market in January 2009, based on the risk of serious psychiatric disorders. Similar results were also found with other CB1 receptor antagonists such as Taranabant. Negative side effects like depression and anxiety63 forced Merck & Co in 2008 to stop their clinical trials even though patients treated with Taranabant decreased their BMI significantly. Nowadays the pharmaceutical industry is focusing more on the development of potential CB2R antagonists as they were found to produce less undesirable psychoactive effects.64 Nevertheless, the Rimonabant scaffold continues to provide a useful building block for the development of tools to probe CB1 receptor function. Further the pyrazole scaffold has been used to develop CB2-selective receptor ligands and probes.65

1.4 Dopamine receptors and their distribution

Since the discovery of 3,4-dihydroxyphenethylamine (dopamine)23 by Carlsson et al. in 195766 and the discovery of the dopamine receptors in 197967 by Kebabin and Calne this neurotransmitter and the dopaminergic system itself has attracted a lot attention, as dopamine is the dominant neurotransmitter in mammalian brain. It is involved in a variety of critical functions, hence many human disorders have been related to dopaminergic dysfunctions. Parkinson’s disease caused by a loss of stratial dopaminergic innervations in the brain is by far the most recognised dopamine-related disorder.68 Furthermore the dopaminergic system was found to modulate maintenance of emotional stability and the regulation of prolactin secretion.69

The fact that almost all clinically effective antipsychotic drugs block the D2 receptor is a basis for the dopaminergic hypothesis of schizophrenia.70 Dopamine dysregulation also plays a crucial role in the pathogenesis of Huntington’s disease71 and Tourette’s syndrome.72

So far five different dopamine receptors have been found that are divided into two different major families, D1R and D2R, based on their ability to modulate cyclic adenosine monophosphate production.73 The D1-class receptors comprise of the D1R and the D5R and were found to bind to Gs-proteins which results in the accumulation of cAMP upon ligand binding while the D2 receptors comprise
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the D2R, D3R and D5R and are linked to Gi-proteins, similar to the CB1 receptor, and inhibits adenyl cyclase activity.\(^{67, 74}\)

Dopamine receptors are found throughout the entire central nervous system, predominantly in the negrostriatal, mesombolic, and tuberoinfundibular tracts.\(^{75}\) The highest levels of D2 receptors are found in the striatum, the nucleus accumbens, and the olfactory tubercle. D2 receptors are also expressed at significant levels in the substantia nigra, ventral tegmental area, hypothalamus, cortical areas, septum, amygdala, and hippocampus.\(^{76}\) The human dopamine D2 receptor (hD2R) comprises of 443 amino acids, while the hD1R contains of 446 amino acids.\(^{77}\) Even though the density of D1 receptors is higher than that of D2 receptors in the human brain, it is the D2R that has been specifically implicated in the physiology of most of the dopamine-associated disorders. Thus, for investigation and development of new dopamine receptor targeting drugs the focus is mainly on the D2R.\(^{69}\)

Dopamine activates the respective receptors with a different affinity ranging from nanomolecular to micromolecular range. In general, different subtypes of dopamine receptors vary significantly in their sensitivity to dopamine receptor agonists and antagonists.\(^{78}\)

1.4.1 Dopamine biosynthesis

Dopamine 23 occurs as an intermediate in the biosynthesis of the stress hormone epinephrine 25 and gets formed within the body from tyrosine 21:

![Scheme 2: Dopamine is produced within the biosynthetic pathway of adrenaline.](image)

The enzyme tyrosine hydroxylase catalyses the hydroxylation of tyrosine 21 to levodopa 22, which then subsequently forms dopamine 23 through an enzyme catalysed decarboxylation. Hereinafter dopamine-b-hydroxylase converts dopamine to norepinephrine 24 by inserting a hydroxyl group in
benzylic position. Finally, phenylethanolamine N-methyltransferase (PNMT) alkylates the primary amine to give epinephrine 25 (Scheme 2).

1.4.2 Dopamine D2 receptor ligands

The range of structural diverse dopamine D2 receptor ligands is highly diverse and there is no generally accepted single model or pharmacophore for the receptors which precisely explains the many different types of dopamine analogues with either agonistic or antagonistic activity.\textsuperscript{79} Different types of models for the D2R have been suggested to date. One of the models proposes a distance of approximately 7Å between the nitrogen atom and the meta-hydroxy group in dopamine, or a corresponding OH in related congeners, respectively.\textsuperscript{79a, 80} For example 2-(N-phenethyl-N-propyl)amino-5-hydroxytetralin (PPHT) 26 comprises a 2-amino-5-hydroxy substituted tetralin motif and was found to act as a potent dopamine D2 receptor agonist. The absence of the 5-hydroxy group resulted in significantly less affinity towards the receptor, whilst a N-propyl group was found to increase the affinity in most of the cases towards a secondary amine function.\textsuperscript{81} PPHT-NH2 NK242 is another potent D2 receptor agonist. Because of the aniline ring this ligand can easily get chemically ligated to other motifs and is a valuable ligand for i.e. dimerisation studies.\textsuperscript{82} Rotigotine 27 is a commercially available D2 receptor agonist, developed by Schwarz Pharma in 1998. It comprises a thiophene moiety instead of a benzene ring in 26 and is sold as a potent anti-Parkinsonian drug.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{tetralin_motif.png}
\caption{Examples of D2R-agonists comprising of a tetralin motif.}
\end{figure}

Dopamine receptor agonists are mainly used in the treatment of diseases that appear due to a lack of dopamine within the body, such as: Parkinson’s disease and restless-legs-syndrome. The structure diversity of dopamine receptor ligands is large and many heterocyclic compounds are used as drugs:
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Figure 14: Selective D2 receptor ligands with different structure motifs.

Pirebidil 28 for example is another D2R selective agonist that contains a piperazine moiety connected to a pyrimidine and a heliotropin motif, while Pramipexol 29 has a tetrahydrobenzothiazol moiety and Ropirinol 30 contains an oxindole structure. Quinpirole 31 shows agonism towards the D2R and is often used in scientific research such as Parkinson's disease. It contains a N-propyl-quinoline core and is a psychoactive drug. Sulpiride 32 is a D2R selective antagonist comprising a benzamide motif and used for example in the treatment of schizophrenia. Haloperidol 33 is another D2R selective antagonist, containing a p-fluorinated butyrophenone core. It is mostly used in antipsychotic medication and listed on the WHO Model List of Essential Medicines.

1.5 Coexpression and a hypothetical CB1-D2 receptor heteromer

Canabinoid CB1 receptors and dopamine D2 receptors have both been shown to be expressed within the same regions of the striatum and both receptors are also known to couple to G-proteins, which inhibits adenylate cyclase. Based on these facts, Glass and Felder in 1997 investigated a correlation between both receptors. They treated rat striatum with forskolin, a diterpene known to increase the cAMP levels in cells, and exposed the activated cells with either a cannabinoid CB1R and/or a dopamine D2R ligand, respectively.

As might be expected, the augmentation of cAMP accumulation in the activated cells was decreased upon exposition with either the CB1 receptor agonist HU210 4 or the D2R agonist quinpirole 31.
Interestingly, they discovered an accumulation of cAMP in forskolin-stimulated rat cells when those were treated simultaneously with the CB1 receptor agonist HU210 and the dopamine D2R agonist quinpirole. The augmentation of cAMP accumulation was blocked completely by either the CB1R inverse agonist Rimonabant or the D2R antagonist sulpiride.

Figure 15: Effect of CB1R and D2R agonists on forskolin-stimulated cAMP accumulation in rat striatum.
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Figure 16: Effect of concurrent activation of CB1 and D2 receptors on cAMP accumulation in forskolin-stimulated rat striatum.\(^87\)

The unexpected results suggest the assumption of G\(_s\)-proteins involved, as they are known to stimulate adenylate cyclase. Hence, it was unclear if either the CB1R or the D2R are bearing the capability to activate G\(_s\)-proteins. Glass and Felder treated the cells with pertussis toxin, a protein known to uncouple receptors from G\(_i\)-proteins by catalysing adenosine diphosphate ribosylation of the \(\alpha\)-subunit, thereby preventing the activation of the G protein.\(^88\) After this treatment, the activated cells were again exposed with either HU210 \(4\) or quinpirole \(31\). While the dopamine receptor agonist no longer inhibited cAMP stimulation, only HU210 \(4\) was still found to stimulate an increase of cAMP accumulation in forskolin-stimulated rat cells.

They further investigated a CB1 receptor stimulation in a clonal Chinese hamster ovary (CHO) cell line, where HU210 \(4\) again demonstrated a concentration-dependent inhibition of forskolin-stimulated cAMP accumulation, similar to the results in rat striatum cells. After treating the CHO cell line with pertussis toxin the activation of the CB1R unmasked a dose-dependent increase of cAMP above forskolin levels.
Figure 17: Effect of pertussis toxin on HU210-mediated inhibition of forskolin-stimulated cAMP accumulation in rat striatum cells [A] and in chinese hamster ovary cells [B]. Activating the CB1R with CB1R agonist HU210 results in a dose-dependent inhibition of cAMP. However, activation after uncoupling the CB1R from Gi-proteins resulted in an augmentation of cAMP accumulation in both cell lines.
1.6 Covalent binding ligands

The increased understanding of GPCR signalling pathways is a major challenge for modern pharmacology and crucial for the development of new and efficient drugs. The low expression of receptors in native tissue and their instability in solution after extraction from membranes are two of the biggest problems yet to overcome. Therefore a clear model of a GPCR such as the CB1 receptor in form of a crystal structure would help massively to understand the binding of certain pharmacophores, investigate active and non-active structures thus improving in drug design.

Over the last few decades much progress has been achieved and crystal structures of 29 receptors have been resolved until June 2015.

![Figure 18: Timescale to illustrate the huge achievements of GPCR structure elucidation during the last 2 decades (photo adapted from Prof. Raymond Stevens/Scripps Institute).](image-url)
Introduction

A promising way for a clearer understanding of GPCR signalling pathways is use of bifunctional molecular probes. These probes comprise small molecules with an either agonist or antagonist pharmacophore that has an affinity for a certain GPCR. The pharmacophore itself is connected to a radioactive labelled or fluorescent tag that helps to visualise the GPCR. A different approach involves the attachment of a pharmacophore covalently to a receptor via a functional group that allows irreversible binding. A covalently bound ligand can stabilise the receptor in solution to allow investigation. Another possibility for increased understanding of receptors is to form a dimer with bivalent tags.

Such probes ideally require an electrophilic functional group that shows low or no activity towards nucleophiles in solution however shows a high binding affinity to a suitably positioned amino acid residue within the binding pocket. The development of irreversible binding probes was inspired by the photoreceptor rhodopsin and its native ligand retinal.

The isomer 11-cis-retinal binds to the binding pocket of the receptor forming a covalent bond to lysine 296 of the receptor via an iminium ion 35. Upon absorption of a photon the Schiff base then isomerises to all-trans-retinal, switching the receptor from an inactive to an active conformation.
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Palczewski et al. solved the first GPCR structure of rhodopsin covalently bound to 11-cis-retinal in 2000 (Scheme 3).92

The first structure of an aminergic GPCR was solved by Stevens et al. in 200793 by crystallisation of the inactive state of the β2-adrenoceptor (β2AR) using complex and difficult techniques. However the process of structural elucidation has since made groundbreaking advances,94 and several inactive- and active-state GPCR structures have been solved in the last few years and greatly improved the understanding of GPCRs and their signalling pathways. The crystal structures of the adenosine A2A receptor95, the κ- (KOR), µ- (MOR) and δ-opioid (DOR) receptors96, the histamine 1 (H1) receptor97 and others have been solved. Obtaining crystals to be used in X-ray crystallography however, is still a significant hurdle. Activated states of GPCRs are especially difficult to crystallise. Stabilising the receptor with a high affinity ligand attached to a covalent binding functional group has therefore been applied to aid the formation of crystals.97, 100 Covalent binding probes have also been used in several biochemical studies that required a stable receptor-ligand binding. Hence, reactive agonists and antagonists were used to investigate receptor subtypes and the pharmacological effects.101 Purification of GPCRs was achieved with radioactive labelled covalent ligands and so gave more clarity about the localisation of the binding site.102

1.7 Reactive electrophiles for covalent GPCR ligands

1.7.1 Michael acceptors

α,β-Unsaturated carbonyl containing compounds such as maleimides, fumarates or acrylates are often used in affinity labels. These Michael acceptors have a high affinity for undergoing a Michael-type addition with nucleophilic cysteine, but can also react with lysine or histidine residues.103 For example Porthogese and co-workers104 synthesised the covalent binding opioid-ligand β-funaltrexamine (β-FNA) 38 by attaching a fumarylamido unit to the reversible binding opioid receptor ligand naltrexone 37.

![Scheme 4: Model for the Michael addition of lysine to the fumarylamido unit of β-FNA 38.](image-url)
Introduction

Although 38 shows affinity for all opioid receptor subtypes covalent binding is \(\mu\)-opioid receptor (MOR) specific via lysine 233, as verified by Chen and co-workers in 1996. In 2012, Manglik and co-workers were then able to form a stable ligand-receptor complex which allowed them to determine the inactive crystal structure of the MOR (Scheme 4).

1.7.2 Isothiocyanates

The synthetic availability of isothiocyanates represents a huge benefit compared to other electrophilic groups as they can be easily prepared from primary amines and anilines in excellent to good yields. These functional groups have shown preference towards amine nucleophiles and sulfhydryl groups of cysteine but only show poor reactivity with nucleophiles such as alcohols or water. For example, benzyl quinolone carboxylic acid (BQCA) 39 is known to be a positive allosteric modulator of the \(M_1\) muscarinic acetylcholine receptor.

![Figure 20](image-url): Benzyl quinolone carboxylic acid, a allosteric modulator of the muscarinic \(M_1\) receptor and a model of a covalent analogue binding to a cysteine.

Scammell's and co-workers have recently reported the first covalently binding allosteric ligand NCS-BQCA 40 for the \(M_1\) muscarinic acetylcholine receptor. The linking was promoted through the substitution of the aryl methoxy group to an isothiocyanate. Interestingly, the covalent ligand shows identical pharmacological activity to 39, even though the binding mode has not yet been fully elucidated.

For a better understanding of the cannabinoid receptors Makryannis et al. designed and synthesised the CB1 receptor agonist AM-841 41. This ligand is based on \((-\Delta^9\text{-trans-})\text{tetrahydrocannabinol (THC)}\) 1 and was shown to bind irreversibly to cysteine 355 of the CB1R. Many other ligands have been designed and synthesised to study receptor isolation and expression. For example fentanyl isothiocyanate (FIT) 43 based on the structure of the synthetic opioid agonist fentanyl 42 and covalently bind to the DOR. Spiperone 44 is a common antipsychotic drug used against diseases like schizophrenia acting as a D2R antagonist. Based on this structure is N-\((p\text{-isothiocyanatophenylethyl})\text{spiperone (NIPS)}\) 45 and is a known established commercially available covalent D2R antagonist for studying D2R.
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Figure 21: Examples for reversible binding GPCR ligands (left) and their covalent NCS congeners (right).

Further NCS-containing probes have been designed for the vasopressin receptor,\textsuperscript{101} the adenosine receptor,\textsuperscript{113} the melatonin receptor,\textsuperscript{114} the adrenergic receptor\textsuperscript{115} and the muscarinic receptor.\textsuperscript{116}

1.7.3 Halomethylketones

The reactivity of halomethylketones increases from least reactive fluoro-analogues through chloro- and bromo-analogues to highly reactive iodo-substituted analogues. Fluoro- and chloro-substituted halomethylketones react with cysteine residues only while their bromo- and iodo-congeners are less selective and have been shown to react also with other nucleophiles such as residues of histidine or lysine.\textsuperscript{117} Especially bromoacetamide analogues of known $\beta$-antagonists (beta blockers) were mainly used to investigate the $\beta$-adrenoceptors ($\beta$AR). The covalent binding probe pBABC 47 has been synthesised from $\beta$-antagonist carazolol\textsuperscript{118} 46, the beta blocker alprenolol 48 is the core structure for Br-AlpM\textsuperscript{19} 49 and based on the drug pindolol 50 the analogue Br-AAM-Pindolol\textsuperscript{120} 51 was first synthesised to bind irreversibly to $\beta$ARs:
**Introduction**

![Molecular structures](image)

**Figure 22**: Known beta blockers *left* and their covalently binding derivatives *right*.

**Scheme 5**: Example for a nucleophilic amino acid residue reacting with the covalent binding ligand Br-AAM-Pindolol 51.

Other types of ligands have also been developed for irreversible binding to receptors including photo activated covalent probes.121
1.8 GPCR dimers

After the discovery of GPCRs in the 1960s, this class of membrane proteins were thought to exist as monomers within the plasma membrane only. However, over the past decades a steadily growing amount of studies have been published indicating that GPCRs are able to react among themselves and form dimers and higher ordered oligomers. The GABA<sub>B</sub> receptor, a class C GPCR, was the first discovered receptor showing homodimerisation. Apart from different receptor interactions a dimerisation could also have a huge influence on the ligand pharmacology, cellular trafficking and signal transduction. Therefore, targeting either GPCR homodimers or heterodimers with divalent ligands may result in in more efficient and selective, less side effect causing drugs, although these may not persist ideal drug-like properties according to accepted medicinal chemistry.

1.8.1 Bivalent ligands for the investigation of GPCR dimerisation

1.8.1.1 Homodivalent probes

Homodivalent ligands consist of two identical moieties showing a high affinity for a certain receptor that are linked together via a flexible linker with variable length:

For example, for investigation of the opioid receptor dimerisation, Portoghese et al. developed and synthesised the first known series of homodimeric ligands. Based on their pioneering work in the 1980’s, many other homodimeric probes have been synthesised to target different GPCRs and help to understand the different signalling of dimers, including dopamine receptors, adenosine receptors, serotonin receptors and cannabinoid receptors. It has to be considered that bivalent ligands have two different possibilities in binding to the receptor. One possibility is interaction with two
orthosteric binding pockets from two neighbouring receptors, resulting in the formation of a homodimer. A second possibility would be the binding to the orthosteric target by one ligand and the binding to an allosteric binding site of the same receptor by the other ligand, so called bitopic binding mode. Bivalent probes binding to the orthosteric pocket of two physically interacting receptors could help to stabilise a homodimer and so present a valuable tool for the investigation of general GPCR dimerisation. A bivalent ligand showing a bitopic binding mode would ease the development of subtype-selective compounds as allosteric regions are usually expressed less within a receptor family than the orthosteric binding pocket.

For the development of promising bivalent ligands, several aspects have to be considered. A suitable pharmacophore is required as well as an eligible attachment point to connect the pharmacophore with the linker and also a linker with ideal length and applicable physicochemical properties such as lipophilicity.

A suitable pharmacophore for the investigation of GPCR dimerisation requires low to medium molecular weight as the incorporation of the linker increases the bulkiness considerably. To find a suitable attachment point of linking ligands together, two points have to be taken into account; the ability to conduct chemical modifications at the desired position of the pharmacophore and also the compatibility of these changes with the binding determinants of the receptor. Recently published structures have shown that carboxyl groups, amines and hydroxyl groups are all useful chemical means for connection of the ligand and linker. Additionally alkynes and azides are a feasible way to create 1,2,3-triazole linkers via a copper catalysed 1,3-dipolar cycloaddition, so called click chemistry. However, additional reaction steps are required to install the requisite terminal acetylene or azide functionalities.

Figure 24: The DOR antagonist Naltrindole 52 and the 5-HT4 receptor agonist ML10302 54 and their modified congeners used in opioid dimerisation studies.
In many cases, pharmacophores contain more than one functional group that is suitable as a possible connecting point. For example the DOR-selective antagonist Natrindole 52 has two hydroxyl groups and an aromatic amine as possible attachment points.

The linker ideally has to be attached without impairing the binding affinity or potency of the ligand. To find a good anchor position an excellent understanding of structure-activity relationship is fundamental. The 5-HT$_4$-receptor partial agonist ML10302 54 was identified via molecular modelling studies and development of fluorescent probes showed that bulky substituents in para-position of the piperidine ring are well tolerated. Although the aromatic amine moiety presented an obvious connection point, modifications in this position resulted in the complete loss of affinity. Based on these results Bestel and co-workers successfully developed homodivalent 5-HT$_4$-receptor ligands by incorporating two identical pharmacophores 54 connected through C-4 of the piperidine ring.

Another important parameter for the design of divalent probes is the linker length. In general a shorter linker length prefers the second ligand to target an allosteric binding pocket while longer linkers are more likely to enable dimer formation. Most successful dimerisation studies have demonstrated a correlation between the length of the linker and receptor affinity, and propose an ideal distance range for linker units connecting two orthosteric ligands. Portoghese and co-workers reported a peak agonist activity from a homodivalent ligand of a MOR agonist at a linker length around 18 atoms. Further studies on opioid receptor dimerisation confirmed an optimal linker length between 18 and 25 atoms. As this length is very specific to opioid receptors, it cannot be used as a general rule as the linker length of a dimer-spanning divalent ligand. The ideal length for each receptor depends on many factors such as the structure of the pharmacophores, their topicity and the position of the attachment points.

Capuano and co-workers have investigated dopamine receptor dimerisation with homodivalent ligands 57 based on the structure of clozapine 56 as the pharmacophore unit:

![Clozapine 56 and related homodivalent ligand 57 for dimerisation studies of the dopamine receptor.](image-url)
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Linkers of different lengths were attached at the N4' position and the results of the pharmacological tests indicated a considerable increase of affinity relative to clozapine for the probes with n = 6 and n = 8.\textsuperscript{126c}

Thomas and co-workers developed a compound library for the investigation of cannabinoid dimers based on the structure of the highly selective CB1 receptor ligand Rimonabant 14. The highly selective CB1 receptor inverse agonist was linked with linkers of different lengths and the homodivalent ligands were then investigated in radioligand binding assays, which showed an initially increasing affinity and with elongation of the linker length a decreasing affinity. The highest gain in affinity compared to the monovalent ligand showed the homodivalent ligand 58 with a linker length of 15 atoms\textsuperscript{129} It is interesting to note, however, that the highest affinity compound in this study was a monovalent control.

\textbf{Figure 26}: The pharmacophore of the CB1-selective inverse agonist Rimonabant was used in dimerisation studies for investigating the CB1/CB1 receptor complex.\textsuperscript{129}

Other modes of receptor binding are also known, i.e. the ligand making simultaneous interaction with an orthosteric and an allosteric site. Alternatively, ligands may distribute between orthosteric or allosteric orientations via a ‘flip-flop’ mechanism, or concomitantly by binding cooperatively to each site on a single receptor.\textsuperscript{138}

1.8.1.2 Heterodivalent probes

In contrast to homodivalent ligands, heterodivalent ligands comprise of two different pharmacophores that target different membrane proteins with high affinity. Divalent ligands could potentially exhibit increased receptor subtype selectivity\textsuperscript{125a, 125b} and in case of receptor cross-linking, enhanced affinity. There is a class of compounds where these receptor binding motifs are They may potentially also be used to estimate the distance between GPCRs or their spatial distribution\textsuperscript{139} thus playing an important role in the understanding of receptor function and pharmacology.\textsuperscript{125d, 140} Furthermore, divalent ligands containing an agonist and an antagonist pharmacophore in some cases have been shown to produce partial agonism.\textsuperscript{127, 141}
In a therapeutic context, divalent ligands may possess efficacy or pharmacokinetic advantages compared to other drugs.\textsuperscript{142} Cross-talk between different GPCRs allows the regulation of cellular responses from extracellular mediators. One example for a receptor cross-talk is the bidirectional effects on adenylate cyclase (AC) activity. A variety GPCRs either stimulate or inhibit AC by activation of $G_s$ or $G_i$ proteins, respectively, and there are a few cases known where $G_i$-coupled receptors can inhibit the ability of $G_s$-coupled receptors to stimulate the enzyme.\textsuperscript{143}

For the determination of cross-talking between $\beta_2$-adrenergic ($\beta_2$AR) and adenosine A\textsubscript{1} (A\textsubscript{1}AR) receptors, Scammells and co-workers synthesised a library of heterodivalent ligands, based on the structures of formoterol 60, a $\beta_2$AR agonist and adenosine 59, an endogenous A\textsubscript{1}AR agonist. The chosen linkage was between the side-chain amino of the $\beta_2$AR and the N-6-position of adenosine as large substituents in these positions have previously been shown to be well tolerated.\textsuperscript{144} The heterodimer 61 shown below was observed to exhibit the best pharmacological properties, as it had the best affinity to both receptor subtypes and was the most potent $\beta_2$AR agonist.\textsuperscript{142}

![Heterodimer 61](image)

**Figure 27:** The $\beta_2$AR-A1AR heterodivalent ligand 61 designed by Scammells \textit{et al.} and the pharmacophores that informed its design.\textsuperscript{142}

Heteromerisation between the adenosine A\textsubscript{2A} receptor (A\textsubscript{2A}R) and the dopamine D2 receptor (D2R) has been established in co-immunoprecipitation studies on neuroblastoma cells\textsuperscript{145} and was also confirmed by FRET and BRET analysis.\textsuperscript{146} The exact stoichiometry of an A\textsubscript{2A}-D2 receptor complex has not been determined however, as higher-order oligomers could also possible be formed. These receptors are mainly co-localised in the dendritic spines of the striatum.\textsuperscript{147} A\textsubscript{2A}R antagonists have been proposed as potent drugs against Parkinson’s disease for over 20 years, due to their ability to augment striatal D2R signalling,\textsuperscript{148} enhancing the therapeutic index ratio between the therapeutic benefits and the negative side effects of D2R agonists.\textsuperscript{149} Anti-Parkinsonian therapies combining A\textsubscript{2A}R antagonists with D2R agonists in a co-formulation are currently in clinical trials (Phase II).\textsuperscript{150}
Soriano et al. have designed and synthesised a variety of A2AR-D2R heterodimeric ligands, with different linker length for getting a better understanding of the heteromerisation. They used a precursor of the A2AR antagonist xanthine amine congener (XAC)\(^\text{151}\) \textbf{62} and the D2R agonist \textbf{PPHT-NH2}\(^\text{152}\) \textbf{NK242} as pharmacophores. The linkers were based on trifunctional amino acids with a repeating PEG-polyamide unit giving a total length anywhere from 26 up to 118 atoms. They found that bivalent probes with shorter linker length bound to the D2R or A2AR with higher affinity than their monovalent controls.\(^\text{82}\) No difference in binding affinity was observed between homodivalent controls and heterodivalent ligands, using membranes from cells expressing only the A2AR or D2R, respectively.

\textbf{Figure 28}: Series of heterodivalent ligands for the investigation of the A2AR-D2R heteromerisation synthesised by Soriano \textit{et al.}\(^\text{82}\)
Aim of research

The principle aim of the study is to develop effective bifunctional probes for the CB1 receptor. Such probes represent invaluable tools for the interrogation of receptor properties, such as distribution, binding, and oligomerisation. The methodology developed in the process will not be limited to the specific probes themselves, but will be applicable to the further progress of CB1R molecular probes.

Probes will be based on the parent ligand Rimonabant (14), a CB1R inverse agonist with high affinity and selectivity. The wealth of SAR data on the molecule and its easily modifiable scaffold make it an ideal ligand for this purpose. A known scheme for installing a linker attachment handle at the C3 position of the pyrazole core will be utilised.

This will be achieved by the synthesis and biological evaluation of several series of compounds possessing functional substituents conjugated via a linker attachment at the C3 position. These compounds will be tested in vitro for their affinity at the CB1 receptor in order assess the tolerance for a variety of linkers of varying length and composition, and the feasibility of assembling bifunctional CB1R ligands using this approach. The preparation of high affinity CB1R ligands containing a biotin tag or fluorescent tag would be of high value in the study of GPCR signalling and trafficking.

Further, it is intended to investigate the formation and potentially modulated signalling of the proposed CB1-CB1 receptor homodimer and CB1-D2 receptor heterodimer complexes by linking the Rimonabant CB1R ligand to either a second Rimonabant ligand or a D2R ligand.

The findings of this study will direct the development of effective bifunctional molecular probes for the CB1 receptor. Such probes will represent functional tools for the study of the receptor in order to better understand its role in healthy and pathological states. It is hoped that in turn, this will result in the development of more successful CB1-targeted therapeutics.

Finally, it is intended to synthesise covalent probes incorporating an irreversibly binding functional group. It is hoped that covalent binding to CB1 GPCR will add a current understanding of the exact mode of entry and binding to the receptor. If successful, this approach might lead to pharmacological probes of very high efficacy.
Chapter 2 Discussion

2.1 Synthesis of CB\textsubscript{1} inverse agonist ligands based on SR171416A

Thomas and co-workers reported the synthesis of a series of bivalent ligand probes derived from Rimonabant \textsuperscript{14} with the aim of targeting the putative homodimer of the CB\textsubscript{1} receptor. These compounds appeared to demonstrate good to excellent affinities at CB\textsubscript{1}.

Based on this precedent it seemed reasonable to begin our studies with further development of probes comprising the same CB1R ligand and attaching linkers via the C3 carboxamide.

Several synthesis of Rimonabant \textsuperscript{14} have been published since it was first reported to display high affinities and inverse agonist activity at the cannabinoid receptors.\textsuperscript{153} In order to apply these towards the aims of our study we initially followed the work of Kotagiri and co-workers.\textsuperscript{154} In our hands, however, the reported yields were not easily reproduced and synthesis on a gram scale proved unreliable.

Some optimisation of the literature route was undertaken to allow routine preparation on multigram scales. The reported route commences with acylation of commercially available 4-chloropropiophenone \textsuperscript{63} using LHMDS to give the lithium enolate \textsuperscript{64}, and subsequently $\alpha,\gamma$-ketoester NK\textsuperscript{20} on acidic workup. Ketoester NK\textsuperscript{20} then undergoes cyclisation with 2, 4-dichlorophenylhydrazine \textsuperscript{65} to give ester NK\textsuperscript{22}. Hydrolysis of this ester yields the carboxylic acid NK\textsuperscript{24} in which the free acid is available for linker attachment via amide bond formation.

The initial problem encountered was a very low 25\% yield for the first step in forming the $\alpha,\gamma$-ketoester NK\textsuperscript{20}. This was predominantly due to difficult purification via flash chromatography, making an acceptable synthesis on a gram scale nearly impossible. After some experimentation it was found that the key to success involved a change of the solvent of the LHMDS solution from THF to hexane, to lower the polarity of the reaction mixture. This made a large difference to the ease of workup, as it proved possible to readily filter off the solid lithium enolate \textsuperscript{64} directly, leaving related impurities in the
organic phase. After aqueous acid workup and purification via flash chromatography the product ketoester could be obtained in good to high yield, enabling the required gram scale synthesis to support ongoing studies.

**Scheme 6**: Improved preparation of the Rimonabant scaffold. *Reagents and conditions*: a) LHMDS, Et₂O, diethyl oxalate 65, 0 °C → r.t., 16 h, 63%; b) 2,4-dichlorophenylhydrazine 66, EtOH, reflux, 16 h, 95%; c) aq. KOH (1N), MeOH, reflux, 3 h, 96%.

Literature preparations of acid *NK24* describe the cyclisation of γ-ketoester *NK20* in two steps; initial condensation with aryl hydrazine 66, followed by cyclisation to the give the pyrazole *NK22*. In optimising this route, it was found that reflux in ethanol was sufficient to effect the desired condensation and pyrazole formation in a single step (Scheme 6). ¹⁵⁵

With a robust preparation of carboxylic acid *NK24* established it was now possible to investigate the effect of some short C3 substituents on affinity at CB1. The aim of this small study was to confirm that affinity could be retained with short linkers before embarking on the synthesis of more complex tethers and the planned bifunctional target compounds.
2.1.2 Synthesis of short linker substituents

Three different C3 substituents were investigated. Based on the studies of Merck\textsuperscript{156} (see Section 2.3, Table 1) that showed piperazine moieties were represented in compounds having reasonable functional activity, we decided to test piperazine linkers and the methyl ester of 6-aminocaproic acid for their binding affinities toward cannabinoid receptors. These linkers would readily lend themselves to subsequent ligation for eventual preparation of longer linkers.

\textbf{Scheme 7}: Synthesis of piperazine short linker. \textit{Reagents and conditions}: a) Boc\textsubscript{2}O, CHCl\textsubscript{3}, 0°C to r.t., 14 h, 76%; b) Ethyl chloroacetate, DIPEA, DCM, r.t., 30 min., 82%; c) DCM/TFA 1:1; quant..

Piperazine 67 was mono-Boc protected according to formerly reported procedures\textsuperscript{157} using 5 equivalents of piperazine 67 in chloroform to give NK58 in 76% yield. The following alkylation using ethyl chloroacetate gave NK60 in 82% yield. Deprotection of the alkylated piperazine with TFA in methylene chloride gave NK62 quantitatively (Scheme 7).

\textbf{Scheme 8}: Synthesis of 6-aminocaproic acid methyl ester \textit{Reagents and conditions}: a) SOCl\textsubscript{2}, MeOH, 0°C to reflux, 78%.

The esterification of 6-aminocaproic acid 68 was performed according to formerly reported procedures\textsuperscript{158} and gave the methyl ester NK323 in 78% yield (Scheme 8).

A further linker was synthesised starting from 4-aminomethylbenzoic acid 69 in ethanol using thionyl chloride to give NK71 in 81% yield (Scheme 9):
Discussion

Scheme 9: Synthesis of 4-aminomethylbenzoic acid ethyl ester Reagents and conditions: EtOH, SOCl₂, 0°C → r.t. → rfx. 81%.

2.2 Synthesis of Rimonabant derivatives with short (≤ 6C) C3 substituents

In order to generate high affinity analogues of Rimonabant 14 as bivalent probes of the CB1 receptor, it was first necessary to synthesise an initial series of test compounds in order to confirm that modification of the C3 carboxamide was tolerated with substituent suitable for derivatisation as linkers. A small number of novel analogues were accordingly synthesised and submitted for affinity assay against the cannabinoid receptor.

Scheme 10: Derivatisation of the Rimonabant scaffold. Reagents and conditions: a) (COCl)₂, DMF, CHCl₃, r.t., 20 min., quant.; b) NEt₃, DCM, amine, r.t..

Preparation of the derivatives was initially undertaken using peptide coupling conditions such as HOAt, HOBt, EDCI, however after some initial work it was found to be more convenient and higher yielding to first synthesise the acid chloride NK25 and then introduce the amine substituent. Accordingly, carboxylic acid NK24 was treated with oxalyl chloride and catalytic dimethylformamide to generate the stable acid chloride NK25 that could be isolated and stored if required, or used in the next step without further purification (Scheme 10).
Discussion

Subsequent addition of an amine in the presence of triethylamine then afforded the desired amides:

![Chemical structures]

**Figure 30**: Rimonabant derivatives with short (≤ 6C) C3 linkers. (Yields from NK25, NK76a prepared over 2 steps from NK25 and NK58 and subsequent Boc-deprotection, see page 117)

### 2.3 Synthesis of CB1R-agonists

Along with our investigations into CB1 inverse agonists based on Rimonabant, we wished to establish whether an analogous study could be conducted to generate an agonist series of ligands. This would potentially allow examination of the anomalous signalling behaviour reported by Glass and Felder that was suggested to be mediated by a CB1-D2 receptor heterodimer.

In 2010 and 2011 several studies were published by Merck\textsuperscript{156c, 159} that were concerned with the development and the synthesis of new CB\textsubscript{1} receptor agonists based on indole-3-carboxamide cores with the general structure:
Discussion

![Figure 31: General structure of indole-3-carboxamide synthesised for the investigation of their CB1 receptor agonist activity.](image)

Adam and co-workers from Merck developed and studied the SAR of a piperazine analogues and a few of their bicyclic analogues, too. It was found that the synthesised and investigated indole-3-carboxamides showed very good functional activity values ($\text{pEC}_{50}$) compared to common CB1R agonist structures such as THC 1 or WIN 55,212-2 8, making these structures an interesting alternative due to their higher hydrophilicity and their ready accessibility in comparison to other agonists:

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>R</th>
<th>$\text{pEC}_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>CH$_2$</td>
<td></td>
<td>6.8</td>
</tr>
<tr>
<td>71</td>
<td>CH$_2$</td>
<td></td>
<td>7.6</td>
</tr>
<tr>
<td>72</td>
<td>CH$_2$</td>
<td></td>
<td>7.6</td>
</tr>
<tr>
<td>73</td>
<td>CH$_2$</td>
<td></td>
<td>8.0</td>
</tr>
<tr>
<td>74</td>
<td>CH$_2$</td>
<td></td>
<td>7.9</td>
</tr>
</tbody>
</table>

Table 1: Indole-3-carboxamide CB1 agonists containing a piperazine moiety that have been tested for their CB1 receptor activity. $^{156a, 156b, 159}$

A wide range of further indole-based CB$_1$ receptor agonists were also prepared and tested for their functional activity, in which the indole 3-carboxamide motif was replaced with a range of heterocyclic systems.
As can be taken from the above figure, the reported $p\text{EC}_{50}$-values are similar to the indole-3-carboxamides (table 1). Interestingly it was found that the 7-indole methoxy substituted thiadiazole-containing indoles showed a higher efficacy when connected to a pyrrolidine moiety (75) rather than connected to a piperazine moiety (76), and that substituting the indole 7-methoxy group with a chlorine (77) showed a lower efficacy. Substituting the cyclohexylmethyl moiety (77) for a tetrahydropyranmethyl moiety (78) showed also lower efficacy. The efficacy of oxadiazole-containing indoles (79, 80) was in general lower than the efficacy of the thiadiazoles.

However, the synthesis of potential indole-3-carboxamide agonists would take only 3 steps instead of 8 steps like for the thiadiazoles. Beside the advantage of easier accessibility an indole-3-carboxamide could also easily get connected to a linker via an amide-bond formation.

The reported $p\text{EC}_{50}$ values of the depicted indole-3-carboxamides gave the occasion to synthesise several indole-based 3-carboxamides as potential cannabinoid receptor ligands in the studies of this project. As indole-3-carboxamides in general seem to be tolerated for binding to the CB$_1$ receptor, this could be a prospective way to for connecting a CB$_1$ agonist via a linker to a D$_2$ receptor agonist. Hence, we synthesised and investigated several indole-3-carboxamides for their receptor activity.

### 2.3.1 Synthesis of indole-3-carboxamides

To establish if the results published by Merck were reproducible, we envisaged the synthesis of a series of potential CB1R agonists and to verify their CB1 affinity. The synthesis of potential cannabinoid
receptor ligands started from commercially available 7-methoxy indole-3-carboxylic acid 81 and 7-chloro indole-3-carboxylic acid 82, respectively:

![Scheme 11: Synthesis of proposed CB1 agonists. Reagents and conditions: a) NaH, DCM, NEt₃, cyclohexylmethyl bromide, r.t., 20 min.; b) (COCl)₂, CHCl₃, amine, r.t., 30 min.]

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>R¹</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK73</td>
<td>-OMe</td>
<td>NBOc</td>
<td>71</td>
</tr>
<tr>
<td>NK74</td>
<td>-Cl</td>
<td>NBOc</td>
<td>77</td>
</tr>
<tr>
<td>NK82</td>
<td>-OMe</td>
<td>CO₂Et</td>
<td>73</td>
</tr>
<tr>
<td>NK85</td>
<td>-Cl</td>
<td>CO₂Et</td>
<td>80</td>
</tr>
<tr>
<td>NK83</td>
<td>-OMe</td>
<td>CO₂Me</td>
<td>85</td>
</tr>
<tr>
<td>NK87</td>
<td>-Cl</td>
<td>CO₂Me</td>
<td>80</td>
</tr>
</tbody>
</table>

Table 2: Indole-3-carboxamides that were synthesised for testing of their binding affinities toward the hCB1 receptors. All depicted molecules failed to displace the radioligand well enough to accurately determine a $K_\text{i}$ value.

The alkylation of the indole was performed using cyclohexylmethyl bromide to give the respective indoles in 85 to 89% yield. The following amide formation was performed via an acid chloride using oxalyl chloride with yields of 71% to 85%, respectively (Scheme 11).
2.4 Biotinylated CB₁ receptor ligands

Biotinylation involves the covalent appendage of biotin to proteins, antibodies and less commonly, small molecule GPCR ligands. Biotin 83, also known as vitamin H or vitamin B7, binds tightly to streptavidin with high specificity and on-rate; the complex formed by this is highly resistant to significant change in pH-value, temperature and other typically denaturing conditions.

![Figure 33: Biotin 83 and monomeric Streptavidin (ribbon diagram) with bound biotin (spheres).](image)

The crystal structure of streptavidin with biotin bound was reported by two groups in 1989. The structure was solved using multi wavelength anomalous diffraction by Hendrickson and co-workers¹⁶¹ and using multiple isomorphous replacement by Weber and co-workers¹⁶⁰.

The extremely high bonding results from eight hydrogen bonds directly made to residues in the binding site involving residues Asn23, Tyr43, Ser27, Ser45, Asn49, Ser88, Thr90 and Asp128.¹⁶⁰⁻¹⁶¹ This interaction is utilised by pharmacologists in numerous assays involving streptavidin conjugates in order to purify biomolecules of interest and study their function, distribution and trafficking. Many streptavidin conjugates, such as horse radish peroxidase, are commercially available making biotinylation a versatile labelling method.

In 2012 Ortega-Gutiérrez and co-workers¹⁶² reported the preparation of biotin-based high affinity molecular probes for the visualisation of cannabinoid receptors in native systems. Three probes were
synthesised; two based on the non-selective CB agonist HU-210 4 and one on the CB2-selective agonist HU-308 (84). The probes comprised of the ligand conjugated to biotin via a short linker fragment attached to the free hydroxyl group of the parent structure:

![Chemical Structures](image)

**Figure 34:** HU-210 and HU-308 based biotinylated molecular probes for the CB receptors.162

All three probes were claimed to retain affinities in the low nanomolar range and the HU-308-based ligand 87 retained the CB1/CB2 receptor selectivity of the parent ligand. All three biotin conjugates successfully labelled cannabinoid expressing cells *in vitro*, though 85 and 86 outperformed 87. Based on molecular dynamics simulations of receptor-bound 85 to 87, this was proposed to be due to incomplete exposure of the tag to the extracellular space.162 This highlights the importance of linker length in the development of molecular probes. It is important to note however, that the high affinity and labelling results of this study have not been successfully replicated despite efforts by other groups.162-163

Regardless of the reproducibility of results reported for compounds 85 - 87 above, biotin remains a cornerstone of cell visualisation methodology. In order to investigate the possibility of building a biotin labelled probe, a derivative was prepared as part of our initial analogue series NK76a (Scheme 12).

![Scheme 12](image)

**Scheme 12:** Synthesis of biotin-labeled C3 Rimonabant derivative NK169. Reagents and conditions: i) SOCl₂, CHCl₃, r.t., 30 min.; ii) NK76a, DCM, NEt₃, 0°C, 60 min, 76% over 2 steps.
2.5 Binding affinities of the CB1R (inverse) agonists

The compounds comprising the linker attachment series (see Figure 30) as well as the indole congeners (see Scheme 11) and the biotinylated Rimonabant derivative NK169 were initially screened in a radioligand displacement assay to give an approximate indication of hCB1 receptor binding affinity. The ability of the ligand to displace bound radioligand from the hCB1 receptor at a single high concentration (10 µM) was determined. The extent to which radioactivity is reduced upon treatment with the ligand, and subsequent washing of the membrane, indicated the strength of ligand binding.

Full displacement curves were measured for the ligands capable of at least 75% radioligand displacement in the 10 µM screen. Displacement at multiple dilutions was determined, allowing an accurate assessment of binding affinity ($K_i$) and concentration-response.

In vitro testing was carried out the Glass research group of the University of Auckland.

Unfortunately the measurement of the expected CB1 receptor agonists NK73, NK74, NK82, NK83 and NK87 only gave dose/response curves that were not adequate for determination of useful $K_i$ values. These results showed that the indole-3-carboxamides failed to displace the radioligand well enough to accurately determine a $K_i$ value making them unsuitable as potential ligands for CB1-D2 receptor dimerisation studies. Similar findings have been reported by Yates and co-workers. They reported that the modification of the 3-naphthoyl position of the hCB2R agonist JWH-159 (see figure 9) with a fluorescent dye caused a >250-fold loss in affinity to the hCB2R.

However, all four derivatives of Rimonabant displayed moderate to reasonable affinity. The best ligand NK75 showed a modest 4-fold decrease and NK90 a reasonable 6.5 fold decrease in affinity compared to Rimonabant 14. These findings supported the C3 position as a suitable position for linker attachment (see Figure 30).

The results encouragingly showed that the C3 derivatised Rimonabant analogues retained high hCB1 affinity, except for large hydrophilic groups, such as biotin (NK169). Based on these data it appeared reasonable to pursue our planned study of longer linkers and bifunctional CB1R ligands using C3 as the attachment point.


Discussion

2.6 CB₁ Inverse agonists ligands with long linkers and functional substituents

Divalent ligands for CB1 receptor homodimers

2.6.1 Bivalent probes for CB₁ GPCR dimer and oligomer formation.

Since the discovery that GPCRs form homo- and hetero-oligomers in native systems, there has been a drive to develop bivalent ligands to probe the occurrence and physiological significance of the phenomenon. Thomas and co-workers targeted the homodimers of the CB₁ receptor with Rimonabant-derived bivalent ligand probes from (14). The C3 carboxamide was chosen as the linker attachment position and several linker units were investigated at this position:

![Diagram of Rimonabant derivative with linkers]

It was found that the peptide and polyethylene glycol linkers reduced affinity for the CB1 receptor, yet with the diamine linker affinity appeared to be retained. This is most likely due to the hydrophilicity of the peptide and polyethylene glycol linkers being poorly tolerated during entry to the receptor via the lipid membrane [see section 2.6.3].

Symmetrical bivalent ligands were then constructed by bridging two Rimonabant (14) derived ligands with diamine linkers. The length of the linker fragment was varied to probe the distance needed for concurrent binding of both ligands to the receptor dimer and the affinity of these probes were compared to monovalent controls.

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Figure 35: Assessment of linker composition for the development of homodivalent CB1 probes.
Discussion

Figure 36: Homodivalent probes for CB1R dimers and their respective controls developed by Thomas and co-workers.\textsuperscript{129}

All homobivalent ligands displayed nanomolar affinities and an apparent trend between the linker length and the affinity was observed. The affinity increased with length from $n = 2$, peaking with $n = 7$ and diminishing with increased linker length thereafter. Interestingly, the monovalent controls also showed affinities comparable or better than their bivalent counterparts. However, the same relationship between affinity and linker length was not observed; rather, affinity showed constant increase with linker length and the longest control ($n = 11$) had the highest affinity. Since the homobivalent ligands show no significant increased affinity compared to their monovalent controls, it is unlikely that they are engaging in two separate binding events simultaneously. It is a possibility that the linker itself represents a second receptor recognition site and that, given the linker fragment is long enough, a second ligand is superfluous to this binding.\textsuperscript{42} It is also possible that the lipophilic nature of the linker causes an increased local concentration of the ligand at the receptor by accumulation in the lipid membrane, promoting binding.

However, it is worth noting that with no unambiguous technique to determine the occurrence of bivalent binding, these explanations await more detailed confirmation.

2.6.2 Extended C3 linkers

The optimal linker lengths, or the distances between the binding sites on neighbouring receptors in receptor dimers or oligomers, have been reported on a number of GPCRs. Molecular modelling studies based on the crystal structure of rhodopsin suggested a distance between the individual receptors to be $\sim 35$ Å, although the receptor dimer was in a head-to-tail orientation.\textsuperscript{92} Similarly, molecular modelling on the opioid receptor suggested that the distance between the recognition sites of either the interlocking or contact dimers with a TM5,6-interface is $\sim 27$ Å, while it is greater ($\sim 32$ Å) in dimers with TM4,5-interface.\textsuperscript{166} However, during their studies on opioid bivalent ligands, Portoghese and co-workers discovered that optimal activity was obtained when linkers are about 22 Å ($\sim 19$ atoms).\textsuperscript{125a} On the other hand, Neumeyer and co-workers found that bivalent ligands for the opioid receptors having linkers containing 10 methylene units or less displayed the highest affinities.\textsuperscript{167} On the basis of these findings and others, linkers between 5 and 23 atoms were initially examined in our laboratory to determine
optimal linker length. Three types of linkers have been considered in the design of the bivalent ligands. The first class investigated was polyethylene glycol linkers. The second category is composed of small peptides (Figure 35). These two classes of linkers have been employed in bivalent ligand development by a number of groups.\textsuperscript{166, 168} Not only are these linkers readily available but they also offer the advantage of gradually increasing the linker length.

As the results of Thomas and co-workers suggested a linker length of nearly 17 atoms between both CB1 pharmacophores showed best results when using a hydrophilic amine linker.

### 2.6.3 Lipid pathway for CB-ligand binding

Covalent labelling studies and molecular dynamic simulations of the CB2 receptor suggests ligand entry to the binding pocket occurs from the lipid membrane via the TM6/7 interface, distinct to the entrance from the extracellular space typical of GPCRs (Figure 39). The covalent agonist AM841 (41) selectively labels residue C6.47(257); in Hurst and Reggio’s CB2 model, this residue is located at the TM6/7 interface, facing out to the lipid membrane. The cysteine residue C7.42(288) positioned inside the binding pocket is not labelled by the agonist, suggesting the covalent bonding occurs during entry to the binding pocket from the lipid membrane. Hurst and Reggio undertook molecular dynamic simulations for the entrance of the endocannabinoid 2-arachidonoyl glycerol to the CB2 receptor which predicted the entrance lipid entrance via the TM6/7 interface, corroborating covalent binding studies. C6.47(257) is also found and selectively labelled by AM841 in the CB1 receptor, suggesting the same entrance pathway is characteristic of both CB receptors.\textsuperscript{110}
2.6.4 Synthesis of homodivalent ligands connected via hydrophilic linkers of varying length

A series of bivalent ligands with 3-position linkers of varying lengths were synthesised and evaluated in efforts to optimise the linker length for bridging of the receptors dimers similar to the preliminary published work of Thomas and co-workers\textsuperscript{129} with different linkers. In particular it was firstly envisaged to study the homodimerisation using a hydrophilic, amino acid based linker with varying length and secondly to synthesise homodivalent ligands connected via a hydrophobic, rigid linker.

A suitable hydrophilic linker for the investigation of homodimerisation has to fulfil at least the following requirements:

- two different functionalities on opposite sides were required so the linker could ideally be used to form dimers and trimers for convenient assembly of longer unit, and
- readily available on scale, and
- inexpensive.

Naturally occurring proteinogenic amino acids where found to fulfil all the above requirements, hence Fmoc-Lys(Boc)-OH (90) was chosen for linker-synthesis:
Discussion

Figure 38: Fmoc-Lys(Boc)-OH (90) for hydrophilic linker-synthesis.

For an amide bond formation between the carboxylic function of Rimonabant-derivative (NK24) and the carboxylic group of lysine it was necessary to include an additional amine functional group in NK24. 1,6-diamino hexane 91 was envisaged as a possible tether between the Rimonabant derivative (NK24) and Fmoc-Lys(Boc)-OH (90). Thus, mono-Boc-protected 1,6-diamino hexane NK650 was synthesised (Scheme 13):

Scheme 13: Synthesis of mono-Boc protected 1,6 diamino hexane NK650. Reagents and conditions: CHCl₃, Boc₂O, 0°C, 79%.

The mono-Boc protection was initially completely unsuccessful when using the HCl-salt of 1,6-diamino hexane instead of the free base. The HCl-salt is absolutely insoluble in all common organic solvents, such as chloroform, methylene chloride, acetone, THF, DMSO, DMF and alcohols such as methanol or ethanol and did also not solubilise in neat NEt₃. Hence, it was necessary to add water to the suspension for solubilisation of the HCl-salt. However, every time the reaction was attempted when water was present only the di-Boc protected diamine was obtained.

NK650 was then connected as a tether to the Rimonabant-core NK24 via prior acid-chloride formation (Scheme 14):

Scheme 14: Preparation of NK370. Reagents and conditions: a) (COCl)₂, DMF, CHCl₃, r.t., 20 min.; b) NEt₃, DCM, NK650, r.t.; c) DCM, TFA, r.t., 74% over 3 steps.
Subsequent deprotection of the Boc-group gave the depicted free amine NK370 which was then connected to lysine 90 via an amide-coupling reaction:

![Chemical structure](image)

**Scheme 15**: Preparation of NK381. Reagents and conditions: a) DCM, EDCI, HOAt, NEt₃, 90, r.t.; b) DCM, Et₂NH, r.t., 72% over 2 steps.

Amide bond formation between NK370 and lysine 90 worked with good yields of 72% without any problems. The subsequent α-amino-Fmoc cleavage proceeded in quantitative yield using a 1:1 mixture of methylene chloride and diethyl amine to give NK381 (Scheme 15).

The lipophilic Fmoc-protecting group was then replaced by an acetyl-group using acetyl chloride (Scheme 16):

![Chemical structure](image)

**Scheme 16**: Acetylation and Boc-deprotection of NK381. Reagents and conditions: a) DCM, AcCl, NEt₃, 0°C; b) DCM, TFA, r.t., 74% over 2 steps.

The free amine NK383 was then elongated with another lysine moiety via amide-bond formation (Scheme 17):
Scheme 17: Preparation of free amine NK388. Reagents and conditions: a) DCM, EDCI, HOAt, NET3, 90, r.t.; b) DCM, Et₂NH, r.t.; c) DCM, AcCl, NET3, 0°C; d) MeOH, TFA, r.t., 60% over 4 steps.

The free amine NK388 was then coupled to another equivalent of acid chloride NK25 to give the homodivalent ligand NK412 with a hydrophillic 22 atom linker between both CB1R ligands (Scheme 18):

Scheme 18: Synthesis of homodivalent ligand NK412. Reagents and conditions: a) DCM, NET3, r.t., 75%.

To access a longer linker, the coupling of free amine NK388 to the mini-PEG-like unit 92 was also undertaken:

Figure 39: 2-[2-(Fmoc-amino)ethoxy]ethoxy]acetic acid

The mini-PEG was chosen due to its high hydrophilicity, and its commercial availability. Furthermore the carboxyl function on one end and amine at the opposite end made it a perfect partner for sequential amide couplings (Scheme 19).
Discussion

Scheme 19: Preparation of free amide NK418. *Reagents and conditions:* a) DCM, EDCI, HOAt, NEt$_3$, 92, r.t.; b) DCM, Et$_2$NH, r.t., 88% over 2 steps.

The free amine NK418 was subsequently coupled to acid chloride NK25 to give the homodivalent ligand NK462 with a 31 atom hydrophilic linker (Scheme 20). Linker length is defined as number of atoms between the C3-carbonyl groups.

Scheme 20: Preparation of homodivalent CB1-CB1 receptor ligand NK462. *Reagents and conditions:* a) DCM, NEt$_3$, r.t., 65%.

Finally, elongation of the free amine NK418 with another mini-PEG-unit 92 to obtain a 41 atom linker was also investigated (Scheme 21):
Discussion

Scheme 21: Preparation of free amine NK420. Reagents and conditions: a) DCM, EDCI, HOAt, NEt$_3$, 92, r.t.; b) DCM, Et$_2$NH, r.t., 35%.

The coupling of the second mini-PEG-unit onto the amine NK418 worked only in low yield. Several other methods for the amide bond formation were tried with even less success. Despite this setback, enough of compound NK420 was obtained to couple with a further equivalent of acid chloride NK25, to give the 41 atom linker homodivalent ligand NK533 (Scheme 22):

Scheme 22: Preparation of homodivalent CB1-CB1 receptor ligand NK533. Reagents and conditions: a) DCM, NEt$_3$, r.t., 40%.
2.6.5 Testing results for homodivalent ligands tethered by a hydrophilic linker

It was intended to test the homodivalent ligands NK412, NK462 and NK533 for their affinity at hCB1. Disappointingly, all of these compounds proved unable to be solubilised in DMSO in the Glass lab, even after gentle warming and sonification at an adequate concentration to obtain displacement data versus a radioligand. Hence, no affinity data was obtained.

2.6.6 Synthesis of homodivalent ligands connected via hydrophobic linkers of varying length

In order to further investigate the effect of linker solubility on ligand properties and CB1 affinity, and in particular to investigate the insolubility of the previous compounds, it was decided to synthesise a small number of CB1-CB1 receptor homodivalent ligands tethered by a hydrophobic linker.

A suitable linker building block should have ideally two different functionalities on opposite sites which could be reacted with each other for varying the length of the linker and should also comprise an aromatic moiety which would increase the lipophilicity of the linker. Hence, 4-aminomethyl benzoic acid 93, an inexpensive and readily available aromatic amino acid, was selected as a possible building block for the linker synthesis:

![Figure 40: 4-Aminomethyl benzoic acid.](image)

Both functional groups of 93 were first of all protected separately (Scheme 23):
Discussion

Scheme 23: Preparation of \textit{NK651} and \textit{NK285}. \textit{Reagents and conditions:} a) DCM, NEt\textsubscript{3}, Boc\textsubscript{2}O; b) MeOH, SOCl\textsubscript{2}, 0°C to reflux 98%.

It was then envisaged to dimerise \textit{NK285} and \textit{NK651} via amide bond formation:

Scheme 24: Coupling of \textit{NK285} and \textit{NK651}. \textit{Reagents and conditions:} DMF, HBTU, DIPEA.

The reaction between the building blocks \textit{NK285} and \textit{NK651} was attempted several times under varying conditions. However, the reaction product precipitated out after 3 to 4 hours and was not soluble in any organic solvent (Scheme 24).

It is very likely that this phenomenon occurred due to the structural similarity of the dimer with Kevlar® which is the registered trademark for a para-aramid synthetic fiber:

Scheme 25: The reaction of 1,4-phenylene-diamine (para-phenylenediamine) \textit{94} with terephthaloyl chloride \textit{95} yielding Kevlar® \textit{96}. 

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Discussion

Kevlar® is synthesised in solution from the monomers 1,4-phenylene-diamine (para-phenylenediamine) and terephthaloyl chloride in a condensation reaction yielding hydrochloric acid as a byproduct (Scheme 25). The result has liquid-crystalline behaviour, and mechanical drawing orients the polymer chains in the fiber's direction. Hexamethylphosphoramide (HMPA) was the solvent initially used for the polymerisation.

For decreasing the rigidity of the Kevlar-like structure of we then attempted to couple the lipophilic alkyl chain of Boc-protected 6-aminocaproic acid NK283 to 4-aminomethyl benzoic acid NK285:

![Scheme 26: Preparation of NK311. Reagents and conditions: a) DMF, HATU, DIPEA, r.t., 86%.]

The coupling reaction gave excellent yields up to 86% (Scheme 26). With NK311 in hand it was then envisaged to first saponify the methyl ester of the benzoic acid moiety and subsequently to couple it to a 6-aminocaproic acid methyl ester moiety NK323:

![Scheme 27: Preparation of NK325. Reagents and conditions: a) MeOH, KOH, r.t. to reflux; b) DMF, HATU, DIPEA, NK323, r.t., 84%.]

The coupling reaction gave excellent yields up to 84% (Scheme 27). NK325 was subsequently saponified to give the free acid NK330 (Scheme 28):

![Scheme 28: Preparation of NK330. Reagents and conditions: a) MeOH, KOH, r.t. to reflux, 97%.]
The free acid was then coupled to CB1R-ligand containing free amine NK370:

![Chemical structure of NK370 and NK330](image)

**Scheme 29**: Preparation of NK504. *Reagents and conditions*: a) DMF, HATU, DIPEA, r.t., 79%.

The coupling reaction worked in moderate to good yields of 79% (Scheme 29). NK504 was then deprotected to give the free amine NK505 with a 95% yield and was subsequently coupled to another equivalent of the acid chloride NK25 to give the homodivalent ligand NK509 with a lipophilic 29 atom linker (Scheme 30):
For obtaining a longer lipophilic linker between both CB1R-ligands it was tried to dimerise the linker by coupling the free amine NK505 to a further fragment of NK330:

Scheme 31: Preparation of NK515. Reagents and conditions: a) DCM/DMF, HBTU, DIPEA, r.t.; 63%.
The coupling worked with reasonable 63% yield and gave the Boc-protected amine NK515 (Scheme 31) which was subsequently deprotected to the free amine NK516 and subsequently coupled to the acid chloride NK25 to obtain the homodivalent ligand NK519 with a 50 atom lipophilic linker in 82% yield (Scheme 32):

Scheme 32: Preparation of NK519. Reagents and conditions: a) THF/MeOH, TFA, r.t.; b) DCM, DIPEA, r.t.; 82% over 2 steps.
2.7 Synthesis of the D2R agonist (±)-PPHT-NH2

In 2009 Royo and co-workers published a study on the dimerisation/oligomerisation of the proposed A2A-D2 receptor heteromer/oligomer. Therefore, they conducted a synthesis of a library of a variety of heterobivalent ligands constituted by two pharmacophores, namely XCC (97) and (±)-PPHT-NH2 NK242:

![Chemical Structures](image)

**Figure 41**: XCC, an adenosine A2A antagonist and (±)-PPHT-NH2, a dopamine D2R agonist used as pharmacophores for heterodimerisation studies by Royo and co-workers.

XCC 97 has been widely used in several studies toward the adenosine A2A-receptor and is known to act as an antagonist, while (±)-PPHT-NH2 NK242 shows agonistic effects toward the D2 receptor. The pharmacophores were linked by PEG-based linker units of several length (see introduction). However, it has to be mentioned, that Royo and co-workers did not find solid evidence for the formation of a A2AR-D2R heterodimer. It was established that the heterodimeric ligands demonstrated high affinity at both A2A and D2 receptors.

As (±)-PPHT-NH2 NK242 has been proved to be an effective D2R agonist and the synthesis has already been conducted by several groups, we decided to use this pharmacophore as the D2R ligand in our proposed CB1-D2 receptor heterodivalent ligand series.

The synthesis started from commercially available 1,6-dihydroxynaphthalene 98 which was alkylated using dimethylsulphate. The 1,6-dimethoxynaphtalene NK227 obtained was subsequently reduced in a Birch-type reaction using metallic sodium to give tetralone NK228 (Scheme 33):

![Synthesis Scheme](image)

**Scheme 33**: Preparation of NK228. Reagents and conditions: a) acetone, Me₂SO₄, K₂CO₃, reflux; b) EtOH, Na(s), reflux; 65% over 2 steps.
Tetralone NK228 was then transformed to the secondary amine NK229 via reductive amination using propylamine and subsequent reduction of the resulting iminium-cation 100 using NaBH$_3$CN in one pot (Scheme 34):

Scheme 34: Preparation of NK229. Reagents and conditions: a) DCM, propyl amine, AcOH, 0°C; b) NaBH$_3$CN, r.t.; 70%.

Secondary amine NK229 was subsequently converted to amide NK232 under Schotten-Baumann conditions$^{170}$, using acid chloride NK230, which was obtained from the reaction of 4-nitrophenylacetic acid 101 and oxalyl chloride beforehand (Scheme 35):

Scheme 35: Preparation of NK232. Reagents and conditions: a) DCM, (COCl)$_2$, DMF, r.t.; b) DCM, NaOH (aq.), NK229, 0°C to r.t. 85%.

In the next steps amide NK232 was reduced to the tertiary amine NK231, which was subsequently converted to the aniline NK239 (Scheme 36):

Scheme 36: Preparation of NK239. Reagents and conditions: a) THF, BH$_3$, 0°C to reflux; b) EtOH, N$_2$H$_4$, Raney-Ni, 50°C; 55% over 2 steps.
Aniline **NK239** was then demethylated to the D2R agonist (±)-PPHT-NH2 (**NK242**) (Scheme 37):

![Scheme 37](image_url)

**Scheme 37**: Preparation of **NK242**. Reagents and conditions: a) DCM, BBr₃, 0°C to r.t. 60%.

It was envisaged to ligate the (±)-PPHT-NH2 ligand **NK242** on the aniline site and furthermore to change the amine function to a carboxylic acid function. Hence, it was decided to benzylate succinic acid and to deprotect the benzyl protecting group after coupling to D2R-agonist **NK242**:

![Scheme 38](image_url)

**Scheme 38**: Preparation of **NK192**. Reagents and conditions: a) CHCl₃, DMAP, r.t. 85%.

The monobenzylated succinic acid **NK192** was synthesised from benzylic alcohol 102 and succinic anhydride 103 on a gram scale in good yields of about 85% (Scheme 38). **NK192** was subsequently coupled to (±)-PPHT-NH2 **NK242** (Scheme 39):

![Scheme 39](image_url)

**Scheme 39**: Preparation of **NK421**. Reagents and conditions: a) DMF, HATU, DIPEA, r.t. 68%. 
Discussion

The prolonged D2R-agonist was subsequently hydrogenated using catalytic palladium on carbon to give free acid NK246 (Scheme 40):

Scheme 40: Preparation of NK246. Reagents and conditions: MeOH/EtOAc, Pd/C, HCl, H₂(g), r.t., quant.

2.8 Synthesis of CB1-D2 receptor heterodivalent ligands

The series of A2AR antagonist / D2R agonist bivalent ligands prepared by Royo and co-workers possessed linkers ranging between 26 and 66 atoms. Interestingly, affinities of the bivalent ligands to both receptors stayed almost identical with the elongation of the linkers. The authors suggested that linkers with 26 atoms were of sufficient length to allow the bivalent ligands to bind to receptor dimers according to receptor docking experiments and suggested that the lack of correlation between binding affinity and linker length might be due to the high flexibility of the mixed peptide/polyethylene glycol linkers.

2.8.1 Synthesis of CB1-D2 receptor heterodivalent ligands tethered via hydrophilic and hydrophylic linkers of varying length

Based on the results of Royo and co-workers we synthesised CB1-D2 receptor heterodivalent ligands using the dopamine D2R-agonist ligand NK246 which was coupled to the free amines NK388, NK418, NK420, NK505 and NK516:
Discussion

**Figure 42:** Free amines comprising a cannabinoid CB1-moiety used for couplings to the D2R-ligand **NK246** for synthesising CB1-D2 receptor heterodivalent ligands.

The coupling reactions resulted in a new series of CB1-D2 receptor heterodivalent ligands:
Figure 43: CB1-D2 receptor heterodivalent ligands containing a CB1R-moiety and a D2R-moiety synthesised.
Table 3: Affinity data for hCB1-D2 receptor heterodivalent ligands show extremely high D2R affinity.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ki values (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK416</td>
<td>0.84</td>
</tr>
<tr>
<td>NK461</td>
<td>2.4</td>
</tr>
<tr>
<td>NK487</td>
<td>1.18</td>
</tr>
<tr>
<td>NK507</td>
<td>2.22</td>
</tr>
<tr>
<td>NK517</td>
<td>4.17</td>
</tr>
<tr>
<td>NK242 (PPHT)</td>
<td>6.80</td>
</tr>
</tbody>
</table>

We were very pleased to establish that the CB1-D2 receptor heterodivalent ligands showed extremely high affinity at the D2 receptor, even exceeding that of the parent ligand NK242 which showed an affinity of $K_i = 6.8$ nM.\textsuperscript{152} In corroboration with the findings of Royo and Soriano, this demonstrates that the D2R ligand pharmacology remains robust in the presence of a long linker. This element of the CB1-D2 receptor heterodivalent ligand assembly can be considered optimised and our next efforts focused on achieving CB1 receptor affinity in the presence of a long linker.

2.8.2 CB1 Control ligands

For investigation of the binding affinity of the cannabinoid receptor ligand moiety connected to a linker it was necessary to synthesise monovalent control ligands. Therefore the free amines depicted earlier (see Figure 42) were acetylated using acetyl chloride to give the following compounds:
Figure 44: Terminal acetylated CB1- ligands synthesised as control-ligands.

Until June 2015 none of the control ligands, namely **NK474, NK472, NK534, NK506** and **NK521** have been tested for their affinity to the hCB1R.

### 2.8.3 D2R Control ligands

Similarly, to further confirm the binding affinity of the dopamine moiety connected to a linker without a pendant CB1R ligand it was necessary to synthesise monovalent control ligands. Therefore, the following monovalent D2R-control ligands were synthesised:
Discussion

**Scheme 41:** *Reagents and conditions:* a) DCM, TFA, r.t. quant.; b) DMF, HATU, DIPEA, NK246, r.t. 81%.

Linker fragment NK325 was Boc-deprotected under standard conditions using TFA in methylene chloride and the resulting free amine NK331 was subsequently coupled to the free acid NK246 using HATU in DMF (Scheme 41).

Furthermore, linker fragment NK325 was dimerised to give the depicted dimer NK332 which was subsequently coupled to the free acid of the D2R moiety NK246 (Scheme 42):

**Scheme 42:** *Reagents and conditions:* a) DMF, HATU, DIPEA, r.t. 81%; b) DCM, TFA, r.t.; c) DCM/DMF, HBTU, DIPEA, r.t. 81%.
As was the case for the CB1-D2 receptor heterodivalent ligands, control compounds consisting of the D2R ligand and a linker also displayed excellent affinity at D2 receptor.

### 2.9 Cholesterol containing ligands

As already explained under section 2.4.3 covalent labelling studies and molecular dynamic simulations of the CB2 receptor suggests ligand entry to the binding pocket occurs from the lipid membrane via the TM6/7 interface, distinct to the entrance from the extracellular space typical of GPCRs (see Figure 37). This means that the cannabinoid receptor ligand enters the receptor through the lipophilic membrane. Therefore, it was envisaged to connect the cannabinoid receptor ligand to a lipophilic “anchor” and hereby helping the cannabinoid receptor ligand getting stabilised within the receptor.

In search for a suitable lipophilic “anchor” cholesterol (104) and lithocholic acid methyl ester NK371
Figure 45: Cholesterol 104 and lithocholic acid methyl ester NK371, two highly lipophilic compounds that were envisaged as “anchors”.

were examined to help stabilising the cannabinoid receptor ligand bound to the receptor while the “anchor”, itself stays in the lipophilic membrane.

Cholesterol is a modified steroid, a lipid molecule and is biosynthesised by all animal cells because it is an essential structural component of all animal cell membranes that is required to maintain both membrane structural integrity and fluidity. Cholesterol enables animal cells to dispense with a cell wall (to protect membrane integrity and cell viability) thus allowing animal cells to change shape and animals to move (unlike bacteria and plant cells which are restricted by their cell walls).

In addition to its importance for animal cell structure, cholesterol also serves as a precursor for the biosynthesis of steroid hormones and bile acids. In vertebrates the hepatic cells typically produce greater amounts than other cells. It is absent among prokaryotes (bacteria and archaea), although there are some exceptions such as Mycoplasma, which require cholesterol for growth.

Cholesterol, given that it composes about 30% of all animal cell membranes, is required to build and maintain membranes; and modulates membrane fluidity over the range of physiological temperatures. The hydroxy group on cholesterol interacts with the polar head groups of the membrane phospholipids and sphingolipids, while the bulky steroid and the hydrocarbon chain are embedded in the membrane, alongside the nonpolar fatty-acid chain of the other lipids. Through the interaction with the phospholipid fatty-acid chains, cholesterol increases membrane packing, which both alters membrane fluidity and maintains membrane integrity so that animal cells do not need to build cell walls (like plants and most bacteria) allowing animal cells to change shape and animals to move.

The structure of the tetracyclic ring of cholesterol contributes to the fluidity of the cell membrane as the molecule is in a trans conformation making all but the side chain of cholesterol rigid and planar.

Lithocholic acid (LCA), is a bile acid that acts as a detergent to solubilise fats for absorption. LCA can activate the vitamin D receptor without raising calcium levels as much as vitamin D itself.

Bile acids are steroid acids found predominantly in the bile of mammals and other vertebrates. Primary bile acids are those synthesised by the liver. Secondary bile acids result from bacterial actions in the colon. Bile acids comprise about 80% of the organic compounds in bile (others are phospholipids and cholesterol). An increased secretion of bile acids produces an increase in bile flow. The main function
Discussion

of bile acids is to allow digestion of dietary fats and oils by acting as a surfactant that emulsifies them into micelles, allowing them to be colloidally suspended in the chyme before further processing.

2.9.1 Preparation of the lipophilic anchors

As it was envisaged to couple the lipophilic moiety via amide bond formation to the cannabinoid receptor ligand moieties that have already been synthesised. Therefore, it was necessary to functionalise the steroid scaffolds of cholesterol and litocholic acid. This was achieved via Michael addition using tert-butyl acrylate to achieve tert-butyl ester NK366 which was subsequently deprotected under standard acidic conditions using TFA in methylene chloride to yield free acid NK380 (Scheme 43):

![Scheme 43](image)

Litocholic methyl ester was first synthesised from litocholic acid 105 using an excess of acetyl chloride. The resulting methyl ester NK371 was then used for forming tert-butyl ester NK372 via Michael addition using tert-butyl acrylate and subsequent saponification under standard acidic conditions using TFA in methylene chloride to yield free acid NK376 (Scheme 44):
2.9.2 Coupling to CB1R moieties

Acid NK380 was then coupled to the amines NK383 and NK388, respectively (see section 2.6.4) using EDCI and HOBT in methylene chloride to give NK384 and NK389 in good yields of 71%, respectively:

Figure 46: Cholesterol containing CB1R ligands NK384 and NK389.
**Discussion**

*NK376* was coupled to free amine *NK375* which contained a Fmoc-protected lysine using EDCI and HOBt in methylene chloride to give *NK377* in acceptable yields which was then deprotected due to the bulkiness of the Fluorenylmethyloxycarbonyl group and the resulting free amine was subsequently acetylated (Scheme 45):

![Scheme 45](image)

**Scheme 45: Reagents and conditions**: a) Et₂NH, DCM, 93%; b) AcCl, DIPEA, DCM, 89%.

A further approach for investigating the binding site of the cannabinoid receptor was realised during this PhD project by attaching a biotin moiety onto the above shown structures. The addition of a biotin unit, in theory, would then allow the use of these ligands as pharmacological probes by the addition of streptavidin conjugates containing for example fluorophores for receptor visualisation.
In the first step a hydrophilic linker in form of 8-(Fmoc-amino)-3,6-dioxaoctanoic acid was attached to NK386 using EDCI and HOBt as coupling reagents to give NK390 in excellent yields of 91%, which was subsequently deprotected using TFA in methylene chloride. The resulting free amine was then coupled to NK380 to give NK394 in reasonable yields. Finally, a further deprotection sequence using Et₂NH in methylene chloride and coupling of the free amine to NK30 gave NK397 in low yields (Scheme 46).

Scheme 46: Reagents and conditions: i) 8-(Fmoc-amino)-3,6-dioxaoctanoic acid, EDCI, HOBt, DCM, 91%; ii) TFA, DCM, 82%; iii) NK380, EDCI, HOBt, DCM, 45%; iv) Et₂NH, DCM, 78%; v) DIPEA, DCM, NK30, 55%.
Discussion

2.9.3 Overall CB1R Affinity Results for Synthesised Ligands

Unfortunately, these cholesterol-containing compounds NK377, NK379, NK384, NK389 and NK397 were found to be too lipophilic to allow establishment of \( K_i \) values.\(^{163}\) A large degree of non-specific binding in the membrane was suggested to occur, possibly indiscriminate sequestration into the lipid membrane without association to the CB1 GPCR, due to their high lipophilicity. Although the cholesterol was incorporated in order to localise the ligand in the membrane it had been hoped that this would then lead to long-term association to the CB1 receptor.

In summary, all the candidate CB1R binding compounds prepared in the synthetic sections were submitted for assay of their binding affinity toward the CB1 receptors in the group of A/Prof. Michelle Glass (UoA, FMHS). To our great disappointment, it was found that all of the synthesised CB1R ligands containing a long linker at the C3 position, both, hydrophilic and lipophilic, were found to have only very little affinity for the CB1 receptor, in some cases because of solubility issues. The soluble compounds were therefore excluded from accurate \( K_i \) determination assays by their inability to displace a radiolabelled CB1 standard ligand by at least 60%, a benchmark level that indicates extremely low receptor affinity.

Accordingly, we turned our attention to the next phase of our investigations, the development of covalent CB1R ligands.

2.10 Covalent Ligand Series for the Cannabinoid CB1 Receptor

To date selective targeting of specific pathways and the uncoupling of undesirable psychiatric effects has proven difficult to achieve. Although much progress in understanding the interaction of cannabinoid receptor ligands with CB\(_1\) has been made in recent years, more accurate understanding of the exact molecular determinants that determine affinity, functional activity and CB\(_1\)/CB\(_2\) subtype selectivity is required to design new generations of ligand candidates. Detailed investigation of the CB\(_1\) GPCR at the molecular level has been complicated by the lack of a crystal structure, however complementary methods including classical SAR, homology modelling and molecular dynamics simulations have provided useful insights.

Covalently binding ligands that form an irreversible bond to a receptor are useful probes for clarifying key ligand-receptor interactions. Formation of a covalent adduct offers useful insights into the pharmacologically relevant binding pose(s and receptor entry pathway of the ligand. Importantly for GPCRs in particular, this information can be complementary to that obtained by crystallography, which may require unusual intra-receptor interactions to be engineered for adequate stabilisation of the receptor to allow crystallisation. To be effective, a covalent ligand is required to possess a moderately reactive functional group that selectively functionalises a nucleophilic residue (usually lysine, serine,
threonine or cysteine) when bound to the target ortho- or allosteric site. Reactive functional groups successfully employed to date include \(\alpha\)-halo acetamides, maleimides and isothiocyanates. Ideally this group needs to be incorporated into the ligand without significantly altering the affinity, sub-type selectivity or functional activity of the parent compound.

The Makriyannis group has played a leading role in the development of covalent ligands for the cannabinoid receptors. The first compound deliberately designed to irreversibly bind to the cannabinoid receptor was AM841\(^{177}\) 41 a derivative of the phytocannabinoid receptor agonist \(\Delta^8\)-THC 2 containing an isothiocyanate group at the end of the lipophilic dimethylheptyl chain.\(^{178}\) Using similar reasoning, AM3677 106 based on an endocannabinoid framework also possesses a terminal isothiocyanate group. Aryl isothiocyanate 107 is a positive allosteric modulator for the agonist CP55,940\(^{179}\) 6. The diaryl pyrazole inverse agonist Rimonabant 14 was withdrawn from clinical use but has proven a highly adaptable scaffold for dissection of CB\(_1\) molecular pharmacology.\(^{180}\) In addition to the compounds mentioned above several other pharmacological probes have been developed to irreversibly bind the CB\(_1\) receptor.\(^{121}\)

![Figure 47: Reported covalent ligands for the CB\(_1\) receptor.](image)

Our own interest in covalent cannabinoid receptor ligands stemmed from investigations into the development of bivalent probes for the CB\(_1\) GPCR.\(^{181}\) In order to attach a second functional element to a CB\(_1\) receptor ligand, it is essential that any linker or linker element does not attenuate the affinity or selectivity of the parent ligand. A number of SAR studies have suggested that derivitisation at C3 of the pyrazole ring carboxamide substituent yields compounds that retain useful affinity at CB\(_1\). This site has been probed in some detail by a number of groups, in whole or in part driven by facile synthetic access to C3 analogues through the carboxylic acid. Further, this site has been used in the preparation of CB1-
Discussion

CB1 bivalent ligands by Zhang and Thomas. Recently, however, a growing body of evidence has suggested that both endogenous and synthetic cannabinoid receptor ligands may in fact enter the orthosteric site from the lipid bilayer via a portal between transmembrane (TM) domains VI and VII. Although the exact details of this mechanism remain to be clarified, molecular dynamics simulations suggest that ligand entry is driven by polar interactions at the receptor-membrane interface.

It has been proposed that in the case of the agonist 2-arachidonylglycerol these interactions alone are sufficient for activation of the CB1 receptor. This mode of entry would appear to be mutually exclusive to the use of pyrazole C3 as an attachment point for linkers suitably long to span the distance between GPCRs in a multimeric complex, or to extend a signalling motif such as a fluorophore or biotin tag into the extracellular matrix.

We aimed to

- (a) provide evidence that a relatively small covalently binding C3 substituent should lead to SR derivatives of high affinity,
- (b) further confirm the accepted binding pose of SR suggested by in silico homology modelling,
- (c) develop a foundation for covalent bivalent CB1 probes to elucidate cannabinoid GPCR oligomerisation, recycling and signalling.

2.10.1 Covalent binding ligands of 10 to 20 Å between C3 carboxamide and a covalent binding functional group.

Our approach started from a ligand-bound model of the CB1 receptor that has been computed by Jack Flanagan (FMHS, University of Auckland) that suggested a distance of approximately 10 to 20 Å between the C3-carboxamide of Rimonabant 14 and lysine 192 of the CB1 receptor.

Thus, it was envisaged to synthesise CB1R ligands based on the structure of Rimonabant 14 having a functional moiety in a distance of approximately 8 to 16 atoms between the C3 carboxamide and a irreversibly binding group which would enable the ligand to bind covalently to lysine of the CB1 receptor. Therefore, two further linkers were synthesised having 9 atoms and 16 atoms, respectively:

The synthesis of the new linkers started from 2,2’-(Ethylenedioxy)bis(ethylamine) which was mono-Boc protected to give NK247 in 95% yield, which was subsequently coupled to commercially available Cbz-protected caproic acid 109. Deprotection of the carboxybenzyl-group using H₂ / Pd gave amine NK279 (Scheme 47):
Discussion

Scheme 47: Synthesis of linkers NK247 and NK279. Reagents and conditions: i) DCM, DIPEA, Boc₂O, r.t., 95%; ii) EDCI, HOBt, DCM, 91%; iii) DCM, MeOH, H₂, Pd, qaunt..

NK600 was synthesised from 1,4 diamino butan 110 with 91% yield (Scheme 48):

Scheme 48: Synthesis of NK600. Reagents and conditions: i) CHCl₃, DIPEA, Boc₂O, r.t., 91%.

The synthesis of covalently binding CB1R ligands began from acid chloride NK25 which was connected to the mono-Boc protected diamines NK247, NK279 and NK600, respectively:

Scheme 49: Synthesis of amines NK251, NK289 and NK525. Reagents and conditions: first step: DCM, NEt₃, amine, 0°C; second step: DCM, TFA, r.t.
Discussion

The resulting Boc-protected amines **NK250**, **NK282** and **NK525** were subsequently deprotected using TFA in methylene chloride to give free amines **NK251**, **NK289** and **NK528** (Scheme 49).

Amines **NK251** and **NK289** (Ar = see scheme 49) were then transformed to isothiocynates **NK295** and **NK292** and to maleimides **NK260** and **NK281** (Scheme 50):

**Scheme 50**: Synthesis of **NK260**, **NK295**, **NK281** and **NK292**. Reagents and conditions: i) DCM, maleic anhydride, Ac₂O, reflux; ii) EtOH, CS₂, Boc₂O, DMAP.

**NK528** was coupled to amino acid 90 to give **NK302**. Deprotection of the Fmoc-group and subsequent conversion of free amine **NK303** using bromoacetyl chloride gave bromo-acetamide **NK304** (Scheme 51):
Discussion

Scheme 51: Synthesis of NK304. Reagents and conditions: i) EDCI, HOBt, DCM, 90, 78%; ii) DCM/Meoh, Et₂NH, quant.; iii) DCM, NEt₃, bromo acetylchloride, 80%.

2.10.2 Results

Compounds NK260, NK295, NK281, NK292 and NK304 were screened in the labs of Prof Michelle Glass for their binding affinity toward the hCB1 receptor at 10 μM. However, they did not displace the radioligand more than 50%, indicating that their $K_i$ values would be too low to be useful ligands in further testings. Following this disappointing result, further molecular modelling studies were carried out in collaboration with Dow Hurst to examine if the covalent approach might still be feasible.
2.10.3 Covalent binding ligands of 5 to 10 Å between C3 carboxamide and a covalent binding functional group

A further library of covalently binding CB1R-ligands was then investigated based on a second model of the CB1 receptor by Dow Hurst in the group of Professor Patricia Reggio, based on further refinement of the CB1 homology model. That model suggested a distance of approximately 4 to 8 atoms between the C3 carboxamide and an irreversibly binding group would enable the ligand to bind covalently to an adjacent cysteine of the CB1 receptor. Further, an energetically minimised model of a hypothetical covalent ligand bound via a 2-carbon linker and an isothiocyanate group to C7.42 appeared to support the feasibility of this revised approach (Figure 48).

**Figure 48:** Interatomic distances from the C3 carboxamide nitrogen of 1 to (left) three local cysteine sulfur atoms (gold) in CB1 TM VI and VII, and (right) isothiocyanate derivative NK530 docked at the CB1 orthosteric site, covalently bound though sulfur to cysteine residue C7.42 in CB1 TM VII. The Lys-carboxamide hydrogen bond is present in both models. The in silico structures are docked in the homology model developed by the Reggio group. Docking studies performed by Dr Jack Flannagan (and Mr Dow Hurst).
The synthesis started from acid chloride **NK25** which was coupled to mono-Boc-protected diamines **NK600**, **NK601** and **NK602**, respectively to give **NK523**, **NK524** and **NK525** (not shown) which were subsequently deprotected using a 3:1 mixture of methylene chloride and TFA. The resulting free amines **NK526**, **NK527** and **NK528** were then transformed to the alpha-bromo-acetamides **NK550**, **NK551** and **NK552** using acetyl chloride with reasonable yields between 75 to 85%.

A second batch of the free amines was transformed to the maleimides **NK547**, **NK548** and **NK549** using maleic anhydride and cat. amounts of Boc$_2$O with yields between 65 to 70%.

Finally, a third batch of the free amines was transformed to the isothiocyanates **NK529**, **NK530** and **NK531** using CS$_2$ with yields between 75 to 80% (Scheme 52):

**Scheme 52**: Synthesis of a library of covalent binding CB1R-ligands having an alpha-bromo acetamide moiety (**NK550-552**), a maleimide moiety (**NK547-549**) and a isothiocyanate moiety (**NK529-531**), respectively.

The aforementioned compounds were incubated with membranes over-expressing the CB1 GPCR. The membranes were then washed exhaustively and the amount of ligand remaining bound to the receptors estimated.

The columns marked give the estimated percentage of test compound washed out of the membrane, implying that the amount remaining covalently bound is (100 - compound V)%.
In the above graphs, V-V represents full wash-out (100% radioligand binding) and V-SR represents the maximal possible radioligand displacement by Rimonabant (0%). All the other conditions have been normalised to these conditions.

V-529, V-530, V-531, V-550, V-551 and V-552 represent the maximal radioligand displacements produced by NK529, NK530, NK531, NK550, NK551 and NK552, respectively. NK529, NK530 and NK552 displaced the radioligand at a level comparable with Rimonabant, while NK531 and NK551 both produced an even greater displacement - indicating a higher affinity for the receptor.

529-V, 530-V, 531-V, 550-V, 551-V and 552-V represents the radioligand bound to the membrane following 3 washes/incubations to remove the putative covalent ligands from the membrane. Thus, this
represents the total amount of compound removed – indicating how well the ligands stay bound to the CB1 receptor. It was found that NK530 remained ~70% and NK552 ~50% irreversibly bound to the membrane on average, while NK531 was only ~35% irreversibly bound and NK529 was completely washed out. SR-V represents Rimonabant 14 wash-out, which was ~96% on average.

Table 5: Irreversible CB1 binding of novel ligands NK529, NK530, NK531, NK550, NK551 and NK552.

These results indicate that covalent binding appears to be possible using this final compound design. From Table 5 it can be seen that significant amounts of irreversible binding was able to be achieved with this second series of covalent ligands, with the best result being 70% binding demonstrated for the three-carbon isothiocyanate compounds NK530. Although the maleimides NK547-549 were not observed to bind covalently, promising results were also seen in the bromoacetamide series where NK551 and NK552 showed approximately 50% retention after washout.

The data suggests that the C3 substituent of the pyrazole core of Rimonabant 14 is important for receptor interactions involved in affinity and possibly entry to the orthosteric pocket of the CB1 GPCR. This helps understand why substantially longer or bulkier substituents lead to compounds of reduced affinity, despite the prevailing SAR in the literature at the outset of this study.

As a result, re-evaluation of different linker attachment positions is likely to be more successful for design of divalent or multifunctional CB1R ligands. Further, using different positions may still allow for the possibility of high-affinity or longer duration of action modulators of CB1 activity through the incorporation of covalent binding motifs as demonstrated here.
Summary & future work

One of the main goals of this PhD-project was to investigate the possibility of preparing a CB1-D2 receptor heterodivalent ligand by the linkage of one ligand having CB1 affinity to a second having D2R affinity, via a variable linker of various lengths. This ligand would be used to investigate and/or promote heterodimerisation to form a CB1-D2 receptor complex. It further has to be mentioned that there is no clear literature evidence that such a heterodimer exists.

In summary, a number of synthetic strategies to allow linker attachment to the pyrazole scaffold of CB1 inverse agonist Rimonabant 14 have been investigated. Based on previously published results, the C3 position was thoroughly investigated as the most likely linker attachment point. Initial results, and those of related literature SAR studies showed that short linkers had a higher affinity toward the CB1 receptor than longer linkers:

![Chemical structures](image-url)

**Figure 50:** Initial CB1 inverse agonist ligands synthesised that showed promising binding affinity for the CB1 receptor.

Building from this early work the synthesis of hydrophilic and lipophilic linkers of varying molecular composition having lengths of up to 40 atoms was carried out. These linkers were then used to tether the core structure of Rimonabant 14 to the D2R antagonist NK246 to yield a library of different CB1-D2 receptor heterodivalent ligands and their appropriate control compounds.
Discussion

Figure 51: Representative CB1-D2 receptor heterodivalent ligands synthesised for evaluation during this study.

Binding affinity data showed that the CB1-D2 receptor heterodivalent ligands synthesised had extremely strong affinity for the D2 receptor. Affinity toward the CB1 receptor was poor in all cases, however, most likely due to the lipid entry to the receptor of this ligand family and/or the key involvement of C3 groups in ligand recognition.

Finally, based on different CB1 receptor molecular modelling data, a library of various CB1R ligands designed to covalently bind the CB1 receptor was synthesised. These novel ligands incorporated an isothiocyanate, bromoacetamide or maleimide moiety anticipated to bind covalently to C7.42 of the CB1 GPCR.
The best-performing ligands designed to bind covalently at CB1 showed excellent affinity, comparable to Rimonabant, and data in wash-out experiments that strongly suggests effective irreversible receptor binding of up to 70%.

Currently, the four most promising covalent ligands **NK530, NK531, NK551** and **NK552** are undergoing further affinity screening in the Glass labs, against Cys→Ala 7.42 mutants of the CB1 receptor, to confirm whether or not this is the position of covalent binding.
Taken together, the results of this study show clearly that attachment of long linkers required for formation of multifunctional CB1R ligands using the Rimonabant pharmacophore is unlikely to be achieved using the C3 position of the pyrazole core.

This conclusion is further borne out by work carried out concurrently in the research group by BSc(Hons) student Phillip Grant, who evaluated the attachment of linkers of varying length to either the N1 position or C5 position of the central pyrazole (Scheme 53).

Scheme 53: Reagents and conditions: a) i) LiHMDS, ether, diethyloxalate, rt., 20 h; b) hydrazine, EtOH, rt, 20 h, 78% over two steps; c) KOH, H2O/MeOH, reflux, 16 h, quant.; d) oxalyl chloride, DMF, DCM, 1 aminopiperidine, DIPEA, DCM, rt, 16 h, 74%; e) tert-butyl 6-bromohexanoate, K2CO3, DMF, rt., 24 h, 68%; f) TFA, DCM, rt., 0.5 h, quant.

Pyrazole 702 was prepared by Knorr pyrazole synthesis and, upon alkylation, afforded 703 bearing a carboxylic acid handle to facilitate linker conjugation.
Discussion

Linkers of varying length and composition were appended to acid 703:

![Chemical Structures](image)

**Figure 53:** Different CB1R ligands based on Rimonabant having linkers of varying length at the N1 position synthesised by other researchers in our research group.

The affinities of compounds 704 to 709 were tested in vitro and compared to the parent acid. However, all derivatives had poor affinity of >10,000 nM. This strongly suggests that modification of N1 as per our current approach will not be a productive route for linker attachment. The very low affinities prevented any further comparison of the relative affinities of compounds 704-709.

More success was achieved by functionalisation of the Rimonabant core at the C5 position.
Discussion

Scheme 54: Synthesis of a Rimonabant analogue having a bromine substituent as a functional group for Suzuki couplings. Reagents and Conditions: a) i) LiHMDS, ether, 0 °C, diethyl oxalate, ether, rt., 16 h, 56%; b) 2, 4-dichlorophenylhydrazine HCl, EtOH, reflux, 16 h, 71%; c) KOH, H2O/MeOH, reflux, 16 h, quant.; d) oxalyl chloride, DMF, DCM, 1-aminopiperidine, DIPEA, rt, 16 h, 93%.

The synthesis of 714 started under the before optimised conditions using 4-bromo-propiophenone instead of 4-chloro-propiophenone as for Rimonabant.

Scheme 55: Sonogashira coupling to afford the depicted alkyne for enabling further functionalisation. Reagents and Conditions: a) 3-butyne-1-ol, Pd(OAc)2, PPh3, Cul, NEt3, 80 °C, 16 h, 92%.

Linkers of varying length and composition were then coupled to alcohol 715 and their affinities were determined in vitro and compared with the parent compound:
Figure 54: C5 Modified Rimonabant-derived CB1R ligands having linkers of varying length also synthesised in the research group.

These findings suggested that linkers of various lengths connected to the C5 position of the Rimonabant core are very well tolerated, giving $K_i$ values within an order of magnitude of the parent compound. Remarkably, when the ester substituent of the linker was extended to 10 carbons to give \textit{721}, the
Discussion

presence of an amine group that would be charged at physiological pH did not attenuate the CB1 affinity with respect to the Rimonabant lead compound. These last results indicate that the C5 position is likely to be the most successful in ongoing work towards CB1 divalent or multifunctional compounds.

Future work will focus on extension of these longer C5 linkers to include biotin, fluorophores, D2R agonist(s) and CB1 inverse agonists and evaluation of their affinity and functional behaviour at CB1. These results will finally determine the feasibility of divalent ligand design as a useful modality in cannabinoid pharmacology.
Chapter 3 Experimental

3.1 General Information

All reactions were carried out in flame- or oven-dried glassware under a dry nitrogen atmosphere. All reagents were purchased as reagent grade and used without further purification. Dimethyl formamide was degassed and dried using an LC Technical SP-1 solvent purification system. Ethanol was distilled over Mg(OEt)$_2$. Ethyl acetate, methanol, and petroleum ether were distilled prior to use. All other solvents were used as received unless stated otherwise. RP-HPLC was performed with an Agilent 1100 using a Jupiter C$_{18}$ 300 Å, 5 µm, 2.0 mm x 250 mm column at a flow rate of 0.2 mLmin$^{-1}$ with a DAD Detector operating at 262, 280 and 320 nm. A suitably adjusted gradient of 5% B to 100% B was used, where solvent A was 0.1% HCOOH in H$_2$O and B was 20 % A in MeCN. Flash chromatography was carried out using 0.063-0.1 mm silica gel with the desired solvent. Thin layer chromatography (TLC) was performed using 0.2 mm Kieselgel F254 (Merck) silica plates and compounds were visualised using UV irradiation at 254 or 365 nm and/or staining with a solution of potassium permanganate and potassium carbonate in aqueous sodium hydroxide. Preparative TLC was performed using 500 µm, 20 x 20 cm Uniplate™ (Analtech) silica gel TLC plates and compounds were visualised using UV irradiation at 254 or 365 nm. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Infrared spectra were obtained using a Perkin-Elmer Spectrum 100 FTIR spectrometer on a film ATR sampling accessory. Absorption maxima are expressed in wavenumbers (cm$^{-1}$). NMR spectra were recorded as indicated on either a Bruker Avance 400 spectrometer operating at 400 MHz for $^1$H nuclei and 100 MHz for $^{13}$C nuclei, a Bruker DRX-400 spectrometer operating at 400 MHz for $^1$H nuclei, 100 MHz for $^{13}$C nuclei, a Bruker Avance AVIII-HD 500 spectrometer operating at 500 MHz for $^1$H nuclei, 125 MHz for $^{13}$C nuclei or a Bruker Avance 600 spectrometer operating at 600 MHz for $^1$H nuclei, 150 MHz for $^{13}$C nuclei. $^1$H and $^{13}$C chemical shifts are reported in parts per million (ppm) relative to CDCl$_3$ ($^1$H and $^{13}$C) or (CD$_3$)$_2$SO ($^1$H and $^{13}$C). High resolution mass spectra were recorded on a Bruker micrOTOF-Q II mass spectrometer with ESI ionisation source. Ultraviolet-visible spectra were run as H$_2$O solutions on a Shimadzu UV-2101PC scanning spectrophotometer.
Experimental

General procedure for the mono-Boc-protection of diamines

**GP 1A:**

To an ice-cooled solution of the respective diamine (44.0 mmol, 5.0 equiv.) in chloroform (30 mL) was added Boc₂O (1.92 g, 8.80 mmol, 1 equiv.) in chloroform (15 mL) over 1h under vigorous stirring. The solution was allowed to warm to r.t. and stirred for additional 14 h. The precipitate was filtered, the solvent was removed *in vacuo* and the residue dissolved in ethyl acetate (100 mL). The organic layer was washed with brine (2 x 20 mL), the aqueous layer was extracted with ethyl acetate (1 x 15 mL), the combined organic layers were dried with MgSO₄ filtered and the solvent removed *in vacuo* to afford the respective mono-Boc-protected diamine.

**GP 1B:**

To a solution of the respective diamine (27.5 mmol, 3.0 equiv.) in methylene chloride (50 mL) and DIPEA (1.56 mL, 9.16 mmol, 1.0 equiv.) was added Boc₂O (2.0 g, 9.16 mmol, 1 equiv.) dissolved in methylene chloride (20 mL) over a period of 1 h under vigorous stirring. The mixture was stirred for an additional hour, the solvent was removed *in vacuo* and the crude product was purified over silica to afford the respective mono-Boc-protected diamine.

General procedure for amide formation via acid-chloride NK25:

**GP 2:**

To an ice-cooled solution of a mono-Boc-protected amine (2.17 mmol, 1.2 equiv.) in methylene chloride (20 mL) and NEt₃ (1.25 mL, 9.0 mmol, 5.0 equiv.) was slowly added acid chloride NK25 (720 mg, 1.8 mmol, 1.0 equiv.). The solution was stirred at r.t. for another 30 minutes after which it was diluted with EtOAc (30 mL) and washed with 1 N aq. HCl (10 mL) and brine (10 mL), dried with MgSO₄, filtered and the solvent removed *in vacuo*. Purification over silica afforded the desired Boc-protected amide.

General procedure for Boc deprotection:

**GP 3:**

The Boc-protected amide (1.2 mmol, 1.0 equiv.) was dissolved in a 1:1 mixture of DCM/TFA (10 mL). Upon completion of the deprotection the solvent was removed *in vacuo*, the residue dissolved in EtOAc (20 mL) and washed with a sat. aqueous NaHCO₃ solution (10 mL), followed by drying with MgSO₄ and filtering. The solvent was removed *in vacuo* and the crude product purified via flash chromatography.
**General procedure for the preparation of isothiocyanates:**

**GP 4:**

The free amine (0.5 mmol, 1.0 equiv) was dissolved in abs. EtOH (2 mL) followed by subsequent addition of CS₂ (300 µL, 5.0 mmol, 10.0 equiv.) and NEt₃ (70 µL, 0.5 mmol, 1.0 equiv) at rt. The solution was stirred for 15 minutes after which time Boc₂O(s) (98 mg, 0.45 mmol, 0.9 equiv.) and DMAP (3 mg, 0.03 mmol, 0.05 equiv.) were added. Upon completion of the isothiocyanate formation the solvent was removed *in vacuo* and the crude product was purified via flash chromatography to afford the desired isothiocyanate.

**General procedure for the synthesis of bromoacetamides:**

**GP 5:**

The respective free amine (0.5 mmol, 1.0 equiv.) was dissolved in methylene chloride (3 mL) and NEt₃ (140 µL, 1.0 mmol, 2.0 equiv.). The solution was cooled to 0 °C after which bromoacetyl bromide (53 µL, 0.6 mmol, 1.2 equiv.) was added. Upon completion of the reaction the solvent was removed *in vacuo* and the crude product was purified via flash chromatography to afford the desired bromoacetamide.

**General procedure for the synthesis of maleimides:**

**GP 6A:**

The respective free amine (0.5 mmol, 1.0 equiv.) was dissolved in methylene chloride (3 mL) and maleic anhydride (0.55 mmol, 1.1 equiv.) was added. After 50 minutes the solvent was removed *in vacuo* and the free acid was purified over silica (Step 1). It was then dissolved in acetic anhydride (2 mL) and refluxed for 30 minutes. After cooling to room temperature the solution was carefully quenched with NH₄Cl(s) and stirred at r.t. for another 10 minutes after which time it was diluted with EtOAc (10 mL) and washed with water (10 mL) and brine (10 mL), dried with MgSO₄, filtered and the solvent removed *in vacuo*. Purification over silica afforded the desired maleimide (Step 2).

**GP 6B:**

The respective free amine (0.5 mmol, 1.0 equiv) was dissolved in methylene chloride (3 mL) and maleic anhydride (0.55 mmol, 1.1 equiv.) was added. After 50 minutes the solvent was removed *in vacuo* and the residue was then dissolved in acetic anhydride (2 mL) and refluxed for 30 minutes. After cooling to
room temperature the solution was carefully quenched with NH$_4$Cl(s) and stirred at r.t. for another 10 minutes after which time it was diluted with EtOAc (10 mL) and washed with water (10 mL) and brine (10 mL), dried with MgSO$_4$, filtered and the solvent removed in vacuo. Purification over silica afforded the desired maleimide.

### 1,6 Dimethoxynaphthalene NK227

![NK227](image)

To a suspension of 1.6-dihydroxynaphtalene (7.0 g, 43.7 mmol, 1.0 equiv.) and K$_2$CO$_3$ (22.3 g, 161.7 mmol, 3.7 equiv.) in acetone (200 mL) was added dropwise Me$_2$SO$_4$ (14.5 mL, 152.6 mmol, 3.5 equiv.) under a nitrogen atmosphere. The reaction mixture was then refluxed for 5 h and subsequently cooled to rt and quenched with a 10% aq. ammonia solution (50 mL) and stirred for another 30 min. The insoluble salts were removed by filtration and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography (hexanes/DCM 2:1) to give NK227 as a white solid (7.2 g, 88%).

$^1$H NMR (300 MHz, CDCl$_3$): $\delta = 8.20$ (d, $J = 9.00$ Hz, 1H), 7.42 - 7.32 (m, 2H), 7.19 - 7.11 (m, 2H), 6.71 (dd, $J = 6.40$, 2.20 Hz, 1 H), 4.00 (s, 3 H), 3.93 ppm (s, 3 H).

Spectroscopic data is consistent with that reported in the literature.$^{82}$

### 5-methoxy-3,4-dihyronaphthalen-2(1H)-one NK228

![NK228](image)

To a warm solution (40 °C) of NK227 (7.0 g, 37.2 mmol, 1.0 equiv.) in EtOH (120 mL) was carefully added small pieces of sodium (7.3 g, 320.0 mmol, 8.5 equiv.) over a period of 1 h. After the last addition the reaction mixture was refluxed for 2.5 h and subsequently cooled to rt. It was quenched with conc. HCl to pH < 2 and afterwards refluxed for 1 hour and then diluted with water and extracted with
methylene chloride (3 x 100 mL). The combined organic layers were dried with MgSO₄, filtered and concentrated in vacuo. The crude product was purified by flash chromatography (hexanes/EtOAc 5:1) to give tetralone **NK228** as a brown oil (4.32 g, 66%).

**1H NMR (300 MHz, CDCl₃):**

δ = 7.14 (t, J = 8.1 Hz, 1H), 6.77 (d, J = 8.1 Hz, 1H), 6.71 (d, J = 8.1 Hz, 1H), 3.84 (s, 3H), 3.54 (s, 2H), 3.07 (t, J = 6.7 Hz, 2H), 2.50 ppm (t, J = 6.7 Hz, 2H).

**13C NMR (75 MHz, CDCl₃):**

δ = 210.7, 156.4, 135.0, 128.2, 127.6, 126.9, 126.8, 125.0, 108.5, 55.4, 44.6, 37.9, 21.0 ppm.²⁻¹⁸³

Spectroscopic data is consistent with that reported in the literature.²⁻¹⁸³

**5-methoxy-N-propyl-1,2,3,4-tetrahydronaphthalen-2-amine NK229**

To a solution of tetralone **NK228** (4.0 g, 22.7 mmol, 1.0 equiv.) in methylene chloride (75 mL) was added 1-propylamine (2.05 mL, 25.0 mmol, 1.1 equiv.) and AcOH (2.6 mL, 45.4 mmol, 2.0 equiv.) The mixture was stirred for 30 min at rt under a nitrogen atmosphere. Then NaBH₃CN was added (4.28 g, 68.1 mmol, 3.0 equiv.) and the resulting suspension was stirred for 14h under a nitrogen atmosphere. The reaction mixture was concentrated in vacuo and the residue was dissolved in a mixture of EtOAc (70 mL) and aq. sat. NaHCO₃ (70 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 x 50 mL). The combined organic layers were dried with MgSO₄, filtered and concentrated in vacuo. The crude oil was purified by flash chromatography (EtOAc/ULTRA 9:1) to give amine **NK229** as a yellow oil (3.73 g, 75%).

**1H NMR (300 MHz, CDCl₃):**

δ = 7.10 (t, J = 8.1 Hz, 1 H), 6.68 (d, J = 8.1 Hz, 1 H), 6.71 (d, J = 8.1 Hz, 1 H), 3.79 (s, 3 H), 3.43-3.27 (m, 1 H), 3.26-3.12 (m, 1 H), 3.11-2.93 (m, 4 H), 2.67-2.49 (m, 1 H), 2.43-2.28 (m, 1 H), 1.96-1.74 (m, 3 H), 1.02 ppm (t, J = 7.4 Hz, 3 H).
Experimental

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta = 157.1, 132.9, 127.0, 123.4, 121.2, 107.9, 55.3, 55.2, 47.1, 31.9, 25.3, 21.9, 19.5, 11.1$ ppm.$^{82}$

Spectroscopic data is consistent with that reported in the literature.$^{82}$

2-(4-nitrophenyl)acetyl chloride NK230

To a solution of 4-nitrophenylacetic acid (3.85 g, 21.2 mmol, 1.25 equiv.) in chloroform (40 mL) was added (COCl)$_2$ (3.6 mL, 42.4 mmol, 2.5 equiv.) and DMF (0.1 mL) and the mixture stirred for 30 min. The reaction mixture was concentrated _in vacuo_ and re-dissolved in toluene (3 x 15 mL). The brown solid NK230 was used as obtained in the next step.

5-methoxy-N-(4-nitrophenethyl)-N-propyl-1,2,3,4-tetrahydronaphthalen-2-amine NK231

To an ice-cooled solution of amine NK229 (3.73 g, 17.0 mmol, 1.0 equiv.) in methylene chloride (25 mL) and 1N aq. NaOH (20 mL) was added dropwise a solution of acid chloride NK230 (4.2 g, 21.2 mmol, 1.25 equiv.) in methylene chloride (20 mL). The solution was then warmed to rt and stirred for another 30 min. The reaction mixture was then concentrated _in vacuo_ and the precipitate was dissolved in EtOAc (60 mL) and washed with brine (3 x 30 mL) and NaHCO$_3$ (30 mL). The organic phase was dried with MgSO$_4$, filtered and concentrated _in vacuo_. The crude product was purified by flash chromatography (hexanes/EtOAc 3:2) to give amide NK231 as a yellow foam (6.0 g, 93%).
Experimental

$^1$H NMR (300 MHz, CDCl3):

δ = 8.24 - 8.13 (m, 2H), 7.50 - 7.37 (m, 2H), 7.18 - 7.04 (m, 1H), 6.74 - 6.62 (m, 2H), 3.87 - 3.77 (m, 5H), 3.26 - 3.12 (m, 2H), 3.08 - 2.93 (m, 2H), 2.90 - 2.37 (m, 3H), 1.96 - 1.74 (m, 2H), 1.74 – 1.56 (m, 2H), 0.99 – 0.86 ppm (m, 3H).

Spectroscopic data is consistent with that reported in the literature.$^{82}$

N-(4-aminophenethyl)-5-methoxy-N-propyl-1,2,3,4-tetrahydronaphthalen-2-amine NK239

\[
\begin{align*}
\text{OMe} & \quad \text{N} \\
\text{C} & \quad \text{C} \\
\text{N} & \quad \text{N} \\
\end{align*}
\]

NK239

To an ice-cooled solution of BH$_3$·SMe$_2$ (4.6 mL, 48.6 mmol, 3.1 equiv.) in THF (70 mL) was slowly added a solution of nitroamide NK231 (6.0 g, 15.7 mmol, 1.0 equiv) in THF (20 mL). The reaction mixture was stirred for 15 min at 0 °C and then refluxed for 4h. After cooling to rt the reaction mixture was carefully quenched with 6 N aq. HCl. The resulting solution was dissolved in water and basified with 4 N aq. NaOH to pH > 12. The aqueous solution was extracted with methylene chloride (3 x 40 mL). The combined organic layers were dried with MgSO$_4$, filtered and concentrated in vacuo. The resulting brown oil was dissolved in EtOH (250 mL) and warmed to 50 °C. Hydrazine (10 mL, 205 mmol, 13.0 equiv) and a catalytic amount of Raney-nickel were added and stirred for 3h. After cooling to rt the mixture was filtered over kieselgur and concentrated in vacuo. The resulting yellow oil was purified by flash chromatography (hexanes/EtOAc/NEt$_3$ 50:50:1) to give NK239 as a yellow oil (3.6 g, 68% over 2 steps).

$^1$H NMR (300 MHz, CDCl3):

δ = 8.24 - 8.13 (m, 2H), 7.50 - 7.37 (m, 2H), 7.18 - 7.04 (m, 1H), 6.74 - 6.62 (m, 2H), 3.87 - 3.77 (m, 5H), 3.26 - 3.12 (m, 1H), 3.11 - 2.93 (m, 4H), 2.67 - 2.49 (m, 1H), 2.43 - 2.28 (m, 1H), 1.96 - 1.74 (m, 3H), 1.02 ppm (t, J = 7.4 Hz, 3 H).

$^{13}$C NMR (75 MHz, CDCl3):

δ = 157.1, 144.3, 138.1 130.9, 129.5, 126.1, 125.3, 121.7, 121.6, 115.2, 106.8, 56.8, 55.2, 53.3, 52.7, 35.1, 32.3, 25.8, 23.8, 22.2, 11.1 ppm.

Spectroscopic data is consistent with that reported in the literature.$^{82}$
Experimental

6-((4-aminophenethyl)(propyl)amino)-5,6,7,8-tetrahydronaphthalen-1-ol NK242

To a cooled solution (-78 °C) of NK239 (3.6 g, 10.7 mmol, 1.0 equiv.) in methylene chloride (70 mL) was slowly added a 1M BBr₃ solution (21.4 mL, 21.4 mmol, 2.0 equiv.). The reaction mixture was stirred for 3h at -78 °C and then slowly allowed to warm to rt and stirred for additional 12h. The brown precipitate was dissolved in hot brine solution (150 mL) and the aqueous layer was washed with EtOAc (2 x 50 mL). The aqueous layer was then basified to pH > 9 with 1N aq. NaOH and extracted with methylene chloride (5 x 60 mL). The combined organic layers were dried with MgSO₄, filtered and concentrated in vacuo. The crude product was purified by flash chromatography (hexanes/EtOAc 1:1.5 + 1% NEt₃) to give NK242 as a brown solid (2.5 g, 73%).

³¹H NMR (300 MHz, CDCl₃):  δ = 7.05 - 6.95 (m, 3H), 6.73 - 6.54 (m, 4H), 3.07 - 2.48 (m, 10H), 2.16 - 2.04 (m, 1H), 1.70 - 1.45 (m, 3H), 0.90 ppm (t, J = 7.4 Hz, 3H).

¹³C NMR (75 MHz, CDCl₃):  δ = 153.4, 144.3, 138.5 130.9, 129.5, 126.4, 122.9, 121.7, 115.3, 111.9, 56.7, 53.2, 52.7, 34.9, 32.8, 25.7, 23.6, 22.1, 11.9 ppm.

Spectroscopic data is consistent with that reported in the literature.⁸²

4-(benzyloxy)-4-oxobutanoic acid NK192

To a solution of benzylic alcohol (1.0 g, 9.3 mmol, 1.0 equiv.) DMAP (250 mg, 0.18 mmol, 0.2 equiv.) in chloroform (10 mL) was added succinic anhydride (1.26 g, 1.26 mmol, 1.26 equiv.) and stirred for 15h. 1N HCl was added (5 mL) to the solution followed by extraction with EtOAc (3 x 20 mL). The combined organic layers were dried with Na₂SO₄, filtered and concentrated in vacuo to give NK192 as a white solid (1.6 g, 85%).
**Experimental**

**H NMR (400 MHz, CDCl3):** \( \delta = 7.38 - 7.29 \text{ (m, 5H)}, 5.14 \text{ (s, 2H)}, 2.74 - 2.64 \text{ ppm (m, 4H)}. \)

Spectroscopic data is consistent with that reported in the literature.\(^ {184}\)

**benzyl 4-(4-((5-hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino)ethyl)phenylamino)-4-oxobutanoate NK421**

![NK421](image)

To a solution of **NK192** (703 mg, 3.38 mmol, 1.1 equiv.) in DMF (12 mL) was added HATU (1.22 g, 3.23 mmol, 1.05 equiv.) and NEt\(_3\) (1.28 mL, 9.24 mmol, 3.0 equiv.) and stirred for 20 min under a nitrogen atmosphere. Then **NK242** (1.0 g, 3.08 mmol, 1.0 equiv.) dissolved in DMF (5 mL) was added and stirred for 14h. The reaction mixture was diluted with EtOAc (120 mL) and brine (60 mL) and extracted with Et\(_2\)O (5 x 50 mL). The combined organic layers were dried with MgSO\(_4\), filtered and concentrated in vacuo. The crude product was purified by flash chromatography (EtOAc/hexanes 7:1) to give **NK421** as a brown oil (1.07 g, 68%).

**H NMR (400 MHz, CDCl3):** \( \delta = 7.77 \text{ (br s, 1H)}, 7.36 \text{ (d, } J = 8.1 \text{ Hz, 2H)}, 7.32 - 7.26 \text{ (m, 5H)}, 7.12 \text{ (d, } J = 8.1 \text{ Hz, 2H)}, 6.93 \text{ (t, } J = 8.1 \text{ Hz, 1H)}, 6.63 \text{ (d, } J = 8.1 \text{ Hz, 1H)}, 6.57 \text{ (d, } J = 8.1 \text{ Hz, 1H)}, 5.11 \text{ (s, 2H)}, 3.02 - 2.80 \text{ (m, 3H)}, 2.80 - 2.67 \text{ (m, 7H)}, 2.65 - 2.59 \text{ (m, 2H)}, 2.59 - 2.46 \text{ (m, 3H)}, 2.09 - 2.01 \text{ (m, 1H)}, 1.62 - 1.44 \text{ (m, 3H)}, 0.88 \text{ ppm (t, } J = 7.5 \text{ Hz, 3H)}.\)

**C NMR (100 MHz, CDCl3):** \( \delta = 173.0, 169.8, 153.7, 138.1, 136.7, 135.6, 135.5, 129.1, 128.5, 128.2, 128.1, 126.2, 123.2, 121.3, 120.0, 112.0, 66.7, 56.7, 52.7, 52.5, 34.9, 32.1, 31.9, 29.5, 25.6, 23.6, 21.8, 11.9 \text{ ppm}.\)

**HRMS (EI, 200 °C):** calculated for C\(_{32}\)H\(_{38}\)N\(_2\)O\(_4\)Na [M+Na]: \( m/z = 537.2724 \), found: \( m/z = 537.2712 \).

**IR (Film):** \( \tilde{\nu} = 2927, 1648, 1552, 1516, 1474, 1373 \text{ cm}^{-1}. \)
Experimental

4-(4-(2-((5-hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino)ethyl)phenylamino)-4-oxobutanoic acid NK246

\[
\begin{align*}
\text{NK246}
\end{align*}
\]

**NK421** (1.07 g, 2.09 mmol, 1.0 equiv.) was dissolved in MeOH (13 mL) and conc. HCl (0.5 mL) and a cat. Amount of Pd/C was added. The reaction mixture was then hydrogenated with H\(_2\)(g) for 12h, after which time it was filtered over celite. The filtrate was concentrated in vacuo and the crude product **NK246** was obtained as brown solid (890 mg, quant.).

\[^1\text{H}\text{ NMR (500 MHz, MeOD)}: \delta = 7.47 (d, J = 8.5 \text{ Hz}, 2 \text{H}), 7.16 (d, J = 8.5 \text{ Hz}, 2 \text{H}), 6.93 (d, J = 7.9 \text{ Hz}, 1 \text{H}), 6.62 - 6.57 (m, 2 \text{H}), 3.50 (m, 1 \text{H}), 3.23 - 3.17 (m, 2 \text{H}), 3.08 - 3.01 (m, 3 \text{H}), 3.01 - 2.97 (m, 1 \text{H}), 2.97 - 2.90 (m, 3 \text{H}), 2.58 (m, 5 \text{H}), 2.30 - 2.22 (m, 1 \text{H}), 1.82 - 1.69 (m, 3 \text{H}), 1.00 \text{ ppm (t, } J = 7.5 \text{ Hz, 3H}).\]

\[^{13}\text{C NMR (75 MHz, MeOD)}: \delta = 180.3, 174.1, 156.0, 138.9, 136.0, 134.3, 130.1, 127.7, 123.5, 121.4, 113.2, 60.6, 53.6, 53.4, 34.5, 33.8, 32.7, 31.5, 25.4, 24.0, 20.4, 11.6 \text{ ppm.}\]

**HRMS (EI, 200 °C):**
Calculated for C\(_{25}\)H\(_{33}\)N\(_2\)O\(_4\) [M+H]: m/z = 425.2435, found: m/z = 425.2421.

**IR (Film):**
\(\tilde{\nu} = 2932, 1661, 1538, 1514, 1463, 1393, 1278, 825, 770, 712 \text{ cm}^{-1}\).

**Melting point:**
82 – 85 °C.
Experimental

Methyl 6-aminohexanoate NK323

\[
\text{H}_2\text{N} \xrightarrow[\text{O}]{} \text{O}\text{Me}
\]

NK323

To an ice-cooled slurry of 6-aminocaproic acid (1.97 g, 15.0 mmol, 1.0 equiv.) in methanol (120 mL) was slowly added thionyl chloride (11.0 mL, 150 mmol, 10.0 equiv.). The solution was then allowed to warm to rt and subsequently refluxed for 5 hours. The solvent was removed \textit{in vacuo} to give NK323 as a white precipitate (2.12 g, 78%, HCl-salt) which was used in the next step without further purification.

\(^1\text{H NMR (300 MHz, CDCl3):} \) \(\delta = 8.24 \text{ (br s, 2H)}, 3.64 \text{ (s, 3H)}, 3.14 - 2.86 \text{ (m, 2H)}, 2.31 \text{ (t, } J = 6.5 \text{ Hz, 2H)} 1.88 - 1.72 \text{ (m, 2H)}, 1.71 - 1.56 \text{ (m, 2H)}, 1.50 - 1.36 \text{ ppm (m, 2H).}

Spectroscopic data is consistent with that reported in the literature.\(^ {158}\)

\textit{tert}-butyl piperazine-1-carboxylate NK58

\[
\text{\large{\text{\begin{array}{c}
\text{H} \\
\text{N} \\
\text{O} \\
\text{O}
\end{array}\}}}}
\]

NK58

Mono-boc protected piperazine NK58 was prepared according to \textbf{GP 1A} to afford a white solid (6.23 g, 76%) after purification over silica (EtOAc/ULTRA 1:2).

\(^1\text{H NMR (400 MHz, CDCl3):} \) \(\delta = 3.38 \text{ (t, } J = 5.3 \text{ Hz, 4H)}, 2.80 \text{ (t, } J = 5.3 \text{ Hz, 4H)}, 1.46 \text{ ppm (s, 9H).}

Spectroscopic data is consistent with that reported in the literature.\(^ {157}\)

\textit{tert}-butyl 4-(2-ethoxy-2-oxoethyl)piperazine-1-carboxylate NK60

\[
\text{\large{\text{\begin{array}{c}
\text{O} \\
\text{O}
\end{array}\}}}}
\]

NK60

To an ice-cooled solution of mono-Boc protected piperazine NK58 (5.0 mmol, 931 mg, 1.0 equiv.) in methylene chloride (35 mL) and DIPEA (7.5 mmol, 1.29 mL, 1.5 equiv.) was slowly added ethyl chloroacetate (5.5 mmol, 0.59 mL, 1.1 equiv.). After stirring for further 30 minutes the solution was...
Experimental

quenched with water and diluted with EtOAc (90 mL), washed with 1 N aq. HCl, washed with brine, dried with MgSO₄, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/hexanes 1:3) afforded NK60 as a white solid.

¹H NMR (400 MHz, CDC3):  δ = 4.18 (q, J = 6.7 Hz, 2H), 3.47 (t, J = 5.3 Hz, 4H), 3.22 (s, 2H), 2.52 (t, J = 5.3 Hz, 4H), 1.45 (s, 9H), 1.27 ppm (t, J = 6.7 Hz, 3H).

Spectroscopic data is consistent with that reported in the literature.¹⁸⁵

ethyl 2-(piperazin-1-yl)acetate NK62

NK62 was prepared according to GP 3 to give a white solid (199 mg, 98%) after purification over silica (DCM/MeOH 97:3).

¹H NMR (400 MHz, D₂O):  δ = 4.35 (q, J = 6.7 Hz, 2H), 4.25 (s, 2H), 3.77 – 3.65 (m, 8H), 1.33 ppm (t, J = 6.7 Hz, 3H).

Spectroscopic data is consistent with that reported in the literature.¹⁸⁶

1-(cyclohexylmethyl)-7-methoxy-1H-indole-3-carboxylic acid NK69

To a solution of 7-methoxy-1H-indole-3-carboxylic acid (956 mg, 5.0 mmol, 1.0 equiv.) in methylene chloride (30 mL) was added slowly added NaH (200 mg, 5.0 mmol, 1.0 equiv., 60% dispersion in mineral oil). After stirring for further 20 minutes cyclohexylmethyl bromide (0.76 mL, 5.5 mmol, 1.1 equiv.) was added to the solution. Upon completion of the reaction the solution was carefully quenched with 1 N aq. HCl (30 mL), diluted with EtOAc (50 mL), washed with brine, dried with Na₂SO₄, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/hexanes 1:1) gave NK69 as a pink powder (1.28 g, 89%).

¹H NMR (400 MHz, CDC3):  δ = 4.18 (q, J = 6.7 Hz, 2H), 3.47 (t, J = 5.3 Hz, 4H), 3.22 (s, 2H), 2.52 (t, J = 5.3 Hz, 4H), 1.45 (s, 9H), 1.27 ppm (t, J = 6.7 Hz, 3H).

Spectroscopic data is consistent with that reported in the literature.¹⁸⁵
Experimental

$^{1}$H NMR (400 MHz, MeOD): $\delta = 7.75$ (s, 1H), 7.67 (d, $J = 8.1$ Hz, 1H), 7.03 (t, $J = 8.1$ Hz, 1H), 6.75 (d, $J = 8.1$ Hz, 1H), 4.24 (d, $J = 7.0$ Hz, 2H), 3.94 (s, 3H), 1.87 – 1.75 (m, 1H), 1.75 – 1.62 (m, 3H), 1.62 – 1.51 (m, 2H), 1.28 – 1.13 (m, 3H), 1.10 – 0.97 ppm (m, 2H).

Spectroscopic data is consistent with that reported in the literature.\(^{187}\)

7-chloro-1-(cyclohexylmethyl)-1H-indole-3-carboxylic acid NK72

![NK72](image)

To a solution of 7-chloro-1H-indole-3-carboxylic acid (975 mg, 5.0 mmol, 1.0 equiv.) in methylene chloride (30 mL) was added slowly added NaH (200 mg, 5.0 mmol, 1.0 equiv.). After stirring for further 20 minutes cyclohexylmethyl bromide (0.76 mL, 5.5 mmol, 1.1 equiv.) was added to the solution. Upon completion of the reaction the solution was carefully quenched with 1 N aq. HCl (30 mL), diluted with EtOAc (50 mL), washed with brine, dried with Na$_2$SO$_4$, filtered and the solvent was removed \textit{in vacuo}. Purification over silica (EtOAc/hexanes 1:1) gave NK72 as a pink powder (1.24 g, 85%).

$^{1}$H NMR (400 MHz, MeOD): $\delta = 8.11$ (dd, $J = 8.1$, 1.0 Hz, 1H), 7.91 (d, $J = 8.1$ Hz, 1H), 7.24 (dd, $J = 8.1$, 1.0 Hz, 1H), 7.15 (t, $J = 8.1$ Hz, 1H), 4.38 (d, $J = 7.0$ Hz, 2H), 1.98 – 1.85 (m, 1H), 1.80 – 1.64 (m, 3H), 1.61 – 1.52 (m, 2H), 1.28 – 1.15 (m, 3H), 1.15 – 1.02 ppm (m, 2H).

Spectroscopic data is consistent with that reported in the literature.\(^{188}\)
To a solution of NK69 (120 mg, 0.41 mmol, 1.0 equiv) in chloroform (20 mL) was added (COCl)$_2$ (40 µL, 0.46 mmol, 1.1 equiv.) and DMF (10 µL). After stirring for 30 minutes the solvent was removed in vacuo and the residue was subsequently dissolved in methylene chloride (20 mL). Then a solution of NK58 (91 mg, 0.49 mmol, 1.2 equiv.) and NEt$_3$ (0.27 mL, 2.0 mmol, 5.0 equiv) was slowly added to the acid chloride and the solution was stirred at rt for another 30 minutes after which it was diluted with EtOAc (30 mL) and washed with 1 N aq. HCl (10 mL) and brine (10 mL), dried with MgSO$_4$, filtered and the solvent removed in vacuo. Purification over silica (EtOAc/hexanes 1:3) afforded NK73 as a white foam (132 mg, 71%).

$^1$H NMR (400 MHz, CDCl$_3$): $\delta = 7.28 - 7.23$ (m, 3H), 7.07 (t, $J = 8.1$ Hz, 1H), 6.65 (d, $J = 8.1$ Hz, 1H), 4.18 (d, $J = 7.0$ Hz, 2H), 3.93 (s, 3H), 3.69 – 3.64 (m, 4H), 3.49 – 3.44 (m, 4H), 1.87 – 1.75 (m, 1H), 1.75 – 1.55 (m, 5H), 1.47 (s, 9H), 1.21 – 1.11 (m, 3H), 1.03 – 0.91 ppm (m, 2H).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 167.0, 154.6, 147.6, 132.3, 128.3, 125.6, 121.3, 113.0, 109.6, 103.1, 80.1, 56.1, 55.3, 39.9, 30.6, 28.3, 26.3, 25.7$ ppm.

HRMS (ESI, 200 ºC): calculated for C$_{26}$H$_{37}$N$_3$KO$_4$ [M+K]: $m/z = 494.2416$, found: $m/z = 494.2409$.

IR (Film): $\tilde{\nu} = 3452, 1662, 1476$ cm$^{-1}$.

Melting point: 125 – 127 ºC.
Experimental

**tert-butyl 4-(7-chloro-1-(cyclohexylmethyl)-1H-indole-3-carbonyl)piperazine-1-carboxylate NK74**

![NK74](image)

To a solution of **NK72** (120 mg, 0.41 mmol, 1.0 equiv) in chloroform (20 mL) was added \((\text{COCl})_2\) (40 µL, 0.46 mmol, 1.1 equiv.) and DMF (10 µL). After stirring for 30 minutes the solvent was removed *in vacuo* and the residue was subsequently dissolved in methylene chloride (20 mL). Then a solution of **NK58** (91 mg, 0.49 mmol, 1.2 equiv.) and NEt₃ (0.27 mL, 2.0 mmol, 5.0 equiv) was slowly added to the acid chloride and the solution was stirred at rt for another 30 minutes after which it was diluted with EtOAc (30 mL) and washed with 1 N aq. HCl (10 mL) and brine (10 mL), dried with MgSO₄, filtered and the solvent removed *in vacuo*. Purification over silica (EtOAc/hexanes 1:3) afforded **NK74** as a white foam (145 mg, 77%).

**¹H NMR (400 MHz, CDCl₃):**

δ = 7.61 (dd, \(J = 8.0, 1.0\) Hz, 1H), 7.30 (s, 1H), 7.20 (dd, \(J = 8.0, 1.0\) Hz, 1H), 7.08 (t, \(J = 8.0\) Hz, 1H), 4.29 (d, \(J = 7.0\) Hz, 2H), 3.69 – 3.64 (m, 4H), 3.51 – 3.44 (m, 4H), 1.95 – 1.83 (m, 1H), 1.78 – 1.57 (m, 5H), 1.47 (s, 9H), 1.22 – 1.12 (m, 3H), 1.05 – 0.93 ppm (m, 2H).

**¹³C NMR (100 MHz, CDCl₃):**

δ = 166.1, 154.6, 133.4, 131.1, 129.4, 124.4, 121.5, 119.3, 117.1, 109.7, 80.2, 55.5, 40.0, 30.5, 28.3, 26.2, 25.6 ppm.

**HRMS (ESI, 200 °C):**

Calculated for C₂₂H₃₄ClN₃NaO₃ [M+Na]: \(m/z = 482.2181\), found: \(m/z = 482.2166\).

**IR (Film):**

\(\tilde{\nu} = 3473, 1652, 1494\) cm⁻¹.

**Melting point:**

128 – 130°C.
Experimental

**ethyl 2-(4-(1-(cyclohexylmethyl)-7-methoxy-1H-indole-3-carbonyl)piperazin-1-yl)acetate NK82**

![NK82](image)

To a solution of **NK69** (120 mg, 0.41 mmol, 1.0 equiv) in chloroform (20 mL) was added \((\text{COCl})_2\) (40 µL, 0.46 mmol, 1.1 equiv.) and DMF (10 µL). After stirring for 30 minutes the solvent was removed *in vacuo* and the residue was subsequently dissolved in methylene chloride (20 mL). Then a solution of **NK62** (85 mg, 0.49 mmol, 1.2 equiv.) and NEt₃ (0.27 mL, 2.0 mmol, 5.0 equiv) was slowly added to the acid chloride and the solution was stirred at rt for another 30 minutes after which it was diluted with EtOAc (30 mL) and washed with 1 N aq. HCl (10 mL) and brine (10 mL), dried with MgSO₄, filtered and the solvent removed *in vacuo*. Purification over silica (EtOAc/hexanes 1:3) afforded **NK82** as a white foam (132 mg, 73%).

**¹H NMR (400 MHz, CDCl₃):**

δ = 7.29 – 7.25 (m, 3H), 7.07 (t, \(J = 8.0\) Hz, 1H), 6.65 (d, \(J = 8.0\) Hz, 1H), 4.22 – 4.15 (m, 4H), 3.92 (s, 3H), 3.80 – 3.74 (m, 4H), 3.25 (s, 2H), 2.66 – 2.60 (m, 4H), 1.85 – 1.74 (m, 1H), 1.73 – 1.55 (m, 5H), 1.27 (t, \(J = 6.8\) Hz, 3H), 1.20 – 1.11 (m, 3H), 1.04 – 0.92 ppm (m, 2H).

**¹³C NMR (100 MHz, CDCl₃):**

δ = 170.0, 166.7, 147.6, 132.2, 128.4, 125.6, 121.2, 113.2, 109.8, 103.0, 60.7, 59.3, 56.1, 55.3, 53.1, 40.0, 30.6, 26.3, 25.7, 14.2 ppm.

**HRMS (ESI, 200 °C):** calculated for C₂₆H₃₆N₃KO₄ [M+K]: \(m/z = 480.2259\), found: \(m/z = 480.2276\).

**IR (Film):** \(\tilde{\nu} = 3449, 1629, 1474\) cm⁻¹.

**Melting point:** 131 – 133°C.
Experimental

ethyl 2-(4-(7-chloro-1-(cyclohexylmethyl)-1H-indole-3-carbonyl)piperazin-1-yl)acetate NK85

To a solution of NK72 (120 mg, 0.41 mmol, 1.0 equiv) in chloroform (20 mL) was added (COCl)$_2$ (40 µL, 0.46 mmol, 1.1 equiv.) and DMF (10 µL). After stirring for 30 minutes the solvent was removed in vacuo and the residue was subsequently dissolved in methylene chloride (20 mL). Then a solution of NK62 (85 mg, 0.49 mmol, 1.2 equiv.) and NEt$_3$ (0.27 mL, 2.0 mmol, 5.0 equiv) was slowly added to the acid chloride and the solution was stirred at rt for another 30 minutes after which it was diluted with EtOAc (30 mL) and washed with 1 N aq. HCl (10 mL) and brine (10 mL), dried with MgSO$_4$, filtered and the solvent removed in vacuo. Purification over silica (EtOAc/hexanes 1:3) afforded NK85 as a white foam (146 mg, 80%).

$^1$H NMR (300 MHz, CDCl$_3$): δ = 7.61 (d, $J = 8.0$ Hz, 1H), 7.35 (s, 1H), 7.22 (dd, $J = 8.0$, 1.0 Hz, 1H), 7.10 (t, $J = 8.0$ Hz, 1H), 4.31 – 4.21 (m, 4H), 4.08 – 4.00 (m, 4H), 3.63 (s, 2H), 3.17 – 3.09 (m, 4H), 1.95 – 1.83 (m, 1H), 1.75 – 1.65 (m, 3H), 1.65 – 1.57 (m, 2H), 1.30 (t, $J = 6.8$ Hz, 3H), 1.22 – 1.13 (m, 3H), 1.06 – 0.94 ppm (m, 2H).

$^{13}$C NMR (75 MHz, CDCl$_3$): δ = 166.5, 165.9, 133.8, 131.1, 129.2, 124.4, 121.6, 119.2, 117.1, 108.6, 61.8, 56.5, 55.4, 53.3, 51.9, 39.8, 30.3, 26.1, 25.5, 13.9 ppm.

HRMS (ESI, 200 °C): calculated for C$_{24}$H$_{32}$N$_3$O$_3$ [M+Na]: $m/z = 468.2024$, found: $m/z = 468.2009$.

IR (Film): $\tilde{\nu} = 3437, 1642, 1482$ cm$^{-1}$.

Melting point: 110 – 113°C.
methyl 6-(1-(cyclohexylmethyl)-7-methoxy-1H-indole-3-carboxamido)hexanoate NK83

To a solution of NK69 (120 mg, 0.41 mmol, 1.0 equiv) in chloroform (20 mL) was added (COCl)₂ (40 µL, 0.46 mmol, 1.1 equiv) and DMF (10 µL). After stirring for 30 minutes the solvent was removed in vacuo and the residue was subsequently dissolved in methylene chloride (20 mL). Then a solution of NK323 (89 mg, 0.49 mmol, 1.2 equiv) and NEt₃ (0.27 mL, 2.0 mmol, 5.0 equiv) was slowly added to the acid chloride and the solution was stirred at r.t. for another 30 minutes after which it was diluted with EtOAc (30 mL) and washed with 1 N aq. HCl (10 mL) and brine (10 mL), dried with MgSO₄, filtered and the solvent removed in vacuo. Purification over silica (EtOAc/Hex 1:3) afforded NK83 as a white foam (144 mg, 85%).

¹H NMR (400 MHz, CDCl₃): δ = 7.52 (s, 1H), 7.49 (d, J = 8.0 Hz, 1H), 7.11 (t, J = 8.0 Hz, 1H), 6.67 (d, J = 8.0 Hz, 1H), 6.02 – 5.96 (m, 1H), 4.16 (d, J = 7.5 Hz, 2H), 3.92 (s, 3H), 3.65 (s, 3H), 3.48 (q, J = 6.7 Hz, 2H), 2.33 (t, J = 6.7 Hz, 2H), 1.85 – 1.74 (m, 1H), 1.73 – 1.55 (m, 9H), 1.49 – 1.39 (m, 2H), 1.20 – 1.12 (m, 3H), 1.02 – 0.91 ppm (m, 2H).

¹³C NMR (100 MHz, CDCl₃): δ = 174.0, 165.3, 147.8, 132.9, 127.6, 126.3, 121.7, 112.5, 110.5, 103.1, 56.3, 55.3, 51.4, 39.9, 39.1, 33.8, 30.6, 29.5, 26.4, 26.3, 25.7, 24.5 ppm.

HRMS (ESI, 200 °C): calculated for C₂₄H₃₄N₂O₄ [M+H]: m/z = 415.2591, found: m/z = 415.2581.

IR (Film): ̇ν = 3457, 1624, 1455 cm⁻¹.

Melting point: 115 – 117°C.
methyl 6-(7-chloro-1-(cyclohexylmethyl)-1H-indole-3-carboxamido)hexanoate NK87

To a solution of NK72 (120 mg, 0.41 mmol, 1.0 equiv) in chloroform (20 mL) was added (COCl)$_2$ (40 µL, 0.46 mmol, 1.1 equiv.) and DMF (10 µL). After stirring for 30 minutes the solvent was removed in vacuo and the residue was subsequently dissolved in methylene chloride (20 mL). Then a solution of NK323 (89 mg, 0.49 mmol, 1.2 equiv.) and NEt$_3$ (0.27 mL, 2.0 mmol, 5.0 equiv) was slowly added to the acid chloride and the solution was stirred at rt for another 30 minutes after which it was diluted with EtOAc (30 mL) and washed with 1 N aq. HCl (10 mL) and brine (10 mL), dried with MgSO$_4$, filtered and the solvent removed in vacuo. Purification over silica (EtOAc/hexanes 1:3) afforded NK87 as a white foam (137 mg, 80%).

$^1$H NMR (400 MHz, CDCl$_3$): $\delta = 7.94$ (dd, $J = 8.0$, 1.0 Hz, 1H), 7.53 (s, 1H), 7.21 (dd, $J = 8.0$, 1.0 Hz, 1H), 7.12 (t, $J = 8.0$ Hz, 1H), 4.29 (d, $J = 7.5$ Hz, 2H), 3.66 (s, 3H), 3.48 (q, $J = 6.7$ Hz, 2H), 2.34 (t, $J = 6.7$ Hz, 2H), 1.94 – 1.83 (m, 1H), 1.75 – 1.55 (m, 9H), 1.49 – 1.40 (m, 2H), 1.22 – 1.12 (m, 3H), 1.06 – 0.94 ppm (m, 2H).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 174.1$, 164.7, 133.8, 131.8, 129.1, 124.4, 121.8, 119.4, 117.2, 110.7, 55.6, 51.4, 40.0, 39.1, 33.8, 30.4, 29.5, 26.4, 26.2, 25.6, 24.4 ppm.

HRMS (ESI, 200 °C): calculated for C$_{23}$H$_{31}$ClN$_2$O$_3$Na [M+Na]: $m/z = 441.1915$, found: $m/z = 441.1899$.

IR (Film): $\tilde{\nu} = 3467$, 1627, 1417 cm$^{-1}$.

Melting point: 115 – 117°C.
**ethyl 3-(4-chlorophenyl)-2-methyl-3-oxopropanoate NK20**

![NK20](image)

To a stirred solution of Et$_2$O (15 mL) and LHMDS (13 mL, 13.00 mmol, 1.1 equiv.) was slowly added 4-chloropropiophenone (2.0 g, 11.90 mmol, 1 equiv.) dissolved in Et$_2$O (5 mL). After stirring for further 20 minutes diethyl oxalate (2.4 mL, 17.80 mmol, 1.5 equiv.) was added within one minute. The reaction was stirred for 14h. The precipitate was filtered off, washed several times with a cold mixture of Et$_2$O/hexanes (3:1), redissolved in EtOAc, washed with 1N aq. HCl, washed with brine, dried with MgSO$_4$, filtered and the solvent was removed *in vacuo*. Purification over silica (EtOAc/hexanes, 1:3) gave NK20 (2.0 g, 63%) as a yellow oil.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 7.95 - 7.90 (m, 2H), 7.52 - 7.46 (m, 2H), 4.98 (q, $J$ = 7.0 Hz, 1H), 4.27 (q, $J$ = 7.0 Hz, 1H), 1.45 (d, $J$ = 7.0 Hz, 3H), 1.30 ppm (t, $J$ = 7.0 Hz, 3H).

Spectroscopic data is consistent with that reported in the literature.$^{154}$

**ethyl 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxylate NK22**

![NK22](image)

To a solution of NK20 (2.0 g, 7.44 mmol, 1.0 equiv.) in EtOH (25 mL) was added 2,4-Dichlorophenylhydrazine (1.75 g, 8.18 mmol, 1.1 equiv.) and the solution was stirred for one hour. The solution was refluxed for 14h, cooled to rt and the volume was reduced by ca. 75% *in vacuo*. The residue was diluted with Et$_2$O (40 mL), washed with water (3 x 30 mL), dried with MgSO$_4$, filtered and the solvent was removed *in vacuo*. Purification over silica (EtOAc/hexanes, 1:4) afforded NK22 (2.9 g, 95%), as a yellow oil.
Experimental

$^1$H NMR (400 MHz, CDCl3): $\delta = 7.38 - 7.36$ (m, 1H), 7.35 - 7.32 (m, 1H), 7.31 - 7.36 (m, 3H), 7.08 - 7.05 (m, 2H), 4.44 (q, $J = 7.0$ Hz, 2H), 2.32 (s, 3H), 1.42 ppm (t, $J = 7.0$ Hz, 3H).

Spectroscopic data is consistent with that reported in the literature.$^{154}$

5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxylic acid NK24

Ethyl ester NK22 (2.9 g, 7.07 mmol, 1.0 equiv.) was dissolved in MeOH (20 mL) and a 1N aq. KOH-solution (20 mL) and refluxed for 3h. After cooling to rt the solution was poured onto an ice-cooled 6N aqueous HCl-solution (70 mL). The precipitate was filtered off and dried in vacuo to afford carboxylic acid NK24 (2.6 g, 96 %) as a white solid.

$^1$H NMR (400 MHz, CDCl3): $\delta = 7.42 - 7.40$ (m, 1H), 7.34 - 7.28 (m, 4H), 7.10 - 7.06 (m, 2H), 2.35 ppm (s, 3H).

Spectroscopic data is consistent with that reported in the literature.$^{154}$
Experimental

5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carbonyl chloride NK25

\[
\begin{align*}
\text{O} & \quad \begin{array}{c}
\text{N} \\
\text{Cl} & \quad \text{Cl} & \quad \text{Cl}
\end{array} \\
\end{align*}
\]

NK25

To a solution of carboxylic acid NK24 (1.0 g, 2.62 mmol, 1.0 equiv.) in methylene chloride (20 mL) was added (COCl)\(_2\) (674 µL, 7.86 mmol, 3.0 equiv.) and DMF (10 µL). The solution was stirred for 20 minutes, the solvent was removed \textit{in vacuo} and threefold re-dissolved in methylene chloride (3 x 5mL) to afford acid chloride NK25 as a yellow foam that was used in the next step without further purification.

ethyl 4-(aminomethyl)benzoate NK71

\[
\begin{align*}
H_2N & \quad \begin{array}{c}
\text{O} \\
\end{array} \\
\text{OEt}
\end{align*}
\]

NK71

To an ice-cooled mixture of 4-Aminomethylbenzoic acid (2.26 g, 15.0 mmol, 1.0 equiv.) in ethanol (120 ml) was slowly added thionyl chloride (11.0 mL, 150 mmol, 10.0 equiv.). The solution was then allowed to warm to room temperature and subsequently refluxed for 5 hours. The solvent was removed \textit{in vacuo} to give NK71 as a white precipitate (2.60 g, 81%, HCl-salt) which was used in the next step without further purification.

\(^1\)H NMR (400 MHz, D\(_2\)O): \(\delta = 8.10\ (d, J = 8.0\ Hz, 2H), 7.59\ (d, J = 8.0\ Hz, 2H), 4.42\ (q, J = 7.0\ Hz, 2H), 4.29\ (br\ s, \ 2H), 1.41\ ppm\ (t, J = 7.0\ Hz, 3H).

Spectroscopic data is consistent with that reported in the literature.\(^{189}\)
Experimental

**tert-butyl 4-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carbonyl)piperazine-1-carboxylate**

![Chemical structure of NK76](image)

**NK76** was prepared according to **GP 2** from **NK25** and **NK58** to afford a yellow oil (890 mg, 90%) after purification over silica (EtOAc/hexanes 2:3).

**1H NMR (400 MHz, CDCl3):**
\[
\delta = 7.45 (d, J = 2.1 Hz, 1H), 7.31 - 7.28 (m, 2H), 7.27 - 7.23 (m, 1H), 7.18 - 7.14 (m, 1H), 7.09 - 7.05 (m, 2H), 3.85 - 3.75 (m, 4H), 3.56 - 3.45 (m, 4H), 2.21 (s, 3H), 1.47ppm (s, 9H).
\]

**13C NMR (100 MHz, CDCl3):**
\[
\delta = 163.4, 154.6, 146.1, 142.0, 135.8, 135.7, 134.8, 132.9, 130.6, 130.4, 130.3, 128.9, 127.8, 127.2, 117.1, 80.1, 47.1, 42.0, 28.3, 9.0 ppm.
\]

**HRMS (ESI, 180 °C):**
calculated for C_{26}H_{27}Cl_{3}N_{4}O_{3}K [M+K] m/z = 587.0780, found: m/z = 587.0783.

**IR (Film):**
\[
\tilde{\nu} = 3435, 1665, 1449 \text{ cm}^{-1}.
\]
**NK77** was prepared according to **GP 2** from **NK25** and **NK60** to afford a yellow oil (820 mg, 85%) after purification over silica (EtOAc/hexanes 2:3).

**1H NMR (400 MHz, CDCl3):**

δ = 7.45 (d, J = 2.1 Hz, 1H), 7.31 - 7.28 (m, 2H), 7.27 - 7.23 (m, 1H), 7.18 - 7.14 (m, 1H), 7.09 - 7.05 (m, 2H), 4.19 (q, J = 7.0 Hz, 2H), 3.95 - 3.86 (m, 4H), 3.27 (s, 2H), 2.75 - 2.63 (m, 4H), 2.21 (s, 3H), 1.28 ppm (q, J = 7.0 Hz, 3H).

**13C NMR (75 MHz, CDCl3):**

δ = 169.9, 163.2, 146.3, 141.9, 135.9, 135.7, 134.8, 133.0, 130.6, 130.5, 130.2, 128.9, 127.8, 127.3, 116.8, 60.7, 59.1, 53.4, 52.6, 47.0, 41.8, 14.2, 9.0 ppm.

**HRMS (ESI, 180 °C):** calculated for C_{25}H_{26}Cl_{3}N_{4}O_{3} [M+H] m/z = 535.1065, found: m/z = 535.1045.

**IR (Film):**

ν = 3355, 1674, 1498 cm\(^{-1}\).
**Experimental**

**ethyl 4-[(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)methyl]benzoate NK75**

![Chemical Structure](image)

**NK75** was prepared according to GP 2 from **NK25** and **NK71** to afford a white powder (850 mg, 87%) after purification over silica (EtOAc/hexanes 1:2).

**1H NMR (400 MHz, CDCl3):**

\[ \delta = 8.01 - 7.98 \text{ (m, 2H)}, 7.44 - 7.36 \text{ (m, 3H)}, 7.31 - 7.25 \text{ (m, 4H)}, 7.09 - 7.04 \text{ (m, 2H)}, 4.66 \text{ (d, } J = 6.3 \text{ Hz, 2H)}, 4.35 \text{ (q, } J = 7.0 \text{ Hz, 2H)}, 2.40 \text{ (s, 3H)}, 1.37 \text{ ppm (t, } J = 7.0 \text{ Hz, 3H}). \]

**13C NMR (100 MHz, CDCl3):**

\[ \delta = 166.3, 162.6, 144.6, 143.5, 143.1, 135.9, 135.8, 134.9, 132.9, 130.7, 130.4, 130.2, 129.8, 129.5, 128.8, 127.8, 127.5, 127.1, 117.9, 60.8, 42.6, 14.2, 9.3 \text{ ppm}. \]

**HRMS (ESI, 180 °C):**

calculated for C_{27}H_{22}Cl_{3}N_{3}O_{3}Na [M+Na] \( m/z \) = 564.0619, found: \( m/z \) = 564.0602.

**IR (Film):**

\[ \tilde{\nu} = 3452, 1662, 1476 \text{ cm}^{-1}. \]

**Melting point:**

78 - 80°C.
methyl 6-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)hexanoate NK90

\[
\text{NK90}
\]

NK90 was prepared according to GP 2 from NK25 and NK323 to afford a yellow oil (722 mg, 79%) after purification over silica (EtOAc/Hex 1:2).

\(^1\)H NMR (300 MHz, CDCl\( _3 \)): \( \delta = 7.42 \) (d, \( J = 2.1 \) Hz, 1H), 7.32 - 7.25 (m, 4H), 7.08 - 7.03 (m, 2H), 6.98 - 6.92 (m, 1H), 3.65 (s, 3H), 3.42 (q, \( J = 7.0 \) Hz, 2H), 2.37 (s, 3H), 2.32 (t, \( J = 7.0 \) Hz, 2H), 1.73 - 1.57 (m, 4H), 1.48 - 1.34 ppm (m, 2H).

\(^{13}\)C NMR (75 MHz, CDCl\( _3 \)): \( \delta = 174.0, 162.7, 145.1, 143.0, 135.9, 134.9, 133.0, 130.8, 130.5, 130.3, 128.9, 127.9, 127.3, 117.7, 51.5, 38.8, 33.9, 29.5, 26.5, 24.6, 9.4 \) ppm.

HRMS (ESI, 180 °C): calculated for C\(_{24}\)H\(_{24}\)Cl\(_3\)N\(_3\)O\(_3\)Na [M+Na] \( m/z \) = 530.0775, found: \( m/z \) = 535.0778.

IR (Film): \( \tilde{\nu} = 3424, 1654, 1437 \) cm\(^{-1}\).

5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoyl chloride NK30

\[
\text{NK30}
\]

To a solution of biotin (50 mg, 0.20 mmol, 1.0 equiv.) in chloroform (5 mL) was added SOCl\(_2\) (45 \( \mu \)L, 0.60 mmol, 3.0 equiv.) and stirred for 30 minutes. The solvent was removed \textit{in vacuo} and the biotin acid chloride was used in the next step without further purification.
Experimental

(3aS,4S,6aR)-4-(5-(4-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carbonyl)piperazin-1-yl)-5-oxopentyl)tetrahydro-1H-thieno[3,4-d]imidazol-2(3H)-one NK169

![NK169](image)

To a solution of NK76 (99 mg, 0.18 mmol, 1.0 equiv.) in methylene chloride (5 mL) was added TFA (0.5 mL) and stirred for 30 minutes. The solvent was removed in vacuo and the residue was dissolved in methylene chloride (5 mL) and DIPEA (0.15 mL, 0.9 mmol, 5.0 equiv.) and subsequently added slowly to an ice-cooled solution of biotin-chloride NK30 (45 mg, 0.20 mmol, 1.1 equiv.) in methylene chloride (2 mL). After stirring for 5 minutes the reaction was diluted with EtOAc (20 mL), washed with 1 N aq. HCl, washed with brine, dried with MgSO₄, filtered and the solvent was removed in vacuo. Purification over silica (DCM/MeOH 95:5) gave NK169 as a white solid (90 mg, 76%).

$^1$H NMR (400 MHz, MeOD): $\delta = 7.64$ (d, $J = 2.1$ Hz, 1H), 7.52 – 7.44 (m, 2H), 7.44 – 7.39 (m, 2H), 7.27 - 7.22 (m, 2H), 4.55 - 4.48 (m, 1H), 4.37 - 4.30 (m, 1H), 3.95 - 3.79 (m, 4H), 3.79 - 3.64 (m, 4H), 3.28 - 3.22 (m, 1H), 3.00 - 2.92 (m, 1H), 2.77 - 2.70 (m, 1H), 2.56 - 2.44 (m, 2H), 2.20 (s, 3H), 1.84 - 1.58 (m, 4 H), 1.58 - 1.46 ppm (m, 2H).

$^{13}$C NMR (100 MHz, CDCl3): $\delta = 174.3, 166.1, 147.2, 144.0, 137.4, 137.2, 136.3, 134.3, 132.5, 132.3, 131.2, 130.0, 129.3, 128.6, 63.4, 61.7, 57.0, 41.1, 33.7, 29.9, 29.6, 26.3, 9.0 ppm.

HRMS (EI, 200 °C): calculated for C₃₁H₃₃Cl₃N₆O₃S [M+K]: $m/z = 713.1032$, found: $m/z = 713.1016$.

IR (Film): $\tilde{\nu} = 2932, 1661, 1538, 1514, 1463, 1393, 1278, 825, 770, 712$ cm$^-1$.

Melting point: 120 – 122 °C.
Experimental

tert-butyl 6-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)hexylcarbamate NK369

NK369 was prepared according to GP 2 from NK25 and mono-Boc-protected 1,6-diaminohexane (470 mg, 2.17 mmol, 1.2 equiv.) to yield a white foam (843 mg, 79%) after purification over silica (EtOAc/hexanes 1:1).

$^1$H NMR (300 MHz, CDCl3): \( \delta = 7.42 \ (d, \ J = 2.1 \ Hz, \ 1H), \ 7.31 - 7.26 \ (m, \ 4H), \ 7.08 - 7.03 \ (m, \ 2H), \ 6.95 \ (l, \ J = 6.0 \ Hz, \ 1H), \ 4.55 \ (br \ s, \ 1H), \ 3.41 \ (q, \ J = 7.0 \ Hz, \ 2H), \ 3.14 - 3.03 \ (m, \ 2H), \ 2.37 \ (s, \ 3H), \ 1.65 - 1.55 \ (m, \ 2H), \ 1.53 - 1.35 \ ppm \ (m, \ 15H). \)

$^{13}$C NMR (75 MHz, CDCl3): \( \delta = 162.7, \ 145.1, \ 143.0, \ 136.0, \ 135.9, \ 134.9, \ 133.0, \ 130.8, \ 130.5, \ 130.3, \ 128.9, \ 127.9, \ 127.3, \ 117.7, \ 40.5, \ 38.8, \ 30.0, \ 29.7, \ 28.4, \ 26.6, \ 26.4, \ 9.4 \ ppm. \)

HRMS (ESI, 180 °C): calculated for C_{28}H_{34}Cl_3N_4O_3 [M+H] m/z = 579.1691, found: m/z = 579.1690.

IR (Film): \( \tilde{\nu} = 3123, \ 1524, \ 1274 \ cm^{-1}. \)

Melting point: 78 - 80°C.
Experimental

**N-(6-aminohexyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK370**

![Chemical Structure of NK370](image)

**NK370** was prepared according to **GP 3** from **NK369**. Purification over silica gave (EtOAc/ULTRA 5:1) gave the free amine as a colourless oil (573 mg, quant.).

**¹H NMR (300 MHz, CDCl₃):**

δ = 7.42 (d, J = 2.1 Hz, 1H), 7.31 - 7.25 (m, 4H), 7.07 - 7.02 (m, 2H), 3.39 (q, J = 7.0 Hz, 2H), 3.11 - 2.99 (m, 2H), 2.28 (s, 3H), 1.78 – 1.66 (m, 2H), 1.66 - 1.55 (m, 2H), 1.51 – 1.31 ppm (m, 4H).

**¹³C NMR (75 MHz, CDCl₃):**

δ = 163.8, 144.1, 143.6, 136.3, 135.5, 135.2, 132.9, 130.8, 130.4, 130.3, 129.0, 128.0, 126.7, 117.7, 40.0, 38.7, 29.0, 26.9, 25.4, 24.9, 9.2 ppm.

**HRMS (ESI, 180 °C):**

Calculated for C_{23}H_{26}Cl_{3}N_{4}O [M+H] m/z = 479.1167, found: m/z = 479.1170.

**IR (Film):**

ν = 3118, 1735, 1165, 1045 cm⁻¹.
To a solution of Fmoc-Lys(Boc)-OH (675 mg, 1.44 mmol, 1.2 equiv.) in methylene chloride (25 mL) was added EDCI (264 mg, 1.38 mmol, 1.15 equiv.) and HOBt (186 mg, 1.38 mmol, 1.15 equiv.) and DIPEA (1.0 mL, 6.0 mmol, 5.0 equiv.). After stirring for 25 minutes, NK370 (573 mg, 1.2 mmol, 1.0 equiv.) dissolved in methylene chloride (12 mL) was added and the solution was stirred for further 4h. The reaction mixture was then diluted with EtOAc (50 mL), washed with 1 N aq. HCl (10 mL), washed with brine, dried with Na$_2$SO$_4$, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 3:1) gave NK374 as a white foam (802 mg, 72%).

$^1$H NMR (400 MHz, CDCl$_3$):  δ = 7.76 (d, J = 7.3 Hz, 2H), 7.58 (d, J = 7.3 Hz, 2H), 7.42 - 7.35 (m, 3H), 7.32 - 7.23 (m, 7H), 7.06 - 7.01 (m, 2H), 7.00 - 6.95 (m, 1H), 6.37 (br s, 1H), 5.66 (br s, 1H), 4.67 (br s, 1H), 4.44 - 4.34 (m, 2H), 4.19 (t, J = 7.5 Hz, 1H), 4.16 - 4.08 (m, 1H), 3.46 - 3.32 (m, 2H), 3.27 - 3.18 (m, 2H), 3.14 - 3.03 (m, 2H), 2.36 (s, 3H), 1.93 - 1.72 (m, 3H), 1.69 - 1.30 ppm (m, 20H).

$^{13}$C NMR (125 MHz, CDCl$_3$):  δ = 171.6, 162.8, 145.0, 143.8, 143.0, 141.3, 135.95, 135.93, 134.9, 132.9, 130.8, 130.3, 128.9, 127.9, 127.2, 127.1, 125.0, 119.9, 117.7, 67.0, 54.9, 47.1, 39.9, 39.2, 38.4, 32.2, 29.6, 29.5, 29.1, 28.4, 26.06, 26.02, 22.5, 9.4 ppm.

HRMS (ESI, 180 °C): calculated for C$_{49}$H$_{55}$Cl$_3$N$_6$O$_6$Na [M+Na] $m/z = 951.3141$, found: $m/z = 951.3178$.

IR (Film):  $\tilde{\nu} = 3248, 1753, 1157, 1103$ cm$^{-1}$. 

(9H-fluoren-9-yl)methyl tert-butyl (6-((6-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)hexyl)amino)-6-oxohexane-1,5-diyld(S)-dicarbamate NK374

![NK374](image-url)
Experimental

Melting point: 78 - 80°C.

(S)-tert-butyl 5-amino-6-(6-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)hexylamino)-6-oxohexylcarbamate NK381

To a solution of NK374 (803 mg, 0.86 mmol, 1.0 equiv.) in methylene chloride (15 mL) was added Et₂NH (3 mL) and the solution was stirred for 2 hours. Upon completion of the reaction the solvent was removed *in vacuo*, the residue was dissolved in EtOAc (30 mL), washed with 1 N aq. HCl, washed with brine, dried with MgSO₄, filtered and purified over silica (EtOAc/ULTRA 3:1) to give NK381 as a white foam (578 mg, 95%).

¹H NMR (300 MHz, CDCl₃): δ = 7.42 (d, J = 2.2 Hz, 1H), 7.36 – 7.25 (m, 6H), 7.09 - 7.03 (m, 2H), 7.02 – 6.95 (m, 1H), 4.65 (br s, 1H), 3.45 - 3.31 (m, 3H), 3.23 (q, J = 6.5 Hz, 2H), 3.15 - 3.05 (m, 2H), 2.36 (s, 3H), 1.90 - 1.77 (m, 4H), 1.65 - 1.33 ppm (m, 19H).

¹³C NMR (75 MHz, CDCl₃): δ = 174.8, 162.8, 156.1, 145.0, 143.0, 135.9, 134.9, 133.0, 130.8, 130.5, 130.3, 128.9, 127.9, 127.2, 117.6, 55.0, 38.9, 38.8, 34.6, 29.9, 29.6, 29.4, 28.4, 26.5, 22.9, 9.4 ppm.

HRMS (ESI, 180 °C): calculated for C₃₅H₄₅Cl₃N₆O₄Na [M+Na] m/z = 729.2460 found: m/z = 729.2468.

IR (Film): ν = 3283, 1737, 1209, 1059 cm⁻¹.

Melting point: 80 - 82°C.
(S)-tert-butyl 5-acetamido-6-(6-[(4-chlorophenyl)-1-[(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)hexylamino]-6-oxohexylcarbamate NK382

To an ice-cooled solution of NK381 (578 mg, 0.82 mmol, 1.0 equiv.) in methylene chloride (15 mL) and DIPEA (0.7 mL, 4.1 mmol, 5.0 equiv.) was slowly added acetyl chloride (70 µL, 0.98 mmol, 1.2 equiv.). The solution was stirred for 20 minutes and subsequently diluted with EtOAc (50 mL), washed with 1N aq. HCl, washed with brine, dried with Na$_2$SO$_4$, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 2:1) gave NK382 as a white foam (504 mg, 82%).

$^1$H NMR (400 MHz, CDCl$_3$): $\delta = 7.42$ (d, $J = 2.2$ Hz, 1H), 7.31 – 7.25 (m, 5H), 7.08 – 7.04 (m, 2H), 7.04 – 6.98 (m, 1H), 6.59 – 6.53 (m, 1H), 6.52 – 6.46 (m, 1H), 4.71 (br s, 1H), 4.42 – 4.34 (m, 1H), 3.48 – 3.33 (m, 2H), 3.28 – 3.16 (m, 2H), 3.12 – 3.01 (m, 2H), 2.36 (s, 3H), 2.00 (s, 3H), 1.68 – 1.55 (m, 3H), 1.55 – 1.46 (m, 4H), 1.46 – 1.29 ppm (m, 16H).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 171.7$, 170.3, 162.8, 156.2, 145.1, 143.1, 136.07, 136.04, 134.9, 133.0, 130.8, 130.6, 130.3, 128.9, 127.9, 127.2, 117.7, 53.0, 40.0, 39.2, 38.5, 32.0, 29.7, 29.6, 29.1, 28.5, 26.15, 26.12, 23.2, 22.6, 9.5 ppm.

HRMS (ESI, 180 °C): calculated for C$_{36}$H$_{47}$Cl$_3$N$_6$O$_5$Na [M+Na] $m/z = 771.2566$, found: $m/z = 771.2575$.

IR (Film): $\tilde{\nu} = 3255, 1741, 1351, 1067, 857$ cm$^{-1}$.

Melting point: 75 - 78 °C.
(S)-N-(6-(2-acetamido-6-aminohexanamido)hexyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK383

NK382 (504 mg, 0.67 mmol, 1.0 equiv.) was dissolved in a 1:1 mixture of DCM/TFA (10 mL). Upon completion of the deprotection the solvent was removed *in vacuo*, the residue was redissolved in EtOAc (20 mL) and washed with a sat. aqueous NaHCO₃ solution (10 mL), followed by drying with MgSO₄ and filtering. The solvent was removed *in vacuo* and the crude product purified over silica (EtOAc/ULTRA 1:1) to give NK383 as a colourless oil (435 mg, quant.).

$^1$H NMR (500 MHz, CDCl₃): $\delta$ = 7.42 (d, $J$ = 2.2 Hz, 1H), 7.31 – 7.28 (m, 2H), 7.25 - 7.23 (m, 2H), 7.06 - 7.03 (m, 2H), 4.52 - 4.45 (m, 1H), 3.40 - 3.33 (m, 2H), 3.28 - 3.16 (m, 2H), 3.12 - 3.01 (m, 2H), 2.29 (s, 3H), 2.04 (s, 3H), 1.83 - 1.64 (m, 5H), 1.62 - 1.55 (m, 2H), 1.54 - 1.47 (m, 3H), 1.39 – 1.30 ppm (m, 4H).

$^{13}$C NMR (125 MHz, CDCl₃): $\delta$ = 173.2, 172.5, 163.6, 144.2, 143.6, 136.3, 135.6, 135.2, 132.9, 130.8, 130.47, 130.42, 129.0, 128.0, 126.8, 117.6, 53.1, 39.9, 39.7, 39.1, 31.4, 29.1, 28.5, 26.0, 25.9, 22.1, 21.9, 9.3 ppm.

HRMS (ESI, 180 °C): calculated for C₃₁H₄₀Cl₅N₆O₃ [M+H] m/z = 649.2222, found: m/z = 649.2246.

IR (Film): $\tilde{\nu}$ = 3217, 1853, 1531, 1157, 857 cm$^{-1}$. 
Experimental

(9H-fluoren-9-yl)methyl tert-butyl ((S)-6-(((S)-5-acetamido-6-((6-((5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)hexyl)amino)-6-oxohexyl)amino)-6-oxohexane-1,5-diyl)dicarbamate NK385

![NK385](image)

To a solution of Fmoc-Lys(Boc)-OH (376 mg, 0.80 mmol, 1.2 equiv.) in methylene chloride (20 mL) was added EDCI (147 mg, 0.77 mmol, 1.15 equiv.) and HOBt (103 mg, 0.77 mmol, 1.15 equiv.) and DIPEA (0.55 mL, 3.35 mmol, 5.0 equiv.). After stirring for 25 minutes, NK383 (435 mg, 0.67 mmol, 1.0 equiv.) dissolved in methylene chloride (7 mL) was added and the solution was stirred for further 4h. The reaction mixture was then diluted with EtOAc (30 mL), washed with 1 N aq. HCl (6 mL), washed with brine, dried with Na₂SO₄, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 9:1) gave NK385 as a white foam (480 mg, 65%).

¹H NMR (500 MHz, MeOD):

δ = 7.81 (d, J = 7.3 Hz, 2 H), 7.68 (t, J = 6.7 Hz, 2 H), 7.58-7.56 (m, 2 H), 7.52-7.48 (m, 1 H), 7.46-7.36 (m, 5 H), 7.35-7.30 (m, 2 H), 7.22-7.17 (m, 2 H), 4.48-4.35 (m, 2 H), 4.30-4.21 (m, 2 H), 4.06-3.98 (m, 1 H), 3.42-3.36 (m, 2 H), 3.25-3.15 (m, 4 H), 3.08-3.02 (m, 2 H), 2.32 (s, 3 H), 1.99 (s, 3 H), 1.82-1.70 (m, 2 H), 1.70-1.57 (m, 4 H), 1.57-1.31 ppm (m, 23 H).

¹³C NMR (100 MHz, CDCl₃):

δ = 175.0, 174.4, 173.3, 173.2, 165.0, 164.9, 146.3, 145.3, 145.2, 144.5, 142.6, 137.4, 137.3, 136.1, 134.1, 132.5, 132.4, 131.0, 129.8, 129.2, 128.8, 128.7, 128.2, 126.2, 120.9, 118.3, 79.8, 56.7, 55.0, 41.0, 40.4, 40.0, 39.9, 33.0, 32.8, 30.5, 30.2, 29.9, 28.8, 27.6, 27.5, 24.2, 24.0, 22.6, 22.5, 9.6 ppm.

HRMS (ESI, 180 °C):

Calculated for C₅₇H₆₉Cl₃N₈O₈Na [M+Na] m/z = 1121.4196, found: m/z = 1121.4219.

IR (Film):

$\tilde{\nu} = 3229, 1879, 1627, 1116, 903$ cm⁻¹.
Experimental

Melting point: 145 – 148 °C.

tert-butyl (S)-6-((S)-5-acetamido-6-(6-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)hexylamino)-6-oxohexylamino)-5-amino-6-oxohexylcarbamate NK386

To a solution of NK385 (480 mg, 0.43 mmol, 1.0 equiv.) in methylene chloride (10 mL) was added Et₂NH (2 mL) and the solution was stirred for 8h. Upon completion of the reaction the solvent was removed in vacuo, the residue was dissolved in EtOAc (15 mL) and MeOH (5 mL), washed with 1 N aq. HCl (3 mL), washed with brine, dried with MgSO₄, filtered and purified over silica (EtOAc/ULTRA 1:1) to give NK386 as a white foam (350 mg, 93%).

¹H NMR (500 MHz, MeOD): δ = 7.61-7.60 (m, 1 H), 7.57-7.54 (m, 1 H), 7.50-7.46 (m, 1 H), 7.42-7.37 (m, 2 H), 7.24-7.21 (m, 2 H), 4.30-4.24 (m, 1 H), 3.40 (t, J = 7.0 Hz, 2 H), 3.31-3.15 (m, 5 H), 3.06 (t, J = 7.0 Hz, 3 H), 2.34 (s, 3 H), 2.01 (s, 3 H), 1.84-1.76 (m, 1 H), 1.73-1.61 (m, 4 H), 1.61-1.30 ppm (m, 24 H).

¹³C NMR (100 MHz, CDCl₃): δ = 175.3, 171.8, 170.5, 162.8, 156.2, 145.1, 143.1, 136.0, 134.9, 133.0, 130.8, 130.6, 130.3, 128.9, 127.9, 127.2, 117.6, 79.1, 55.1, 53.0, 40.2, 39.2, 38.7, 38.4, 34.7, 31.9, 29.9, 29.6, 29.2, 28.4, 26.3, 26.2, 23.2, 22.9, 22.6, 9.4 ppm.

HRMS (ESI, 180 °C): calculated for C₄₂H₆₆Cl₅N₈O₆ [M+H] m/z = 877.3696, found: m/z = 877.3720; calculated for C₄₂H₆₅Cl₅N₈O₆Na [M+Na] m/z = 899.3515, found: m/z = 899.3579.

IR (Film): ʋ = 3253, 1781, 1529, 1142, 924 cm⁻¹.

Melting point: 55 - 57 °C.
tert-butyl (S)-5-acetamido-6-((S)-5-acetamido-6-(6-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)hexylamino)-6-oxohexylamino)-6-oxohexylcarbamate NK387

To an ice-cooled solution of NK386 (350 mg, 0.40 mmol, 1.0 equiv.) in methylene chloride (15 mL), DMF (3 mL) and DIPEA (0.3 mL, 2.0 mmol, 5.0 equiv.) was slowly added acetyl chloride (34 µL, 0.48 mmol, 1.2 equiv.). The solution was stirred for 20 minutes and subsequently diluted with EtOAc (20 mL) and MeOH (5 mL), washed with 1N aq. HCl (5 mL), washed with brine, dried with Na$_2$SO$_4$, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 1:1) gave NK387 as a white foam (265 mg, 72%).

$^1$H NMR (500 MHz, MeOD): $\delta = 7.60$-$7.58$ (m, 1 H), 7.57-$7.53$ (m, 1 H), 7.50-$7.45$ (m, 1 H), 7.42-$7.37$ (m, 2 H), 7.25-$7.19$ (m, 2 H), 4.30-$4.21$ (m, 2 H), 3.43-$3.37$ (m, 2 H), 3.24-$3.17$ (m, 4 H), 3.05 (t, $J = 7.0$ Hz, 2 H), 2.34 (s, 3 H), 2.02-$1.99$ (m, 6 H), 1.82-$1.72$ (m, 2 H), 1.70-$1.59$ (m, 4 H), 1.59-$1.30$ ppm (m, 23 H).

$^{13}$C NMR (125 MHz, MeOD): $\delta = 174.5$, 174.4, 174.3, 173.3, 165.0, 164.9, 146.3, 144.6, 137.4, 137.3, 136.2, 134.2, 132.6, 132.4, 131.1, 129.9, 129.2, 128.7, 118.3, 79.9, 55.1, 54.9, 41.1, 40.4, 40.3, 40.1, 40.0, 39.9, 32.8, 32.7, 30.6, 30.5, 30.2, 29.9, 28.8, 27.6, 27.5, 24.2, 24.1, 22.5, 9.6 ppm.

HRMS (ESI, 180 °C): calculated for C$_{44}$H$_{61}$Cl$_3$N$_8$O$_7$Na $[M+Na]$ m/z = 941.3621, found: m/z = 941.3637; calculated for C$_{44}$H$_{62}$Cl$_3$N$_8$O$_7$ [M+H] m/z = 919.3802, found: m/z = 919.3806.

IR (Film): $\tilde{\nu} = 3248$, 1691, 1574, 1129, 902 cm$^{-1}$.

Melting point: 104-$107$ °C.
Experimental

N-(6-((S)-2-acetamido-6-((S)-2-acetamido-6-aminohexanamido)hexanamido)hexyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK388

NK387 (265 mg, 0.29 mmol, 1.0 equiv.) was dissolved in a 1:1 mixture of DCM/TFA (6 mL). Upon completion of the deprotection the solvent was removed *in vacuo*, the residue dissolved in EtOAc (5 mL) and MeOH (2 mL) and washed with a sat. aqueous NaHCO₃ solution (2 mL), followed by drying with MgSO₄ and filtering. The solvent was removed *in vacuo* and the crude product purified over silica (EtOAc/ULTRA 1:2) to give NK388 as a colourless oil (238 mg, quant.).

\[ \text{HNMR (400 MHz, CDCl₃):} \quad \delta = 7.45-7.38 \text{ (m, 1 H)}, 7.38-7.20 \text{ (m, 4 H)}, 7.08-7.00 \text{ (m, 2 H)}, 4.40 \text{ (br s, 2 H)}, 3.44-3.30 \text{ (m, 2 H)}, 3.30-3.12 \text{ (m, 4 H)}, 3.12-2.97 \text{ (m, 2 H)}, 2.28 \text{ (s, 3 H)}, 2.06 \text{ (br s, 6 H)}, 1.86-1.23 \text{ ppm (m, 20 H).} \]

\[ \text{13C NMR (100 MHz, MeOD):} \quad \delta = 174.3, 174.1, 173.4, 173.3, 164.9, 146.3, 144.5, 137.4, 137.3, 136.1, 134.1, 132.6, 132.4, 131.0, 129.9, 129.2, 128.7, 118.2, 55.0, 54.8, 40.5, 40.3, 40.0, 39.9, 32.8, 32.5, 30.5, 30.2, 29.9, 28.1, 27.6, 27.5, 24.2, 23.9, 22.6, 22.5, 9.6 \text{ ppm.} \]

\[ \text{HRMS (ESI, 180 °C):} \quad \text{calculated for } C_{39}H_{54}Cl_3N_8O_5 [M+H] m/z = 819.3277, \text{ found: } m/z = 819.3296; \text{ calculated for } C_{38}H_{53}Cl_3N_8O_5Na [M+Na] m/z = 841.3097, \text{ found: } m/z = 841.3114. \]

\[ \text{IR (Film):} \quad \tilde{\nu} = 3199, 1749, 1421, 1157, 857 \text{ cm}^{-1}. \]

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To a solution of NK388 (52 mg, 0.06 mmol, 1.0 equiv.) in methylene chloride (3 mL), DMF (1 mL) and DIPEA (50 μL, 0.3 mmol, 5.0 equiv.) was added acid chloride NK25 (30 mg, 0.07 mmol, 1.2 equiv.) dissolved in methylene chloride (2 mL). After stirring for 30 minutes, the solvent was removed in vacuo and the residue dissolved in EtOAc (3 mL) and MeOH (1 mL), washed with 1 N aq. HCl (0.5 mL), washed with water, dried with MgSO₄, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 1:3) gave NK412 as a colourless resin (38 mg, 53%).

$^1$H NMR (500 MHz, MeOD) $\delta = 7.60 - 7.58$ (m, 2H), $7.56 - 7.52$ (m, 2H), $7.49 - 7.45$ (m, 2H), $7.41 - 7.37$ (m, 4H), $7.23 - 7.18$ (m, 4H), $4.28 - 4.23$ (m, 2H), $3.42 - 3.36$ (m, 4H), $3.23 - 3.17$ (m, 4H), $2.34 - 2.32$ (m, 6H), $2.01 - 1.98$ (m, 6H), $1.82 - 1.73$ (m, 2H), $1.73 - 1.59$ (m, 6H), $1.58 - 1.50$ (m, 5H), $1.47 - 1.34$ ppm (m, 7H).

$^{13}$C NMR (100 MHz, MeOD): $\delta = 174.4, 174.48, 174.35, 174.33, 165.0, 164.9, 146.9, 146.3, 146.2, 144.65, 144.63, 137.4, 137.4, 137.3, 136.2, 136.1, 134.2, 134.1, 132.5, 132.4, 131.0, 129.9, 129.2, 128.76, 128.73, 118.3, 55.06, 55.02, 40.3, 39.9, 39.7, 32.87, 32.85, 30.5, 30.29, 30.24, 29.9, 27.6, 27.5, 24.3, 24.1, 22.56, 22.53, 9.66, 9.62 ppm.

HRMS (ESI, 180 °C): calculated for C₅₆H₆₂Cl₆N₁₀O₆Na [M+Na] $m/z = 1203.2877$, found: $m/z = 1203.2900$.

IR (Film): $\tilde{\nu} = 3253, 1781, 1489, 1422, 949$ cm$^{-1}$. 
To a solution of NK388 (52 mg, 0.06 mmol, 1.0 equiv.) in methylene chloride (3 mL), DMF (1 mL) and DIPEA (50 µL, 0.3 mmol, 5.0 equiv.) was added acid chloride NK30 (19 mg, 0.07 mmol, 1.2 equiv.) dissolved in methylene chloride (2 mL). After stirring for 30 minutes, the solvent was removed in vacuo and the residue dissolved in EtOAc (3 mL) and MeOH (1 mL), washed with 1 N aq. HCl (0.5 mL), washed with water, dried with MgSO₄, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 1:6) gave NK456 as a colourless resin (30 mg, 48%).

**¹H NMR (500 MHz, MeOD)**

δ = 7.60 (d, J = 2.2 Hz, 1H), 7.57 – 7.54 (m, 1H), 7.49 – 7.46 (m, 1H), 7.42 – 7.38 (m, 2H), 7.24 – 7.20 (m, 2H), 4.53 – 4.49 (m, 1H), 4.34 – 4.30 (m, 1H), 4.28 – 4.23 (m, 2H), 3.39 (t, J = 7.5 Hz, 2H), 3.25 – 3.17 (m, 7H), 2.97 – 2.92 (m, 1H), 2.75 – 2.73 (m, 1H), 2.34 (s, 3H), 2.22 (t, J = 7.5 Hz, 2H), 2.02 – 2.00 (m, 6H), 1.82 – 1.72 (m, 3H), 1.72 – 1.59 (m, 7H), 1.59 – 1.50 (m, 6H), 1.50 – 1.35 ppm (m, 10H).

**¹³C NMR (125 MHz, MeOD):**

δ = 176.0, 174.48, 174.43, 173.3, 166.1, 165.0, 146.3, 144.6, 137.4, 137.3, 136.2, 134.2, 132.6, 132.5, 131.1, 129.9, 129.2, 128.7, 118.3, 63.4, 61.6, 57.0, 55.1, 55.0, 41.0, 40.3, 40.08, 40.06, 40.01, 36.8, 32.8, 30.6, 30.3, 30.1, 29.9, 29.8, 29.5, 27.66, 27.62, 26.9, 24.3, 24.2, 22.5, 9.6 ppm.

**HRMS (ESI, 180 °C):**

calculated for C₄₉H₅₇Cl₅N₁₀O₇NaS [M+Na] m/z = 1067.3873, found: m/z = 1067.3858.

**IR (Film):**

ν = 3188, 1697, 1529, 1448, 963 cm⁻¹.
Experimental

N1-((S)-5-acetamido-6-((S)-5-acetamido-6-(6-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)hexylamino)-6-oxohexylamino)-6-oxohexyl)-N4-(4-((5-hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino)ethyl)phenyl)succinamide NK416

To a solution of NK421 (30 mg, 0.07 mmol, 1.2 equiv.) in methylene chloride (2 mL) was added EDCI (14 mg, 0.07 mmol, 1.15 equiv.) and HOBt (10 mg, 0.07 mmol, 1.15 equiv.) and DIPEA (50 µL, 0.3 mmol, 5.0 equiv.). After stirring for 25 minutes, NK388 (52 mg, 0.06 mmol, 1.0 equiv.) in methylene chloride (3 mL), and DMF (1 mL) was added and the solution was stirred for 14 h. The solvent was removed in vacuo and the residue dissolved in EtOAc (3 mL) and MeOH (1 mL), washed with 1 N aq. HCl (0.5 mL), washed with water, dried with MgSO₄, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 1:6) gave NK416 as a brown oil (28 mg, 39%).

¹H NMR (500 MHz, MeOD) δ = 7.58 – 7.56 (m, 1H), 7.55 – 7.46 (m, 3H), 7.46 – 7.43 (m, 1H), 7.39 – 7.35 (m, 2H), 7.21 – 7.16 (m, 4H), 6.94 (t, J = 7.6 Hz, 1H), 6.60 – 6.56 (m, 2H), 4.29 – 4.19 (m, 2H), 3.37 (t, J = 6.8 Hz, 2H), 3.25 – 3.12 (m, 6H), 3.10 – 3.02 (m, 1H), 3.02 – 2.95 (m, 1H), 2.92 – 2.83 (m, 3H), 2.82 – 2.74 (m, 3H), 2.74 – 2.64 (m, 4H), 2.60 – 2.48 (m, 3H), 2.33 (s, 3H), 2.17 – 2.10 (m, 1H), 2.01 – 1.97 (m, 6H), 1.82 – 1.71 (m, 2H), 1.71 – 1.60 (m, 7H), 1.60 – 1.50 (m, 6H), 1.47 – 1.30 (m, 9H), 0.96 (t, J = 7.6 Hz, 3H).

¹³C NMR (125 MHz, MeOD): δ = 174.6, 174.4, 173.3, 173.3, 172.8, 164.9, 155.9, 146.3, 144.6, 138.1, 137.4, 137.3, 136.1, 134.1, 132.5, 132.4, 131.0, 130.1, 129.9, 129.2, 128.7, 127.3, 124.2, 121.5, 121.3, 118.3, 112.8, 55.1, 55.0, 53.9, 53.8, 40.4, 40.3, 40.07, 40.04, 33.0, 32.8, 32.7, 31.9, 30.5, 30.2, 30.0, 29.9, 27.66, 27.62, 26.6, 24.6, 24.27, 24.23, 22.5, 22.3, 12.2, 9.6 ppm.

HRMS (ESI, 180 °C): calculated for C₆₄H₆₃Cl₅N₁₀O₉Na [M+Na] m/z = 1247.5353, found: m/z = 1247.5331.
IR (Film): \[ \tilde{\nu} = 3144, 1623, 1574, 1185, 913 \text{ cm}^{-1}. \]

**N,N'-(S)-6-(S)-5-acetamido-6-(6-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)hexylamino)-6-oxohexylamino)-6-oxohexane-1,5-diyl)diacetamide NK474**

![Chemical structure of NK474](image)

To a solution of **NK388** (52 mg, 0.06 mmol, 1.0 equiv.) in methylene chloride (3 mL), DMF (1 mL) and DIPEA (50 µL, 0.3 mmol, 5.0 equiv.) was added acetyl chloride (5 µL, 0.07 mmol, 1.2 equiv.). After stirring for 30 minutes, the solvent was removed *in vacuo* and the residue dissolved in EtOAc (3 mL) and MeOH (1 mL), washed with 1 N aq. HCl (0.5 mL), washed with water, dried with MgSO\(_4\), filtered and the solvent was removed *in vacuo*. Purification over silica (EtOAc/ULTRA 1:4) gave **NK474** as a white foam (27 mg, 52%).

**\( ^1\)H NMR (400 MHz, MeOD)**

\[ \delta = 7.60 (d, J = 2.2 \text{ Hz}, 1H), 7.57–7.53 (m, 1H), 7.49–7.45 (m, 1H), 7.41–7.37 (m, 2H), 7.24–7.20 (m, 2H), 4.29–4.20 (m, 2H), 3.39 (m, 2H), 3.28 (t, J = 7.5 \text{ Hz}, 2H), 3.23–3.15 (m, 6H), 2.34 (s, 3H), 2.03–2.00 (m, 6H), 1.95 (s, 3H), 1.82–1.73 (m, 2H), 1.68–1.61 (m, 4H), 1.57–1.50 (m, 6H), 1.50–1.35 ppm (m, 8H). \]

**\( ^{13}\)C NMR (100 MHz, MeOD):**

\[ \delta = 174.5, 174.4, 173.4, 173.3, 164.9, 146.3, 144.6, 137.4, 137.3, 136.2, 134.2, 132.6, 132.4, 131.1, 129.9, 129.2, 128.7, 118.3, 55.1, 55.0, 40.4, 40.3, 40.2, 40.1, 39.9, 32.88, 32.84, 30.5, 30.3, 30.21, 29.9, 27.6, 27.5, 24.3, 24.2, 22.6, 22.5, 9.6 ppm. \]

**HRMS (ESI, 180 °C):** calculated for C\(_{41}\)H\(_{55}\)Cl\(_3\)N\(_8\)O\(_6\)Na [M+Na] \(m/z = 883.3202\), found: \( m/z = 883.3193 \).

IR (Film):

\[ \tilde{\nu} = 3157, 1689, 1549, 1171, 925 \text{ cm}^{-1}. \]

**Melting point:** 153 – 155 °C.
Experimental

(9H-fluoren-9-yl)methyl (11S,18S)-11,18-diacetamido-1-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl)-1,10,17,24-tetraoxo-26,29-dioxa-2,9,16,23-tetraazahentriacontan-31-ylcarbamate NK417

\[
\text{NK417}
\]

To a solution of 8-(Fmoc-amino)-3,6-dioxaoctanoic acid (207 mg, 0.54 mmol, 1.2 equiv.) in methylene chloride (15 mL) was added EDCI (96 mg, 0.48 mmol, 1.15 equiv.) and HOBt (69 mg, 0.48 mmol, 1.15 equiv.) and DIPEA (355 µL, 2.1 mmol, 5.0 equiv.). After stirring for 25 minutes, NK388 (340 mg, 0.42 mmol, 1.0 equiv.) in methylene chloride (12 mL), and DMF (4 mL) was added and the solution was stirred for 14h. The solvent was removed \textit{in vacuo} and the residue dissolved in EtoAc (20 mL) and MeOH (4 mL), washed with 1 N aq. HCl (10 mL), washed with water, dried with MgSO\textsubscript{4}, filtered and the solvent was removed \textit{in vacuo}. Purification over silica (EtoAc/ULTRA 1:2) gave NK417 as a colourless resin (343 mg, 69%).

\[\text{1H NMR (300 MHz, MeOD)}\]
\[\delta = 7.81 \text{ (d, } J = 7.5 \text{ Hz, 2H)} , 7.67 \text{ (d, } J = 7.5 \text{ Hz, 2H)} , 7.57 \text{ (d, } J = 2.2 \text{ Hz, 1H)} , 7.54 – 7.50 \text{ (m, 1H)} , 7.49 – 7.29 \text{ (m, 7H)} , 7.23 – 7.18 \text{ (m, 2H)} , 4.39 \text{ (d, } J = 6.7 \text{ Hz, 2H)} , 4.30 – 4.19 \text{ (m, 3H)} , 4.00 \text{ (br s, 2H)} , 3.97 \text{ (s, 2H)} , 3.73 – 3.63 \text{ (br s, 4H)} , 3.63 – 3.54 \text{ (m, 2H)} , 3.44 – 3.34 \text{ (m, 4H)} , 3.30 – 3.15 \text{ (m, 6H)} , 2.33 \text{ (s, 3H)} , 2.04 – 1.99 \text{ (m, 6H)} , 1.85 – 1.72 \text{ (m, 2H)} , 1.72 – 1.60 \text{ (m, 4H)} , 1.60 – 1.50 \text{ (m, 6H)} , 1.47 – 1.32 \text{ ppm (m, 8H)} .\]

\[\text{13C NMR (75 MHz, MeOD):}\]
\[\delta = 174.4 , 174.3 , 174.2 , 173.2 , 172.6 , 164.8 , 146.3 , 145.3 , 144.5 , 142.6 , 137.3 , 137.2 , 136.1 , 134.1 , 132.5 , 132.4 , 131.0 , 129.8 , 129.2 , 128.8 , 128.6 , 128.1 , 126.1 , 120.9 , 118.3 , 72.0 , 71.2 , 71.1 , 70.9 , 67.6 , 55.0 , 54.9 , 54.8 , 41.6 , 40.4 , 40.2 , 40.1 , 39.9 , 39.7 , 39.6 , 32.8 , 32.7 , 30.5 , 30.2 , 30.1 , 29.9 , 27.6 , 27.5 , 24.2 , 24.1 , 22.5 , 9.6 \text{ ppm}.\]

HRMS (ESI, 180 °C): calculated for C\textsubscript{60}H\textsubscript{74}Cl\textsubscript{3}N\textsubscript{9}O\textsubscript{10} [M+H] \textit{m/z} = 1186.4697, found: \textit{m/z} = 1186.4721.

IR (Film): \[\tilde{\nu} = 3174 , 1598 , 1534 , 1233 , 957 \text{ cm}^{-1}.\]
5-(4-chlorophenyl)-N-((14S,21S)-14,21-diacetamido-1-amino-8,15,22-trioxo-3,6-dioxa-9,16,23-triazanonacosan-29-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK418

NK417 (343 mg, 0.29 mmol, 1.0 equiv.) was dissolved in a 1:1 mixture of DCM/TFA (12 mL). Upon completion of the deprotection the solvent was removed *in vacuo*, the residue dissolved in EtOAc (5 mL) and MeOH (2 mL) and washed with a 1 N aqueous NaOH solution (2 mL), followed by drying with MgSO₄ and filtering. The solvent was removed *in vacuo* and the crude product purified over silica (EtOAc/ULTRA 1:2) to give NK418 as a colourless oil (278 mg, quant.).

1H NMR (500 MHz, MeOD): δ = 7.60 (d, J = 2.2 Hz, 1H), 7.57 - 7.54 (m, 1H), 7.50 - 7.46 (m, 1H), 7.41 - 7.38 (m, 2H), 7.24 - 7.20 (m, 2H), 4.30 - 4.21 (m, 2H), 4.02 (br s, 2H), 3.74 - 3.67 (m, 4H), 3.60 (t, J = 5.3 Hz, 2H), 3.40 (t, J = 7.0 Hz, 2H), 3.28 (t, J = 7.0 Hz, 2H), 1.85-1.73 (m, 2H), 1.70-1.51 (m, 10 H), 1.49-1.33 ppm (m, 8 H).

13C NMR (125 MHz, MeOD): δ = 174.4, 174.3, 173.38, 173.32, 172.5, 164.9, 146.3, 144.5, 137.4, 137.3, 136.1, 134.1, 132.6, 132.4, 131.0, 129.9, 129.2, 128.7, 118.3, 72.6, 71.9, 71.3, 71.2, 55.0, 54.9, 41.8, 40.3, 40.0, 39.9, 39.6, 32.8, 32.7, 30.5, 30.28, 30.25, 29.9, 27.6, 27.5, 24.27, 24.21, 22.5, 9.6 ppm.

HRMS (ESI, 180 °C): calculated for C₄₅H₆₄Cl₃N₉O₈Na [M+Na] m/z = 986.3836, found: m/z = 986.3830.

IR (Film): ʋ = 3237, 1690, 1489, 1434, 968 cm⁻¹.
Experimental

5-(4-chlorophenyl)-N-((11S,18S)-11,18-diacetamido-1-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl)-1,10,17,24-tetraoxo-26,29-dioxa-2,9,16,23-tetraazahentriacontan-31-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK462

To a solution of NK418 (65 mg, 0.06 mmol, 1.0 equiv.) in methylene chloride (3 mL), DMF (1 mL) and DIPEA (50 µL, 0.3 mmol, 5.0 equiv.) was added acid chloride NK25 (30 mg, 0.07 mmol, 1.2 equiv.) dissolved in methylene chloride (2 mL). After stirring for 30 minutes, the solvent was removed in vacuo and the residue dissolved in EtOAc (3 mL) and MeOH (1 mL), washed with 1 N aq. HCl (0.5 mL), washed with water, dried with MgSO₄, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 1:4) gave NK462 as a colourless resin (44 mg, 49%).

$^1$H NMR (500 MHz, MeOD) δ = 7.59 – 7.56 (m, 2H), 7.56 – 7.52 (m, 2H), 7.48 – 7.45 (m, 2H), 7.40 – 7.37 (m, 4H), 7.23 – 7.18 (m, 4H), 4.30 – 4.21 (m, 2H), 4.00 (s, 2H), 3.98 (s, 2H), 3.74 – 3.68 (m, 6H), 3.64 - 3.60 (m, 2H), 3.39 (t, $J = 7.6$ Hz, 2H), 3.25 – 3.17 (m, 6H), 2.34 (s, 3H), 2.33 (s, 3H), 2.01 (br s, 6H), 1.82 – 1.73 (m, 2H), 1.69 – 1.60 (m, 4H), 1.58 – 1.50 (m, 6H), 1.47 – 1.34 ppm (m, 8H).

$^{13}$C NMR (100 MHz, MeOD): δ = 174.48, 174.43, 173.3, 173.2, 172.6, 165.0, 164.9, 146.3, 146.0, 144.6, 144.5, 137.4, 137.38, 137.35, 137.31, 136.2, 136.1, 134.15, 134.11, 132.56, 132.54, 132.4, 131.1, 131.0, 129.99, 129.92, 129.3, 129.2, 128.7, 128.6, 118.4, 118.3, 72.0, 71.3, 71.1, 70.6, 55.1, 55.09, 55.06, 55.02, 40.4, 40.1, 39.99, 39.92, 39.7, 32.9, 32.89, 32.85, 32.81, 30.5, 30.29, 30.21, 29.9, 27.6, 27.5, 24.2, 24.1, 22.6, 22.5, 9.7, 9.6 ppm.

HRMS (ESI, 180 °C): calculated for C₆₂H₇₃Cl₆N₁₁O₉Na [M+Na] $m/z = 1348.3616$, found: $m/z = 1348.3621$.

IR (Film): $\tilde{\nu} = 3137, 1576, 1489, 1217, 931$ cm$^{-1}$. 
Experimental

5-(4-chlorophenyl)-N-((20S,27S)-20,27-diacetamido-5,14,21,28-tetraoxo-1-((4S)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-9,12-dioxo-6,15,22,29-tetraazapentriacontan-35-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK464

To a solution of NK418 (65 mg, 0.06 mmol, 1.0 equiv.) in methylene chloride (3 mL), DMF (1 mL) and DIPEA (50 µL, 0.3 mmol, 5.0 equiv.) was added acid chloride NK30 (19 mg, 0.07 mmol, 1.2 equiv.) dissolved in methylene chloride (2 mL). After stirring for 30 minutes, the solvent was removed in vacuo and the residue dissolved in EtOAc (3 mL) and MeOH (1 mL), washed with 1 N aq. HCl (0.5 mL), washed with water, dried with MgSO$_4$, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 1:7) gave NK464 as a colourless resin (32 mg, 45%).

$^1$H NMR (500 MHz, MeOD) \[ \delta = 7.60 \text{ (d, } J = 2.2 \text{ Hz, 1H), 7.57 - 7.53 \text{ (m, 1H), 7.50 - 7.46 \text{ (m, 1H), 7.42 - 7.37 \text{ (m, 2H), 7.23 - 7.18 \text{ (m, 2H), 4.54 - 4.50 \text{ (m, 1H), 4.35 - 4.31 \text{ (m, 1H), 4.29 - 4.23 \text{ (m, 2H), 4.02 \text{ (br s, 2H), 3.73 - 3.67 \text{ (m, 4H), 3.63 \text{ (t, } J = 5.5 \text{ Hz, 2H), 3.43 - 3.37 \text{ (m, 4H), 3.28 \text{ (t, } J = 7.5 \text{ Hz, 2H), 3.24 - 3.19 \text{ (m, 5H), 2.97 - 2.93 \text{ (m, 1H), 2.76 - 2.72 \text{ (m, 1H), 2.34 \text{ (s, 3H), 2.26 \text{ (t, } J = 7.5 \text{ Hz, 2H), 2.03 - 2.01 \text{ (m, 6H), 1.82 - 1.72 \text{ (m, 3H), 1.71 - 1.60 \text{ (m, 7H), 1.60 - 1.50 \text{ (m, 7H), 1.50 - 1.35 ppm (m, 9H).}} \]

$^{13}$C NMR (125 MHz, MeOD): \[ \delta = 176.2, 174.4, 174.3, 173.3, 172.6, 166.1, 164.9, 146.3, 144.6, 137.4, 137.3, 136.1, 134.2, 132.6, 132.4, 131.1, 129.9, 129.2, 128.7, 118.3, 72.0, 71.3, 71.2, 70.6, 63.4, 61.6, 57.0, 55.05, 55.02, 41.0, 40.2, 40.08, 40.04, 39.6, 36.7, 32.89, 32.82, 30.6, 30.29, 30.21, 29.9, 29.8, 29.5, 27.66, 27.62, 26.9, 24.3, 24.2, 22.5, 9.6 ppm. \]

HRMS (ESI, 180 °C): calculated for C$_{55}$H$_{78}$Cl$_3$N$_{11}$O$_{10}$NaS [M+Na] $m/z = 1212.4612$, found: $m/z = 1212.4658$.

IR (Film): \[ \tilde{\nu} = 3174, 1598, 1534, 1233, 957 \text{ cm}^{-1}. \]
To a solution of **NK421** (30 mg, 0.07 mmol, 1.2 equiv.) in methylene chloride (2 mL) was added EDCI (14 mg, 0.07 mmol, 1.15 equiv.) and HOBt (10 mg, 0.07 mmol, 1.15 equiv.) and DIPEA (50 µL, 0.3 mmol, 5.0 equiv.). After stirring for 25 minutes, **NK418** (65 mg, 0.06 mmol, 1.0 equiv.) in methylene chloride (3 mL), and DMF (1 mL) was added and the solution was stirred for 16h. The solvent was removed *in vacuo* and the residue dissolved in EtOAc (3 mL) and MeOH (1 mL), washed with 1 N aq. HCl (0.5 mL), washed with water, dried with MgSO$_4$, filtered and the solvent was removed *in vacuo*. Purification over silica (EtOAc/ULTRA 1:8) gave **NK461** as a brown oil (29 mg, 35%).

**1H NMR (500 MHz, MeOD)**

$$\delta = 7.58 - 7.56 (m, 1H), 7.55 - 7.48 (m, 3H), 7.46 - 7.43 (m, 1H), 7.40 - 7.35 (m, 2H), 7.23 - 7.18 (m, 4H), 6.93 (t, J = 7.6 Hz, 1H), 6.62 - 6.56 (m, 2H), 4.27 - 4.22 (m, 2H), 3.98 (br s, 2H), 3.69 - 3.64 (m, 4H), 3.58 (t, J = 5.5 Hz, 2H), 3.43 - 3.35 (m, 4H), 3.25 (t, J = 6.8 Hz, 2H), 3.22 - 3.16 (m, 5H), 3.05 - 2.90 (m, 4H), 2.90 - 2.77 (m, 5H), 2.72 - 2.67 (m, 2H), 2.63 - 2.50 (m, 3H), 2.33 (s, 3H), 2.21 - 2.13 (m, 1H), 2.00 (s, 6H), 1.82 - 1.71 (m, 2H), 1.71 - 1.60 (m, 7H), 1.60 - 1.50 (m, 6H), 1.47 - 1.30 (m, 10H), 0.98 ppm (t, J = 7.6 Hz, 3H).

**13C NMR (125 MHz, MeOD):**

$$\delta = 174.8, 174.4, 174.3, 173.35, 173.33, 172.8, 172.6, 164.9, 156.0, 146.3, 144.6, 138.3, 137.4, 137.3, 136.1, 134.1, 132.6, 132.4, 131.0, 130.1, 129.9, 129.2, 128.7, 127.4, 124.0, 121.5, 121.3, 118.3, 112.9, 72.0, 71.3, 71.2, 70.6, 55.0, 54.9, 53.8, 40.4, 40.3, 40.0, 39.9, 39.6, 33.0, 32.88, 32.84, 32.6, 31.9, 30.5, 30.27, 30.25, 29.9, 27.6, 27.5, 26.3, 24.5, 24.3, 24.2, 22.5, 21.9, 12.0, 9.6 ppm.$$

**HRMS (ESI, 180 °C):** calculated for C$_{70}$H$_{94}$Cl$_{3}$N$_{11}$O$_{11}$Na [M+Na] $m/z = 1392.6092$, found: $m/z = 1392.6110$. 

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**N1-((11S,18S)-11,18-diacetamido-1-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl)-1,10,17,24-tetraoxo-26,29-dioxo-2,9,16,23-tetraazahentriacontan-31-yl)-N4-(4-((5-hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino)ethyl)phenyl)succinamide NK461**

![Chemical Structure](image)
IR (Film): \( \tilde{\nu} = 3215, 1693, 1529, 1214, 918 \) cm\(^{-1}\).

5-(4-chlorophenyl)-N-((17S,24S)-17,24-diacetamido-2,11,18,25-tetraoxo-6,9-dioxa-3,12,19,26-tetraazadotriacontan-32-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK472

![Chemical Structure of NK472](image)

To a solution of NK418 (65 mg, 0.06 mmol, 1.0 equiv.) in methylene chloride (3 mL), DMF (1 mL) and DIPEA (50 µL, 0.3 mmol, 5.0 equiv.) was added acetyl chloride (5 µL, 0.07 mmol, 1.2 equiv.). After stirring for 90 minutes, the solvent was removed \textit{in vacuo} and the residue dissolved in EtOAc (3 mL) and MeOH (1 mL), washed with 1 N aq. HCl (0.5 mL), washed with water, dried with MgSO\(_4\), filtered and the solvent was removed \textit{in vacuo}. Purification over silica (EtOAc/ULTRA 1:4) gave NK472 as a colourless resin (29 mg, 48%).

\(^1\)H NMR (400 MHz, MeOD) \( \delta = 7.60 \) (d, \( J = 2.2 \) Hz, 1H), 7.57 – 7.53 (m, 1H), 7.50 – 7.46 (m, 1H), 7.42 – 7.37 (m, 2H), 7.23 – 7.18 (m, 2H), 4.30 – 4.21 (m, 2H), 4.02 (s, 2H), 3.74 – 3.65 (m, 4H), 3.59 (t, \( J = 5.5 \) Hz, 2H), 3.40 (t, \( J = 5.5 \) Hz, 4H), 3.28 (t, \( J = 7.5 \) Hz, 2H), 3.23 – 3.17 (m, 4H), 2.34 (s, 3H), 2.03 – 2.00 (m, 6H), 1.98 (s, 3H), 1.82 – 1.73 (m, 2H), 1.73 – 1.61 (m, 4H), 1.61 – 1.50 (m, 6H), 1.50 – 1.34 ppm (m, 8H).

\(^{13}\)C NMR (100 MHz, MeOD): \( \delta = 1.74.4, 174.3, 173.4, 173.3, 172.6, 164.9, 146.3, 144.5, 137.4, 137.3, 136.1, 134.1, 132.6, 132.4, 131.0, 129.9, 129.2, 128.7, 118.3, 71.9, 71.3, 71.2, 70.6, 55.0, 54.9, 40.4, 40.3, 40.0, 39.9, 39.6, 32.8, 32.7, 30.5, 30.29, 30.21, 29.9, 27.6, 27.5, 24.3, 24.2, 22.6, 22.5, 9.6 ppm.

HRMS (ESI, 180 °C): calculated for C\(_{47}\)H\(_{66}\)Cl\(_3\)N\(_9\)O\(_9\)Na: [M+Na] \( m/z = 1028.3941 \), found: \( m/z = 1028.3908 \).

IR (Film): \( \tilde{\nu} = 3157, 1689, 1549, 1171, 925 \) cm\(^{-1}\).
Experimental

(9H-fluoren-9-yl)methyl (11S,18S)-11,18-diacetamido-1-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl)-1,10,17,24,33-pentaoxo-26,29,35,38-tetraoxa-2,9,16,23,32-pentaazatetracontan-40-ylcarbamate NK419

To a solution of 8-(Fmoc-amino)-3,6-dioxaoctanoic acid (143 mg, 0.37 mmol, 1.2 equiv.) in methylene chloride (12 mL) was added EDCI (66 mg, 0.33 mmol, 1.15 equiv.) and HOBt (48 mg, 0.33 mmol, 1.15 equiv.) and DIPEA (244 µL, 1.45 mmol, 5.0 equiv.). After stirring for 25 minutes, NK418 (280 mg, 0.29 mmol, 1.0 equiv.) in methylene chloride (12 mL), and DMF (2 mL) was added and the solution was stirred for 16h. The solvent was removed in vacuo and the residue dissolved in EtOAc (20 mL) and MeOH (4 mL), washed with 1 N aq. HCl (10 mL), washed with water, dried with MgSO₄, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 1:2) gave NK419 as a colourless resin (247 mg, 64%).

1H NMR (400 MHz, MeOD)  δ = 7.81 (d, J = 7.5Hz, 2H), 7.67 (d, J = 7.5Hz, 2H), 7.57 (d, J = 2.2 Hz, 1H), 7.54 – 7.50 (m, 1H), 7.47 – 7.29 (m, 7H), 7.23 – 7.18 (m, 2H), 4.40 (d, J = 6.7Hz, 2H), 4.30 – 4.19 (m, 3H), 4.01 (s, 2H), 3.97 (s, 2H), 3.71 – 3.60 (m, 8H), 3.59 – 3.53 (m, 2H), 3.43 (t, J = 5.5 Hz, 2H), 3.41 – 3.36 (m, 2H), 3.24 (t, J = 7.5 Hz, 2H), 3.20 (t J = 6.7Hz, 4H), 2.33 (s, 3H), 2.01 (br s, 6H), 1.82 – 1.71 (m, 2H), 1.71 – 1.60 (m, 4H), 1.60 - 1.50 (m, 6H), 1.46 – 1.32 ppm (m, 8H).

13C NMR (100 MHz, MeOD):  δ = 174.38, 174.32, 173.2, 172.8, 172.4, 164.8, 158.8, 146.3, 145.3, 144.5, 142.6, 137.4, 137.2, 136.1, 134.1, 132.5, 132.4, 131.0, 129.8, 129.2, 128.8, 128.6, 128.2, 126.1, 121.0, 118.3, 72.0, 71.9, 71.2, 71.19, 71.13, 70.5, 67.6, 55.0, 54.9, 54.8, 41.7, 40.2, 39.9, 39.69, 39.63, 32.8, 32.7, 30.5, 30.29, 30.21, 29.9, 28.2, 27.6, 27.5, 24.2, 24.1, 22.5, 9.6 ppm.
**Experimental**

5-(4-chlorophenyl)-N-((23S,30S)-23,30-diacetamido-1-amino-8,17,24,31-tetraoxo-3,6,12,15-tetraoxa-9,18,25,32-tetraazaoctatriacontan-38-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK420

NK419 (247 mg, 0.19 mmol, 1.0 equiv.) was dissolved in a 1:1 mixture of DCM/TFA (12 mL). Upon completion of the deprotection the solvent was removed in vacuo and subsequently purified over silica (EtOAc/ULTRA 1:8) to give NK420 as a colourless gum (209 mg, 98%).

$^1$H NMR (400 MHz, MeOD) δ = 7.60 (d, $J = 2.2$ Hz, 1H), 7.57 – 7.53 (m, 1H), 7.50 – 7.46 (m, 1H), 7.42 – 7.37 (m, 2H), 7.23 – 7.18 (m, 2H), 4.30 – 4.21 (m, 2H), 4.03 (s, 2H), 4.00 (s, 2H), 3.75 – 3.66 (m, 8H), 3.66 – 3.60 (m, 4H), 3.49 (t, $J = 5.5$ Hz, 2H), 3.43 – 3.37 (m, 2H), 3.27 (t, $J = 7.5$ Hz, 2H), 3.23 – 3.17 (m, 4H), 3.01 – 2.94 (m, 2H), 2.35 (s, 3H), 2.03 – 1.99 (m, 6H), 1.82 – 1.71 (m, 2H), 1.71 – 1.60 (m, 4H), 1.60 - 1.50 (m, 6H), 1.50 – 1.34 ppm (m, 8H).

$^{13}$C NMR (100 MHz, MeOD): δ = 174.4, 174.3, 173.3, 172.7, 172.5, 164.9, 146.3, 144.6, 137.4, 137.3, 136.1, 134.1, 132.6, 132.4, 131.0, 129.9, 129.2, 128.7, 118.3, 71.9, 71.39, 71.32, 71.29, 71.21, 70.5, 55.08, 55.03, 41.5, 40.3, 40.0, 39.9, 39.7, 39.6, 32.8, 32.7, 30.5, 30.29, 30.21, 29.9, 27.6, 27.5, 24.3, 24.2, 22.5, 9.6 ppm.

HRMS (ESI, 180 °C): calculated for C$_{51}$H$_{76}$Cl$_3$N$_{10}$O$_{11}$ [M+H] $m/z$ = 1109.4755, found: $m/z$ = 1109.4795.

IR (Film): $\tilde{\nu}$ = 3202, 1678, 1539, 1214, 937 cm$^{-1}$.
5-(4-chlorophenyl)-N-((11S,18S)-11,18-diacetamido-1-(5-(4-chlorophenyl)-1-(2,4-
dichlorophenyl)-4-methyl-1H-pyrazol-3-yl)-1,10,17,24,33-pentaoxo-2,9,16,23,32-pentaazatetracontan-40-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-
carboxamide NK533

To a solution of NK419 (50 mg, 0.05 mmol, 1.0 equiv.) in methylene chloride (3 mL), DMF (1 mL) and 
DIPEA (50 µL, 0.3 mmol, 5.0 equiv.) was added acid chloride NK25 (30 mg, 0.07 mmol, 1.2 equiv.) 
dissolved in methylene chloride (2 mL). After stirring for 30 minutes, the solvent was removed in vacuo 
and the residue subsequently purified over silica (EtOAc/ULTRA 1:7) to give NK533 as a colourless 
resin (30 mg, 41%).

$^1$H NMR (400 MHz, MeOD)  \( \delta = 7.61 - 7.53 (m, 4H), 7.50 - 7.45 (m, 2H), 7.42 - 7.37 (m, 
4H), 7.23 - 7.18 (m, 4H), 4.30 - 4.21 (m, 2H), 4.00 (s, 2H), 
3.98 (s, 2H), 3.75 - 3.60 (m, 12H), 3.58 (t, \( J = 5.5 \) Hz, 2H), 
3.43 - 3.35 (m, 4H), 3.29 - 3.17 (m, 6H), 2.35 (s, 3H), 2.33 (s, 
3H), 2.00 (br s, 6H), 1.82 - 1.71 (m, 2H), 1.71 - 1.60 (m, 4H), 
1.60 - 1.50 (m, 6H), 1.47 - 1.34 ppm (m, 8H).

$^{13}$C NMR (100 MHz, MeOD):  \( \delta = 174.4, 174.3, 173.3, 173.2, 172.6, 172.5, 165.0, 164.9, 
146.3, 146.0, 144.7, 144.6, 137.46, 137.41, 137.3, 137.32, 
136.2, 136.1, 134.2, 134.1, 132.6, 132.4, 131.19, 131.13, 
129.99, 129.92, 129.3, 129.2, 128.7, 128.6, 118.4, 118.3, 
72.0, 71.9, 71.39, 71.32, 71.19, 71.13, 70.7, 70.5, 55.0, 54.9, 
40.3, 40.0, 39.99, 39.92, 39.7, 39.6, 32.88, 32.82, 30.6, 30.29, 
30.21, 29.9, 27.6, 27.5, 24.3, 24.2, 22.6, 22.5, 9.69, 9.62 ppm.

HRMS (ESI, 180 °C): calculated for C\(_{68}\)H\(_{86}\)Cl\(_4\)N\(_{12}\)O\(_{12}\)Na \([M+Na] m/z = 1493.4355, 
found: \( m/z = 1493.4414. 

IR (Film):  \( \tilde{\nu} = 3254, 1721, 1540, 1186, 942 \) cm\(^{-1}\).
Experimental

5-(4-chlorophenyl)-N-((29S,36S)-29,36-diacetamido-5,14,23,30,37-pentaooxo-1-((4S)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-9,12,18,21-tetraoxa-6,15,24,31,38-pentaazatetratetracontan-44-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK536

To a solution of NK419 (50 mg, 0.05 mmol, 1.0 equiv.) in methylene chloride (3 mL), DMF (1 mL) and DIPEA (50 µL, 0.3 mmol, 5.0 equiv.) was added acid chloride NK30 (19 mg, 0.07 mmol, 1.2 equiv.) dissolved in methylene chloride (2 mL). After stirring for 45 minutes, the solvent was removed in vacuo and the residue subsequently purified over silica (EtOAc/ULTRA 1:7) to give NK536 as a colourless resin (26 mg, 39%).

$^{1}$H NMR (400 MHz, MeOD) $\delta = 7.60$ (d, $J = 2.2$ Hz, 1H), 7.57 – 7.53 (m, 1H), 7.50 – 7.46 (m, 1H), 7.42 – 7.37 (m, 2H), 7.23 – 7.18 (m, 2H), 4.54 – 4.48 (m, 1H), 4.35 – 4.31 (m, 1H), 4.30 – 4.21 (m, 2H), 4.03 (s, 2H), 4.00 (s, 2H), 3.75 – 3.66 (m, 8H), 3.63 (t, $J = 5.5$ Hz, 2H), 3.58 (t, $J = 5.5$ Hz, 2H), 3.49 (t, $J = 5.5$ Hz, 2H), 3.43 – 3.35 (m, 4H), 3.27 (t, $J = 7.5$ Hz, 2H), 3.23 – 3.17 (m, 5H), 2.98 – 2.91 (m, 1H), 2.75 – 2.70 (m, 1H), 2.33 (s, 3H), 2.25 (t, $J = 7.5$ Hz, 2H), 2.03 – 2.00 (m, 6H), 1.82 – 1.72 (m, 3H), 1.71 – 1.60 (m, 7H), 1.60 – 1.50 (m, 7H), 1.50 – 1.35 ppm (m, 9H).

$^{13}$C NMR (100 MHz, MeOD): $\delta = 176.1, 174.4, 174.3, 173.3, 172.8, 172.5, 166.0, 164.9, 146.3, 144.6, 137.4, 137.3, 136.1, 134.1, 132.6, 132.4, 131.0, 129.9, 129.2, 128.7, 118.3, 72.0, 71.3, 71.29, 71.21, 70.6, 70.5, 63.4, 61.6, 57.0, 55.0, 54.9, 41.1, 40.3, 40.0, 39.9, 39.7, 39.6, 36.7, 32.89, 32.82, 30.6, 30.29, 30.21, 29.9, 29.8, 29.5, 27.6, 27.5, 26.8, 24.3, 24.2, 22.5, 9.6 ppm.

HRMS (ESI, 180 °C): calculated for C$_{61}$H$_{80}$Cl$_{3}$N$_{12}$O$_{13}$NaS [M+Na] $m/z = 1357.5351$, found: $m/z = 1357.5394$.

IR (Film): $\tilde{\nu} = 3216, 1714, 1548, 1149, 913$ cm$^{-1}$. 
Experimental

N1-((11S,18S)-11,18-diacetamido-1-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl)-1,10,17,24,33-pentaoxo-26,29,35,38-tetraoxa-2,9,16,23,32-pentaazatetracontan-40-yl)-N4-(4-(2-((5-hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino)ethyl)phenyl)succinamide NK487

To a solution of NK24 (30 mg, 0.07 mmol, 1.2 equiv.) in methylene chloride (2 mL) was added EDCI (14 mg, 0.07 mmol, 1.15 equiv.) and HOBt (10 mg, 0.07 mmol, 1.15 equiv.) and DIPEA (50 µL, 0.3 mmol, 5.0 equiv.). After stirring for 25 minutes, NK419 (50 mg, 0.05 mmol, 1.0 equiv.) in methylene chloride (3 mL), and DMF (1 mL) was added and the solution was stirred for 16 hours. The solvent was removed in vacuo and the residue subsequently purified over silica (EtOAc/ULTRA 1:8) to give NK487 as a brown oil (27 mg, 35%).

$^1$H NMR (400 MHz, MeOD) $\delta = 7.58 - 7.56$ (m, 1H), $7.55 - 7.46$ (m, 4H), $7.40 - 7.35$ (m, 2H), $7.24 - 7.20$ (m, 4H), $6.93$ (t, $J = 7.6$ Hz, 1H), $6.60$ (t, $J = 7.6$ Hz, 2H), $4.27 - 4.22$ (m, 2H), $4.03 - 3.98$ (m, 4H), $3.71 - 3.63$ (m, 8H), $3.63 - 3.56$ (m, 4H), $3.50 - 3.46$ (m, 2H), $3.43 - 3.37$ (m, 4H), $3.25$ (t, $J = 7.6$ Hz, 2H), $3.22 - 3.16$ (m, 5H), $3.05 - 2.96$ (m, 4H), $2.90 - 2.77$ (m, 5H), $2.72 - 2.67$ (m, 2H), $2.63 - 2.50$ (m, 3H), $2.33$ (s, 3H), $2.24 - 2.16$ (m, 1H), $2.00$ (br s, 6H), $1.82 - 1.71$ (m, 2H), $1.71 - 1.60$ (m, 8H), $1.60 - 1.50$ (m, 7H), $1.47 - 1.30$ (m, 8H), $0.98$ ppm (t, $J = 7.6$ Hz, 3H).

$^{13}$C NMR (100 MHz, MeOD): $\delta = 174.8, 174.49, 174.42, 173.3, 172.9, 172.6, 165.0, 156.0, 146.3, 144.6, 138.4, 137.4, 137.3, 136.2, 134.2, 132.6, 132.5, 131.1, 130.2, 129.9, 129.3, 128.7, 127.5, 121.5, 121.4, 118.3, 113.0, 72.0, 71.39, 71.33, 71.2, 70.6, 70.5, 55.09, 55.03, 54.8, 53.88, 53.81, 40.4, 40.3, 40.0, 39.7, 39.6, 33.0, 32.8, 32.7, 31.9, 30.6, 30.3, 30.2, 29.9, 27.6, 27.5, 24.3, 24.2, 22.6, 11.9, 9.6 ppm.

HRMS (ESI, 180 °C): calculated for C$_{76}$H$_{105}$Cl$_3$N$_{12}$O$_{14}$Na [M+Na] $m/z$ = 1537.6831, found: $m/z$ = 1537.6870.
Experimental

IR (Film): $\tilde{\nu} = 3237, 1673, 1519, 1234, 914$ cm$^{-1}$.

5-(4-chlorophenyl)-N-((26S,33S)-26,33-diacetamido-2,11,20,27,34-pentaoxo-6,9,15,18-tetraoxa-3,12,21,28,35-pentaazahentetracontan-41-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK534

To a solution of NK419 (50 mg, 0.05 mmol, 1.0 equiv.) in methylene chloride (3 mL), DMF (1 mL) and DIPEA (50 µL, 0.3 mmol, 5.0 equiv.) was added acetyl chloride (5 µL, 0.07 mmol, 1.2 equiv.). After stirring for 90 minutes, the solvent was removed in vacuo and the residue subsequently purified over silica (EtOAc/ULTRA 1:4) to give NK534 as a colourless resin (23 mg, 40%).

$^1$H NMR (400 MHz, MeOD) $\delta = 7.60$ (d, $J = 2.2$ Hz, 1H), 7.57 – 7.53 (m, 1H), 7.50 – 7.46 (m, 1H), 7.42 – 7.37 (m, 2H), 7.23 – 7.18 (m, 2H), 4.30 – 4.21 (m, 2H), 4.03 (s, 2H), 4.00 (s, 2H), 3.75 – 3.66 (m, 8H), 3.63 (t, $J = 5.5$ Hz, 2H), 3.58 (t, $J = 5.5$ Hz, 2H), 3.49 (t, $J = 5.5$ Hz, 2H), 3.43 – 3.35 (m, 4H), 3.27 (t, $J = 7.5$ Hz, 2H), 3.23 – 3.17 (m, 4H), 2.35 (s, 3H), 2.03 – 2.00 (m, 6H), 1.98 (s, 3H), 1.82 – 1.71 (m, 2H), 1.71 – 1.50 (m, 10H), 1.50 – 1.34 ppm (m, 8H).

$^{13}$C NMR (100 MHz, MeOD): $\delta = 174.3, 173.4, 173.39, 173.32, 172.8, 172.6, 164.9, 146.3, 144.6, 137.4, 137.3, 136.1, 134.2, 132.6, 132.4, 131.1, 129.9, 129.2, 128.7, 118.3, 72.0, 71.3, 71.29, 71.21, 70.6, 70.5, 55.0, 54.9, 40.4, 40.3, 40.0, 39.9, 39.7, 39.6, 32.89, 32.82, 30.6, 30.29, 30.21, 29.9, 27.6, 27.5, 24.3, 24.2, 22.6, 22.5, 9.6 ppm.

HRMS (ESI, 180 °C): calculated for C$_{53}$H$_{77}$Cl$_3$N$_{10}$O$_{12}$Na [M+Na] $m/z = 1173.4680$, found: $m/z = 1173.4715$.

IR (Film): $\tilde{\nu} = 3188, 1756, 1499, 1152, 935$ cm$^{-1}$.
Experimental

6-(tert-butoxycarbonylamino)hexanoic acid NK283

\[
\begin{align*}
\text{HO} & \quad \text{N} & \quad \text{O} \\
\text{C} & \quad \text{C} & \quad \text{C} \\
\text{C} & \quad \text{C} & \quad \text{C} \\
\text{C} & \quad \text{C} & \quad \text{C}
\end{align*}
\]

To an ice-cooled solution of aminocaproic acid (650 mg, 3.0 mmol, 1.0 equiv.) in chloroform (30 mL) was added Boc\(_2\)O (990 mg, 4.50 mmol, 1.5 equiv.) in chloroform (15 mL) over 1 h under vigorous stirring. The solution was allowed to warm to r.t. and stirred for additional 14 h. The precipitate was filtered, the solvent was removed \textit{in vacuo} and the residue dissolved in ethyl acetate (100 mL). The organic layer was washed with brine (2 x 20 mL), the aqueous layer was extracted with ethyl acetate (1 x 15 mL), the combined organic layers were dried with MgSO\(_4\) filtered and the solvent removed \textit{in vacuo} to afford NK283 as a white solid (750 mg, 92%).

\[^{1}\text{H NMR (300 MHz, CDCl}_3\text{):} \quad \delta = 4.57 \text{ (br s, 1H), 3.70 (s, 3H), 3.18 – 3.02 (m, 2H), 2.33 (t, } J = 6.5 \text{ Hz, 2H) 1.71 – 1.59 (m, 2H), 1.56 – 1.30 ppm (m, 13H).} \]

Spectroscopic data is consistent with that reported in the literature.\(^{190}\)

Methyl 4-(aminomethyl)benzoate NK285

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{O} \\
\text{C} & \quad \text{C}
\end{align*}
\]

To an ice-cooled slurry of 4-(Methylamino)benzoic acid (2.25 g, 15.0 mmol, 1.0 equiv.) in methanol (120 mL) was slowly added thionyl chloride (11.0 mL, 150 mmol, 10.0 equiv.). The solution was then allowed to warm to rt and subsequently refluxed for 5 hours. The solvent was removed \textit{in vacuo} to give NK285 as a white powder (5.85 g, 98%) which was used in the next step without further purification.

\[^{1}\text{H NMR (300 MHz, CDCl}_3\text{):} \quad \delta = 8.01 \text{ (d, } J = 8.0 \text{ Hz, 2H), 7.38 (d, } J = 8.0 \text{ Hz, 2H), 3.93 (br s, 2H),} \\
3.91 \text{ ppm (s, 3H).} \]

Spectroscopic data is consistent with that reported in the literature.\(^{191}\)
Experimental

Methyl 4-((6-(tert-butoxycarbonylamino)hexanamido)methyl)benzoate NK311

![NK311](attachment:image)

To a solution of NK285 (703 mg, 3.38 mmol, 1.1 equiv.) in DMF (12 mL) was added HATU (1.22 g, 3.23 mmol, 1.05 equiv.) and NEt₃ (1.28 mL, 9.24 mmol, 3.0 equiv.) and stirred for 20 min under a nitrogen atmosphere. Then NK283 (1.0 g, 3.08 mmol, 1.0 equiv.) dissolved in DMF (5 mL) was added and stirred for 14h. The reaction mixture was diluted with EtOAc (120 mL) and brine (60 mL) and extracted with Et₂O (5 x 50 mL). The combined organic layers were dried with MgSO₄, filtered and concentrated in vacuo. The crude product was purified by flash chromatography (EtOAc/hexanes 7:1) to give NK311 as a brown oil (1.07 g, 68%).

¹H NMR (300 MHz, CDCl₃):  δ = 7.99 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 8.2 Hz, 2H), 5.59 – 5.88 (m, 1H), 4.64 – 4.51 (m, 1H), 4.49 (d, J = 6.0 Hz, 2H), 3.90 (s, 3H), 3.10 (q, J = 6.5 Hz, 2H), 2.24 (t, J = 7.2 Hz, 2H), 1.74 - 1.63 (m, 3H), 1.55 – 1.35 ppm (m, 12H).

¹³C NMR (75 MHz, CDCl₃):  δ = 172.8, 162.3, 143.7, 130.0, 129.3, 127.5, 52.1, 43.2, 40.3, 36.4, 29.8, 28.4, 26.3, 25.2 ppm.

HRMS (ESI, 180 °C): calculated for C₂₀H₃₀N₂O₅Na [M+Na]: m/z = 401.2047, found: m/z = 401.2053.

IR (Film):  ν = 3174, 1598, 1534, 1233, 957 cm⁻¹.

Melting point:  158 – 160 °C
Experimental

4-((6-(tert-butoxycarbonylamino)hexanamido)methyl)benzoic acid NK314

Methyl ester **NK311** (1.0 g, 2.30 mmol, 1.0 equiv.) was dissolved in MeOH (20 mL) and a 1N aq. KOH-solution (20 mL) and refluxed for 3h. After cooling to rt the solution was poured onto an ice-cooled 6N aqueous HCl-solution (70 mL). The precipitate was filtered off and dried *in vacuo* to afford carboxylic acid **NK314** (900 mg, 96 %) as a white solid.

\[\text{NK314}\]

\[
\begin{align*}
\text{HO-} & \quad \text{N} \\
& \quad \text{O} \\
& \quad \text{N} \\
& \quad \text{O}
\end{align*}
\]

\[\text{NK314}\]

\[\begin{align*}
\text{δ} &= 7.99 \text{ (d, } J = 8.2 \text{ Hz, 2H)}, \quad 7.39 \text{ (d, } J = 8.2 \text{ Hz, 2H)}, \quad 4.43 \text{ (br s, 2H)}, \quad 3.03 \text{ (t, } J = 7.2 \text{ Hz, 2H)}, \quad 2.27 \text{ (t, } J = 7.2 \text{ Hz, 2H)}, \quad 1.73 - 1.60 \text{ (m, 2H)}, \quad 1.56 - 1.28 \text{ ppm (m, 14H).}
\end{align*}\]

\[\begin{align*}
\text{δ} &= 176.1, \quad 169.6, \quad 163.7, \quad 145.6, \quad 131.0, \quad 128.4, \quad 79.8, \quad 43.7, \quad 41.2, \quad 36.9, \quad 30.6, \quad 28.8, \quad 27.4, \quad 26.6 \text{ ppm.}
\end{align*}\]

\[\begin{align*}
\text{HRMS (ESI, 180 °C):} \quad \text{calculated for C}_{19}\text{H}_{28}\text{N}_{2}\text{O}_{5}\text{Na}[M+Na]: m/z = 387.1890, \text{ found:} \quad m/z = 387.1882.
\end{align*}\]

\[\begin{align*}
\text{IR (Film):} \quad \tilde{\nu} &= 3188, \quad 1756, \quad 1499, \quad 1152, \quad 935 \text{ cm}^{-1}.
\end{align*}\]

\[\text{Melting point:} \quad 158 - 160 \text{ °C}\]
Experimental

Methyl 6-((6-((tert-butoxycarbonylamino)hexanamido)methyl)benzamido)hexanoate NK325

To a solution of NK314 (703 mg, 3.38 mmol, 1.1 equiv.) in DMF (12 mL) was added HATU (1.22 g, 3.23 mmol, 1.05 equiv.) and NEt₃ (1.28 mL, 9.24 mmol, 3.0 equiv.) and stirred for 20 min under a nitrogen atmosphere. Then NK283 (1.0 g, 3.08 mmol, 1.0 equiv.) dissolved in DMF (5 mL) was added and stirred for 14h. The reaction mixture was diluted with EtOAc (120 mL) and brine (60 mL) and extracted with Et₂O (5 x 50 mL). The combined organic layers were dried with Na₂SO₄, filtered and concentrated in vacuo. The crude product was purified by flash chromatography (EtOAc) to give NK325 as a colourless oil (1.07 g, 68%).

¹H NMR (500 MHz, CDCl₃): δ = 7.70 (d, J = 8.2 Hz, 2H), 7.26 (d, J = 8.2 Hz, 2H), 6.71 – 6.64 (m, 1H), 6.53 – 6.45 (m, 1H), 4.67 – 4.59 (m, 1H), 4.45 – 4.42 (m, 2H), 3.66 (s, 3H), 3.45 – 3.40 (m, 1H), 3.06 – 3.00 (m, 2H), 2.32 (t, J = 7.2 Hz, 2H), 2.22 (t, J = 7.2 Hz, 2H), 1.69 – 1.60 (m, 6H), 1.45 – 1.40 (m, 13H), 1.32 - 1.27 ppm (m, 2H).

¹³C NMR (125 MHz, CDCl₃): δ = 176.1, 175.0, 169.2, 158.1, 144.1, 135.7, 129.5, 129.3, 81.1, 53.5, 44.9, 42.3, 41.7, 38.3, 35.8, 31.7, 31.2, 30.4, 28.4, 28.3, 27.3, 26.4 ppm.

HRMS (ESI, 180 °C): calculated for C₂₆H₄₁N₃O₆Na [M+Na]: m/z = 514.2888, found: m/z = 514.2877.

IR (Film): "v = 3174, 1598, 1534, 1233, 957 cm⁻¹."
6-(4-((6-(tert-butoxycarbonylamino)hexanamido)methyl)benzamido)hexanoic acid NK330

Methyl ester NK325 (1.07 g, 2.30 mmol, 1.0 equiv.) was dissolved in MeOH (20 mL) and a 1N aq. KOH-solution (20 mL) and refluxed for 3h. After cooling to rt the solution was poured onto an ice-cooled 6N aqueous HCl-solution (70 mL). The precipitate was filtered off and dried *in vacuo* to afford carboxylic acid NK330 (900 mg, 96 %) as a white solid.

\[
\begin{align*}
\text{NK330} \\
\end{align*}
\]

\[\text{1H NMR (500 MHz, MeOD): } \delta = 7.80 (d, J = 8.2 \text{ Hz}, 2H), 7.39 (d, J = 8.2 \text{ Hz}, 2H), 4.46 - 4.42 (m, 2H), 3.43 - 3.37 (m, 2H), 3.05 (t, J = 7.2 \text{ Hz}, 2H), 2.34 (t, J = 7.2 \text{ Hz}, 2H), 2.29 (t, J = 7.2 \text{ Hz}, 2H), 1.73 - 1.63 (m, 6H), 1.52 - 1.42 (m, 12H), 1.42 - 1.28 \text{ ppm (m, 3H).}
\]

\[\text{13C NMR (125 MHz, MeOD): } \delta = 177.5, 176.2, 176.1, 170.0, 143.9, 134.7, 128.55, 128.53, 79.8, 43.8, 43.7, 41.2, 41.0, 40.8, 37.06, 37.02, 34.8, 30.6, 30.2, 28.8, 27.6, 27.5, 26.7, 25.8 \text{ ppm.}
\]

\[\text{HRMS (ESI, 180 °C): calculated for C}_{25}\text{H}_{39}\text{N}_{3}\text{O}_{6}\text{Na } [M+Na]: m/z = 500.2731, \text{ found: m/z = 500.2738.}
\]

\[\text{IR (Film): } \tilde{\nu} = 3237, 1673, 1519, 1234, 914 \text{ cm}^{-1}.
\]
Experimental

tert-buty l 6-(6-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)hexylamino)-6-oxohexylcarbamoylbenzylamino)-6-oxohexylcarbamate NK504

To a solution of NK330 (535 mg, 1.15 mmol, 1.5 equiv.) in methylene chloride (15 mL) was added EDCI (242 mg, 1.32 mmol, 1.15 equiv.) and HOBT (220 mg, 1.32 mmol, 1.15 equiv.) and DIPEA (0.9 mL, 3.83 mmol, 5.0 equiv.). After stirring for 25 minutes, NK370 (483 mg, 0.77 mmol, 1.0 equiv.) dissolved in methylene chloride (7 mL) was added and the solution was stirred for further 8 h. The reaction mixture was then diluted with EtOAc (20 mL), washed with 1 N aq. HCl (15 mL), washed with brine, dried with Na₂SO₄, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 5:1) gave NK504 as a colourless oil (780 mg, 73%).

¹H NMR (400 MHz, MeOD) δ = 7.84 (d, J = 8.5 Hz, 2H), 7.55 – 7.51 (m, 2H), 7.46 – 7.43 (m, 1H), 7.40 – 7.35 (m, 4H), 7.23 – 7.18 (m, 4H), 7.21 (d, J = 8.5 Hz, 2H), 4.47 – 4.42 (m, 2H), 3.44 – 3.36 (m, 4H), 3.18 (t, J = 7.0 Hz, 2H), 3.06 (t, J = 7.0 Hz, 2H), 2.34 (br s, 3H), 2.30 (t, J = 7.0 Hz, 2H), 2.23 (t, J = 7.0 Hz, 2H), 1.73 – 1.59 (m, 8H), 1.54 – 1.34 (m, 21H) ppm.

¹³C NMR (100 MHz, MeOD): δ = 176.0, 175.5, 169.7, 164.8, 158.5, 146.4, 144.6, 144.0, 137.4, 137.3, 136.2, 134.6, 134.2, 132.6, 132.5, 131.2, 130.0, 129.3, 128.7, 128.6, 128.5, 118.4, 79.8, 43.9, 43.8, 41.3, 41.0, 40.4, 40.1, 37.2, 37.1, 30.8, 30.7, 30.4, 30.3, 29.1, 27.8, 27.7, 27.6, 26.9, 26.8, 10.0 ppm.

HRMS (ESI, 180 °C): calculated for C₄₈H₆₂Cl₄N₇O₆Na [M+Na] m/z = 960.3719, found: m/z = 960.3745.

IR (Film): ν = 3188, 1756, 1499, 1152, 935 cm⁻¹.

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N-(6-(4-((6-aminohexanamido)methyl)benzamido)hexanamido)hexyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK505

To a solution of NK504 (780 mg, 0.56 mmol, 1.0 equiv.) in methylene chloride (12 mL) was added TFA (3 mL) and stirred for 90 minutes. The solution was carefully quenched using sat. aqeous NaHCO₃ solution and the solvent was removed in vacuo to give NK505 as a yellow oil (680 mg, 96%).

¹H NMR (400 MHz, MeOD) δ = 7.72 (d, J = 8.5 Hz, 2H), 7.50 – 7.47 (m, 1H), 7.46 – 7.43 (m, 1H), 7.40 – 7.36 (m, 1H), 7.30 (d, J = 8.5 Hz, 4H), 7.12 (d, J = 8.5 Hz, 2H), 4.35 (s, 2H), 3.34 – 3.28 (m, 4H), 3.10 (t, J = 7.0 Hz, 2H), 2.86 (t, J = 7.0 Hz, 2H), 2.26 – 2.21 (m, 5H), 2.14 (t, J = 7.0 Hz, 2H), 1.67 – 1.50 (m, 10H), 1.48 – 1.40 (m, 2H), 1.40 – 1.29 ppm (m, 8H).

¹³C NMR (100 MHz, MeOD): δ = 176.1, 175.8, 169.9, 165.0, 146.3, 144.6, 143.9, 137.4, 137.3, 136.2, 134.7, 132.5, 132.4, 131.1, 129.9, 129.2, 128.7, 128.6, 118.3, 43.7, 40.9, 40.5, 40.3, 40.0, 37.0, 36.6, 30.6, 30.3, 30.2, 28.3, 27.7, 27.6, 27.0, 26.8, 26.3, 10.0 ppm.

HRMS (ESI, 180 °C): calculated for C₄₃H₅₄Cl₃N₇O₄Na [M+Na] m/z = 860.3195, found: m/z = 860.3221.

IR (Film): ν = 3237, 1673, 1519, 1234, 914 cm⁻¹.
Experimental

5-(4-chlorophenyl)-N-(6-(6-((6-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)hexanamido)methyl)benzamido)hexanamido)hexyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK509

To a solution of NK505 (52 mg, 0.06 mmol, 1.0 equiv.) in methylene chloride (3 mL), DMF (1 mL) and DIPEA (50 µL, 0.3 mmol, 5.0 equiv.) was added acid chloride NK25 (30 mg, 0.07 mmol, 1.2 equiv.) dissolved in methylene chloride (2 mL). After stirring for 30 minutes, the solvent was removed in vacuo and the residue dissolved in EtOAc (3 mL) and MeOH (1 mL), washed with 1 N aq. HCl (0.5 mL), washed with water, dried with Na₂SO₄, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 1:3) gave NK509 as a colourless resin (38 mg, 53%).

¹H NMR (400 MHz, MeOD) δ = 7.81 – 7.76 (m, 2H), 7.57 (t, J = 2.5 Hz, 2H), 7.54 – 7.50 (m, 2H), 7.47 – 7.43 (m, 2H), 7.40 – 7.35 (m, 6H), 7.23 – 7.18 (m, 4H), 4.42 (br s, 2H), 3.41 – 3.36 (m, 6H), 3.17 (t, J = 6.8 Hz, 2H), 2.34 – 2.32 (m, 6H), 2.29 (t, J = 7.5 Hz, 2H), 2.21 (t, J = 7.5 Hz, 2H), 1.76 – 1.60 (m, 10H), 1.53 – 1.36 ppm (m, 10H).

¹³C NMR (100 MHz, MeOD): δ = 176.06, 176.03, 169.8, 164.95, 164.92, 146.3, 144.6, 144.0, 137.4, 137.3, 136.1, 134.6, 134.1, 132.5, 132.4, 131.0, 129.9, 129.2, 128.7, 128.5, 118.3, 43.7, 40.8, 40.2, 40.0, 39.9, 37.0, 36.9, 30.6, 30.3, 30.2, 27.67, 27.62, 27.5, 26.78, 26.74, 9.6 ppm.

HRMS (ESI, 180 °C): calculated for C₆₀H₅₃Cl₆N₉O₅Na [M+Na] m/z = 1222.2976, found: m/z = 1222.2992.

IR (Film): ̇ν = 3187, 1622, 1514, 1274, 932 cm⁻¹.
Experimental

tert-butyl 6-(4-(6-(6-(6-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)hexylamino)-6-oxohexylcarbamoyl)benzylamino)-6-oxohexylamino)-6-oxohexylcarbamate NK515

To a solution of NK505 (105 mg, 0.29 mmol, 1.5 equiv.) in methylene chloride (5 mL) was added EDCI (42 mg, 0.22 mmol, 1.15 equiv.) and HOBT (41 mg, 0.22 mmol, 1.15 equiv.) and DIPEA (0.2 mL, 0.95 mmol, 5.0 equiv.). After stirring for 25 minutes, NK330 (96 mg, 0.19 mmol, 1.0 equiv.) dissolved in methylene chloride (3 mL) was added and the solution was stirred for further 8h. The reaction mixture was then diluted with EtOAc (5 mL), washed with 1 N aq. HCl (3 mL), washed with brine, dried with Na$_2$SO$_4$, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 5:1) gave NK504 as a white powder (98 mg, 59%).

$^1$H NMR (400 MHz, MeOD) $\delta = 7.88 - 7.84$ (m, 4H), 7.59 - 7.57 (m, 1H), 7.55 - 7.51 (m, 1H), 7.50 - 7.46 (m, 1H), 7.46 - 7.41 (m, 6H), 7.25 - 7.21 (m, 2H), 4.52 - 4.48 (m, 4H), 3.51 - 3.43 (m, 6H), 3.29 - 3.21 (m, 4H), 3.15 - 3.09 (m, 2H), 2.41 (s, 3H), 2.38 - 2.32 (m, 4H), 2.34 - 2.25 (m, 4H), 1.79 - 1.67 (m, 15H), 1.67 - 1.55 (m, 7H), 1.55 - 1.42 ppm (m, 19H).

$^{13}$C NMR (100 MHz, MeOD): $\delta = 175.6, 175.5, 169.4, 145.8, 144.2, 143.4, 136.97, 136.94, 135.8, 134.2, 133.7, 131.9, 130.8, 129.6, 128.9, 128.25, 128.23, 118.1, 79.6, 43.6, 43.5, 40.7, 40.5, 40.1, 40.0, 39.8, 36.86, 36.82, 36.75, 36.72, 30.2, 30.0, 29.98, 29.85, 29.7, 28.8, 27.35, 27.31, 27.2, 27.1, 26.4, 26.3, 9.6 ppm.

HRMS (ESI, 180 °C): calculated for C$_{69}$H$_{91}$Cl$_3$N$_{10}$O$_{9}$Na $[M+Na]$ $m/z =$ 1319.5928, found: $m/z =$ 1319.5915.

IR (Film): $\tilde{\nu} =$ 3199, 1652, 1528, 1211, 952 cm$^{-1}$.

Melting point: 111 – 113 °C.
N-(6-(6-(4-((6-(6-((6-aminohexanamido)methyl)benzamido)hexanamido)hexanamido)methyl)benzamido)hexanamido)methyl)benzamido)hexanamido)hexyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK516

To a solution of NK515 (98 mg, 0.17 mmol, 1.0 equiv.) in methylene chloride (3 mL) was added TFA (0.1 mL) and stirred for 40 minutes. The solution was carefully quenched using sat. aqueous NaHCO₃ solution and the solvent was removed in vacuo to give NK516 as a colourless oil (89 mg, 96%).

$^1$H NMR (400 MHz, MeOD) $\delta$ = 7.82 (d, $J$ = 8.5 Hz, 4H), 7.59 – 7.57 (m, 1H), 7.54 – 7.50 (m, 1H), 7.48 – 7.44 (m, 1H), 7.43 – 7.37 (m, 6H), 7.24 – 7.19 (m, 2H), 4.46 (br s, 4H), 3.46 – 3.39 (m, 6H), 3.24 – 3.18 (m, 4H), 2.78 (t, $J$ = 7.0 Hz, 2H), 2.37 (s, 3H), 2.35 – 2.27 (m, 4H), 2.27 – 2.21 (m, 4H), 1.78 – 1.64 (m, 14H), 1.64 – 1.51 (m, 6H), 1.51 – 1.36 ppm (m, 13H).

$^{13}$C NMR (100 MHz, MeOD): $\delta$ = 175.8, 169.6, 164.7, 146.0, 144.4, 143.6, 137.1, 136.0, 134.4, 133.9, 132.2, 132.1, 131.0, 129.7, 128.3, 118.2, 43.6, 41.5, 40.7, 40.1, 40.0, 39.8, 36.9, 36.8, 36.6, 31.3, 30.4, 30.1, 30.0, 29.9, 27.5, 27.4, 27.1, 26.5, 26.45, 26.42, 9.6 ppm.

HRMS (ESI, 180 °C): calculated for C₆₃H₆₃Cl₃N₁₀O₇Na [M+Na] $m/z$ = 1219.5404, found: $m/z$ = 1219.5452.

IR (Film): $\tilde{\nu}$ = 3187, 1622, 1514, 1274, 932 cm⁻¹.
Experimental

5-(4-chlorophenyl)-N-(6-(6-(4-((6-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)hexanamido) methyl)benzamido)hexanamido)hexanamido) methyl)benzamido)hexyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK519

To a solution of NK516 (20 mg, 0.06 mmol, 1.0 equiv.) in methylene chloride (3 mL), DMF (1 mL) and DIPEA (50 µL, 0.3 mmol, 5.0 equiv.) was added acid chloride NK25 (30 mg, 0.07 mmol, 1.2 equiv.) dissolved in methylene chloride (2 mL). After stirring for 30 minutes, the solvent was removed in vacuo and the residue dissolved in EtOAc (3 mL) and MeOH (1 mL), washed with 1 N aq. HCl (0.5 mL), washed with water, dried with Na₂SO₄, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 3:7) gave NK519 as a colourless oil (15 mg, 68%).

\[ \begin{align*}
\text{1H NMR (400 MHz, MeOD)} & : \ \\
\delta & = 7.81 - 7.76 \ (m, \ 4H), \ 7.57 \ (t, \ J = 2.5 \ Hz, \ 2H), \ 7.54 - 7.50 \ (m, \ 2H), \ 7.47 - 7.43 \ (m, \ 2H), \ 7.40 - 7.33 \ (m, \ 8H), \ 7.23 - 7.18 \ (m, \ 4H), \ 4.44 - 4.39 \ (m, \ 4H), \ 3.41 - 3.36 \ (m, \ 8H), \ 3.20 - 3.12 \ (m, \ 4H), \ 2.34 - 2.31 \ (m, \ 6H), \ 2.30 - 2.24 \ (m, \ 4H), \ 2.24 - 2.17 \ (m, \ 4H), \ 1.74 - 1.60 \ (m, \ 15H), \ 1.55 - 1.46 \ (m, \ 5H), \ 1.46 - 1.34 \ ppm \ (m, \ 12H).
\end{align*} \]

\[ \begin{align*}
\text{13C NMR (100 MHz, MeOD):} & \ \\
\delta & = 176.1, \ 176.0, \ 169.9, \ 169.8, \ 165.05, \ 165.02, \ 146.3, \ 144.6, \ 144.0, \ 137.4, \ 137.3, \ 136.2, \ 134.7, \ 134.2, \ 132.6, \ 132.5, \ 131.1, \ 129.9, \ 129.3, \ 128.7, \ 128.5, \ 118.3, \ 43.7, \ 40.8, \ 40.3, \ 40.2, \ 40.0, \ 39.9, \ 37.0, \ 36.9, \ 30.6, \ 30.3, \ 30.2, \ 30.1, \ 27.7, \ 27.6, \ 26.8, \ 26.74, \ 26.71, \ 9.6 \ ppm.
\end{align*} \]

HRMS (ESI, 180 °C): calculated for C₈₀H₉₃Cl₆N₁₂O₈ [M+H] \( m/z \) = 1559.5365, found: \( m/z \) = 1559.5395.

IR (Film): \( \tilde{\nu} = 3277, \ 1542, \ 1506, \ 1252, \ 947 \ cm^{-1} \).
N1-(6-(4-(6-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)hexylamino)-6-oxohexylcarbamoyl)benzylamino)-6-oxohexyl-N4-(4-(2-((5-hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino)ethyl)phenyl)succinamide NK507

To a solution of NK24 (10 mg, 0.02 mmol, 1.2 equiv.) in methylene chloride (2 mL) was added EDCI (5 mg, 0.02 mmol, 1.15 equiv.) and HOBt (4 mg, 0.02 mmol, 1.15 equiv.) and DIPEA (15 µL, 0.1 mmol, 5.0 equiv.). After stirring for 25 minutes, NK505 (11 mg, 0.05 mmol, 1.0 equiv.) in methylene chloride (3 mL), and DMF (1 mL) was added and the solution was stirred for 16 hours. The solvent was removed in vacuo and the residue subsequently purified over silica (EtOAc/ULTRA 1:4) to give NK507 as a brown oil (13 mg, 45%).

$^1$H NMR (400 MHz, MeOD) $\delta = 7.80 - 7.76$ (m, 2H), 7.56 – 7.54 (m, 1H), 7.53 – 7.49 (m, 3H), 7.45 – 7.41 (m, 1H), 7.39 – 7.33 (m, 4H), 7.23 – 7.17 (m, 4H), 6.94 (t, $J = 7.6$ Hz, 1H), 6.63 – 6.58 (m, 2H), 4.40 (br s, 2H), 3.40 – 3.34 (m, 6H), 3.17 (q, $J = 6.8$ Hz, 4H), 3.12 – 3.03 (m, 2H), 3.03 – 2.98 (m, 1H), 2.98 – 2.94 (m, 1H), 2.94 – 2.83 (m, 5H), 2.67 (t, $J = 7.0$ Hz, 2H), 2.62 – 2.52 (m, 3H), 2.32 (s, 3H), 2.28 – 2.17 (m, 5H), 1.75 – 1.56 (m, 11H), 1.56 – 1.47 (m, 4H), 1.46 – 1.32 (m, 9H), 0.99 ppm (t, $J = 7.6$ Hz, 3H).

$^{13}$C NMR (100 MHz, MeOD): $\delta = 176.1, 176.0, 174.5, 172.9, 169.8, 165.0, 156.0, 146.3, 144.6, 144.0, 138.5, 137.4, 137.3, 136.2, 134.6, 134.2, 132.6, 132.5, 131.1, 130.2, 129.9, 129.2, 128.7, 128.56, 128.53, 127.6, 123.8, 121.5, 118.3, 113.1, 54.8, 53.8, 53.7, 43.7, 40.8, 40.3, 40.0, 37.0, 36.9, 33.1, 32.0, 30.6, 30.3, 30.2, 30.1, 27.7, 27.6, 27.5, 26.8, 26.7, 26.0, 24.3, 21.4, 11.9, 9.6 ppm.

HRMS (ESI, 180 °C): calculated for C$_{68}$H$_{84}$Cl$_3$N$_9$O$_7$Na [M+Na] $m/z = 1266.5451$, found: $m/z = 1266.5492$.

IR (Film): $\tilde{\nu} = 3152, 1633, 1584, 1274, 938$ cm$^{-1}$.  

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To a solution of **NK24** (10 mg, 0.02 mmol, 1.2 equiv.) in methylene chloride (2 mL) was added EDCI (5 mg, 0.02 mmol, 1.15 equiv.) and HOBt (4 mg, 0.02 mmol, 1.15 equiv.) and DIPEA (15 µL, 0.1 mmol, 5.0 equiv.). After stirring for 25 minutes, **NK519** (15 mg, 0.05 mmol, 1.0 equiv.) in methylene chloride (3 mL), and DMF (1 mL) was added and the solution was stirred for 16 hours. The solvent was removed in vacuo and the residue subsequently purified over silica (EtOAc/ULTRA 1:4) to give **NK517** as a brown oil (13 mg, 36%).

**$^1$H NMR (400 MHz, MeOD)**
\[
\delta = 7.81 - 7.75 \text{ (m, 4H)}, 7.58 - 7.56 \text{ (m, 1H)}, 7.54 - 7.47 \text{ (m, 3H)}, 7.47 - 7.43 \text{ (m, 1H)}, 7.40 - 7.33 \text{ (m, 6H)}, 7.23 - 7.17 \text{ (m, 4H)}, 6.93 \text{ (t, } J = 7.6 \text{ Hz, 1H)}, 6.62 - 6.57 \text{ (m, 2H)}, 4.43 - 4.39 \text{ (m, 4H)}, 3.40 - 3.34 \text{ (m, 6H)}, 3.20 - 3.13 \text{ (m, 6H)}, 3.03 - 2.98 \text{ (m, 3H)}, 2.98 - 2.94 \text{ (m, 1H)}, 2.94 - 2.78 \text{ (m, 4H)}, 2.67 \text{ (t, } J = 7.0 \text{ Hz, 2H)}, 2.62 - 2.52 \text{ (m, 3H)}, 2.32 \text{ (s, 3H)}, 2.29 - 2.23 \text{ (m, 4H)}, 2.23 - 2.15 \text{ (m, 5H)}, 1.72 - 1.57 \text{ (m, 18H)}, 1.56 - 1.47 \text{ (m, 6H), 1.46 - 1.32 \text{ (m, 13H)}, 0.99 ppm (t, } J = 7.6 \text{ Hz, 3H)}.\]

**$^{13}$C NMR (100 MHz, MeOD):**
\[
\delta = 176.0, 174.5, 172.9, 169.8, 164.9, 156.0, 146.3, 144.6, 144.0, 137.4, 137.3, 136.1, 134.6, 134.1, 132.5, 132.4, 131.0, 130.1, 129.9, 129.2, 128.7, 128.5, 127.5, 121.5, 121.4, 118.3, 112.9, 53.86, 53.83, 43.7, 40.8, 40.3, 40.2, 40.0, 37.0, 36.9, 33.1, 32.0, 30.6, 30.3, 30.2, 30.15, 30.12, 27.6, 27.57, 27.53, 26.7, 26.6, 24.4, 12.0, 9.6 ppm.\]

**HRMS (ESI, 180 °C):** calculated for C$_{88}$H$_{114}$Cl$_3$N$_{12}$O$_{10}$ [M+H] $m/z = 1603.7841$, found: $m/z = 1603.7885$.  

**IR (Film):** \[\tilde{\nu} = 3144, 1654, 1538, 1214, 982 \text{ cm}^{-1}.\]
N-(6-(6-(4-((6-acetamidohexanamido)methyl)benzamido)hexamido)hexyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK506

To an ice-cooled solution of NK505 (11 mg, 0.02 mmol, 1.0 equiv.) in methylene chloride (2 mL) and DIPEA (0.1 mL, 0.1 mmol, 5.0 equiv.) was slowly added acetyl chloride (16 µL, 0.02 mmol, 1.2 equiv.). The solution was stirred for 20 minutes and subsequently diluted with EtOAc (3 mL), washed with 1N aq. HCl, washed with brine, dried with Na₂SO₄, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 1:4) gave NK506 as a colourless oil (9 mg, 82%).

¹H NMR (400 MHz, MeOD)  δ = 7.82 – 7.78 (m, 2H), 7.59 – 7.58 (m, 1H), 7.55 – 7.52 (m, 1H), 7.48 – 7.44 (m, 1H), 7.41 – 7.36 (m, 4H), 7.23 – 7.19 (m, 2H), 4.43 (s, 2H), 3.42 – 3.36 (m, 4H), 3.20 – 3.14 (m, 4H), 2.33 (s, 3H), 2.28 (t, J = 7.5 Hz, 2H), 2.22 (t, J = 7.5 Hz, 2H), 1.94 (s, 3H), 1.72 – 1.60 (m, 8H), 1.59 – 1.48 (m, 4H), 1.47 – 1.35 ppm (m, 8H).

¹³C NMR (100 MHz, MeOD):  δ = 176.0, 173.2, 169.8, 165.0, 146.3, 144.6, 144.0, 137.4, 137.3, 136.2, 134.7, 134.2, 132.6, 132.5, 131.1, 129.9, 129.2, 128.7, 128.5, 118.3, 43.7, 40.8, 40.36, 40.32, 40.0, 37.0, 36.9, 30.6, 30.3, 30.2, 30.1, 27.67, 27.62, 26.8, 26.7, 22.6, 9.6 ppm.

HRMS (ESI, 180 °C): calculated for C₄₅H₅₆Cl₃N₇O₅Na [M+Na] m/z = 902.3301, found: m/z = 902.3330.

IR (Film):  ν = 3216, 1684, 1579, 1256, 981 cm⁻¹.
Experimental

N-(6-(6-(4-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide)hexyl)-5-(4-acetamidoheptanamido)methyl)benzamido)hexanamido)hexanamido)hexanamido)methyl)benzamido)hexyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK521

To an ice-cooled solution of NK515 (11 mg, 0.02 mmol, 1.0 equiv.) in methylene chloride (2 mL) and DIPEA (0.1 mL, 0.1 mmol, 5.0 equiv.) was slowly added acetyl chloride (16 μL, 0.02 mmol, 1.2 equiv.). The solution was stirred for 20 minutes and subsequently diluted with EtOAc (3 mL), washed with 1N aq. HCl, washed with brine, dried with Na2SO4, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 1:4) gave NK521 as a colourless oil (11 mg, 71%).

1H NMR (400 MHz, MeOD) δ = 7.82 – 7.78 (m, 4H), 7.59 – 7.58 (m, 1H), 7.55 – 7.52 (m, 1H), 7.48 – 7.44 (m, 1H), 7.44 – 7.36 (m, 6H), 7.23 – 7.19 (m, 2H), 4.43 (br s, 2H), 3.44 – 3.37 (m, 6H), 3.22 – 3.15 (m, 6H), 2.34 (s, 3H), 2.32 – 2.26 (m, 4H), 2.26 – 2.19 (m, 4H), 1.96 (s, 3H), 1.73 – 1.62 (m, 14H), 1.58 – 1.49 (m, 5H), 1.47 – 1.35 ppm (m, 13H).

13C NMR (100 MHz, (CD3)2SO): δ = 172.4, 172.1, 169.2, 166.1, 162.1, 145.1, 142.9, 142.6, 135.9, 135.1, 133.8, 133.25, 133.22, 132.0, 131.4, 129.7, 128.8, 128.4, 127.3, 127.2, 126.9, 116.4, 48.7, 41.8, 38.55, 38.53, 38.4, 35.5, 35.4, 29.3, 29.2, 29.0, 26.2, 25.2, 25.1, 22.7, 9.2 ppm.

HRMS (ESI, 180 °C): calculated for C_{65}H_{85}Cl_{3}N_{10}O_{8}Na [M+Na] m/z = 1261.5510, found: m/z = 1261.5545.

IR (Film): $\tilde{\nu} = 3224, 1670, 1537, 1219, 951 \text{ cm}^{-1}$. 
Methyl 6-((4-((6-aminohexanamido)methyl)benzamido)hexanoate NK331

To a solution of NK330 (780 mg, 0.56 mmol, 1.0 equiv.) in methylene chloride (12 mL) was added TFA (3 mL) and stirred for 90 minutes. The solution was carefully quenched with sat. aqueous NaHCO₃ solution and the solvent was removed in vacuo to give NK331 as a colourless oil (680 mg, 96%).

¹H NMR (500 MHz, MeOD): \( \delta = 7.66 (d, J = 8.2 \text{ Hz}, 2H), 7.26 (d, J = 8.2 \text{ Hz}, 2H), 4.31 (s, 2H), 3.53 (s, 3H), 3.26 (t, J = 7.2 \text{ Hz}, 2H), 2.80 (t, J = 7.2 \text{ Hz}, 2H), 2.24 (t, J = 7.2 \text{ Hz}, 2H), 2.19 (t, J = 7.2 \text{ Hz}, 2H), 1.61 - 1.48 (m, 8H), 1.36 – 1.26 ppm (m, 4H).

¹³C NMR (125 MHz, MeOD): \( \delta = 177.5, 176.2, 176.1, 170.0, 143.9, 134.7, 128.55, 128.53, 79.8, 43.8, 43.7, 41.2, 41.0, 40.8, 37.06, 37.02, 34.8, 30.6, 30.2, 28.8, 27.6, 27.5, 26.7, 25.8 \text{ ppm}.

HRMS (ESI, 180 °C): calculated for C₂₅H₃₉N₃O₆Na [M+Na]: \( m/z = 500.2731 \), found: \( m/z = 500.2738 \).

IR (Film): \( \tilde{\nu} = 3169, 1624, 1559, 1207, 942 \text{ cm}^{-1} \).
Experimental

methyl 6-((6-(4-(4-(2-(5-hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino)ethyl)phenylamino)-4-oxobutanamido)hexanamido)methyl)benzamido)hexanoate NK511

To a solution of NK246 (35 mg, 0.02 mmol, 1.2 equiv.) in methylene chloride (2 mL) was added EDCI (21 mg, 0.02 mmol, 1.15 equiv.) and HOBt (21 mg, 0.02 mmol, 1.15 equiv.) and DIPEA (15 µL, 0.1 mmol, 5.0 equiv.). After stirring for 25 minutes, NK331 (11 mg, 0.05 mmol, 1.0 equiv.) in methylene chloride (3 mL), and DMF (1 mL) was added and the solution was stirred for 10 hours. The solvent was removed in vacuo and the residue subsequently purified over silica (EtOAc/ULTRA 1:9) to give NK511 as a brown oil (52 mg, 81%).

\[ \delta = 7.78 \text{ (d, } J = 8.5 \text{ Hz, } 2\text{H}), 7.47 \text{ (d, } J = 8.5 \text{ Hz, } 2\text{H}), 7.35 \text{ (d, } J = 8.5 \text{ Hz, } 2\text{H}), 7.16 \text{ (d, } J = 8.5 \text{ Hz, } 2\text{H}), 6.90 \text{ (t, } J = 7.6 \text{ Hz, } 1\text{H}), 6.60 \text{ – 6.54 (m, } 2\text{H}), 4.40 \text{ (br s, } 2\text{H}), 3.64 \text{ (br s, } 3\text{H}), 3.36 \text{ (t, } J = 7.0 \text{ Hz, } 2\text{H}), 3.17 \text{ (t, } J = 7.0 \text{ Hz, } 2\text{H}), 3.08 \text{ – 2.93 (m, } 2\text{H}), 2.92 \text{ – 2.82 (m, } 3\text{H}), 2.80 \text{ – 2.73 (m, } 2\text{H}), 2.73 \text{ – 2.63 (m, } 4\text{H}), 2.58 \text{ – 2.46 (m, } 3\text{H}), 2.34 \text{ (t, } J = 7.5 \text{ Hz, } 2\text{H}), 2.24 \text{ (t, } J = 7.5 \text{ Hz, } 2\text{H}), 2.16 \text{ – 2.08 (m, } 1\text{H}), 1.71 \text{ – 1.47 (m, } 11\text{H}), 1.44 \text{ – 1.29 (m, } 5\text{H}), 0.94 \text{ ppm (t, } J = 7.6 \text{ Hz, } 3\text{H}). \]

\[ \delta = 176.0, 175.8, 174.5, 172.8, 169.8, 155.9, 144.0, 138.2, 138.0, 137.1, 134.6, 130.1, 128.5, 127.3, 124.2, 121.5, 121.3, 112.8, 58.7, 53.9, 53.7, 52.0, 43.7, 40.8, 40.2, 36.9, 35.1, 34.7, 33.1, 32.0, 30.1, 30.0, 27.5, 26.6, 25.7, 24.6, 22.3, 12.2 \text{ ppm}. \]

HRMS (ESI, 180 °C): calculated for C_{46}H_{64}N_{5}O_{7} [M+H] m/z = 798.4800, found: m/z = 798.4804.

IR (Film): \[ \tilde{\nu} = 3129, 1588, 1520, 1207, 924 \text{ cm}^{-1}. \]
methyl 6-(4-((6-(tert-butoxycarbonylamino)hexanamido)methyl)benzamido)-hexanamido)hexanamido)methyl)benzamido)hexanoate NK332

To a solution of NK330 (35 mg, 0.02 mmol, 1.2 equiv.) in methylene chloride (2 mL) was added EDCI (21 mg, 0.02 mmol, 1.15 equiv.) and HOBt (21 mg, 0.02 mmol, 1.15 equiv.) and DIPEA (15 µL, 0.1 mmol, 5.0 equiv.). After stirring for 25 minutes, NK331 (11 mg, 0.05 mmol, 1.0 equiv.) in methylene chloride (3 mL), and DMF (1 mL) was added and the solution was stirred for 10 hours. The solvent was removed in vacuo and the residue subsequently purified over silica (EtOAc/ULTRA 1:9) to give NK332 as a white precipitate (52 mg, 81%).

$^1$H NMR (500 MHz, (CD$_3$)$_2$SO):
\[
\delta = 7.80 - 7.74 \text{ (m, 4H)}, 7.73 - 7.67 \text{ (m, 1H)}, 7.32 - 7.22 \text{ (m, 4H)}, 6.78 - 6.71 \text{ (m, 1H)}, 4.32 - 4.24 \text{ (m, 4H)}, 3.57 \text{ (br s, 3H)}, 3.26 - 3.17 \text{ (m, 4H)}, 3.03 - 2.96 \text{ (m, 2H)}, 2.91 - 2.84 \text{ (m, 2H)}, 2.31 - 2.26 \text{ (m, 2H)}, 2.17 - 2.09 \text{ (m, 4H)}, 2.07 - 1.99 \text{ (m, 2H)}, 1.55 - 1.46 \text{ (m, 11H)}, 1.38 - 1.34 \text{ (m, 11H)}, 1.34 - 1.21 \text{ ppm (m, 11H)}.
\]

$^{13}$C NMR (125 MHz, (CD$_3$)$_2$SO):
\[
\delta = 173.3, 172.1, 171.8, 165.8, 155.5, 142.8, 133.1, 127.1, 126.8, 77.3, 51.1, 41.7, 38.3, 35.4, 35.3, 33.2, 29.2, 28.9, 28.8, 28.3, 26.2, 26.1, 26.0, 25.9, 25.1, 25.0, 24.2 \text{ ppm}.
\]

HRMS (ESI, 180 °C):
calculated for C$_{46}$H$_{70}$N$_6$O$_9$Na [M+Na]: m/z = 873.5096, found: m/z = 873.5088.

IR (Film):
\[\tilde{\nu} = 1690, 851, 721, 620 \text{ cm}^{-1} \]

Melting point:
197 - 199 °C.
methyl 6-(4-((6-(6-(6-(6-((6-aminohexanamido)methyl)benzamido)hexanamido)hexanamido)methyl)benzamido)hexanamido)hexanoate

NK337

To a solution of NK332 (52 mg, 0.02 mmol, 1.2 equiv.) in methanol (2 mL) was added TFA (200 µL). After stirring for 50 minutes the solvent was removed *in vacuo* to give NK337 as a clear oil (45 mg, 90%).

${}^1$H NMR (300 MHz, MeOD): $\delta = 7.75$ (d, $J = 8.5$ Hz, 4H), 7.34 (d, $J = 8.5$ Hz, 4H), 4.39 (br s, 4H), 3.62 (s, 3H), 3.35 (t, $J = 7.2$ Hz, 3H), 3.13 (t, $J = 7.2$ Hz, 2H), 2.94 - 2.85 (m, 2H), 2.38 - 2.11 (m, 8H), 1.71 - 1.54 (m, 13H), 1.54 - 1.28 ppm (m, 11H).

${}^{13}$C NMR (100 MHz, MeOD): $\delta =$ 176.1, 175.9, 175.8, 169.9, 169.8, 144.1, 144.0, 134.7, 128.6, 128.5, 52.0, 43.7, 40.5, 40.2, 37.0, 36.9, 36.6, 34.7, 30.3, 30.2, 30.1, 28.3, 27.6, 27.5, 27.0, 26.8 ppm.

HRMS (ESI, 180 °C): calculated for C$_{41}$H$_{63}$N$_6$O$_7$ [M+H]$^+$: $m/z = 751.4766$, found: $m/z = 751.4777$.

IR (Film): $\tilde{\nu} = 2928, 2457, 1706, 1672, 1441, 1115, 722$ cm$^{-1}$. 
methyl 6-{4-(6-{4-(6-{4-(2-((5-hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino)ethyl)phenylamino)-4-oxobutanamido)hexanamido)methyl)benzamido)hexanamido)methyl]benzamido}hexanoate NK493

To a solution of NK246 (35 mg, 0.02 mmol, 1.2 equiv.) in methylene chloride (2 mL) was added EDCI (21 mg, 0.02 mmol, 1.15 equiv.) and HOBt (21 mg, 0.02 mmol, 1.15 equiv.) and DIPEA (15 µL, 0.1 mmol, 5.0 equiv.). After stirring for 25 minutes, NK337 (26 mg, 0.05 mmol, 1.0 equiv.) in methylene chloride (3 mL), and DMF (1 mL) was added and the solution was stirred for 10 hours. The solvent was removed in vacuo and the residue subsequently purified over silica (EtOAc/ULTRA 1:9) to give NK493 as a brown oil (45 mg, 79%).

$^1$H NMR (500 MHz, (CD$_3$)$_2$SO) δ = 7.82 (t, J = 5.4 Hz, 1H), 7.77 (d, J = 8.5 Hz, 6H), 7.70 (t, J = 5.4 Hz, 1H), 7.28 (d, J = 8.5 Hz, 6H), 6.84 (t, J = 7.6 Hz, 1H), 6.54 (d, J = 8.5 Hz, 1H), 6.49 (d, J = 8.5 Hz, 1H), 4.31 – 4.25 (m, 4H), 3.56 (br s, 3H), 3.26 – 3.17 (m, 6H), 3.05 – 2.96 (m, 5H), 2.85 – 2.76 (m, 2H), 2.69 – 2.56 (m, 5H), 2.34 (t, J = 7.5 Hz, 2H), 2.29 (t, J = 7.5 Hz, 3H), 2.16 – 2.10 (m, 6H), 2.03 (t, J = 7.5 Hz, 3H), 1.93 – 1.87 (m, 1H), 1.57 – 1.46 (m, 15H), 1.40 – 1.34 (m, 5H), 1.30 – 1.22 (m, 10H), 0.82 ppm (t, J = 7.6 Hz, 3H).

$^{13}$C NMR (125 MHz, (CD$_3$)$_2$SO): δ = 173.3, 172.1, 171.8, 171.0, 170.2, 165.8, 154.7, 142.8, 137.7, 137.1, 135.2, 133.1, 128.8, 127.1, 126.8, 125.8, 123.0, 119.8, 118.8, 56.0, 52.3, 51.7, 51.1, 41.7, 38.4, 38.3, 35.4, 35.3, 34.7, 33.2, 32.0, 31.7, 30.4, 28.9, 28.8, 26.2, 26.1, 25.9, 25.4, 25.1, 25.0, 24.2, 23.5, 11.7 ppm.

HRMS (ESI, 180 °C): calculated for C$_{66}$H$_{93}$N$_8$O$_{10}$ [M+H] m/z = 1157.7009, found: m/z = 1157.7052.

IR (Film): $\tilde{\nu}$ = 1670, 817, 728, 630 cm$^{-1}$. 
(R)-methyl 4-((3R,5R,8S,9S,10S,13R,14S,17R)-3-hydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate NK371

To an ice-cooled slurry of lithocholic acid (5.65 g, 15.0 mmol, 1.0 equiv.) in methanol (120 mL) was slowly added thionyl chloride (11.0 mL, 150 mmol, 10.0 equiv.). The solution was then allowed to warm to rt and subsequently refluxed for 5 hours. The solvent was removed in vacuo to give NK371 as a white powder (5.85 g, 98%) which was used in the next step without further purification.

$^1$H NMR (300 MHz, CDCl$_3$): $\delta = 3.66$ (s, 3H), 3.64 – 3.55 (m, 1H), 2.42 – 2.29 (m, 1H), 2.28 – 2.14 (m, 1H), 1.99 – 1.72 (m, 7H), 1.70 – 1.46 (m, 3H), 1.46 – 0.99 (m, 16H), 0.93 – 0.88 (m, 6H), 0.64 ppm (s, 3H).

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta = 174.7, 71.7, 56.4, 55.9, 51.4, 42.6, 42.0, 40.3, 40.1, 36.3, 35.7, 35.3, 34.5, 31.0, 30.9, 30.4, 28.1, 27.1, 26.3, 24.1, 23.3, 20.7, 18.2, 11.9$ ppm.

Spectroscopic data is consistent with that reported in the literature.$^{192}$
(R)-methyl 4-((3R,5R,8R,9S,10S,13R,14S,17R)-3-(3-tert-butoxy-3-oxopropoxy)-10,13-
dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate NK372

To a solution of NK371 (3.92 g, 10.0 mmol, 1.0 equiv.) in THF (50 mL) was added NaH (440 mg, 11.0 mmol, 60% dispersion in mineral oil, 1.1 equiv.) and stirred for 30 minutes. Then tert-butyl-acrylate (1.35 g, 11.0 mmol, 1.1 equiv.) dissolved in THF (7 mL) was added. After stirring for 6 hours the solution was carefully quenched with water, the solvent was removed in vacuo, the residue was re-dissolved in methylene chloride (30 mL) and washed with water (3 x 10 mL), dried with MgSO₄, filtered and solvent was removed in vacuo. Purification over silica (hexanes/EtOAc 95:5) gave NK372 as a clear oil (4.50 g, 87%).

¹H NMR (300 MHz, CDCl₃): δ = 3.69 (t, J = 6.7 Hz, 2H), 3.66 (s, 3H), 3.31 – 3.19 (m, 1H), 2.46 (t, J = 6.7 Hz, 2H), 2.40 – 2.29 (m, 1H), 2.28 – 2.14 (m, 1H), 1.97 – 1.68 (m, 7H), 1.62 – 1.50 (m, 2H), 1.50 – 1.17 (m, 20H), 1.17 – 0.98 (m, 5H), 0.94 – 0.83 (m, 7H), 0.64 ppm (s, 3H).

¹³C NMR (75 MHz, CDCl₃): δ = 174.7, 171.1, 80.3, 79.3, 63.7, 56.4, 55.9, 51.4, 42.7, 42.0, 40.2, 40.1, 36.8, 35.8, 35.3, 34.8, 33.1, 31.0, 30.9, 28.1, 27.2, 27.1, 26.3, 24.1, 23.3, 20.7, 18.2, 12.0 ppm.

HRMS (ESI, 180 °C): calculated for C₃₂H₆₄O₆Na [M+Na] m/z = 541.3863, found: m/z = 541.3874.

IR (Film): ν = 1690, 851, 721, 620 cm⁻¹.
Experimental

3-((3R,5R,8R,9S,10S,13R,14S,17R)-17-((R)-5-methoxy-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-3-yloxy)propanoic acid NK376

To a solution of NK372 (2.0 g, 3.85 mmol, 1.0 equiv.) in methylene chloride (25 mL) was added TFA (3 mL) and stirred for 90 minutes. The solution was carefully quenched using sat. aqueous NaHCO₃ solution and the solvent was removed in vacuo to give NK376 as a clear oil (1.76 g, 96%).

$^1$H NMR (300 MHz, CDCl₃): $\delta$ = 3.86 (t, $J = 6.7$ Hz, 2H), 3.71 (s, 3H), 3.50 – 3.37 (m, 1H), 2.70 (t, $J = 6.7$ Hz, 2H), 2.47 – 2.35 (m, 1H), 2.33 – 2.19 (m, 1H), 2.00 – 1.70 (m, 7H), 1.64 – 1.52 (m, 2H), 1.48 – 1.02 (m, 16H), 0.97 – 0.86 (m, 7H), 0.64 ppm (s, 3H).

$^{13}$C NMR (75 MHz, CDCl₃): $\delta$ = 177.7, 177.2, 80.8, 62.9, 56.3, 55.8, 52.2, 42.7, 42.1, 40.3, 40.0, 35.8, 35.3, 35.1, 34.7, 32.5, 31.2, 31.0, 28.1, 27.1, 26.7, 26.2, 24.1, 23.2, 20.8, 18.1, 11.9 ppm.

HRMS (ESI, 180 °C): calculated for C_{28}H_{46}O_{5}Na [M+Na] $m/z = 485.3237$, found: $m/z = 485.3219$.

IR (Film): $\tilde{\nu} = 1720, 981, 773, 712$ cm$^{-1}$. 
(R)-methyl 4-(((3R,5R,9S,10S,13R,14S,17R)-3-((S)-5-(((9H-fluoren-9-yl)methoxy)carbonylamino)-6-((5-(4-chlorophenyl)-1(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)hexylamino)-6-oxohexylamino)-3-oxopropyloxy)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate NK377

To a solution of NK376 (70 mg, 0.15 mmol, 1.5 equiv.) in methylene chloride (5 mL) was added EDCI (22 mg, 0.12 mmol, 1.15 equiv.) and HOBt (20 mg, 0.12 mmol, 1.15 equiv.) and DIPEA (85 µL, 5.0 mmol, 5.0 equiv.). After stirring for 25 minutes, NK375 (83 mg, 0.1 mmol, 1.0 equiv.) dissolved in methylene chloride (2 mL) was added and the solution was stirred for further 8 h. The reaction mixture was then diluted with EtOAc (9 mL), washed with 1 N aq. HCl (2 mL), washed with brine, dried with Na₂SO₄, filtered and the solvent was removed in vacuo. Purification over silica (DCM/EtOAc/ULTRA 7:2:1) gave NK374 as a white foam (80 mg, 62%).

¹H NMR (500 MHz, CDCl₃):

δ = 7.74 (d, J = 7.3 Hz, 2H), 7.59 (t, J = 6.5 Hz, 1H), 7.42 (d, J = 2.1 Hz, 1H), 7.38 (t, J = 7.3 Hz, 2H), 7.31 – 7.24 (m, 5H), 7.06 – 7.02 (m, 2H), 6.99 (t, J = 6.2 Hz, 1H), 6.54 – 6.47 (m, 2H), 5.84 – 5.78 (m, 1H), 4.41 – 4.34 (m, 2H), 4.19 (t, J = 7.2 Hz, 1H), 3.68 (t, J = 7.2 Hz, 1H), 3.65 (s, 3H), 3.44 – 3.33 (m, 2H), 3.29 – 3.17 (m, 5H), 2.41 (t, J = 5.3 Hz, 1H), 2.36 (s, 3H), 2.34 – 2.30 9m, 1H), 2.25 – 2.15 (m, 1H), 1.96 – 1.64 (m, 10H), 1.61 – 1.45 (m, 9H), 1.43 – 1.28 (m, 15H), 1.23 – 1.16 (m, 3H), 1.14 – 0.97 (m, 6H), 0.94 – 0.84 (m, 8H), 0.62 ppm (s, 3H).
Experimental

$^{13}$C NMR (125 MHz, CDCl$_3$): δ = 174.4, 172.0, 171.7, 162.8, 156.4, 145.0, 143.8, 143.0, 141.3, 135.9, 134.9, 133.0, 130.8, 130.6, 130.3, 128.9, 127.9, 127.7, 127.2, 127.1, 125.1, 120.0, 117.7, 79.6, 77.3, 77.1, 76.8, 67.0, 64.1, 60.4, 56.4, 56.1, 56.0, 54.9, 53.4, 51.5, 47.2, 42.7, 42.0, 40.4, 40.1, 39.2, 38.6, 37.4, 35.8, 35.4, 35.2, 34.9, 33.1, 32.0, 31.1, 31.0, 29.6, 29.2, 28.2, 27.3, 27.2, 26.4, 26.3, 26.2, 26.1, 24.2, 23.4, 22.5, 21.0, 20.8, 18.3, 14.2, 12.0, 9.5 ppm.

HRMS (ESI, 180 °C): calculated for C$_{72}$H$_{91}$Cl$_3$N$_6$O$_8$Na [M+Na] $m/z$ = 1295.5856, found: $m/z = 1295.5878$.

IR (Film): $\tilde{\nu} = 1751, 1021, 752, 699$ cm$^{-1}$.

Melting point: 78 - 80°C.

(R)-methyl 4-((3R,5R,8R,9S,10S,13R,14S,17R)-3-((3R,5R,8R,9S,10S,13R,14S,17R)-3-((3S)-5-amino-6-(6-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)hexylamino)-6-oxohexylamino)-3-oxopropoxy)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate NK378

To a solution of NK377 (80 mg, 0.06 mmol, 1.0 equiv.) in methylene chloride (4 mL) was added Et$_2$NH (0.5 mL) and the solution was stirred for 2 hours. Upon completion of the reaction the solvent was removed in vacuo, the residue was dissolved in EIOAc (4 mL), washed with 1 N aqueous HCl (1 mL), washed with brine, dried with MgSO$_4$, filtered and purified over silica (EIOAc/ULTRA 1:1) to give NK378 as a white foam (58 mg, 93%).
Experimental

1H NMR (400 MHz, CDCl3): δ = 7.42 (d, J = 2.1 Hz, 1H), 7.36 (t, J = 6.0 Hz, 1H), 7.31 – 7.26 (m, 4H), 7.08 – 7.02 (m, 2H), 6.98 (t, J = 6.0 Hz, 1H), 6.50 (t, J = 6.0 Hz, 1H), 3.70 (t, J = 7.2 Hz, 2H), 3.65 (s, 3H), 3.44 – 3.33 (m, 3H), 3.32 – 3.18 (m, 4H), 2.42 (t, J = 6.0 Hz, 2H), 2.36 (s, 3H), 2.34 – 2.30 (m, 1H), 2.25 – 2.15 (m, 1H), 1.99 – 1.46 (m, 23H), 1.46 – 1.00 (m, 26H), 0.97 – 0.84 (m, 7H), 0.62 ppm (s, 3H).

13C NMR (100 MHz, CDCl3): δ = 174.4, 174.6, 171.8, 162.7, 145.0, 143.0, 135.9, 134.9, 132.9, 130.8, 130.5, 130.3, 128.8, 127.8, 127.2, 117.6, 79.6, 64.0, 56.4, 55.9, 54.9, 51.4, 42.7, 42.0, 40.4, 40.1, 38.9, 38.7, 37.3, 35.8, 35.3, 35.2, 34.8, 34.5, 33.1, 31.05, 31.02, 29.6, 29.46, 29.41, 28.1, 27.25, 27.23, 26.5, 26.4, 24.2, 23.4, 22.9, 20.8, 18.2, 12.0, 9.4 ppm.

HRMS (ESI, 180 °C): calculated for C57H81Cl3N6O6Na [M+Na] m/z = 1073.5157, found: m/z = 1073.5173.

IR (Film): ν = 1732, 1009, 741, 706 cm⁻¹.

Melting point: 74 - 76 °C.
To an ice-cooled solution of **NK378** (58 mg, 0.06 mmol, 1.0 equiv.) in methylene chloride (4 mL) and DIPEA (50 µL, 0.3 mmol, 5.0 equiv.) was slowly added acetyl chloride (5 µL, 0.07 mmol, 1.1 equiv.). The solution was stirred for 20 minutes and subsequently diluted with EtOAc (2 mL), washed with 1N aqueous HCl (1 mL), washed with brine, dried with Na$_2$SO$_4$, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 4:1) gave **NK379** as a white foam (57 mg, 89%).

**$^1$H NMR (300 MHz, CDCl$_3$):**
\[ \delta = 7.44 - 7.40 \text{ (m, 1H)}, 7.33 - 7.25 \text{ (m, 4H)}, 7.10 - 6.99 \text{ (m, 3H)}, 6.76 - 6.66 \text{ (m, 2H)}, 6.58 \text{ (t, } J = 6.0 \text{ Hz, 1H)}, 4.40 - 4.27 \text{ (m, 1H)}, 3.70 \text{ (t, } J = 7.2 \text{ Hz, 2H)}, 3.65 \text{ (s, 3H)}, 3.48 - 3.33 \text{ (m, 2H)}, 3.32 - 3.13 \text{ (m, 5H)}, 2.42 \text{ (t, } J = 6.0 \text{ Hz, 2H)}, 2.36 \text{ (s, 3H)}, 2.34 - 2.05 \text{ (m, 4H)}, 2.00 \text{ (s, 3H)}, 1.98 - 1.45 \text{ (m, 19H)}, 1.45 - 0.95 \text{ (m, 28H)}, 0.94 - 0.84 \text{ (m, 8H)}, 0.64 \text{ ppm (s, 3H)}.\]

**$^{13}$C NMR (75 MHz, CDCl$_3$):**
\[ \delta = 174.4, 172.0, 171.7, 170.5, 162.7, 145.0, 143.0, 135.9, 134.9, 132.9, 130.8, 130.5, 130.3, 128.8, 127.8, 127.2, 117.6, 79.6, 64.0, 56.4, 55.9, 54.9, 53.0, 51.4, 42.7, 42.0, 40.4, 40.1, 39.1, 38.6, 38.4, 37.3, 35.8, 35.3, 35.2, 34.8, 33.1, 31.5, 31.0, 30.9, 29.6, 29.5, 29.2, 29.1, 28.1, 27.25, 27.23, 26.3, 26.16, 26.12, 24.1, 23.3, 23.1, 22.4, 20.8, 18.2, 12.0, 9.4 \text{ ppm.}\]
Experimental

HRMS (ESI, 180 °C): calculated for C\textsubscript{59}H\textsubscript{83}Cl\textsubscript{3}N\textsubscript{6}O\textsubscript{7}Na [M+Na] \textit{m/z} = 1115.5281, found: \textit{m/z} = 1115.5324.

IR (Film): \bar{\nu} = 1699, 1112, 787, 728 cm\textsuperscript{-1}.

Melting point: 84 – 86 °C.

tert-butyl 3-((8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yloxy)propanoate NK366

To a solution of cholesterol (3.87 g, 10.0 mmol, 1.0 equiv.) in THF (50 mL) was added NaH (440 mg, 11.0 mmol, 60% dispersion in mineral oil, 1.1 equiv.) and stirred for 30 minutes. Then tert-butyl-acrylate (1.35 g, 11.0 mmol, 1.1 equiv.) dissolved in THF (7 mL) was added. After stirring for 6 hours the solution was carefully quenched with water, the solvent was removed \textit{in vacuo}, the residue was re-dissolved in methylene chloride (30 mL) and washed with water (3 x 10 mL), dried with Na\textsubscript{2}SO\textsubscript{4}, filtered and solvent was removed \textit{in vacuo}. Purification over silica (hexanes/EtOAc 95:5) gave NK366 as a clear oil (4.22 g, 82%).

\textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): \delta = 5.36 – 5.30 (m, 1H), 3.70 (t, \textit{J} = 6.7 Hz, 2H), 3.22 – 3.09 (m, 1H), 2.47 (t, \textit{J} = 6.7 Hz, 2H), 2.40 – 2.29 (m, 1H), 2.24 – 2.11 (m, 1H), 2.06 – 1.75 (m, 5H), 1.60 – 0.83 (m, 42H), 0.67 ppm (s, 3H).

Spectroscopic data is consistent with that reported in the literature.\textsuperscript{193}
Experimental

3-((8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yloxy)propanoic acid NK380

To a solution of NK366 (2.0 g, 3.88 mmol, 1.0 equiv.) in methylene chloride (25 mL) was added TFA (3 mL) and stirred for 75 minutes. The solution was carefully quenched using sat. aqueous NaHCO₃ solution and the solvent was removed in vacuo to give NK380 as a white powder (1.72 g, 97%).

¹H NMR (300 MHz, CDCl₃): δ = 5.37 – 5.31 (m, 1H), 3.76 (t, J = 6.7 Hz, 2H), 3.28 – 3.14 (m, 1H), 2.63 (t, J = 6.7 Hz, 2H), 2.41 – 2.31 (m, 1H), 2.27 – 2.14 (m, 1H), 2.06 – 1.75 (m, 5H), 1.62 – 0.83 (m, 33H), 0.68 ppm (s, 3H).

Spectroscopic data is consistent with that reported in the literature.¹⁹³

N-(6-((2S)-2-acetamido-6-((2S)-2-acetamido-6-((3S,10R,13R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yloxy)propanamido)hexanamido)hexanamido)hexyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK384

To a solution of NK360 (75 mg, 0.15 mmol, 1.5 equiv.) in methylene chloride (5 mL) was added EDCI (22 mg, 0.12 mmol, 1.15 equiv.) and HOBt (20 mg, 0.12 mmol, 1.15 equiv.) and DIPEA (85 µL, 5.0 mmol, 5.0 equiv.). After stirring for 25 minutes, NK383 (83 mg, 0.1 mmol, 1.0 equiv.) dissolved in
methylene chloride (2 mL) was added and the solution was stirred for further 8h. The reaction mixture was diluted with EtOAc (9 mL), washed with 1 N aq. HCl (2 mL), washed with brine, dried with Na$_2$SO$_4$, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 4:1) gave NK384 as a colourless oil (78 mg, 71%).

$^1$H NMR (500 MHz, CDCl$_3$): $\delta = 7.43 - 7.41$ (m, 1H), $7.31 - 7.26$ (m, 4H), $7.08 - 7.04$ (m, 2H), $7.02$ (t, $J = 6.0$ Hz, 1H), $6.76 - 6.65$ (m, 2H), $6.58$ (t, $J = 6.0$ Hz, 1H), $5.36 - 5.30$ (m, 1H), $4.42 - 4.31$ (m, 1H), $3.70$ (t, $J = 6.0$ Hz, 2H), $3.48 - 3.34$ (m, 2H), $3.31 - 3.11$ (m, 5H), $2.42$ (t, $J = 6.0$ Hz, 2H), $2.36$ (s, 3H), $1.33 - 2.28$ (m, 1H), $2.23 - 2.09$ (m, 1H), $2.05 - 1.92$ (m, 5H), $1.90 - 1.76$ (m, 4H), $1.76 - 1.44$ (m, 10H), $1.44 - 1.22$ (m, 14H), $1.22 - 0.82$ (m, 22H), 0.67 ppm (s, 3H).

$^{13}$C NMR (125 MHz, CDCl$_3$): $\delta = 171.9, 171.7, 170.5, 162.7, 145.1, 143.0, 140.4, 135.9, 134.9, 133.0, 130.9, 130.8, 130.6, 130.3, 128.9, 127.9, 127.2, 121.9, 117.6, 79.4, 64.0, 56.7, 56.2, 53.0, 50.1, 42.3, 39.7, 39.5, 39.2, 39.0, 38.6, 38.5, 37.3, 37.1, 36.8, 36.2, 35.8, 31.95, 31.92, 31.6, 29.6, 29.2, 29.1, 28.4, 28.2, 28.0, 26.26, 26.23, 24.3, 23.8, 23.1, 22.8, 22.55, 22.53, 21.1, 19.4, 18.7, 11.8, 9.4 ppm.

HRMS (ESI, 180 °C): calculated for C$_{61}$H$_{87}$Cl$_3$N$_6$O$_5$Na [M+Na] $m/z = 1111.5696$, found: $m/z = 1111.5716$.

IR (Film): $\tilde{\nu} = 1732, 1009, 741, 706$ cm$^{-1}$.
N-(6-((2S)-2-acetamido-6-((2S)-2-acetamido-6-((3S,10R,13R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yloxy)propanamido)hexanamido)hexanamido)hexyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK389

To a solution of NK380 (75 mg, 0.15 mmol, 1.5 equiv.) in methylene chloride (5 mL) was added EDCI (22 mg, 0.12 mmol, 1.15 equiv.) and HOBt (20 mg, 0.12 mmol, 1.15 equiv.) and DIPEA (85 µL, 5.0 mmol, 5.0 equiv.). After stirring for 25 minutes, NK388 (94 mg, 0.1 mmol, 1.0 equiv.) dissolved in methylene chloride (2 mL) was added and the solution was stirred for further 8h. The reaction mixture was diluted with EtOAc (9 mL), washed with 1 N aq. HCl (2 mL), washed with brine, dried with Na₂SO₄, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 4:1) gave NK389 as a colourless oil (78 mg, 71%).

¹H NMR (500 MHz, CDCl₃): δ = 7.43 – 7.41 (m, 1H), 7.31 – 7.26 (m, 4H), 7.08 – 7.04 (m, 2H), 7.03 – 7.01 (m, 1H), 6.89 – 6.85 (m, 2H), 6.80 (d, J = 7.6 Hz, 1H), 6.65 (t, J = 6.0 Hz, 1H), 5.35 – 5.31 (m, 1H), 4.42 – 4.36 (m, 1H), 4.36 – 4.31 (m, 1H), 3.70 (t, J = 6.0 Hz, 2H), 3.44 - 3.35 (m, 2H), 3.33 - 3.12 (m, 8H), 2.42 (t, J = 6.0 Hz, 2H), 2.34 – 2.30 (m, 1H), 2.20 – 2.12 (m, 1H), 2.04 – 1.97 (m, 8H), 1.86 - 1.23 (m, 36H), 1.55 – 0.83 (m, 20H), 0.67 ppm (s, 3H).

¹³C NMR (125 MHz, CDCl₃): δ = 172.1, 171.9, 171.8, 170.7, 170.5, 162.7, 145.0, 143.1, 140.4, 136.0, 135.9, 134.9, 133.0, 130.8, 130.6, 130.3, 128.9, 127.9, 127.2, 122.0, 117.6, 79.4, 64.0, 56.7, 56.1, 53.3, 53.9, 50.1, 42.3, 39.7, 39.6, 39.2, 38.9, 38.8, 38.75, 38.72, 38.4, 37.3, 37.1, 36.8, 36.2, 35.8, 32.1, 31.95, 31.92, 31.4, 29.7, 29.6, 29.2, 29.1, 28.4, 28.3, 28.2, 28.0, 26.27, 26.22, 24.3, 23.8, 23.1, 22.8, 22.5, 22.4, 21.0, 19.4, 18.7, 11.8, 9.4 ppm.
Experimental

HRMS (ESI, 180 °C): calculated for C_{69}H_{101}Cl_3N_8O_7Na [M+Na] m/z = 1281.6751, found: m/z = 1281.6703.

IR (Film): \bar{\nu} = 1785, 1064, 752, 726 cm^{-1}.

(9H-fluoren-9-yl)methyl tert-butyl (6-(6-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)hexyl)amino)-6-oxohexane-1,5-diyl)(S)-dicarbamate NK390

To a solution of 8-(Fmoc-amino)-3,6-dioxoaoctanoic acid (105 mg, 0.27 mmol, 1.2 equiv.) in DMF (14 mL) was added EDCI (48 mg, 0.27 mmol, 1.15 equiv.) and HOBr (46 mg, 0.27 mmol, 1.15 equiv.) and DIPEA (0.2 mL, 1.13 mmol, 5.0 equiv.). After stirring for 25 minutes, NK386 (200 mg, 0.23 mmol, 1.0 equiv.) dissolved in methylene chloride (3 mL) was added and the solution was stirred for further 4h. The reaction mixture was then diluted with EtOAc (20 mL), washed with 1 N aq. HCl (10 mL), washed with brine, dried with Na_2SO_4, filtered and the solvent was removed \textit{in vacuo}. Purification over silica (EtOAc/ULTRA 7:3) gave NK390 as a colourless resin (255 mg, 91%).

^1H NMR (500 MHz, CDCl3):

\delta = 7.74 (d, J = 7.0 Hz, 2H), 7.65 – 7.48 (m, 3H), 7.43 – 7.41 (m, 1H), 7.37 (t, J = 7.0 Hz, 2H), 7.31 – 7.26 (m, 6H), 7.07–7.04 (m, 2H), 7.03 – 7.01 (m, 1H), 6.99 – 6.94 (m, 1H), 6.82 – 6.69 (m, 1H), 6.50 – 6.43 (m, 1H), 4.86 – 4.76 (m, 1H), 4.56 – 4.42 (m, 1H), 4.39 – 4.27 (m, 2H), 4.27 – 4.16 (m, 1H), 4.06 – 3.94 (m, 2H), 3.73 – 3.55 (m, 5H), 3.55 – 3.45 (m, 1H), 3.45 – 3.31 (m, 3H), 3.31 – 3.13 (m, 4H), 3.13 – 2.96 (m, 2H), 2.35 (s, 3H), 1.97 (s, 3H), 1.92 – 1.79 (m, 1H), 1.79 – 1.62 (m, 3H), 1.63 – 1.53 (m, 3H), 1.53 – 1.25 ppm (m, 22H).
Experimental

\[ ^{13}\text{C NMR (125 MHz, CDCl}_3\text{):} \]
\[ \delta = 171.8, 171.5, 170.5, 170.2, 162.8, 156.9, 156.1, 145.0, 144.14, 144.12, 143.1, 141.2, 136.0, 135.9, 134.9, 133.0, 130.8, 130.6, 130.3, 128.9, 127.9, 127.7, 127.0, 125.3, 119.9, 117.6, 79.1, 71.2, 70.6, 70.2, 70.0, 66.7, 47.3, 41.0, 40.2, 39.2, 38.7, 29.7, 29.6, 28.5, 26.3, 23.1, 9.4 \text{ ppm.} \]

HRMS (ESI, 180 °C): calculated for C\text{63}H\text{80}Cl\text{3}N\text{9}O\text{11}Na [M+Na] \[ m/z = 1266.4935, \]
found: \[ m/z = 1266.4951. \]

IR (Film): \[ \tilde{\nu} = 1720, 981, 773, 712 \text{ cm}^{-1}. \]

(9H-fluoren-9-yl)methyl \text{(11S,18S)-11-acetamido-18-(4-aminobutyl)-1-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl)-1,10,17,20-tetraoxo-22,25-dioxa-2,9,16,19-tetraazaheptacosan-27-ylcarbamate NK391}

To a solution of NK90 (255 mg, 0.20 mmol, 1.0 equiv.) in methylene chloride (10 mL) was added TFA (2 mL) and the solution was stirred for 3 hours and subsequently quenched with sat. aqueous NaHCO\text{3} solution, extracted with EtOAc (3 \* 5 mL), dried with MgSO\text{4}, filtered, and the solvent was removed \text{in vacuo} to give NK391 as a colourless resin (187 mg, 82%) which was used in the next step without further purification.
Experimental

(9H-fluoren-9-yl)methyl (11S,18S)-11-acetamido-1-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-
methyl-1H-pyrazol-3-yl)-18-(4-((8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-
-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-
cyclopenta[ajphenanthren-3-yl)oxy)propanamido)butyl)-1,10,17,20-tetraoxo-22,25-dioxa-
2,9,16,19-tetraazaheptacosan-27-ylcarbamate NK394

To a solution of NK380 (105 mg, 0.20 mmol, 1.2 equiv.) in methylene chloride (8 mL) was added EDCI (38 mg, 0.20 mmol, 1.15 equiv.) and HOBT (36 mg, 0.20 mmol, 1.15 equiv.) and DIPEA (0.1 mL, 1.0 mmol, 5.0 equiv.). After stirring for 25 minutes, NK391 (188 mg, 0.16 mmol, 1.0 equiv.) dissolved in methylene chloride (3 mL) was added and the solution was stirred for further 4h. The reaction mixture was then diluted with EtOAc (10 mL), washed with 1 N aq. HCl (5 mL), washed with brine, dried with Na₂SO₄, filtered and the solvent was removed in vacuo. Purification over silica (DCM/MeOH 96:4) gave NK394 as a colourless resin (140 mg, 45%).

¹H NMR (500 MHz, CDCl₃): δ = 7.74 (d, J = 7.0 Hz, 2H), 7.64 – 7.60 (m, 2H), 7.43 – 7.41 (m, 1H), 7.37 (t, J = 7.0 Hz, 2H), 7.30 – 7.25 (m, 6H), 7.05 (d, J = 7.0 Hz, 2H), 5.33 – 5.29 (m, 1H), 4.55 – 4.48 (m, 1H), 4.40 – 4.30 (m, 3H), 4.25 – 4.19 (m, 1H), 4.06 – 3.95 (m, 2H), 3.72 – 3.58 (m, 8H), 3.56 – 3.44 (m, 2H), 3.43 – 3.35 (m, 3H), 3.34 – 3.26 (m, 1H), 3.26 – 3.09 (m, 7H), 2.42 – 2.36 (m, 2H), 2.35 (s, 3H), 2.34 – 2.28 (m, 1H), 2.18 – 2.10 (m, 1H), 2.02 – 1.91 (m, 6H), 1.89 – 1.77 (m, 5H), 1.73 - 1.64 (m, 2H), 1.60 – 1.24 (m, 25H), 1.16 – 0.87 (m, 20H), 0.66 ppm (s, 3H).

¹³C NMR (125 MHz, CDCl₃): δ = 171.6, 171.3, 170.4, 169.9, 162.5, 156.7, 144.9, 144.0, 143.9, 142.9, 141.1, 140.3, 135.8, 134.8, 132.8, 130.7, 130.4, 130.1, 128.7, 127.8, 127.5, 126.9, 125.1, 121.8, 119.8, 117.5, 79.2, 71.1, 70.4, 70.0, 69.8, 66.6, 63.9, 56.6, 56.0, 53.3, 52.2, 50.0, 47.1, 42.2, 40.7, 39.6, 39.4, 39.0, 38.9, 38.7, 38.4, 37.1, 36.9, 36.7, 36.0, 35.6, 32.3, 32.0, 31.8, 31.7, 29.4, 29.0, 28.9, 28.3, 28.1, 27.9, 24.1, 23.7, 22.9, 22.7, 22.4, 20.9, 19.2, 18.6, 11.7, 9.3 ppm.
**Experimental**

**HRMS (ESI, 180 °C):** calculated for C_{88}H_{120}Cl_{3}N_{9}O_{11}Na [M+Na] m/z = 1606.8065, found: m/z = 1606.8057.

**IR (Film):** $\tilde{\nu} = 1730, 991, 784, 720 \text{ cm}^{-1}$.

N-(((10S,17S)-17-acetamido-1-amino-10-(4-((3,6-dioxa-9,12,19-triazapentacosan-25-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK395

To a solution of NK394 (140 mg, 0.07 mmol, 1.0 equiv.) in methylene chloride (5 mL) was added Et$_2$NH (1 mL) and the solution was stirred for 4 hours. Upon completion of the reaction the solvent was removed in vacuo, the residue was dissolved in EtOAc (5 mL), washed with 1 N aq. HCl, washed with brine, dried with MgSO$_4$, filtered and purified over silica (DCM/MeOH 96:4) to give NK395 as a colourless resin (76 mg, 78%).

$^1$H NMR (500 MHz, CDCl3):

$\delta = 7.54 - 7.49$ (m, 1H), $7.45 - 7.40$ (m, 1H), $7.34 - 7.27$ (m, 5H), $7.16 - 7.11$ (m, 1H), $7.09 - 7.03$ (m, 3H), 6.83 (t, $J = 6.0$ Hz, 1H), $5.34 - 5.31$ (m, 1H), $4.52 - 4.45$ (m, 1H), $4.44 - 4.37$ (m, 1H), $4.09 - 3.93$ (m, 2H), $3.78 - 3.57$ (m, 9H), $3.57 - 3.44$ (m, 3H), $3.43 - 3.35$ (m, 3H), $3.32 - 3.11$ (m, 8H), $3.01 - 2.92$ (m, 2H), $2.46 - 2.40$ (m, 2H), $2.35$ (s, 3H), $2.34 - 2.28$ (m, 1H), $2.18 - 2.10$ (m, 1H), $2.04 - 1.92$ (m, 6H), $1.89 - 1.02$ (m, 40H), $1.01 - 0.96$ (m, 4H), $0.91$ (d, $J = 6.0$ Hz, 4H), $0.87$ (dd, $J = 6.0, 2.4$ Hz, 6H), 0.67 ppm (s, 3H).
Experimental

$^{13}$C NMR (125 MHz, CDCl$_3$): $\delta = 172.0$, 171.8, 171.6, 170.6, 170.1, 162.7, 145.0, 143.0, 140.5, 135.9, 134.9, 132.9, 130.8, 130.6, 130.3, 128.9, 127.9, 127.2, 121.9, 117.6, 79.4, 72.0, 71.1, 70.3, 70.0, 64.1, 56.7, 56.1, 53.4, 53.1, 52.5, 50.1, 42.3, 41.2, 39.7, 39.5, 39.3, 39.1, 38.9, 38.7, 37.3, 37.1, 36.8, 36.2, 35.8, 32.4, 32.1, 31.97, 31.94, 29.6, 29.2, 29.0, 28.6, 28.4, 28.2, 28.0, 26.4, 24.3, 23.8, 23.2, 22.8, 22.7, 22.65, 22.62, 21.1, 19.4, 18.7, 11.9, 9.4 ppm.

HRMS (ESI, 180 °C): calculated for C$_{73}$H$_{111}$Cl$_3$N$_9$O$_9$ [M+H] $m/z = 1362.7565$, found: $m/z = 1362.7571$.

IR (Film): $\tilde{\nu} = 1755$, 953, 728, 682 cm$^{-1}$.

N-((16S,23S)-23-acetamido-16-(4-(3-((8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)propanamido)butyl)-5,14,17,24-tetraoxo-1-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-9,12-dioxo-6,15,18,25-tetraazahentriacontan-31-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK397

To a solution of NK395 (76 mg, 0.07 mmol, 1.0 equiv.) in methylene chloride (5 mL) and DIPEA (30 µL) was added biotin-chloride NK30 (20 mg, 0.08 mmol, 1.15 equiv.) in methylene chloride (2 mL). After stirring for 60 minutes the reaction was diluted with EtOAc (3 mL), washed with 1 N aq. HCl, washed with brine, dried with MgSO$_4$, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 9:1.5) gave NK397 as a colourless resin (60 mg, 55%).
Experimental

$^1$H NMR (400 MHz, CDCl₃): $\delta = 7.72 – 7.66$ (m, 1H), $7.53 – 7.48$ (m, 1H), $7.42 – 7.39$ (m, 1H), $7.30 – 7.26$ (m, 4H), $7.07 – 7.02$ (m, 2H), $5.35 – 5.30$ (m, 1H), $4.74 – 4.47$ (m, 2H), $4.33 – 4.15$ (m, 4H), $4.08 – 3.84$ (m, 4H), $3.74 – 3.50$ (m, 10H), $3.42 – 3.33$ (m, 2H), $3.27 – 2.84$ (m, 12H), $2.45 – 2.26$ (m, 7H), $2.20 – 2.10$ (m, 2H),

$^{13}$C NMR (100 MHz, CDCl₃): $\delta = 173.4, 173.3, 171.8, 171.0, 170.7, 170.6, 167.7, 162.6, 145.0, 143.0, 140.4, 135.9, 134.9, 132.9, 132.4, 132.3, 130.8, 128.9, 127.9, 121.9, 117.6, 79.4, 68.1, 65.5, 64.0, 56.7, 56.1, 50.1, 42.3, 39.7, 39.5, 39.0, 38.7, 37.1, 36.8, 36.1, 35.7, 31.9, 31.8, 30.5, 30.3, 29.6, 28.9, 28.4, 28.2, 28.0, 26.3, 24.2, 23.8, 23.7, 22.9, 22.8, 22.5, 21.0, 19.3, 19.1, 18.7, 14.0, 13.7, 11.8, 11.1, 10.9, 9.4 ppm.

HRMS (ESI, 180 °C): calculated for C₈₃H₁₂₄Cl₃N₁₁O₁₁SNa [M+Na] $m/z = 1610.8160$, found: $m/z = 1610.8153$.

IR (Film): $\tilde{\nu} = 1780, 1005, 817, 706$ cm$^{-1}$.

**tert-butyl 4-aminobutylcarbamate NK600**

\[
\text{H}_2\text{N} - \text{C} - \text{O} - \rightleftharpoons \\
\text{NK600}
\]

**NK600** was prepared from 1,4 diaminobutane according to **GP 1A** to afford a white solid (1.55 g, 93%) after purification over silica (EtOAc/ULTRA 3:7).

$^1$H NMR (300 MHz, CDCl₃): $\delta 3.18 – 3.06$ (m, 2H), $2.72$ (t, $J = 6.5$ Hz, 2H), $1.63 – 1.39$ ppm (m, 13H).

$^{13}$C NMR (75 MHz, CDCl₃): $\delta = 156.0, 79.0, 41.7, 40.4, 30.7, 28.4, 27.5$ ppm.

Spectroscopic data is consistent with that reported in the literature.$^{194}$
tert-butyl 2-(2-(2-aminoethoxy)ethoxy)ethylcarbamate NK247

\[
\begin{array}{c}
\text{H}_2\text{N} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{N} \quad \text{O} \\
\text{NK247}
\end{array}
\]

Mono-Boc-protected 1,2-bis(2-aminoethoxy)ethane NK247 was prepared from 2,2′-(ethane-1,2-diylbis(oxy))diethanamine according to GP 1B to afford a colourless oil (2.16 g, 95%) after purification over silica (EtOAc/ULTRA 3:7).

\[^1\text{H} \text{ NMR (400 MHz, CDCl}_3\text{):} \]
\[\delta = 3.59 \text{ (br s, 4H), 3.54 – 3.47 (m, 4H), 3.32 – 3.25 (m, 2H), 2.85 (t, J = 5.5 Hz, 2H), 1.42 ppm (br s, 9H).}\]

\[^{13}\text{C} \text{ NMR (75 MHz, CDCl}_3\text{):} \]
\[\delta = 155.9, 79.1, 73.4, 70.1, 41.7, 40.3, 28.3 \text{ ppm.}\]

Spectroscopic data is consistent with that reported in the literature.\(^{195}\)

tert-butyl 2-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)ethylcarbamate NK523

\[
\begin{array}{c}
\text{NK523}
\end{array}
\]

NK523 was prepared according to GP 2 from acid-chloride NK25 and 1-Boc-ethylenediamine to afford a colourless oil (845 mg, 89%) after purification over silica (EtOAc/hexanes 2:1).

\[^1\text{H} \text{ NMR (400 MHz, MeOD):} \]
\[\delta = 7.58 – 7.53 \text{ (m, 2H), 7.46 – 7.43 (m, 1H), 7.40 – 7.35 (m, 2H), 7.23 – 7.18 (m, 2H), 3.53 – 3.45 (m, 2H), 3.33 – 3.27 (m, 2H), 2.34 (s, 3H), 1.43 ppm (br s, 9H).}\]

\[^{13}\text{C} \text{ NMR (100 MHz, MeOD):} \]
\[\delta = 165.3, 158.7, 146.2, 144.6, 137.5, 137.3, 136.2, 134.2, 132.6, 132.5, 131.1, 130.0, 129.3, 128.7, 118.4, 80.2, 41.0, 40.6, 28.8, 9.7 \text{ ppm.}\]
Experimental

HRMS (ESI, 180 °C): calculated for C_{24}H_{25}Cl_{3}N_{4}O_{3}Na [M+Na] \text{ m/z } = 545.0884, found: \text{ m/z } = 545.0882.

IR (Film): \tilde{\nu} = 3417, 1684, 1422, 984 \text{ cm}^{-1}

tert-butyl 3-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)propylcarbamate NK524

NK524 was prepared according to GP 2 from acid-chloride NK25 and 1-Boc-propylenediamine to afford a colourless oil (822 mg, 85%) after purification over silica (EtOAc/hexanes 2:1).

\textsuperscript{1}H NMR (400 MHz, MeOD) \delta = 7.58 – 7.53 (m, 2H), 7.46 – 7.43 (m, 1H), 7.40 – 7.35 (m, 2H), 7.23 – 7.18 (m, 2H), 3.53 – 3.45 (m, 2H), 3.33 – 3.27 (m, 2H), 2.34 (s, 3H), 1.82 – 1.73 (m, 2H), 1.43 ppm (br s, 9H).

\textsuperscript{13}C NMR (100 MHz, MeOD): \delta = 165.0, 158.4, 146.1, 144.4, 137.3, 137.2, 136.1, 134.0, 132.4, 132.3, 131.0, 129.8, 129.1, 128.6, 118.3, 79.9, 38.8, 37.4, 30.9, 28.8, 9.7 ppm.

HRMS (ESI, 180 °C): calculated for C_{25}H_{27}Cl_{3}N_{4}O_{3}Na [M+Na] \text{ m/z } = 559.1041, found: \text{ m/z } = 559.1030.

IR (Film): \tilde{\nu} = 3419, 1678, 1454, 835 \text{ cm}^{-1}
Experimental

tert-butyl 4-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)butylcarbamate NK525

![NK525](image)

**NK525** was prepared according to GP 2 from acid-chloride **NK25** and 1-Boc-amino-1,4-butanediamine **NK600** to afford a colourless oil (824 mg, 83%) after purification over silica (EtOAc/hexanes 2:1).

$^1$H NMR (400 MHz, CDCl3) $\delta = 7.43 - 7.41$ (m, 1H), 7.32 – 7.26 (m, 4H), 7.09 – 7.00 (m, 3H), 4.75 (br s, 1H), 3.47 – 3.39 (m, 2H), 3.19 – 3.09 (m, 2H), 2.34 (s, 3H), 1.68 – 1.53 (m, 4H), 1.43 ppm (br s, 9H).

$^{13}$C NMR (100 MHz, CDCl3): $\delta = 164.6, 157.8, 146.8, 144.9, 137.8, 136.7, 134.8, 132.7, 132.4, 132.1, 130.7, 129.7, 129.1, 119.5, 80.8, 42.1, 40.4, 30.3, 29.4, 29.0, 11.2$ ppm.

HRMS (ESI, 180 °C): calculated for C$_{26}$H$_{29}$Cl$_3$N$_4$O$_3$Na [M+Na] $m/z = 573.1197$, found: $m/z = 573.1189$.

IR (Film): $\tilde{\nu} = 3421, 1668, 1432, 931$ cm$^{-1}$

tert-butyl 2-(2-(5-(4-chlorophenyl))-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)ethoxy)ethoxy)ethylcarbamate NK250

![NK250](image)

**NK250** was prepared according to GP 2 from acid-chloride **NK25** and **NK247** to afford a colourless oil (791 mg, 72%) after purification over silica (EtOAc/ULTRA 3:7).
Experimental

$^1$H NMR (300 MHz, CDCl$_3$):  $\delta = 7.42 - 7.40$ (m, 1H), 7.31 – 7.26 (m, 5H), 7.07 – 7.03 (m, 2H), 5.03 (br s, 1H), 3.69 – 3.56 (m, 8H), 3.50 (t, $J = 5.5$ Hz, 2H), 3.27 – 3.18 (m, 2H), 2.37 (s, 3H), 1.42 ppm (s, 9H).

$^{13}$C NMR (100 MHz, CDCl$_3$):  $\delta = 162.8, 144.9, 143.0, 135.9, 134.9, 132.9, 130.8, 130.5, 130.3, 128.9, 127.9, 127.2, 117.7, 70.2, 70.0, 41.0, 38.7, 28.4, 9.4$ ppm.

HRMS (ESI, 200 °C): calculated for C$_{28}$H$_{33}$Cl$_3$N$_4$NaO$_5$ [M+Na]: $m/z = 633.1409$, found: $m/z = 633.1387$.

IR (Film):  $\tilde{\nu} = 3421, 1668, 1432, 783$ cm$^{-1}$.

tert-butyl 1-(5-(chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl)-1,8-dioxo-12,15-dioxa-2,9-diazahexadecan-17-ylcarbamate NK282

![Chemical Structure](image)

NK282 was prepared according to GP 2 from acid-chloride NK25 and NK279 to afford a colourless oil (900 mg, 69%) after purification over silica (EtOAc/ULTRA 3:7).

$^1$H NMR (300 MHz, CDCl$_3$):  $\delta = 7.43 - 7.41$ (m, 1H), 7.33 – 7.25 (m, 4H), 7.09 – 7.02 (m, 2H), 6.98 (m, 1H), 6.03 (br s, 1H), 5.02 (br s, 1H), 3.65 – 3.50 (m, 8H), 3.48 – 3.36 (m, 4H), 3.36 – 3.24 (m, 2H), 2.37 (s, 3H), 2.19 (t, $J = 7.7$ Hz, 2H), 1.75 – 1.56 (m, 6H), 1.46 – 1.40 ppm (m, 9H).

HRMS (EI, 200 °C): calculated for C$_{34}$H$_{44}$Cl$_3$N$_5$O$_6$Na [M+Na]: $m/z = 746.2249$, found: $m/z = 746.2258$.

IR (Film):  $\tilde{\nu} = 3423, 1654, 1474, 848$ cm$^{-1}$.
Experimental

N-(2-aminoethyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK526

NK526 was prepared according to GP 3 from NK523 to afford a colourless oil (498 mg, 98%) after purification over silica (DCM/ULTRA 1:1).

$^1$H NMR (400 MHz, CDCl3) $\delta = 7.37 - 7.33$ (m, 1H), $7.33 - 7.22$ (m, 5H), $7.05 - 6.99$ (m, 2H), $6.91$ (br s, 2H), $4.75$ (br s, 1H), $3.70 - 3.59$ (m, 2H), $3.19 - 3.09$ (m, 2H), $2.27$ ppm (s, 3H).

$^{13}$C NMR (100 MHz, CDCl3): $\delta = 166.0$, $146.3$, $145.0$, $137.9$, $137.7$, $136.8$, $134.6$, $132.7$, $132.6$, $130.8$, $129.9$, $129.0$, $119.6$, $62.3$, $16.1$, $11.3$ ppm.

HRMS (ESI, 180 °C): calculated for C$_{19}$H$_{17}$Cl$_3$N$_4$O$_1$ [M+H] $m/z = 423.0541$, found: $m/z = 423.0544$.

IR (Film): $\tilde{\nu} = 3438$, $1698$, $1456$, $892$ cm$^{-1}$.

N-(3-aminopropyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK527

NK527 was prepared according to GP 3 from NK524 to afford a colourless oil (508 mg, 97%) after purification over silica (DCM/ULTRA 1:1).
Experimental

$^1$H NMR (400 MHz, CDCl₃) $\delta = 7.47 - 7.43 \text{ (m, 1H)}, 7.40 - 7.34 \text{ (m, 1H)}, 7.33 - 7.22 \text{ (m, 4H)}, 7.05 - 6.99 \text{ (m, 2H)}, 3.58 - 3.49 \text{ (m, 2H)}, 3.19 - 3.09 \text{ (m, 2H)}, 2.27 \text{ (s, 3H)}, 2.19 - 2.05 \text{ ppm (m, 2H)}$.

$^{13}$C NMR (100 MHz, CDCl₃): $\delta = 166.1, 146.1, 145.1, 137.9, 137.7, 136.9, 134.6, 132.7, 132.5, 132.1, 130.8, 129.8, 128.9, 119.7, 38.8, 37.4, 29.5, 11.2 \text{ ppm}$.

HRMS (ESI, 180°C): calculated for $C_{20}H_{20}Cl_3N_4O$ [M+H] $m/z = 437.0697$, found: $m/z = 437.0706$.

IR (Film): $\tilde{\nu} = 3437, 1697, 1467, 968 \text{ cm}^{-1}$.

**N-(4-aminobutyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK528**

![Chemical structure of NK528]

**NK528** was prepared according to **GP 3** from **NK525** to afford (520 mg, 96%) of a colourless oil after purification over silica (DCM/ULTRA 1:1).

$^1$H NMR (400 MHz, CDCl₃) $\delta = 7.37 - 7.33 \text{ (m, 1H)}, 7.33 - 7.22 \text{ (m, 5H)}, 7.05 - 6.99 \text{ (m, 2H)}, 3.49 - 3.35 \text{ (m, 2H)}, 3.13 - 3.02 \text{ (m, 2H)}, 2.27 \text{ (s, 3H)}, 1.90 - 1.80 \text{ (m, 2H)}, 1.75 - 1.63 \text{ ppm (m, 2H)}$.

$^{13}$C NMR (100 MHz, CDCl₃): $\delta = 163.2, 144.7, 143.1, 137.9, 136.0, 135.8, 134.9, 132.8, 130.8, 130.6, 130.2, 128.9, 127.9, 127.1, 117.5, 39.6, 38.4, 26.8, 24.8, 9.4 \text{ ppm}$.

HRMS (ESI, 180°C): calculated for $C_{21}H_{21}Cl_3N_4ONa$ [M+Na] $m/z = 473.0673$, found: $m/z = 473.0679$.

IR (Film): $\tilde{\nu} = 3413, 1694, 1464, 1265, 984 \text{ cm}^{-1}$.
Experimental

**N-(2-(2-((2-aminoethoxy)ethoxy)ethyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK251**

![NK251](image)

**NK251** was prepared according to **GP 3** from **NK250** to afford (595 mg, 97%) of a colourless oil after purification over silica (DCM/ULTRA 1:1).

\[
\begin{align*}
\delta &= 7.44 - 7.42 \text{ (m, 1H)}, \ 7.34 - 7.26 \text{ (m, 4H)}, \ 7.07 - 7.03 \text{ (m, 2H)}, \ 3.79 - 3.60 \text{ (m, 10H)}, \ 3.19 - 3.07 \text{ (m, 2H)}, \ 2.28 \text{ ppm (s, 3H).} \\
\delta &= 162.3, 136.6, 135.4, 132.7, 130.8, 130.49, 130.45, 129.0, 128.2, 126.5, 117.8, 70.4, 70.2, 69.9, 66.6, 39.8, 39.2, 9.1 \text{ ppm.} \\
\text{HRMS (ESI, 200 °C):} & \text{ calculated for } C_{23}H_{25}Cl_3N_4NaO_3: \ m/z = 533.0884, \text{ found: } m/z = 533.0864. \\
\text{IR (Film):} & \tilde{\nu} = 3423, 1654, 1474, 968 \text{ cm}^{-1}. 
\end{align*}
\]

**N-(6-((2-(2-((2-aminoethoxy)ethoxy)ethylamino)-6-oxohexyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK289**

![NK289](image)

**NK289** was prepared according to **GP 3** from **NK282** to afford (697 mg, 93%) of a colourless oil after purification over silica (DCM/ULTRA 1:1.3).
Experimental

$^1$H NMR (300 MHz, CDCl3): $\delta = 7.44 - 7.40$ (m, 1H), 7.33 - 7.25 (m, 4H), 7.10 - 6.98 (m, 3H), 6.59 (m, 1H), 3.69 - 3.51 (m, 8H), 3.47 - 3.35 (m, 4H), 3.24 (br s, 2H), 2.96 - 2.89 (m, 2H), 2.36 (s, 3H), 2.21 (t, $J = 7.7$ Hz, 2H), 1.74 - 1.56 (m, 4H), 1.48 - 1.35 ppm (m, 2H).

$^{13}$C NMR (75 MHz, CDCl3): $\delta = 173.1, 162.6, 145.0, 143.0, 135.9, 134.8, 132.9, 130.7, 130.5, 130.2, 128.8, 127.8, 127.2, 117.5, 72.0, 70.1, 70.0, 69.9, 41.2, 39.1, 38.7, 36.3, 29.4, 26.5, 25.3, 9.3$ ppm.

HRMS (EI, 200 °C): calculated for $C_{29}H_{36}Cl_{3}N_{5}O_{4}$ [M+Na]: $m/z = 646.1725$, found: $m/z = 646.1700$.

IR (Film): $\tilde{\nu} = 3487, 1667, 1484, 951$ cm$^{-1}$.

5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-N-(2-isothiocyanatoethyl)-4-methyl-1H-pyrazole-3-carboxamide NK529

![NK529](image)

NK529 was prepared according to GP 4 from free amine NK526 to afford (183 mg, 79%) a colourless foam after purification over silica (DCM/ULTRA 96:4).

$^1$H NMR (400 MHz, MeOD) $\delta = 7.61 - 7.52$ (m, 2H), 7.49 - 7.44 (m, 1H), 7.41 - 7.36 (m, 2H), 7.25 - 7.19 (m, 2H), 3.79 (t, $J = 6.0$ Hz, 2H), 3.66 (t, $J = 6.0$ Hz, 2H), 2.35 ppm (s, 3H).

$^{13}$C NMR (100 MHz, MeOD): $\delta = 165.3, 145.8, 144.6, 137.4, 137.3, 136.2, 134.2, 132.5, 132.4, 131.1, 129.9, 129.2, 128.6, 118.6, 45.6, 39.8, 9.6$ ppm.

HRMS (ESI, 180 °C): calculated for $C_{20}H_{15}Cl_{3}N_{5}OS$ [M+Na] $m/z = 486.9924$, found: $m/z = 486.9931$.

IR (Film): $\tilde{\nu} = 3398, 1647, 1447$ cm$^{-1}$.

Melting point: $55 - 57$ °C.
5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-N-(3-isothiocyanatopropyl)-4-methyl-1H-pyrazole-3-carboxamide NK530

NK530 was prepared according to GP 4 from free amine NK527 to afford (177 mg, 74%) of a white solid after purification over silica (DCM/ULTRA 96:4).

$^1$H NMR (400 MHz, MeOD) \[ \delta = 7.61 - 7.52 \text{ (m, 2H)}, 7.49 - 7.44 \text{ (m, 1H)}, 7.41 - 7.36 \text{ (m, 2H)}, 7.25 - 7.19 \text{ (m, 2H)}, 3.79 \text{ (t, } J = 6.0 \text{Hz, 2H)}, 3.66 \text{ (t, } J = 6.0 \text{Hz, 2H)}, 2.35 \text{ (s, 3H)}, 2.05 \text{ ppm (m, 2H)}. \]

$^{13}$C NMR (100 MHz, MeOD): \[ \delta = 165.1, 146.0, 144.5, 137.3, 137.2, 136.1, 134.1, 132.4, 132.3, 131.0, 129.8, 129.2, 128.6, 118.4, 43.8, 37.3, 31.1, 9.6 \text{ ppm}. \]

HRMS (ESI, 180 °C): calculated for C$_{21}$H$_{17}$Cl$_3$N$_4$OS [M+Na] \( m/z = 501.0081 \), found: \( m/z = 501.0087 \).

IR (Film): \[ \tilde{\nu} = 3423, 1654, 1474 \text{ cm}^{-1}. \]

Melting point: \[ 79 - 81 \text{ °C} \]
5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-N-(4-isothiocyanatobutyl)-4-methyl-1H-pyrazole-3-carboxamide NK531

\[ \text{NK531} \]

\[ \text{NK531} \]

NK531 was prepared according to GP 4 from free amine NK528 to afford (187 mg, 76%) of a white solid after purification over silica (DCM/ULTRA 96:4).

\(^1\text{H NMR (400 MHz, MeOD)}\)  
\[ \delta = 7.61 - 7.52 (m, 2H), 7.49 - 7.44 (m, 1H), 7.41 - 7.36 (m, 2H), 7.25 - 7.19 (m, 2H), 3.63 (t, J = 6.0Hz, 2H), 3.43 (t, J = 6.0Hz, 2H), (m, 2H), 2.33 (s, 3H), 1.84 - 1.68 ppm (m, 4H). \]

\(^{13}\text{C NMR (100 MHz, MeOD)}\):  
\[ \delta = 164.9, 146.1, 144.5, 137.3, 137.2, 136.1, 134.1, 132.48, 132.43, 131.0, 129.8, 129.2, 128.6, 118.3, 45.7, 39.1, 28.5, 27.8, 9.6 \text{ ppm.} \]

\(\text{HRMS (ESI, 180 °C):} \) calculated for C\(_{22}\)H\(_{18}\)Cl\(_3\)N\(_4\)OSNa \([\text{M+Na]} \) \(m/z = 515.0237\), found: \(m/z = 515.0231\).

\(\text{IR (Film):} \)  
\[ \tilde{\nu} = 3403, 1649, 1448 \text{ cm}^{-1}. \]

\(\text{Melting point:} \)  
85 – 87 °C.
Experimental

5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-N-(2-(2-isothiocyanatoethoxy)ethoxy)ethyl)-4-methyl-1H-pyrazole-3-carboxamide NK295

\[ \text{NK295} \]

NK295 was prepared according to GP 4 from free amine NK250 to afford (192 mg, 79%) of a colourless oil after purification over silica (DCM/ULTRA 1:2).

\[ ^1H \text{ NMR (300 MHz, CDCl}_3\text{):} \quad \delta = 7.44 - 7.42 (m, 1H), 7.33 - 7.26 (m, 4H), 7.09 - 7.03 (m, 2H), 3.71 - 3.63 (m, 10H), 3.60 - 3.54 (m, 2H), 2.37 ppm (s, 3H). \]

\[ ^{13}C \text{ NMR (75 MHz, CDCl}_3\text{):} \quad \delta = 162.7, 162.3, 144.9, 143.0, 135.9, 134.9, 132.9, 130.8, 130.5, 130.3, 128.8, 127.9, 127.2, 117.7, 70.7, 70.3, 70.0, 69.3, 45.1, 38.7, 9.4 ppm. \]

HRMS (ESI, 200 °C): calculated for C_{24}H_{23}Cl_3N_4NaSO_3 [M+Na]: \( m/z = 575.0449 \), found: \( m/z = 575.0433 \).

IR (Film): \( \bar{\nu} = 3419, 1641, 1473, 1186 \text{ cm}^{-1} \).
Experimental

5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-N-(6-(2-(2-isothiocyanatoethoxy)ethoxy)ethylamino)-6-oxohexyl-4-methyl-1H-pyrazole-3-carboxamide NK292

NK292 was prepared according to GP 4 from free amine NK289 to afford (245 mg, 74%) of a colourless oil after purification over silica (DCM/ULTRA 1:2).

$^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 7.43 - 7.41 (m, 1H), 7.32 - 7.25 (m, 4 H), 7.09 - 7.03 (m, 2H), 6.98 (m, 1H), 6.01 (m, 1H), 3.72 - 3.60 (m, 8H), 3.59 - 3.52 (m, 2H), 3.49 - 3.36 (m, 4H), 2.36 (s, 3H), 2.23 (t, $J = 7.7$ Hz, 2H), 1.75 - 1.56 (m, 4H), 1.48 - 1.35 ppm (m, 2H).

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ = 174.9, 164.6, 147.0, 145.0, 137.9, 136.8, 134.9, 132.8, 132.5, 132.3, 130.8, 129.8, 129.2, 119.6, 72.6, 72.2, 72.0, 71.2, 47.2, 41.1, 40.8, 38.5, 31.5, 28.6, 27.3, 11.4 ppm.

HRMS (EI, 200 °C): calculated for C$_{30}$H$_{34}$Cl$_3$N$_5$O$_4$S [M+Na]: $m/z$ = 688.1289, found: $m/z$ = 688.1308.

IR (Film): $\bar{\nu}$ = 3422, 1656, 1486, 948 cm$^{-1}$.
Experimental

N-(2-(2-bromoacetamido)ethyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK550

NK550 was prepared according to GP 5 from free amine NK526 to afford (209 mg, 77%) of a white foam after purification over silica (EA/Hex 4:1).

$^1$H NMR (500 MHz, MeOD) $\delta =$ 7.59 (d, $J = 2.5$ Hz, 1H), 7.56 – 7.53 (m, 1H), 7.48 – 7.46 (m, 1H), 7.41 – 7.37 (m, 2H), 7.23 – 7.20 (m, 2H), 3.86 (s, 2H), 3.57 – 3.52 (m, 2H), 3.48 – 3.43 (m, 2H), 2.34 ppm (s, 3H).

$^{13}$C NMR (100 MHz, MeOD): $\delta =$ 169.9, 165.5, 146.1, 144.6, 137.4, 137.3, 136.2, 134.2, 132.5, 132.4, 131.1, 129.9, 129.2, 128.7, 118.4, 40.8, 39.4, 28.7, 9.6 ppm.

HRMS (ESI, 180 °C): calculated for C$_{21}$H$_{18}$Cl$_{3}$BrN$_{4}$O$_{3}$Na [M+Na] $m/z$ = 564.9571, found: $m/z$ = 564.9575.

IR (Film): $\tilde{\nu} =$ 3433, 1645, 1470, 1195 cm$^{-1}$.

Melting point: 77 – 79 °C.
N-(3-(2-bromoacetamido)propyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK551

NK551 was prepared according to GP 5 from free amine NK527 to afford (225 mg, 81%) of a white foam after purification over silica (EA/hexanes 4:1).

\[ \delta = 7.59 \ (d, \ J = 2.5 \ Hz, \ 1H), \ 7.56 - 7.53 \ (m, \ 1H), \ 7.48 - 7.46 \ (m, \ 1H), \ 7.41 - 7.37 \ (m, \ 2H), \ 7.23 - 7.20 \ (m, \ 2H), 3.86 \ (s, \ 2H), \ 3.47 - 3.42 \ (m, \ 2H), 3.37 - 3.31 \ (m, \ 2H), 2.34 \ (s, \ 3H), 1.88 - 1.80 \ ppm \ (m, \ 2H). \]

\[ \delta = 169.6, 165.0, 146.1, 144.5, 137.4, 137.2, 136.1, 134.1, \]

\[ 132.5, 132.4, 131.0, 129.8, 129.2, 128.6, 118.3, 38.6, 37.4, \]

\[ 30.3, 29.0, 9.6 \ ppm. \]

HRMS (ESI, 180 °C): calculated for C\textsubscript{22}H\textsubscript{20}Cl\textsubscript{3}BrN\textsubscript{4}O\textsubscript{2}Na [M+Na] \( m/z \) = 578.9727, found: \( m/z \) = 578.9727.

IR (Film): \( \tilde{\nu} = 3426, 1684, 1484, 1189, 983 \ cm^{-1}. \)

Melting point: 81 – 83 °C.
**Experimental**

**N-(4-(2-bromoacetamido)butyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide NK552**

![Chemical Structure](image)

**NK552** was prepared according to **GP 5** from free amine **NK528** to afford (226 mg, 79%) of a white foam after purification over silica (EA/Hex 4:1).

**$^1$H NMR (400 MHz, MeOD)**:\[\delta = 7.57 (d, J = 2.5 Hz, 1H), 7.56 – 7.53 (m, 1H), 7.48 – 7.44 (m, 1H), 7.41 – 7.37 (m, 2H), 7.23 – 7.20 (m, 2H), 3.84 (s, 2H), 3.44 – 3.40 (m, 2H), 3.30 – 3.25 (m, 2H), 2.34 (s, 3H), 1.72 – 1.59 ppm (m, 4H).\]

**$^{13}$C NMR (100 MHz, MeOD):**\[\delta = 169.3, 164.9, 146.2, 144.5, 137.3, 137.2, 136.1, 134.1, 132.5, 132.4, 131.0, 129.8, 129.2, 128.6, 118.3, 40.6, 39.6, 28.9, 28.0, 27.5, 9.6 ppm.\]

**HRMS (ESI, 180 °C):** calculated for C$_{23}$H$_{22}$Cl$_3$BrN$_4$O$_2$Na $[M+Na]$ $m/z = 592.9884$, found: $m/z = 592.9883$.

**IR (Film):**\[\tilde{\nu} = 3426, 1671, 1421, 1197, 968 \text{ cm}^{-1}.\]

**Melting point:** 82 – 84 °C.
Experimental

(E)-4-(2-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)ethylamino)-4-oxobut-2-enoic acid NK544

\[
\begin{align*}
\text{NK544}
\end{align*}
\]

NK544 was prepared according to GP 6A (Step 1) from free amine NK526 to afford (211 mg, 81%) of a colourless oil after purification over silica (EtOAc/ULTRA 3:7).

\[^1\text{H} \text{NMR (500 MHz, MeOD)} \quad \delta = 7.57 - 7.54 (m, 2H), 7.47 - 7.43 (m, 1H), 7.38 - 7.35 (m, 2H), 7.21 - 7.17 (m, 2H), 6.27 (d, J = 12.5 Hz, 1H), 5.89 (d, J = 12.5 Hz, 1H), 3.56 - 3.52 (m, 2H), 3.50 - 3.46 (m, 2H), 2.30 ppm (s, 3H).\]

\[^{13}\text{C} \text{NMR (125 MHz, MeOD):} \quad \delta = 172.9, 168.7, 165.3, 146.2, 144.5, 137.4, 137.2, 136.1, 134.1, 134.2, 132.6, 132.4, 131.0, 129.8, 129.2, 128.8, 118.3, 40.1, 39.7, 9.5 \text{ppm.}\]

HRMS (ESI, 180 °C): calculated for C\textsubscript{23}H\textsubscript{19}Cl\textsubscript{3}N\textsubscript{4}O\textsubscript{4}Na [M+Na] \textit{m/z} = 543.0364, found: \textit{m/z} = 543.0363.

IR (Film): \quad \tilde{\nu} = 3426, 1671, 1421, 874 \text{ cm}^{-1} \text{.}
Experimental

(E)-4-(3-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)propylamino)-4-oxobut-2-enoic acid NK545

NK545 was prepared according to GP 6A (Step 1) from free amine NK527 to afford (222 mg, 83%) of a colourless oil after purification over silica (EtOAc/ULTRA 3:7).

\[^1\text{H} \text{NMR (500 MHz, MeOD)}\]
\[\delta = 7.55 (d, J = 2.5 \text{ Hz}, 1 \text{H}), 7.54 - 7.52 (m, 1 \text{H}), 7.46 - 7.43 (m, 1 \text{H}), 7.38 - 7.34 (m, 2 \text{H}), 7.21 - 7.17 (m, 2 \text{H}), 6.25 (d, J = 12.5 \text{ Hz}, 1 \text{H}), 5.92 (d, J = 12.5 \text{ Hz}, 1 \text{H}), 3.44 (t, J = 6.5 \text{ Hz}, 2 \text{H}), 3.35 (t, J = 6.5 \text{ Hz}, 2 \text{H}), 2.30 (s, 3 \text{H}), 1.88 - 1.82 \text{ ppm (m, 2H).}
\]

\[^{13}\text{C} \text{NMR (125 MHz, MeOD):}\]
\[\delta = 172.5, 168.3, 165.1, 146.2, 144.5, 137.4, 137.2, 136.9, 136.1, 134.1, 132.6, 132.4, 131.0, 129.8, 129.2, 128.7, 118.2, 38.0, 37.7, 30.2, 9.5 \text{ ppm.}
\]

HRMS (ESI, 180 °C): calculated for C\text{_{24}}H\text{_{22}}Cl\text{_{3}}N\text{_{4}}O\text{_{4}} [M+H] m/z = 535.0701, found: m/z = 535.0684.

IR (Film):
\[\tilde{\nu} = 3438, 1662, 1417, 886 \text{ cm}^{-1}.
\]

(E)-4-(4-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)butylamino)-4-oxobut-2-enoic acid NK546

NK546
**Experimental**

NK546 was prepared according to GP 6A (Step 1) from free amine NK528 to afford (225 mg, 82%) of a colourless oil after purification over silica (EtOAc/ULTRA 3:7).

$^{1}$H NMR (500 MHz, MeOD) \(\delta = 7.59 (d, J = 2.5 \text{ Hz}, 1\text{H}), 7.56 - 7.53 (m, 1\text{H}), 7.48 - 7.46 (m, 1\text{H}), 7.41 - 7.37 (m, 2\text{H}), 7.24 - 7.20 (m, 2\text{H}), 6.47 (d, J = 12.5 \text{ Hz}, 1\text{H}), 6.26 (d, J = 12.5 \text{ Hz}, 1\text{H}), 3.46 - 3.41 (m, 2\text{H}), 3.40 - 3.36 (m, 2\text{H}), 2.30 (s, 3\text{H}), 1.72 - 1.67 \text{ ppm (m, 4H).}

$^{13}$C NMR (125 MHz, MeOD): \(\delta = 168.0, 167.7, 165.1, 146.2, 144.6, 137.4, 137.3, 136.2, 134.1, 133.6, 132.5, 132.4, 131.0, 129.9, 129.2, 128.7, 118.3, 40.6, 39.5, 28.0, 27.2, 9.5 \text{ ppm.}

HRMS (ESI, 180 °C): calculated for C$_{25}$H$_{23}$Cl$_3$N$_4$O$_4$Na [M+Na] \(m/z = 571.0677\), found: \(m/z = 571.0664\).

IR (Film): \(\tilde{\nu} = 3416, 1691, 1452, 854 \text{ cm}^{-1}\).

5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-N-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)-4-methyl-1H-pyrazole-3-carboxamide NK547

![NK547](attachment:image.png)

NK547 was prepared according to GP 6A (Step 2) to afford (146 mg, 71%) of a white foam after purification over silica (EtOAc/hexanes 1:1).

$^{1}$H NMR (400 MHz, MeOD) \(\delta = 7.59 (d, J = 2.5 \text{ Hz}, 1\text{H}), 7.56 - 7.53 (m, 1\text{H}), 7.48 - 7.46 (m, 1\text{H}), 7.41 - 7.37 (m, 2\text{H}), 7.24 - 7.20 (m, 2\text{H}), 6.83 (s, 2\text{H}), 3.79 - 3.75 (m, 2\text{H}), 3.61 - 3.56 (m, 2\text{H}), 2.30 \text{ ppm (s, 3H).}

$^{13}$C NMR (100 MHz, MeOD): \(\delta = 172.6, 165.4, 146.0, 144.5, 137.4, 137.3, 136.2, 135.5, 134.2, 132.55, 132.52, 131.1, 129.9, 129.2, 128.7, 118.4, 38.7, 38.4, 9.6 \text{ ppm.}
Experimental

HRMS (ESI, 180 °C): calculated for C_{23}H_{17}Cl_{3}N_{4}O_{3}Na [M+Na] m/z = 525.0258, found: m/z = 525.0253.

IR (Film): \bar{\nu} = 3458, 1642, 1439, 851 cm^{-1}.

Melting point: 72 – 74 °C.

5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-N-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propyl)-4-methyl-1H-pyrazole-3-carboxamide NK548

\[
\begin{align*}
\text{Cl} & \text{N} & \text{O} & \text{O} \\
\text{Cl} & \text{N} & \text{C} & \text{C} \\
\text{Cl} & \text{N} & \text{C} & \text{C}
\end{align*}
\]

NK548 was prepared according to GP 6A (Step 2) to afford (150 mg, 70%) of a white foam after purification over silica (EtOAc/hexanes 1:1).

\(^1\text{H NMR (500 MHz, MeOD)}\) \(\delta = 7.59 (\text{d, } J = 2.5 \text{ Hz, 1H}), 7.56 – 7.53 (\text{m, 1H}), 7.48 – 7.46 (\text{m, 1H}), 7.41 – 7.37 (\text{m, 2H}), 7.24 – 7.20 (\text{m, 2H}), 6.84 (\text{s, 2H}), 3.66 (\text{t, } J = 6.5 \text{ Hz, 2H}), 3.44 (\text{q, } J = 6.5 \text{ Hz, 2H}), 2.37 (\text{s, 3H}), 1.98 – 1.91 \text{ ppm (m, 2H).}

\(^{13}\text{C NMR (125 MHz, MeOD)}\): \(\delta = 172.4, 164.8, 145.9, 144.4, 137.15, 137.12, 136.0, 135.2, 133.9, 132.25, 132.21, 131.0, 129.7, 129.0, 128.4, 118.3, 37.4, 36.2, 29.5, 9.6 \text{ ppm.}

HRMS (ESI, 180 °C): calculated for C_{24}H_{19}Cl_{3}N_{4}O_{3}Na [M+Na] m/z = 539.0415, found: m/z = 539.0410.

IR (Film): \bar{\nu} = 3454, 1637, 1472, 781 cm^{-1}.

Melting point: 72 – 74 °C.

5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-N-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butyl)-4-methyl-1H-pyrazole-3-carboxamide NK549
NK549 was prepared according to GP 6A (Step 2) to afford (140 mg, 65%) of a white foam after purification over silica (EtOAc/Hex 1:1).

$^1$H NMR (500 MHz, MeOD)  
$\delta = 7.57$ (d, $J = 2.5$ Hz, 1H), 7.56 – 7.54 (m, 1H), 7.48 – 7.44 (m, 1H), 7.41 – 7.37 (m, 2H), 7.24 – 7.20 (m, 2H), 6.81 (s, 2H), 3.56 (t, $J = 6.5$ Hz, 2H), 3.44 – 3.38 (m, 2H), 2.33 (s, 3H), 1.71 – 1.57 ppm (m, 4H).

$^{13}$C NMR (125 MHz, MeOD):  
$\delta = 172.5$, 164.8, 146.1, 144.5, 137.3, 137.2, 136.1, 135.3, 134.1, 132.5, 132.4, 131.0, 129.8, 129.2, 128.6, 118.3, 39.5, 38.2, 27.9, 27.0, 9.6 ppm.

HRMS (ESI, 180 °C):  
Calculated for C$_{25}$H$_{21}$Cl$_3$N$_4$O$_3$Na [M+Na] $m/z = 553.0571$, found: $m/z = 553.0571$.

IR (Film):  
$\tilde{\nu} = 3398, 1674, 1385, 759$ cm$^{-1}$.

5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-N-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethyl)-4-methyl-1H-pyrazole-3-carboxamide NK260

NK260 was prepared according to GP 6B from NK251 to afford (240 mg, 81%) of a brown oil after purification over silica (EtOAc/Hex 2:1).
Experimental

$^{1}$H NMR (300 MHz, CDCl₃): $\delta = 7.42 - 7.39$ (m, 1H), $7.32 - 7.26$ (m, 4H), $7.09 - 7.03$ (m, 2H), $6.67$ (s, 2H), $3.70 - 3.56$ (m, 12H), $2.36$ ppm (s, 3H).

$^{13}$C NMR (75 MHz, CDCl₃): $\delta = 170.6, 162.8, 145.0, 142.9, 136.0, 135.9, 134.8, 134.1, 132.9, 130.8, 130.6, 130.2, 128.8, 127.9, 127.3, 117.7, 70.3, 70.05, 70.03, 67.8, 38.7, 37.1, 9.4$ ppm.

HRMS (ESI, 200 °C): calculated for C$_{27}$H$_{25}$Cl$_{3}$N$_{2}$NaO$_{4}$: $m/z$ = 613.0783, found: $m/z$ = 613.0770.

IR (Film): $\tilde{\nu} = 3415, 1688, 1403, 764$ cm$^{-1}$.

5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-N-(6-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethylamino)-6-oxohexyl)-4-methyl-1H-pyrazole-3-carboxamide NK281

NK281 was prepared according to GP 6B from NK289 to afford (266 mg, 76%) of a brown oil after purification over silica (EtOAc/Hex 2:1).

$^{1}$H NMR (300 MHz, CDCl₃): $\delta = 7.43 - 7.41$ (m, 1H), $7.33 - 7.25$ (m, 4H), $7.09 - 7.03$ (m, 2H), $6.98$ (m, 1H), $6.70$ (s, 2H), $6.17$ (m, 1H), $3.78 - 3.70$ (m, 2H), $3.69 - 3.62$ (m, 2H), $3.62 - 3.57$ (m, 2H), $3.57 - 3.52$ (m, 2H), $3.52 - 3.46$ (m, 2H), $3.46 - 3.35$ (m, 4H), $2.37$ (s, 3H), $2.23$ (t, $J = 7.7$ Hz, 2H), $1.72 - 1.55$ (m, 4H), $1.48 - 1.35$ ppm (m, 2H).

$^{13}$C NMR (75 MHz, CDCl₃): $\delta = 175.0, 172.7, 164.7, 147.0, 145.0, 137.9, 136.9, 136.2, 134.9, 132.8, 132.5, 132.3, 130.8, 129.8, 129.2, 119.6, 72.1, 71.92, 71.89, 69.8, 41.1, 40.8, 39.2, 38.4, 31.5, 28.6, 27.3, 11.4$ ppm.

HRMS (EI, 200 °C): calculated for C$_{33}$H$_{36}$Cl$_{6}$N$_{5}$O$_{6}$ [M+Na]: $m/z$ = 726.1623, found: $m/z$ = 726.1649.
Experimental

IR (Film): \( \tilde{\nu} = 3379, 1695, 1417, 752 \text{ cm}^{-1} \).

**NK266**

![NK266](image)

To a solution of Cbz-protected caproic acid (1.2 g, 4.4 mmol, 1.1 equiv.) in methylene chloride (20 mL) was added EDCI (524 mg, 4.4 mmol, 1.1 equiv.) and HOBt (508 mg, 4.4 mmol, 1.10 equiv.) and DIPEA (1.0 mL, 6.0 mmol, 5.0 equiv.). After stirring for 25 minutes, NK247 (992 mg, 4.0 mmol, 1.0 equiv.) dissolved in methylene chloride (8 mL) was added and the solution was stirred for further 4h. The reaction mixture was then diluted with EtOAc (20 mL), washed with 1 N aq. HCl (10 mL), washed with brine, dried with Na\(_2\)SO\(_4\), filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/ULTRA 3:1) gave NK266 as a white solid (1.78 g, 91%).

\(^1\)H NMR (300 MHz, MeOD): \( \delta = 7.41 - 7.29 \text{ (m, 5H)}, 5.09 \text{ (br s, 2H)}, 3.63 \text{ (s, 4H)}, 3.58 - 3.52 \text{ (m, 4H)}, 3.38 \text{ (t, } J = 5.5 \text{ Hz, 2H)}, 3.25 \text{ (t, } J = 5.5 \text{ Hz, 2H)}, 3.14 \text{ (t, } J = 5.5 \text{ Hz, 2H)}, 2.23 \text{ (t, } J = 7.7 \text{ Hz, 2H}), 1.69 - 1.61 \text{ (m, 2H)}, 1.58 - 1.50 \text{ (m, 2H)}, 1.50 - 1.43 \text{ (m, 9H)}, 1.43 - 1.28 \text{ ppm (m, 2H)} \).

\(^{13}\)C NMR (125 MHz, MeOD): \( \delta = 176.2, 138.5, 129.4, 128.9, 128.7, 71.3, 71.1, 70.6, 67.3, 41.6, 41.2, 40.3, 36.9, 30.6, 28.8, 27.3, 26.6 \text{ ppm} \).

HRMS (EI, 200 °C): calculated for C\(_{25}\)H\(_{41}\)N\(_3\)O\(_7\)Na [M+Na]: \( m/z = 518.2837 \), found: \( m/z = 518.2837 \).

IR (Film): \( \tilde{\nu} = 2884, 1678, 1526, 931 \text{ cm}^{-1} \).

Melting point: 65 – 67 °C.
Experimental

tert-butyl 2-(2-(6-aminohexanamido)ethoxy)ethoxy)ethylcarbamate NK279

To a solution of NK266 (1.7 g, 3.96 mmol, 1.0 equiv.) in methylene chloride (20 mL) and MeOH (10 mL) was added palladium on carbon (150 mg) and the solution was hydrogenated using H₂ for 6h. The reaction mixture was then filtered, washed with brine, dried with Na₂SO₄, filtered and the solvent was removed in vacuo to give NK279 (1.4 g, quant.) as a yellow oil which was used without further purification.

¹H NMR (500 MHz, MeOD):  δ = 3.72 (s, 4H), 3.64 (t, J = 5.5 Hz, 2H), 3.62 (t, J = 5.5 Hz, 2H), 3.46 (t, J = 5.5 Hz, 2H), 3.33 (t, J = 5.5 Hz, 2H), 2.72 (t, J = 7.7 Hz, 2H), 2.31 (t, J = 7.7 Hz, 2H), 1.77 - 1.69 (m, 2H), 1.63 - 1.51 (m, 11H), 1.49 - 1.43 ppm (m, 2H).

¹³C NMR (125 MHz, MeOD): δ = 176.1, 158.3, 80.0, 71.2, 71.0, 70.6, 42.4, 41.2, 40.2, 36.9, 33.6, 28.8, 27.5, 26.8 ppm.

HRMS (EI, 200 °C): calculated for C₁₇H₃₅N₃O₅ [M+Na]: m/z = 384.2469, found: m/z = 384.2475.

IR (Film):  ν = 2765, 1829, 1457, 1006 cm⁻¹.
To a solution of lysine 90 (520 mg, 1.1 mmol, 1.1 equiv.) in methylene chloride (20 mL) was added EDCI (106 mg, 1.1 mmol, 1.1 equiv.) and HOBT (100 mg, 1.1 mmol, 1.1 equiv.) and DIPEA (0.3 mL, 2.5 mmol, 2.5 equiv.). After stirring for 25 minutes, NK528 (450 mg, 1.0 mmol, 1.0 equiv.) dissolved in methylene chloride (12 mL) was added and the solution was stirred for further 3h. The reaction mixture was then diluted with EtOAc (20 mL), washed with 1 N aq. HCl (10 mL), washed with brine, dried with Na$_2$SO$_4$, filtered and the solvent was removed in vacuo. Purification over silica (EtOAc/hexanes 1:3) gave NK302 as a white solid (700 mg, 78%).

$^1$H NMR (300 MHz, CDCl$_3$): $\delta = 7.75$ (d, $J = 7.5$ Hz, 2H), 7.58 (d, $J = 7.5$ Hz, 2H), 7.43 - 7.40 (m, 1H), 7.37 (d, $J = 7.5$ Hz, 2H), 7.33 - 7.20 (m, 6H), 7.09 (m$_c$, 1H), 7.07 - 7.01 (m, 2H), 6.64 (br s, 1H), 5.70 (m$_c$, 1H), 4.70 (br s, 1H), 4.45 - 4.32 (m, 2H), 4.20 (t, $J = 6.9$ Hz, 1H), 4.16 (m, 2H), 3.51 - 3.36 (m, 2H), 3.36 - 3.20 (m, 2H), 3.17 - 3.00 (m, 2H), 2.36 (s, 3H), 2.00 - 1.77 (m, 2H), 1.72 - 1.55 (m, 5H), 1.55 - 1.29 ppm (m, 13H).

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta = 171.7, 163.0, 156.3, 156.2, 144.9, 143.8, 143.1, 141.3, 136.0, 135.9, 135.0, 133.0, 130.8, 130.5, 130.3, 128.9, 127.9, 127.7, 127.1, 127.0, 125.1, 120.0, 117.7, 79.1, 67.0, 54.9, 47.2, 40.0, 39.2, 38.4, 32.4, 29.6, 27.5, 26.3, 22.6, 9.4 ppm.

HRMS (ESI, 200 °C): calculated for C$_{47}$H$_{51}$Cl$_{3}$N$_{6}$NaO$_{6}$: $m/z = 923.2828$, found: $m/z = 923.2805$.

IR (Film): $\tilde{\nu} = 3241, 2655, 1539, 985$ cm$^{-1}$.

Melting point: 99 - 101 °C.
Experimental

(R)-tert-butyl 5-amino-6-(4-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)butylamino)-6-oxohexylcarbamate NK303

To a solution of NK302 (700 mg, 0.78 mmol, 1.0 equiv.) in methylene chloride (20 mL) was added Et₂NH (5 mL) and the solution was stirred for 4 hours. Upon completion of the reaction the solvent was removed in vacuo, the residue was dissolved in EtOAc (30 mL), washed with 1 N aq. HCl, washed with brine, dried with MgSO₄, filtered and purified over silica (EtOAc/ULTRA 3:1) to give NK303 as a colourless oil (530 mg, 99%).

¹H NMR (300 MHz, CDCl₃): δ = 7.44 - 7.36 (m, 2H), 7.33 - 7.24 (m, 4H), 7.10 - 6.98 (m, 3H), 4.66 (br s, 1 H), 3.50 - 3.39 (m, 2H), 3.37 - 3.24 (m, 3H), 3.15 - 3.03 (m, 2H), 2.36 (s, 3 H), 1.92 - 1.32 ppm (m, 19H).

¹³C NMR (75 MHz, CDCl₃): δ = 174.8, 162.7, 156.0, 144.9, 143.0, 135.96, 135.92, 134.9, 132.9, 130.8, 130.5, 130.3, 128.8, 127.8, 127.2, 117.6, 79.0, 55.0, 53.4, 40.1, 38.7, 38.5, 34.6, 29.9, 28.4, 27.3, 27.0, 22.8, 9.3 ppm.

HRMS (El, 200 °C): calculated for C₃₂H₄₂Cl₃N₆O₄ [M+H]: m/z = 679.2328, found: m/z = 679.2343.

IR (Film): ν = 2952, 2914, 1785, 939 cm⁻¹.
(S)-tert-butyl 5-(2-bromoacetamido)-6-(4-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamido)butylamino)-6-oxohexylcarbamate NK304

NK304 was prepared according to GP 5 from free amine NK303 to afford (320 mg, 80%) of a white foam after purification over silica (EA/Hex 4:1).

$^1$H NMR (300 MHz, CDCl$_3$): $\delta = 7.46$-$7.41$ (m, 1 H), 7.35-$7.24$ (m, 4 H), 7.12 (m, 1 H), 7.09-$7.03$ (m, 2 H), 6.86 (t, $J = 5.5$ Hz, 1 H), 4.76 (t, $J = 6.0$ Hz, 1 H), 4.50-$4.37$ (m, 1 H), 3.86 (s, 2 H), 3.54-$3.38$ (m, 2 H), 3.38-$3.21$ (m, 2 H), 3.17-$2.99$ (m, 2 H), 2.36 (s, 3 H), 2.07-$1.79$ (m, 2 H).

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta = 171.7$, 163.0, 156.3, 156.2, 144.9, 143.8, 143.1, 141.3, 136.0, 135.9, 135.0, 133.0, 130.8, 130.5, 130.3, 128.9, 127.9, 127.7, 127.1, 127.0, 125.1, 120.0, 117.7, 79.1, 67.0, 54.9, 47.2, 40.0, 39.2, 38.4, 32.4, 29.6, 27.5, 26.3, 22.6, 9.4 ppm.

HRMS (ESI, 180 °C): calculated for C$_{34}$H$_{43}$BrCl$_3$N$_6$O$_5$ [M+H] $m/z = 799.1538$, found: $m/z = 799.1536$.

IR (Film): $\tilde{\nu} = 3214$, 3009, 2249, 1420 cm$^{-1}$. 
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Spectra of novel compounds