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UMI
ANALOGUES OF METHYLLYACONITINE (MLA)
AS ANTAGONISTS OF NICOTINIC RECEPTORS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By
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* * * * *

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2002

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ABSTRACT

Nicotinic acetylcholine receptors (nAChRs) are very important in the physiology of the human body. They are involved in transmitting nerve signals and in the control of neurotransmitter release. Among the physiological functions affected are memory, learning and pain transduction. Several different subtypes of receptors are known, and they appear to have distinct physiological functions. Even though a variety of ligands for nicotinic receptors is known, there is a lack of ligands binding selectively to specific subtypes. Such ligands would be very useful as pharmacological tools in determining the roles of specific subtypes as well as in manipulations of the activity of those subtypes.

One ligand has caught the eye of researchers due to its selectivity and high potency towards α7-receptors. This is methyllycaconitine (MLA), which is a norditerpenoid alkaloid isolated primarily from plants of Delphinium species. MLA is the most potent non-protein antagonist of the α7-subtype of nicotinic receptors, and is therefore ideal for studies on structure-activity relationships. The main drawback is the complexity of the molecule, which makes synthesis of
very similar analogues very difficult. Therefore, most work in this area has been
directed at simpler analogues.

Initially a series of ring E analogues of MLA had been prepared. The
biological results from that series showed promise for the prospects of
developing other analogues with even higher potency towards \( \alpha_3\beta_4^* \) receptors.
The synthesis of another series of analogues was in progress and the completion
of that series is described as well as the synthesis of four additional series of
analogues. A problem with one step in the initial synthesis was solved, and
finally, a ring DE analogue of MLA was designed and its synthesis carried out.

The results obtained from biological assays indicate high potency towards
\( \alpha_3\beta_4^* \) receptors, even exceeding that of MLA. Although MLA is selective
towards \( \alpha_7 \)-subtype of receptors, it is still quite potent towards \( \alpha_3\beta_4^* \). However,
all the analogues, that have been tested so far, have been found to be non-
competitive antagonists. This means that these analogues are not binding to the
agonist binding site on the receptor, but are affecting the receptor in some other
way.
Dedicated to my wife, who has supported me through thick and thin
ACKNOWLEDGMENTS

I would like to thank my research adviser, Dr. Stephen C. Bergmeier, for his help and guidance through the years.

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 CHAPTER 1
 INTRODUCTION

Nicotinic acetylcholine receptors (nAChRs) are very important in the physiology of the human body. They are involved in transmitting nerve signals and in the control of neurotransmitter release. Several different subtypes of receptors are known, and they appear to have distinct physiological functions. Some of those functions have been elucidated, but some have not been attributed to specific subtypes yet. The manipulations of nAChRs are considered to be viable as potential treatments for Alzheimer’s disease, Parkinson’s disease as well as for the control of pain. Even though a variety of ligands for nicotinic receptors is known, there is a lack of ligands binding selectively to specific subtypes. Such ligands would be very useful as pharmacological tools in determining the roles of specific subtypes as well as in manipulations of the activity of those subtypes.

One ligand has caught the eye of researchers due to its selectivity and high potency towards α7-receptors. This is methyllycaconitine (MLA, see Figure 1.1), which is a norditerpenoid alkaloid isolated from plants of Delphinium and Aconitum species. MLA is the most potent non-peptide antagonist of the α7-
subtype of nicotinic receptors, and is therefore ideal for studies on structure-activity relationships. The main drawback is the complexity of the molecule, which makes synthesis of very similar analogues very difficult. Therefore, work in this area has primarily been directed at simpler analogues. Several research groups have worked on syntheses of parts of the norditerpenoid skeleton of MLA, but few reports have been published on the binding affinity and potency of those analogues.

Our research group initially prepared a series of \( N \)-substituted ring \( E \) analogues of MLA containing the methylsuccinimidobenzoate side chain present in MLA (see Figure 1.2).\(^1\) The biological results from that series showed promise for developing other analogues with high potency towards \( \alpha 3\beta 4^* \) receptors.

![Figure 1.1: Structure of methyllycaconitine (MLA).](image)

\( \text{MLA} \)
Figure 1.2: General structure of \(N\)-substituted ring E analogues.

The synthesis of the four diastereomers of the most active compound from the first series was also completed.\(^2\) Work on a series of analogues with varying substitution on the succinimide ring (see Figure 1.3) was in progress when I joined the project. I completed that series, adding two analogues with disubstituted succinimides.

Figure 1.3: General structure of analogues from succinimide series.
I also prepared a series of compounds containing substitution, other than a succinimide, on the benzoate ring.

During the initial work, one step in the synthesis gave very low yields, even using an expensive reagent. After examining the process, I discovered the reason for this problem. An alternative route was constructed, giving higher yields using a much less expensive reagent.

Based on the results from previous series of analogues, I prepared three series containing different \( N \)-substituents (see Figure 1.4).

![General structure of three \( N \)-substitution series.](image)

The biological results completed at this time indicate that several of the analogues prepared, have a potency towards \( \alpha3\beta4^* \)-receptors, similar to, or greater than that of MLA. Although MLA is selective towards \( \alpha7 \)-subtype of receptors, it is still quite potent towards \( \alpha3\beta4^* \). However, while MLA is a
competitive antagonist, all our analogues are non-competitive antagonists. This means that probably some part of the MLA-skeleton, other than ring E and the methylsuccinimidobenzoate side chain, is conferring competitiveness on the molecule. In order to prepare selective analogues, we need to have competitive antagonists first. To that end, I have designed and synthesized an analogue containing ring E connected to what looks like ring D of MLA, although the connectivity is slightly different.

![MLA and Ring DE Analogue](image)

**Figure 1.5: Structures of MLA and ring DE analogue.**
CHAPTER 2
NEURONAL NICOTINIC ACETYLCOLINE RECEPTORS

2.1. Introduction

The pharmacology of nicotinic acetylcholine receptors has been extensively studied and a wide variety of ligands are known. Many activate or block the receptors indiscriminately, but only a few are selective for individual or a group of subtypes. Nicotinic receptors are involved in transmitting nerve signals as well as in neurotransmitter release and are thought to be viable targets in treatments of disorders such as Alzheimer's disease, Parkinson's disease, control of pain and others. If single subtypes are responsible for these disorders, selective ligands are needed to effect the disorder with minimal side-effects elsewhere in the body. Selective ligands are also needed as pharmacological tools to better characterize different subtypes and their roles.

2.1.1. Classification of acetylcholine receptors

Acetylcholine receptors (AChRs) are divided into two categories, muscarinic and nicotinic receptors. Muscarinic receptors are so named due to their activation by the agent muscarine. Nicotinic acetylcholine receptors
(nAChRs) on the other hand are activated by nicotine, and may be classified according to their location, either at the neuromuscular junction or in the nervous system (neuronal nAChRs). Neuronal nAChRs can then be classified further according to their composition (see also Section 2.1.4). Those which express only one type of \( \alpha \)-subunit are termed homomeric receptors, and those which combine one or more types of \( \alpha \)-subunits with one or more types of \( \beta \)-subunits are called heteromeric receptors.

![Structures of acetylcholine, muscarine and nicotine.](image)

**Figure 2.1:** Structures of acetylcholine, muscarine and nicotine.

### 2.1.2. Role of nicotinic ACh receptors

Nicotinic receptors are ligand-gated ion channels,\(^4-6\) which upon activation, propagate nerve signals through the nervous system. The conductance of the ion channels is independent of the agonists which activate them.\(^7\) Some nicotinic receptors modulate the release of neurotransmitters, such as acetylcholine, norepinephrine, serotonin and dopamine.\(^8\) They are also known to be involved in processes such as attention, memory, nociception,
reward and temperature control. Some information about the role of different subtypes has been elucidated. The α9-subtype appears to be involved in encoding auditory stimuli. The α3-subtype has been connected with norepinephrine and dopamine-release. A decrease in α3-receptors has been connected with age-related memory loss, and anabaseine as well as related compounds have been shown to have some memory-enhancing effects. Other nicotinic agonists have been found to increase cognitive function in patients suffering from Alzheimer's disease. These receptors are also involved in pain transduction from the periphery. Agonists such as nicotine and epibatidine have analgesic properties, which appear to be mediated through α3β4-receptors in the spinal cord and through α4β2-receptors in the brain.

Figure 2.2: Structures of anabaseine and epibatidine.
2.1.3. Composition of nAChRs

Nicotinic receptors are composed of receptor subunits. As can be seen in Figure 2.3, those subunits have a relatively hydrophilic extracellular amino terminal portion, followed by three hydrophobic transmembrane domains (M1-M3), a large intracellular loop followed by a fourth hydrophobic transmembrane domain (M4) and finally an extracellular carboxy terminal portion. Kinetic studies indicate that there are two ACh binding sites on each receptor. In the amino terminal extracellular domain of the α-subunits, two Cys-residues (Cys-192 and Cys-193) are located near the ACh-binding site and have been shown to be involved in the binding of a ligand. Those cysteine-residues form a disulfide bond that is not present in β-subunits. Five subunits come together to form the pentameric structure of the receptor. Heteromeric neuronal nAChRs usually consist of two α-subunits and three β-subunits, whereas homomeric subunits are made up of five α-subunits.

Eleven genes coding for nAChR subunits have been cloned. The eleven subunits have been classified into eight α (α2-α9) and three β (β2-β4) subunits. Receptors at the neuromuscular junction consist of α1- and β1-subunits as well as γ (or ε) and δ.
Figure 2.3: Diagram of (A) heteromeric and (B) homomeric nicotinic acetylcholine receptors.
2.1.4. Subtypes

The $\alpha_2$-$\alpha_5$ subunits generally join together with $\beta_2$-$\beta_4$ subunits to form heteromeric receptors, whereas the $\alpha_7$-$\alpha_9$ subunits usually form homomeric receptors. The $\alpha$-subunits participate in the formation of agonist binding sites, but $\beta_3$ are considered structural subunits, whereas $\beta_2$ and $\beta_4$ are thought to contribute towards pharmacological specificity. Subtypes containing $\alpha_3$-subunits also contain $\beta_4$ and vice versa. Most $\alpha_5$-subunits in fully assembled receptors are associated with both $\alpha_3$ and $\beta_4$. Receptor subtypes containing $\beta_2$-subunits have been shown to have considerably greater affinity for nicotine, than subtypes containing the $\beta_4$-subunits. The $\alpha_8$-subtypes have been shown to be more sensitive to agonists than $\alpha_7$, but less sensitive to antagonists.

2.1.4.1. Distribution of subtypes

In vertebrate brain $\alpha_4\beta_2$ is the major heteromeric subtype, in autonomic neurons the $\alpha_3$-subunit is most abundant, along with $\alpha_5$, $\beta_4$ and sometimes $\beta_2$. In the nervous systems (both central and peripheral), $\alpha_7$ is the major homomeric subtype.
2.1.4.2. Differentiation of subtypes

Although there are several different receptor subtypes known, there are few selective pharmacological tools available for research on these subtypes \textit{in vivo}.\textsuperscript{27}

The most common compounds used for the pharmacological differentiation of subtypes are the snake venom neurotoxins (bungarotoxins),\textsuperscript{28} marine snail conotoxins\textsuperscript{29-31} and the plant alkaloid, methyllycaconitine (MLA).\textsuperscript{32,33} MLA (\(\alpha_4\beta_2\)-binding IC\(_{50} = 0.9 \text{ \mu M}, \alpha_7\)-binding IC\(_{50} = 10 \text{ nM}\)),\textsuperscript{34} \(\alpha\)-bungarotoxin and \(\alpha\)-conotoxin-Iml are selective for homomeric receptors, while neuronal bungarotoxin (or \(\kappa\)-bungarotoxin) and \(\alpha\)-conotoxin-MII are selective for heteromeric receptors.

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<th>(\alpha_9)-function (IC(_{50}))\textsuperscript{a}</th>
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<td>(\kappa)-Bungarotoxin</td>
<td>~ 10 nM\textsuperscript{c}</td>
<td>ND\textsuperscript{b}</td>
<td>ND\textsuperscript{b}</td>
</tr>
<tr>
<td>(\alpha)-Conotoxin Iml</td>
<td>&gt; 5 \mu M</td>
<td>220 nM</td>
<td>1.80 \mu M</td>
</tr>
<tr>
<td>(\alpha)-Conotoxin MII</td>
<td>0.5 nM</td>
<td>&gt; 0.2 \mu M</td>
<td>&gt; 0.2 \mu M</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Determined by the ability to block ACh-induced current in \textit{Xenopus} oocytes expressing the appropriate mammalian nAChRs.

\textsuperscript{b} ND = Not determined.

\textsuperscript{c} Reported as "substantial blockade at 10 nM"\textsuperscript{28}

\textbf{Table 2.1: Functional data for nicotinic antagonists.}
2.2. Nicotinic agonists

Nicotinic agonists hold promise for potential therapeutic use, such as analgesics, and as treatments in Alzheimer's disease and Parkinson's disease.

Classical nicotinic agonists, like nicotine and acetylcholine, have some selectivity for α4β2-subtypes, but very little selectivity for homomeric or heteromeric receptor subtypes in general (see Table 2.2).

Epibatidine is a very potent agonist isolated from the skin of Ecuadorean poisonous frogs. It binds to α4β2 receptors in the brain with high affinity (Kᵢ = 55 pM), but much lower to α7 receptors (Kᵢ = 25 nM). It inhibits α4β2 receptors in rat brain with an IC₅₀ of 0.60 nM. It does not have much selectivity for specific receptor subtypes, but it is selective for heteromeric subtypes over homomeric ones (see Table 2.2). In spite of its lack of selectivity for specific subtypes, it has been used to identify the subtypes of human heteromeric receptors and is frequently used in binding studies due to its high potency. Epibatidine has been shown to have strong analgesic activity (EC₅₀ = 4 nmol/kg, i.p.), yet it is unlikely to be used clinically as such, due to its very narrow therapeutic index. The doses required for analgesia are close to those causing severe hypertension. A dose of 4.8 nmol/kg increased arterial pressure by 39 ± 11 mm Hg. A related compound, epiboxidine (see Figure
2.4) has slightly lower potency than epibatidine on \( \alpha 4\beta 2 \) receptors (\( K_i = 0.60 \) nM).\(^{38}\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \alpha 4\beta 2 ) EC(_{50})^{a}</th>
<th>( \alpha 3\beta 4 ) EC(_{50})^{b}</th>
<th>( \alpha 7 ) EC(_{50})^{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td>0.48 ( \mu )M</td>
<td>160 ( \mu )M</td>
<td>79 ( \mu )M</td>
</tr>
<tr>
<td>(-)-Nicotine</td>
<td>0.35 ( \mu )M</td>
<td>110 ( \mu )M</td>
<td>40 ( \mu )M</td>
</tr>
<tr>
<td>(-)-Epibatidine</td>
<td>4.2 nM</td>
<td>21 nM</td>
<td>1.1 ( \mu )M</td>
</tr>
</tbody>
</table>

\(^{a}\) Determined on chicken receptors expressed in Xenopus oocytes
\(^{b}\) Determined on human receptors expressed in Xenopus oocytes

Table 2.2: Potency of nicotinic agonists.\(^{39}\)

Selective agonists can be very useful for examining the effect of individual subtypes. There is a large variety of ligands available that bind with high affinity to \( \alpha 4\beta 2 \)-receptors. However, there are only a few which bind selectively to these receptors, \( i.e. \) have much lower affinity towards other subtypes.

Cytisine (Figure 2.4) is a full agonist of receptors containing \( \beta 4 \)-subunits, but a potent inhibitor of \( \beta 2 \)-containing subtypes. It has much higher affinity for \( \alpha 4\beta 2 \) (\( K_i = 0.2 \) nM in rat brain, 0.3 nM in human cells) than for \( \alpha 7 \) (\( K_i = 3.9 \) \( \mu \)M in human cells).\(^{18,37}\)

Dimethylphenylpiperazinium (DMPP, Figure 2.4) is a full agonist for \( \alpha 8 \), but a partial agonist for \( \alpha 7 \).\(^{18}\) In competition assays, its IC\(_{50}\) at \( \alpha 4\beta 2 \)
(competition with \[^{3}\text{H}]-\text{nicotine}\) was 0.11 \(\mu\text{M}\), and at \(\alpha 7\) (competition with \[^{125}\text{I}]-\alpha-\text{bungarotoxin}\) it was 7.6 \(\mu\text{M}\).\(^{42}\)

![Chemical structures]

**Figure 2.4: Structures of epiboxidine, cytisine and DMPP.**

The affinity of (-)-nicotine has been determined to be 1 nM for human \(\alpha 4\beta 2\), but much higher (1.61 \(\mu\text{M}\)) for human \(\alpha 7\).\(^{37}\) The potency is greater for \(\alpha 3\beta 4\) (\(\text{EC}_{50} = 0.89 \text{ \(\mu\text{M}\)}\)), than for \(\alpha 4\beta 2\) (\(\text{EC}_{50} = 14 \text{ \(\mu\text{M}\)}\)) or \(\alpha 7\) (\(\text{EC}_{50} = 47 \text{ \(\mu\text{M}\)}\)).\(^{43}\)

Isoarecolones and arecolones (Figure 2.5) have also been found to be agonists of nicotinic receptors, although agonistic activity towards muscarinic receptors limits their usefulness in examining nAChRs.\(^{44}\) Both arecolone and isoarecolone bind to \(\alpha 4\beta 2\)-receptors with high affinity (see Table 2.3).\(^{45}\)
Figure 2.5: Structures of arecolone and isoarecolone.

Table 2.3: Potency of arecolone and isoarecolone.

<table>
<thead>
<tr>
<th>Compound</th>
<th>α4β2-binding (Kj)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine</td>
<td>8.2 nM</td>
</tr>
<tr>
<td>Arecolone</td>
<td>33 nM</td>
</tr>
<tr>
<td>Isoarecolone</td>
<td>48 nM</td>
</tr>
</tbody>
</table>

\(^a\) Determined in a competitive binding study with \([3H]\)-cytisine in rat brain

Anatoxin-a (AnTX-a, Figure 2.6) is an alkaloid found in freshwater cyanobacteria. It is an agonist for nicotinic receptors. It has been found that (+)-AnTX-a has much higher affinity than (-)-AnTX-a (IC\(_{50}\) = 4.5 nM vs. 750 nM, respectively) in a competitive binding study with \([3H]\)-ACh in rat brain.\(^{46}\) The affinity of anatoxin-a is much stronger for α4β2-receptors (K\(_i\) = 0.6 nM in rat brain, 0.5 nM in human cells) than for α7-receptors (K\(_i\) = 20 nM in rat brain, 63 nM in human cells).\(^{37}\)
Anabaseine (see Figure 2.2) has been isolated from marine worms as well as from certain species of ants.\textsuperscript{43} It is contains a pyridine-ring, like nicotine does, but has a tetrahydropyridine rather than the methylpyrrolidine of nicotine. It is an agonist for both $\alpha 4\beta 2$- and $\alpha 7$-receptors ($EC_{50} = 4.2$ and 6.7 $\mu$M, respectively),\textsuperscript{43} although it binds $\alpha 7$-receptors with lower affinity (see Table 2.4).\textsuperscript{37} It has stronger potency on ganglionic nicotinic receptors with $EC_{50} = 0.66$ $\mu$M.

A derivative of anabaseine, 3-(4)-dimethylaminocinnamyldine (DMAC) anabaseine is a more potent agonist and has selectivity for the $\alpha 7$-receptors, while having very little activity on $\alpha 4\beta 2$.\textsuperscript{18}

GTS-21 (DMXB-anabaseine) is a synthetic derivative of anabaseine. GTS-21 binds with greater affinity to $\alpha 4\beta 2$-receptors than to $\alpha 7$-receptors (see Table 2.4).\textsuperscript{37} It is only a partial agonist, and at lower concentrations, can be used as an inhibitor of the actions of ACh.\textsuperscript{19,47}

\begin{center}
\includegraphics[width=\textwidth]{structures.png}
\end{center}

\textit{Figure 2.6: Structures of anatoxin-a and anabaseine derivatives.}

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<table>
<thead>
<tr>
<th>Compound</th>
<th>$\alpha_4\beta_2$-binding ($K_i$)$^a$</th>
<th>$\alpha_7$-binding ($K_i$)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-Nicotine</td>
<td>3.47 nM</td>
<td>820 nM</td>
</tr>
<tr>
<td>ACh</td>
<td>14.52 nM</td>
<td>4.00 μM</td>
</tr>
<tr>
<td>Cytisine</td>
<td>0.46 nM</td>
<td>1.40 μM</td>
</tr>
<tr>
<td>Anabaseine</td>
<td>74.9 nM</td>
<td>347.1 nM</td>
</tr>
<tr>
<td>DMXB-Anabaseine</td>
<td>84.46 nM</td>
<td>211.5 nM</td>
</tr>
<tr>
<td>DMAC-Anabaseine</td>
<td>347.2 nM</td>
<td>33.6 nM</td>
</tr>
</tbody>
</table>

$^a$ Determined in a competitive binding study with [$^3$H]-cytisine in rat brain  
$^b$ Determined in a competitive binding study with [$^{125}$I]-$\alpha$-Bgt in rat brain

Table 2.4: Binding data for anabaseine and related analogues.$^{14}$

Lobeline has much greater affinity for $\alpha_4\beta_2$ than $\alpha_7$-receptors. The affinity ($K_i$) for $\alpha_4\beta_2$ in human cells is 2 nM, compared with >10 μM for $\alpha_7$. The same situation applies to rat brain ($K_i$ = 1.4 nM and 9 μM, respectively).$^{37}$ In competitive studies with [$^3$H]-nicotine, the affinity ($K_i$) was found to be 4.4 nM.$^{48}$

ABT-418 is an isoxazole analogue of nicotine. It is a selective agonist for $\alpha_4\beta_2$-receptors over $\alpha_7$-receptors. Its $K_i$ for $\alpha_4\beta_2$ receptors is 7.9 nM in cells expressing human receptors (see Table 2.5).$^{49}$

ABT-089 is an isomer of nicotine and has lower affinity for $\alpha_4\beta_2$-receptors than does ABT-418 ($K_i$ of 16.7 nM).$^{37,38}$
<table>
<thead>
<tr>
<th>Compound</th>
<th>$\alpha 4\beta2$-binding ($K_i$)$^a$</th>
<th>$\alpha 4\beta2$-function ($EC_{50}$)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td>2.66 nM</td>
<td>43.79 µM</td>
</tr>
<tr>
<td>(+)-Nicotine</td>
<td>26.25 nM</td>
<td>152.6 µM</td>
</tr>
<tr>
<td>(-)-Nicotine</td>
<td>1.05 nM</td>
<td>3.99 µM</td>
</tr>
<tr>
<td>(±)-Epibatidine</td>
<td>70 pM</td>
<td>17 nM</td>
</tr>
<tr>
<td>DMPP</td>
<td>10.71 nM</td>
<td>2.51 µM</td>
</tr>
<tr>
<td>(-)-Cytisine</td>
<td>0.43 nM</td>
<td>38.15 µM</td>
</tr>
<tr>
<td>ABT-418</td>
<td>7.89 nM</td>
<td>10.55 µM</td>
</tr>
<tr>
<td>(±)-Anabasine</td>
<td>37.63 nM</td>
<td>ND$^c$</td>
</tr>
<tr>
<td>(+)-Anatoxin A</td>
<td>0.55 nM</td>
<td>ND$^c$</td>
</tr>
<tr>
<td>$\alpha$-Bungarotoxin</td>
<td>4.7 µM</td>
<td>ND$^c$</td>
</tr>
<tr>
<td>Chlorisondamine</td>
<td>&gt;10 µM</td>
<td>ND$^c$</td>
</tr>
<tr>
<td>DHβE</td>
<td>60.13 nM</td>
<td>ND$^c$</td>
</tr>
<tr>
<td>d-Tubocurarine</td>
<td>3.48 µM</td>
<td>ND$^c$</td>
</tr>
<tr>
<td>Decamethonium</td>
<td>962 nM</td>
<td>ND$^c$</td>
</tr>
<tr>
<td>(−)-Lobeline</td>
<td>1.92 nM</td>
<td>ND$^c$</td>
</tr>
<tr>
<td>Mecamylamine</td>
<td>&gt;10 µM</td>
<td>ND$^c$</td>
</tr>
<tr>
<td>Methyllycaconitine</td>
<td>3.2 µM</td>
<td>ND$^c$</td>
</tr>
</tbody>
</table>

$^a$ Determined in a competitive binding study with $[^3]$H-cytisine on human $\alpha 4\beta2$-receptors  
$^b$ Determined by the measurement of $^{86}$Rb$^+$ efflux resulting from binding to human $\alpha 4\beta2$-receptors  
$^c$ ND = Not determined

Table 2.5: Affinities and potency of nAChR ligands to human $\alpha 4\beta2$.49
Several 3-pyridyl ethers have been prepared and found to have significant agonistic activity.\textsuperscript{50} One of the more active is ABT-594 (see Figure 2.8), which is a selective $\alpha_{4\beta2}$-agonist and has been shown to have analgesic properties equal in efficacy to that of morphine, but without the physical dependence and withdrawal symptoms.\textsuperscript{24,51,52}

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\alpha_{4\beta2}$-binding ($K_i$)\textsuperscript{a}</th>
<th>$\alpha_{4\beta2}$-function ($EC_{50}$)\textsuperscript{b}</th>
<th>$\alpha_7$-binding ($K_i$)\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-Nicotine</td>
<td>1.0 nM</td>
<td>4.20 $\mu$M</td>
<td>4.0 $\mu$M</td>
</tr>
<tr>
<td>(±)-Epibatidine</td>
<td>43 pM</td>
<td>17 nM</td>
<td>230 nM</td>
</tr>
<tr>
<td>ABT-594</td>
<td>37 pM</td>
<td>140 nM</td>
<td>13.8 $\mu$M</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Determined in a competitive binding study with $[^3]$H-cytisine in rat brain
\textsuperscript{b} Determined by measurement of $^{86}$Rb$^+$ efflux through human $\alpha_{4\beta2}$-receptors expressed in K177 cells
\textsuperscript{c} Determined in a competitive binding study with $[^{125}]$-Bgt in rat brain

Table 2.6: Assay data for ABT-594 compared with known agonists.\textsuperscript{51,52}
A-85380 has similar receptor selectivity as ABT-594. This is not surprising as these two compounds are very similar structurally. A-85380 has a very high affinity for α4β2-receptors, with a $K_i$ measured ranging from 40 to 52 pM.\(^{37,38}\) The affinity for α7 is much lower ($K_i = 180$ nM in rat brain).\(^{37}\) It has been found to have some analgesic activity (ED\(_{50} = 0.11\) μmol/kg, i.p.), although that is 33-fold weaker than that of epibatidine.\(^{40}\)

![Chemical structures of ABT-594 and A-85380.](image)

**Figure 2.8: Structures of ABT-594 and A-85380.**

RJR-2403 is a metabolite of nicotine (see Figure 2.9), and is an agonist selective for α4β2-subtype of receptors, of similar potency and selectivity as ABT-418.\(^{53}\) It appears to have some cognition-enhancing effects.\(^{19}\)
<table>
<thead>
<tr>
<th>Compound</th>
<th>$\alpha_4\beta_2$-binding $(K_i)^a$</th>
<th>$\alpha_4\beta_2$-function $(EC_{50})^b$</th>
<th>$\alpha_3\beta_4$-function $(EC_{50})^b$</th>
<th>$\alpha_7$-binding $(K_i)^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine</td>
<td>4 nM</td>
<td>0.59 $\mu$M</td>
<td>20 $\mu$M</td>
<td>0.6 $\mu$M</td>
</tr>
<tr>
<td>RJR-2403</td>
<td>26 nM</td>
<td>0.73 $\mu$M</td>
<td>$&gt;$1 mM</td>
<td>36 $\mu$M</td>
</tr>
</tbody>
</table>

$^a$ Determined in a competitive binding study with $[^3]$H-nicotine in rat brain  
$^b$ Determined by measurement of $^{86}$Rb$^+$ efflux from rat brain thalamic synaptosomes ($\alpha_4\beta_2$) or from PC-12 cells  
$^c$ Determined in a competitive binding study with $[^{125}]$I-\alpha-Bgt

Table 2.7: Comparison of data for nicotine and RJR-2403.\textsuperscript{53}

![Structure of RJR-2403, SIB-1765F, and Altinicline](image)

Figure 2.9: Structures of RJR-2403, SIB-1765F and altinicline.

SIB-1765F (Figure 2.9) is an agonist for nicotinic receptors. It is selective for $\alpha_4\beta_2$ in rat brain ($K_i = 2.8$ nM) over $\alpha_7$ ($K_i > 100$ $\mu$M). The potency of SIB-1765F against $\alpha_4\beta_2$ is 10-fold that against $\alpha_4\beta_4$ (2.62 $\mu$M vs. 25.0 $\mu$M, respectively).\textsuperscript{54}
<table>
<thead>
<tr>
<th>Compound</th>
<th>$\alpha_4\beta_2$-binding $(K_i)^a$</th>
<th>$\alpha_4\beta_2$-function $(EC_{50})^b$</th>
<th>$\alpha_4\beta_4$-binding $(K_i)^a$</th>
<th>$\alpha_4\beta_4$-function $(EC_{50})^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-Nicotine</td>
<td>4.4 nM</td>
<td>2.1 $\mu$M</td>
<td>14.3 nM</td>
<td>7.1 $\mu$M</td>
</tr>
<tr>
<td>SIB-1765F</td>
<td>7.5 nM</td>
<td>2.62 $\mu$M</td>
<td>235 nM</td>
<td>25.0 $\mu$M</td>
</tr>
</tbody>
</table>

$^a$ Determined in a competitive binding study with $[^3]$H-epibatidine using HEK293 cells expressing human nAChRs

$^b$ Determined by measurement of Ca$^{2+}$ flux in HEK293 cells expressing human nAChRs

Table 2.8: Comparison of nicotine and SIB-1765F.$^{54}$

Altinicline (SIB-1508Y, see Figure 2.9) is the (S)-enantiomer of SIB-1765F. It is an agonist selective for heteromeric receptors. It has a higher affinity for the $\alpha_4\beta_2$-subtype over the $\alpha_4\beta_4$. It is under investigation as a potential treatment of Parkinson's disease.$^{19}$
Table 2.9: Data for enantiomers of SIB-1765F.

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \alpha 4\beta 2 )-binding (IC(_{50})) (^a)</th>
<th>( \alpha 4\beta 2 )-function (EC(_{50})) (^b)</th>
<th>( \alpha 4\beta 4 )-function (EC(_{50})) (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine</td>
<td>4 nM</td>
<td>2 ( \mu )M</td>
<td>0.8 ( \mu )M</td>
</tr>
<tr>
<td>SIB-1765F</td>
<td>4.6 nM</td>
<td>0.2 ( \mu )M</td>
<td>3.16 ( \mu )M</td>
</tr>
<tr>
<td>Altinicline</td>
<td>3 nM</td>
<td>0.32 ( \mu )M</td>
<td>2 ( \mu )M</td>
</tr>
<tr>
<td>((R)-SIB-1765F)</td>
<td>75 nM</td>
<td>12.5 ( \mu )M</td>
<td>NA (^c)</td>
</tr>
</tbody>
</table>

\(^a\) Determined in a competitive binding study with \([^3]H\)-nicotine in rat brain
\(^b\) Determined by measurement of \(Ca^{2+}\) flux in HEK293 cells expressing human nAChRs
\(^c\) NA = No activity

A series of 3-pyridylamines were also prepared and their affinities for \(\alpha 4\beta 2\)-receptors determined. The amine was substituted with a methylazetidine, -pyrrolidine or -piperidine. Only one of the analogues prepared had a similar binding affinity as nicotine (see Table 2.10 for those analogues with \(K_i < 100 \text{ nM}\)). Several compounds were also tested for analgesic activity, showing all to be less active than nicotine.\(^{55}\)
Figure 2.10: Structure of 3-pyridylamines.

![Structure of 3-pyridylamines](image)

Table 2.10: Binding of 3-pyridylamine analogues to \( \alpha 4\beta 2\)-receptors.

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>X</th>
<th>R(^1)</th>
<th>R(^2)</th>
<th>( \alpha 4\beta 2)-binding ((K_i))(^b)</th>
<th>Analgesia (ED(_{50}))(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.91 nM</td>
<td>1.96 mg/kg</td>
</tr>
<tr>
<td>1a</td>
<td>1</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>S</td>
<td>97.2 nM</td>
</tr>
<tr>
<td>1b</td>
<td>1</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>R</td>
<td>78.1 nM</td>
</tr>
<tr>
<td>1c(^d)</td>
<td>1</td>
<td>Cl</td>
<td>H</td>
<td>H</td>
<td>S</td>
<td>88.3 nM</td>
</tr>
<tr>
<td>1d</td>
<td>2</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>R</td>
<td>89.0 nM</td>
</tr>
<tr>
<td>1e</td>
<td>2</td>
<td>Cl</td>
<td>H</td>
<td>H</td>
<td>R</td>
<td>54.3 nM</td>
</tr>
<tr>
<td>1f</td>
<td>2</td>
<td>H</td>
<td>Me</td>
<td>Me</td>
<td>R</td>
<td>8.9 nM</td>
</tr>
</tbody>
</table>

\(^a\) Absolute stereochemistry  
\(^b\) Determined in a competitive binding study with \(^3\)H-cytisine in rat brain  
\(^c\) Determined in a tail-flick test  
\(^d\) Amino-isostere of ABT-594

A conformationally restricted analogue of acetylcholine (AR-R17779)\(^56\) has been prepared and found to be very selective for \( \alpha 7\)-receptors over \( \alpha 4\beta 2\)-

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receptors, with the potency towards $\alpha 7$ higher than that of nicotine. Data from binding studies indicate that most of this is down to the (-)-enantiomer.

![AR-R17779](image)

**Figure 2.11:** Structure of AR-R17779.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\alpha$4$\beta$2-binding ($K_i^a$)</th>
<th>$\alpha$7-binding ($K_i^b$)</th>
<th>$\alpha$7-function ($EC_{50}^c$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine</td>
<td>2.3 nM</td>
<td>480 nM</td>
<td>43 mM</td>
</tr>
<tr>
<td>AR-R17779</td>
<td>16 $\mu$M</td>
<td>190 nM</td>
<td>ND$^d$</td>
</tr>
<tr>
<td>(+)-AR-R17779</td>
<td>30 $\mu$M</td>
<td>9.4 $\mu$M</td>
<td>ND$^d$</td>
</tr>
<tr>
<td>(-)-AR-R17779</td>
<td>16 $\mu$M</td>
<td>92 nM</td>
<td>21 mM</td>
</tr>
</tbody>
</table>

$^a$ Determined in a competitive binding study with $[^3H]$-nicotine in rat brain  
$^b$ Determined in a competitive binding study with $[^{125}\text{I}]$-$\alpha$-Bgt in rat brain  
$^c$ Determined by measurement of current activation in *Xenopus* oocytes expressing rat $\alpha 7$-receptors  
$^d$ ND = Not determined

**Table 2.11:** Biological data for AR-R17779.

A 5-HT$_3$-receptor antagonist, tropisetron, has also been found to be a selective, partial agonist of $\alpha 7$ nAChRs. Quaternized tropisetron and even a
quinuclidine analogue are more potent than the parent compound (see Figure 2.12 and Table 2.12).57

![Figure 2.12: Structures of tropisetron and its quinuclidine analogue.](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>(\alpha_3)-binding ((K_i))^a</th>
<th>(\alpha_4\beta_2)-binding ((K_i))^b</th>
<th>(\alpha_7)-binding ((K_i))^c</th>
<th>(\alpha_7)-function ((EC_{50}))^d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine</td>
<td>220 nM</td>
<td>2.3 nM</td>
<td>480 nM</td>
<td>38 (\mu)M</td>
</tr>
<tr>
<td>Tropisetron</td>
<td>16 (\mu)M</td>
<td>55 (\mu)M</td>
<td>6.9 nM</td>
<td>1.30 (\mu)M</td>
</tr>
<tr>
<td>4° tropisetron</td>
<td>2.30 (\mu)M</td>
<td>7.80 (\mu)M</td>
<td>6.4 nM</td>
<td>280 nM</td>
</tr>
<tr>
<td>Quinuclidine analogue</td>
<td>ND^e</td>
<td>2.60 (\mu)M</td>
<td>2.3 nM</td>
<td>580 nM</td>
</tr>
</tbody>
</table>

^a Determined in a competitive binding study with [\(^3\)H]-epibatidine in PC12 cell membranes
^b Determined in a competitive binding study with [\(^3\)H]-nicotine in rat brain
^c Determined in a competitive binding study with [\(^{125}\)I]-\(\alpha\)-Bgt in rat brain
^d Determined by measurement of current activation in \textit{Xenopus} oocytes expressing rat \(\alpha_7\) receptors
^e ND = Not determined

**Table 2.12: Binding and functional data for tropisetron and derivatives.**

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2.3. Nicotinic antagonists

Nicotinic antagonists can be divided according to their usefulness in examining the roles of different subtypes of nAChRs. Nonselective antagonists are not very useful for pharmacological investigations of that type, but selective antagonists can be very useful and can be classified further according to their selectivity.

Competitive antagonists connect to the ACh-binding site, and thereby prevent agonists from binding, but do not elicit any action from the receptors. Noncompetitive antagonists can either block the ion channel itself or bind elsewhere on the receptor and stabilize the receptor in a state that does not bind agonists as well.19

Classical antagonists used in early pharmacological characterization of receptors include hexamethonium, decamethonium, mecamylamine, d-tubocurarine, dihydro-β-erythroidine (DHβE), MLA (see Figure 2.13) and snake toxins such as α-bungarotoxin.36
2.3.1. Nonselective antagonists

There are three primary antagonists that are nonselective. \(d\)-Tubocurarine (curare) is an alkaloid that has been used as arrow poison for hunting. It has a positively charged nitrogen and is therefore not suitable for studying nAChRs, since it can not cross the blood-brain barrier. It binds to all nicotinic subtypes with relatively low affinity.\(^{18,30}\) Compounds related to \(d\)-tubocurarine are still used as muscle relaxants for surgery, and as antihypertensives.\(^{58}\)

Hexamethonium has low affinity for \(\alpha 4\beta 2\)-receptors (IC\(_{50} = 150\ \mu M\), in a competitive binding study with \([^3H]\)-nicotine in rat brain).\(^{59}\)
Dihydro-β-erythroidine (DHβE) is an alkaloid with some selectivity for heteromeric receptors. It has much higher affinity for α4β2 than α7 (see Table 2.13). It can be used to distinguish between subtypes due to its slight selectivity based on subunit composition. It has higher affinity for α4β4 than for α3β4 (IC$_{50}$ = 0.19 μM vs. 23 μM in oocytes expressing rat nAChRs). Likewise, it binds with higher affinity to α3β2 than α3β4 (IC$_{50}$ = 0.41 μM for α3β2). It is a competitive antagonist of both homomeric and heteromeric receptors and binds strongest to α7 of homomeric receptors and to α4 of heteromeric receptors, rather than α3 containing subtypes.$^{18}$

A related compound, erysodine (Figure 2.14), does not bind well to α7-receptors, but binds α4β2-receptors with higher affinity than DHβE.$^{37,61}$

![Chemical Structures](image)

**Figure 2.14:** Structures of DHβE and erysodine.
<table>
<thead>
<tr>
<th>Compound</th>
<th>$\alpha4\beta2$-binding $(K_i)^a$</th>
<th>$\alpha7$-binding $(K_i)^b$</th>
<th>$\alpha3\beta2$-function $(IC_{50})^c$</th>
<th>$\alpha3\beta4$-function $(IC_{50})^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHβE</td>
<td>35 nM</td>
<td>9.0 μM</td>
<td>58 nM</td>
<td>84 μM</td>
</tr>
<tr>
<td>Erysodine</td>
<td>5 nM</td>
<td>4.0 μM</td>
<td>58 nM</td>
<td>7 μM</td>
</tr>
</tbody>
</table>

- $^a$ Determined in a competitive binding study with $[^3H]$-cytisine in rat brain
- $^b$ Determined in a competitive binding study with $[^{125}I]$-$\alpha$-Bgt in rat brain
- $^c$ Determined by measuring inhibition of nicotine-induced release of $[^3H]$-dopamine from rat striatal slices
- $^d$ Determined by measuring the inhibition of nicotine-stimulated $^{86}$Rb$^+$ efflux from IMR-32 cells

Table 2.13: Binding and function of DHβE and erysodine.

Mecamylamine (see Figure 2.13) is a noncompetitive antagonist, which is thought to act by physically blocking the ion channel. It does have selectivity for neuronal nAChRs over muscle-type receptors, but very limited selectivity between neuronal receptors.$^{19,27}$

Another noncompetitive antagonist, which is believed to block the ion channel is chlorisondamine (see Figure 2.15). It has very low affinity for $\alpha4\beta2$-receptors ($K_i > 100 \mu M$ in a competitive study with $[^3H]$-cytisine in rat brain), but is still a potent inhibitor of those receptors ($IC_{50} = 200 \text{nM}$ determined by measurement of effect on $^{86}$Rb$^+$ efflux).$^{62}$
Catestatin is a 21-amino acid residue oligopeptide, made by the proteolysis of chromogranin A, which is a protein contained in vesicles and released with catecholamines (such as norepinephrine). Catestatin inhibits the nicotine-induced release of $[^3]$H-norepinephrine from bovine chromaffin cells ($IC_{50} = 200 \text{ nM}$). Therefore, the physiological role of catestatin may be negative feedback to control catecholamine release.

### 2.3.2. Selective antagonists

Some antagonists are selective for specific subtypes of nicotinic receptors. However, many of those antagonists exhibiting some selectivity, are not selective for specific subtypes, but rather for either homomeric or heteromeric receptors. These can be very useful, but in order to elucidate the exact function of different subtypes, selective antagonists for specific subtypes are very helpful and more variety in those is needed.
2.3.2.1. Selectivity for homomeric receptors

α-Bungarotoxin (α-Bgt) is a major component of venom from a Southeast Asian snake. It is a peptide toxin, consisting of 74 amino acids, and blocks the activation of homomeric α7, α8 and α9-receptors (IC$_{50}$ = 0.30 nM, 1.4 nM and 100 nM, respectively, for inhibition of ACh-induced current), but does not block heteromeric receptors.18,65

Methyllycaconitine (MLA) is an alkaloid isolated from plants, primarily from Delphinium-species. It is the strongest non-peptide inhibitor of α7-subtypes known (K$_{i}$ = 4 nM in rat brain), but is weaker towards α3β2 and α4β2 (K$_{i}$ = 2.5 μM in rat brain).18,37 MLA has no effect on nicotinic receptors at the neuromuscular junction, and is the only available antagonist that distinguishes between α-Bgt sensitive sites on neuronal and muscle-type nicotinic receptors.27

Strychnine (see Figure 2.16) is an alkaloid which has been used as a specific blocker of glycine receptors, but has also been found to inhibit α7 (IC$_{50}$ = 0.52 μM), α8 (IC$_{50}$ = 0.8 μM) and α9 (IC$_{50}$ = 20 nM) in studies using inhibition of ACh-induced current as a measure of the potency.6,18

F3 and MG 624 (see Figure 2.16) are synthetic stilbene-derivatives containing a quaternary nitrogen. They are potent inhibitors of α7-receptors, weak for β4-containing receptors and very weak antagonists of α4β2-receptors. The two enantiomers of F3 do not show a difference in activity.3
Figure 2.16: Structures of strychnine, F3 and MG 624.

<table>
<thead>
<tr>
<th>Compound</th>
<th>β2-binding (K)_β</th>
<th>α7-binding (IC₅₀)_β</th>
<th>α7-function (IC₅₀)_β</th>
</tr>
</thead>
<tbody>
<tr>
<td>F3</td>
<td>39 µM</td>
<td>50 nM</td>
<td>119 nM</td>
</tr>
<tr>
<td>MG 624</td>
<td>69.5 µM</td>
<td>27 nM</td>
<td>94 nM</td>
</tr>
</tbody>
</table>

a Determined in a competitive binding study with [³H]-epibatidine
b Determined in a competitive binding study with [¹²⁵I]-α-Bgt
c Determined by measurement of inhibition of ACh-stimulated current in oocytes expressing chick α7-receptors

Table 2.14: Assay data for F3 and MG 624.

α-Conotoxin Imi is a peptide toxin (12 amino acids) from a marine snail. It inhibits α7 (IC₅₀ = 220 nM in oocytes expressing rat α7-receptors⁶⁰) and α9 subtypes reversibly, but has no effect on heteromeric receptors (1 µM does not block nicotine-stimulated dopamine release in rat striatal synaptosomes⁶⁰).¹⁸,³¹

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2.3.2.2. Selectivity for heteromeric receptors

Several compounds are selective for heteromeric receptors. Neuronal bungarotoxin (NBT, κ-bungarotoxin) is a minor component of snake venom. It is a peptide toxin consisting of 66 amino acid residues. It has strong inhibitory activity against α3β2 (IC$_{50}$ = 3 nM at receptors expressed in *Xenopus* oocytes$^{60}$), but much weaker against α4β2, and does not block α2β2. It only blocks α3β4 with rapid onset and recovery, indicating that the β-subunits determine the kinetics of binding.$^{18,28,66}$ In a competitive binding study in bovine chromaffin cells, it inhibited the binding of [$^3$H]-nicotine (IC$_{50}$ = 300 nM).$^{67}$ It also inhibits ACh-induced currents in α9-receptors (IC$_{50}$ = 100 nM).$^{10}$ Limited availability of the snake venom makes the use of this toxin in research problematic, although recombinant κ-bungarotoxin has been prepared using *Escherichia coli* or a yeast expression system.$^{30,60}$

α-Conotoxin MIL is a peptide toxin (16 amino acids) from a marine snail and is a potent inhibitor of the α3β2 subtype (IC$_{50}$ = 0.5 nM in receptors expressed in *Xenopus* oocytes$^{60}$), but 2-4 orders of magnitude weaker against other subunits.$^{30}$

Lophotoxin is a diterpene found in sea whips, that inhibits α2β2, α3β2 and α4β2 irreversibly by forming a covalent bond to a tyrosine residue in the receptor. It also has this effect in muscle-type nicotinic receptors.$^{30,68}$
Lophotoxin, analog-1 (LTX-1) is a close relative of Lophotoxin, and can also be isolated from Pacific sea whips.\textsuperscript{69} It is a strong inhibitor of $\alpha$3$\beta$2 and $\alpha$4$\beta$2, but weaker against $\alpha$2$\beta$2.\textsuperscript{28}

![Lophotoxin and LTX-1](image)

**Figure 2.17: Structures of lophotoxin and its analogue, LTX-1.**

Neosurugatoxin (NSTX) is a glycoside from a Japanese ivory shell and inhibits $\alpha$2$\beta$2, $\alpha$3$\beta$2 and $\alpha$4$\beta$2 with similar potency.\textsuperscript{28,30} In a competitive study with $[^3H]$-nicotine in rat brain, it was found to have high affinity ($IC_{50} = 83$ nM) for $\alpha$4$\beta$2-receptors,\textsuperscript{59} but it does not inhibit the binding of $[^{125}I]$$\alpha$-Bgt.\textsuperscript{60}

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Trimethaphan is a competitive ganglionic antagonist. It contains a sulfonium functionality with a permanent positive charge (see Figure 2.19), which indicates that trimethaphan will have very limited penetration into the central nervous system. In a study on chicken lateral spiriform nucleus (located in the optic lobe), the antagonistic activity of trimethaphan was found to cover a wide range (see Table 2.15). The much narrower range of activity for dihydro-β-erythroidine confirmed that this was not a flaw in the experiment. Rather, this was thought to be caused by the selective action of trimethaphan on specific subtypes, although it was not determined what those subtypes were. In addition to its ganglionic blocking actions, trimethaphan also has direct vasodilating actions and has been used clinically to control blood pressure.
<table>
<thead>
<tr>
<th>Compound</th>
<th>(\alpha 4\beta 2)-binding ((K_i)^a)</th>
<th>Inhibition ((K_i)^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethaphan</td>
<td>390 µM</td>
<td>4-66 µM</td>
</tr>
<tr>
<td>DH(\beta)E</td>
<td>3.0 µM</td>
<td>0.09-0.16 µM</td>
</tr>
</tbody>
</table>

\(^a\) Determined in a competitive binding study with \([^3\text{H}]-\text{nicotine}\) in chick optic lobe homogenates

\(^b\) Determined as the inhibition of receptor activation by carbachol (carbamylcholine chloride, see Figure 2.19)

**Table 2.15: Comparison of trimethaphan and DH\(\beta\)E.**

![Chemical Structures](image)

**Figure 2.19: Structures of trimethaphan and carbachol.**

GTS-21 (DMXB-Anabaseine) is a partial agonist (see Section 2.2), and can be used to inhibit \(\beta_2\)-containing receptors.

### 2.4. Measurements of activity of antagonists

In order to be able to compare different ligands, we need ways to quantify the interaction of a ligand with a receptor. There are two basic ways of doing...
this, either by measuring the binding of the ligand to the receptor, or by measuring the product of the receptor's action upon binding to a ligand, i.e. the function of the receptor.

2.4.1. Binding studies

Binding studies can be performed to determine the affinity of a ligand for a receptor. This does not indicate what the effect of that binding is, so these are often used in conjunction with a functional study.

Usually, a radioactive ligand is used to determine the binding to the receptor. The assay is set up to see how well the ligand to be measured competes with ligands of known binding strength. Immunoprecipitation is carried out to isolate the receptors, and the radioactivity is used to determine the amount of radioligand still bound to the receptors.

For α4β2-receptors, a tritiated ligand is used. The most commonly used are nicotine,42,54 and cytisine,52,54,72 but N-methylcarbamylcholine can also be used.73

![Structure of N-methylcarbamylcholine](image)

**Figure 2.20:** Structure of N-methylcarbamylcholine.
Another tritiated ligand, $[^3H]$-epibatidine, is the primary ligand used on α3-containing receptors in ganglionic synapses.

The best-known ligand selective for α7-receptors is α-bungarotoxin (α-Bgt), and it is used for studies on that subtype as $[^{125}\text{I}]$-α-Bgt.\(^{19,52,54}\)

2.4.2. Functional studies

Behavioral studies can be performed, and those can give important information about the effects of certain receptors. The difficulty with these studies lies in the fact that they require a significant amount of compound to be tested and they also cause a problem with interpreting results for SAR. Another method is to measure the effect of binding, for example the release of neurotransmitters or the flux of ions through an ion-channel in response to a ligand. The receptors used in functional studies are either recombinant receptor systems or endogenous receptors. Results from these do not always correlate due to the variety of subunit combinations in endogenous receptors. There is also some species variability, such that results from endogenous receptors in rats can not be assumed to be the same as they would be in human endogenous receptors.\(^{19}\)

As there is much variety in the actions of nicotinic receptors, there is more variety to the functional studies than the binding studies.
2.4.2.1. Knock out mice

One method to identify the role of a particular subtype of receptors is the use of knock out mice. These are mice that have been developed lacking specific nAChR subunits. This can reveal important information about the role of these subtypes. One problem with this approach, is that the mice develop without the specific subtype, where the mice may adapt to living without that subtype.24

2.4.2.2. Release of [³H]-neurotransmitters

If the cells can take up the tritiated neurotransmitter, the rate of its release can be measured in response to a receptor stimulus. Most commonly used are adrenal catecholamines (such as norepinephrine), dopamine, acetylcholine, and γ-aminobutyric acid (GABA).8,34

2.4.2.3. Studies on ion-transport

Ligand-gated ion channels can also be examined for their effect on ion movements. The response can be measured electrophysiologically as the flux of radioactive ions (such as ⁸⁶Rb⁺ or ²₂Na⁺)⁴,³¹,⁵⁰,⁵² or as calcium influx, which can be detected using calcium-sensitive dyes.⁴,¹⁹,⁵⁴
2.4.2.4. Fluorescence Resonance Energy Transfer (FRET)

One method is available to determine the distance between a ligand and the lipid membrane surrounding the receptor. This is called fluorescence resonance energy transfer (or Förster-type resonance energy transfer, FRET), and is based on the principle that the rate of excitation energy transfer between a fluorescence donor and a suitable acceptor is related to the distance separating the two molecules. The exact location of the binding site cannot be determined directly by this method, but rather, by determining the distance between a molecule bound to the receptor and the lipid membrane surface (surrounding the receptor), some conclusions can be reached regarding its location. A fluorescence acceptor, containing a fatty acid side-chain is embedded in the lipid membrane. Excitation of the ligand, bound to the receptor, results in emission of fluorescence which is quenched by the acceptor. An example of this, is a report where the location of a binding site for ethidium on the nicotinic receptor is determined, using C₁₂-Texas Red as the fluorescence acceptor.⁷⁴
2.5. Conclusions

Several ligands have been discovered, that bind selectively to specific subtypes of nicotinic receptors. Selective agonists for α4β2-receptors in the brain include A-84543, RJR-2403 and ABT-418, while for α7-receptors, the primary selective agonist is DMAC-anabaseine and as antagonists α-bungarotoxin and MLA have been used. MLA has the advantage over α-Bgt, that it reaches the brain in significant concentrations after peripheral administration. Ganglionic α3-containing receptors can be blocked with hexamethonium and κ-Bgt (NBT), but better antagonists are needed in this area.

Figure 2.21: Structures of ethidium and C_{12}-Texas Red.\textsuperscript{74}
Drug discovery directed at nicotinic receptors is focusing primarily on three areas, cognition-enhancers (for Alzheimer's disease), dopamine-release stimulators (for Parkinson's disease) and analgesic agents.19
CHAPTER 3
ANALOGUES OF METHYLLYCACONITINE (MLA)

3.1. Introduction

Methyllycaconitine (MLA, also known as delartine, delsemidine, and mellictine\textsuperscript{76}) is a hexacyclic norditerpenoid alkaloid which has been isolated primarily from Delphinium species.\textsuperscript{77,78} The structure can be seen in Figure 3.1, where R represents 2-methylsuccinimidobenzoate ester. The absolute stereochemistry of the methyl-group has been determined to be (S).\textsuperscript{79} Either one of the two representations shown can be used, but I will be using the one on the left.

![MLA structure](image)

Figure 3.1: Structure of methyllycaconitine (MLA).
The rings of the skeleton have been labelled (A-F) and the atoms numbered (1-19). These designations can be seen in Figure 3.2.

![Diagram of molecular structure]

Figure 3.2: Designations of rings A-F and atoms 1-19.

MLA has been found to be a very potent antagonist of nicotinic receptors, especially the \( \alpha_7 \)-subtype. The actions of MLA and related compounds on nAChRs are the reason *Delphinium* plants cause more cattle deaths in North America than any other poisonous plant.\(^8^0 \) Although it is not certain that MLA is the main cause in all those plants, it has been shown to be the primary toxin of *Delphinium brownii* Rydb., which is one of those plants causing cattle poisoning.\(^8^1 \)

Since nAChRs are the primary receptors in insect central nervous systems, MLA causes mortality in insects. Extracts from *Delphinium* have long been used as insecticides. The earliest report is from AD 77, when Pliny the
Elder described its use as a parasiticide. It has been confirmed that MLA is the primary insecticidal compound in *Delphinium*-extracts. If the antagonistic activity of MLA on mammalian nAChRs could be separated from its effects on insects this might be a viable option for new insecticides, even though a recent report indicates that nicotinic agonists have better insecticidal properties than do antagonists. A few compounds, related to MLA, have been identified with higher (mammalian IC$_{50}$)/(insect IC$_{50}$) ratios than MLA (see Table 3.1).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rat IC$_{50}$</th>
<th>House fly IC$_{50}$</th>
<th>Rat IC$<em>{50}$/Fly IC$</em>{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Bgt</td>
<td>2 nM</td>
<td>1 nM</td>
<td>2</td>
</tr>
<tr>
<td>MLA</td>
<td>1.7 nM</td>
<td>0.64 nM</td>
<td>3</td>
</tr>
<tr>
<td>Glaudelsine</td>
<td>16 nM</td>
<td>42 pM</td>
<td>381</td>
</tr>
<tr>
<td>Delphenine</td>
<td>&gt;10 μM</td>
<td>32 nM</td>
<td>&gt;333</td>
</tr>
</tbody>
</table>

* Determined in a competitive binding study with [$_{3}^{3}$H]-α-Bgt in rat brain
*b Determined in a competitive binding study with [$_{125}^{1}$]-α-Bgt in house fly brain

Table 3.1: Binding to mammalian and insect receptors.
Figure 3.3: Promising compounds for insecticides.

MLA has also been used as a neuromuscular blocking agent to induce muscle relaxation when necessary to inhibit muscle spasms, e.g. to counteract increased muscle tone caused by traumatic injuries to the brain.\textsuperscript{86}

These interesting activities make MLA a feasible model for the synthesis of a variety of analogues. It could also be useful in studying subtypes of nicotinic receptors due to its subtype selectivity.

3.2. Structure-activity relationships

In order to find a starting point for the design of simple analogues, we need to look at what is known about essential parts of the molecule.

It is known that the 3-methylsuccinimidobenzoate moiety is essential for activity. The parent compound lycoctonine is virtually devoid of all activity (see 48...
Figure 3.4, and an investigation into several different norditerpenoid alkaloids has revealed that this appears to be a general principle, which applies both to α7 and α4β2 subtypes of receptors. Even a sodium channel activator (aconitine, Figure 3.4) turns into a nicotinic antagonist by the substitution of a methylsuccinimidobenzoate ester for the methyl ether. A comparison of several norditerpenoid alkaloids, using a mouse bioassay, showed that the benzoate moiety is important for toxicity, and that an amide or an imide on the benzoate increases the toxicity (see Table 3.2 and Figure 3.4).
<table>
<thead>
<tr>
<th>Compound</th>
<th>( \text{LD}_{50} ) (mg/kg)(^{a})</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLA</td>
<td>7.5</td>
</tr>
<tr>
<td>Nudicauline</td>
<td>2.7</td>
</tr>
<tr>
<td>14-Deacetylnudicauline</td>
<td>4.0</td>
</tr>
<tr>
<td>Elatine</td>
<td>9.2</td>
</tr>
<tr>
<td>Delavaine A+B</td>
<td>3.3</td>
</tr>
<tr>
<td>Anthranoyllycocotonine</td>
<td>20.8</td>
</tr>
<tr>
<td>Deltaline</td>
<td>200.5</td>
</tr>
<tr>
<td>N-Desethyldeltaline</td>
<td>210</td>
</tr>
<tr>
<td>Dictyocarpine</td>
<td>282.9</td>
</tr>
<tr>
<td>Lycoctonine</td>
<td>443.5</td>
</tr>
</tbody>
</table>

\(^{a}\) Determined after intravenous injection into mice

Table 3.2: Toxicity of norditerpenoid alkaloids.\(^{91}\)
Figure 3.4: Structures of various norditerpenoid alkaloids.

A look at a set of nicotinic agonists indicated a requirement for a cationic center (the piperidine nitrogen in MLA) and and electronegative atom, which is thought to form hydrogen bonds with residues in the binding site (the oxygen of the benzoate ester).\(^9^2\)

The methyl-group on the succinimide has also been found to increase the binding affinity of those compounds, both towards \(\alpha 7\) and \(\alpha 4\beta 2\) receptors. In a
comparison of MLA (which has a methyl group on the succinimide ring) and lycaconitine (which does not have the methyl group, see Figure 3.5), MLA had more than twice the affinity of lycaconitine for α4β2-receptors, while there was a 40-fold difference in the affinity for α7-subtype of receptors (see Table 3.3).93

<table>
<thead>
<tr>
<th>Compound</th>
<th>α4β2-binding (Kᵢ)ᵃ</th>
<th>α7-binding (Kᵢ)ᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyllycaconitine</td>
<td>400 nM</td>
<td>4 nM</td>
</tr>
<tr>
<td>Lycaconitine</td>
<td>1 μM</td>
<td>80 nM</td>
</tr>
</tbody>
</table>

ᵃ Determined in a competitive binding study with [³H]-cytisine in rat brain
ᵇ Determined in a competitive binding study with [¹²⁵I]-α-Bgt in rat brain

Table 3.3: Binding data for MLA and lycaconitine.⁹³

Figure 3.5: Structures of MLA and lycaconitine.

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3.3. Analogues of MLA

The synthesis of simpler analogues of MLA is important, because it may furnish more active analogues, as well as provide information about structure-activity relationships for MLA.

3.3.1. Acyclic or monocyclic analogues

Not much work has been done on very simple analogues of MLA. Apart from the work done in our lab, only one report can be found in the literature. To gain information on the pharmacophore of MLA, four acyclic esters were prepared as analogues of MLA (see Equation 3.1). Those were prepared by reacting $N, N$-dimethylethanolamine (2a) or $N, N$-dimethylpropanolamine (2b) with either benzoyl ($R = H$) or 2-chlorobenzoyl chloride ($R = Cl$). This gave esters (3a-d) in moderate yield. All of these compounds were inactive ($IC_{50} > 1$ mM).

\[
\begin{align*}
\text{2a: } n & = 1 \\
\text{2b: } n & = 2 \\
R & = H, Cl
\end{align*}
\]

Six monocyclic ring E analogues were also prepared. Either 3-hydroxymethylpiperidine (4) or 4-hydroxypiperidine (6) was coupled with the
same benzoate derivatives as before, as well as the 2-
methylsuccinimidobenzoate found in MLA.\textsuperscript{94}

Scheme 3.1: Synthesis of simple ring E analogues.
Table 3.4: Binding data for simple ring E analogues.\textsuperscript{94}

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\alpha$7-binding (IC\textsubscript{50})\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLA</td>
<td>7.6 nM</td>
</tr>
<tr>
<td>5a</td>
<td>800 $\mu$M</td>
</tr>
<tr>
<td>5b</td>
<td>100 $\mu$M</td>
</tr>
<tr>
<td>5c</td>
<td>50 $\mu$M</td>
</tr>
<tr>
<td>7a</td>
<td>100 $\mu$M</td>
</tr>
<tr>
<td>7b</td>
<td>50 $\mu$M</td>
</tr>
<tr>
<td>7c</td>
<td>500 $\mu$M</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Determined in a competitive binding study with $[^{125}\text{I}]$-\textalpha-{bungarotoxin in rat brain

It was found that none of the monocyclic analogues had activity towards $\alpha$4$\beta$2-receptors. However, some of the monocyclic analogues had weak antagonistic activity against $\alpha$7-receptors, compared with MLA. The methylsuccinimide-substituted analogue of ring E (5c) and chloro-substituted analogue 7b had the highest potency. Given the information from this study of acyclic and monocyclic analogues, it appears that the piperidine ring is required for any activity against nicotinic receptors.\textsuperscript{94}
Independently from this previous study, our research group looked at different nicotinic agonists and antagonists, and found that many of them contain an ester-function 2 or 3 carbons removed from an electronegative atom. We also see this in MLA, where the benzoate ester is 3 carbons away from the piperidine nitrogen. The choice was made to prepare a series of \( N \)-alkylsubstituted ring E analogues (see Figure 3.6).\(^1\)

![MLA and Ring E Analogue](image)

**Figure 3.6: Structure of MLA and selected ring E analogue.**

For the synthesis of those analogues, two pieces were needed. One of those was 2-(3'-methyl)succinimidobenzoic acid (10), which was prepared by the fusion of anthranilic acid (8) and methylsuccinic anhydride (9).\(^95\)
The piperidine was prepared from hydroxymethylpyridine (11), which was first alkylated, and the resulting pyridinium salt (12a-h) then hydrogenated to give the salt of N-substituted hydroxymethylpiperidines 13a-h.

Scheme 3.2: Synthesis of hydroxymethylpiperidines 13a-h.

The benzoate esters (14a-h) were formed by using a coupling agent to combine the acid (10) with alcohols 13a-h (see Equation 3.3). This reaction generally gave a very poor yield. The best results were received using an expensive coupling reagent, TBTU (O-(1H-Benzotriazol-1-yl)-N, N, N', N'-tetramethyluronium tetrafluoroborate, see Figure 3.7).
Figure 3.7: Structure of TBTU.

Most of the analogues prepared had fairly low affinity, but the inhibitory activity of the most active was approaching the potency of MLA against nAChR-stimulated catecholamine release from adrenal chromaffin cells ($\alpha_3\beta_4^*\)$. It is interesting to note that the $N$-ethyl analogue (14b) had fairly low potency, while larger alkyl chains had higher potency and the most active analogue in this series (14g) has $N$-substitution that is considerable different from that of MLA.
<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>α7-binding(^a) IC(_{50}) (µM)</th>
<th>α4-binding(^b) IC(_{50}) (µM)</th>
<th>α3-function(^c) IC(_{50}) (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLA</td>
<td>-</td>
<td>0.01</td>
<td>0.9</td>
<td>2.6</td>
</tr>
<tr>
<td>14a</td>
<td>Me</td>
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<td>&gt;300</td>
<td>63.9</td>
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<tr>
<td>14b</td>
<td>Et</td>
<td>&gt;300</td>
<td>&gt;300</td>
<td>62.5</td>
</tr>
<tr>
<td>14c</td>
<td>i-Pr</td>
<td>&gt;300</td>
<td>&gt;300</td>
<td>37.1</td>
</tr>
<tr>
<td>14d</td>
<td>n-Bu</td>
<td>&gt;300</td>
<td>&gt;300</td>
<td>27.4</td>
</tr>
<tr>
<td>14e</td>
<td>EtO(CH(_2))(_2)</td>
<td>ND</td>
<td>ND</td>
<td>59.6</td>
</tr>
<tr>
<td>14f</td>
<td>Ph(CH(_2))(_2)</td>
<td>&gt;300</td>
<td>&gt;300</td>
<td>53.0</td>
</tr>
<tr>
<td>14g</td>
<td>Ph(CH(_2))(_3)</td>
<td>177</td>
<td>&gt;300</td>
<td>11.4</td>
</tr>
<tr>
<td>14h</td>
<td>PhO(CH(_2))(_2)</td>
<td>&gt;300</td>
<td>&gt;300</td>
<td>20.2</td>
</tr>
</tbody>
</table>

\(^a\) Determined in a competitive binding study with \(^{[125]}\)-\(\alpha\)-Bgt in rat brain

\(^b\) Determined in a competitive binding study with \(^{3}H\)-nicotine in rat brain

\(^c\) Determined in a study of the inhibition of nAChR-stimulated catecholamine release in bovine adrenal chromaffin cells

Table 3.5: Binding and antagonistic activity of ring E analogues 14a-h.

The stereochemistry of receptor ligands in biological systems is often important. Since the receptors are three-dimensional molecules, binding pockets can be lined up such that ligands need to have a particular stereochemistry in order to bind to the receptors. After finding the promising inhibitory activity of compound 14g, our group was interested in examining the effect of the 4 different diastereomers of 14g on nicotinic receptors.\(^2\)
The (R)- and (S)-enantiomers of methylsuccinic acid were converted to the corresponding enantiomers of 2-(3'-methyl)succinimidobenzoic acid in two steps as shown in Scheme 3.3.

Scheme 3.3: Preparation of (R)- and (S)-methylsuccinimidobenzoic acid.

For the synthesis of the piperidine-portion of the molecule, racemic nipecotic acid was used as the starting material. The two enantiomers were crystallized as salts of camphorsulfonic acid (CSA). That gave (R)-nipecotic acid as the (R)-CSA-salt (18a) and (S)-nipecotic acid as the (S)-CSA-salt (18b). These were then reacted with hydrocinnamoyl chloride (19) to form amide 20, which was reduced with LiAlH₄ to give the desired hydroxymethylpiperidines (21, see Scheme 3.4).
Scheme 3.4: Preparation of enantiomers of hydroxymethylpiperidine.

The acids (17a-b) were finally coupled to hydroxymethylpiperidines 21a-b to provide the four different diastereomers of compound 14g. Results from the biological evaluation indicate that there is not a significant difference in the potency of the four diastereomers. This would suggest that the binding of these compounds to the receptor is not stereospecific.

3.3.2. AE analogues

The general structure for AE analogues of MLA is shown in Figure 3.7.

Figure 3.8: General structure of AE-ring analogues of MLA.
Most of the work on these types of analogues belongs to two research groups. Fukumoto and co-workers had synthesized 24 as an intermediate in the total synthesis of atisine.\(^{96,97}\) In order to use this same compound as an intermediate for the synthesis of alkaloids from Aconitum species, they improved on their previous synthesis of monoacetate 24 by using diol 23\(^{96,97}\) and Candida cylindracea lipase (CCL).\(^{98}\) Ethyl ester 26 was also prepared in 4 steps (from 23) in an overall yield of 58%. Biological data for these compounds was not reported.

Blagbrough and co-workers have done a lot of work on this type of analogues. Starting from β-keto ester 27, they prepared two bicyclic compounds (28a and 28b) through a double Mannich reaction with formaldehyde and the appropriate alkylamine. These were then used for the synthesis of two analogues from each. The alcohols (29a-b and 30a-b) were reacted with isatoic anhydride (31) and the resulting anthranilate ester was fused with methylsuccinic anhydride to give esters 32a-d (see Equation 3.4). The ester-formation has since been improved upon, to allow the formation of anthranilate esters of secondary and tertiary alcohols if necessary.

Scheme 3.6: Synthesis of bicyclic AE-skeleton.
A pair of analogues of MLA, with rings A and E in place and a connection to what resembles ring D, has also been prepared. Lithium acetylide was reacted with cyclohexanone 33 to form propargylic alcohol 34 in a 12:1 ratio of diastereomers. After reacting the acetylide anion with ketone 28a, the alkyne was reduced to an alkane, and the ethyl ester reduced to the alcohol to give triol 36. This was then converted to the methylsuccinimidobenzoate ester in the same way as shown in Equation 3.4.
Scheme 3.7: Synthesis of AE analogue with D-ring connection.

The same group also prepared analogues containing a double bond between the A- and D-rings, both with Z- and E-geometry. These were prepared from the alkyne (35), by controlling the reaction conditions to give the desired geometry selectively.

Scheme 3.8: Reduction of alkyne to Z- and E-alkenes.
A structure similar to the AE-ring of MLA with contraction of the piperidine ring ([3.2.1]-azabicycle, 44) has also been prepared, with the key step being a Horner-Emmons olefination followed by a conjugate addition to form a pyrrolidine ring. Starting from \( \alpha,\beta \)-unsaturated ketone 39, the ethoxy group was first exchanged for a sulfide. Cyanide addition, followed by protection of the ketone and reduction of the cyanide gave amine 41. This amine was protected and the ketone deprotected to set up the olefination and conjugate addition to form the bicyclic compound (43). Manipulations of the existing groups then gave the AE analogue with ring contraction (44) in 5 steps.

Scheme 3.9: Synthesis of [3.2.1]-azabicycle.
One more bicyclic AE analogue (45) has been prepared. The preparation is not described but this analogue was found to have very little antagonistic activity at neuronal nicotinic receptors (see Table 3.6 below for binding data).

![Figure 3.9: Structure of AE analogue 45.](image)

**Figure 3.9: Structure of AE analogue 45.**

### 3.3.3. ABE analogues

The ABE-ring system has not been extensively studied, and only one report can be found of the activity of an analogue, although the synthesis is not described. As can be seen in Figure 3.9, this analogue is perhaps not strictly an ABE analogue, since the connections for the "B"-ring appear to be a mixture of the B- and F-rings. The stereochemistry of each of the diastereomers is not described, but what the authors designated as tricycle Y is 46a, and tricycle X is 46b.
Figure 3.10: Structure of tricyclic ABE analogues 46a-b.

Compound 46b had significantly stronger affinity at $\alpha 7$-subtype of receptors, than the 46a or the corresponding bicyclic analogue (see Table 3.6), but still much weaker than MLA (about 200-fold). None of these analogues showed affinity towards $\alpha 4\beta 2$-receptors.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\alpha 7$-binding (IC$_{50}$)$^a$</th>
<th>$\alpha 4\beta 2$-binding (IC$_{50}$)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLA</td>
<td>2.18 nM</td>
<td>ND</td>
</tr>
<tr>
<td>45</td>
<td>107 $\mu$M</td>
<td>&gt; 1 mM</td>
</tr>
<tr>
<td>46a</td>
<td>111 $\mu$M</td>
<td>&gt; 1 mM</td>
</tr>
<tr>
<td>46b</td>
<td>478 nM</td>
<td>&gt; 1 mM</td>
</tr>
</tbody>
</table>

$^a$ Determined in a competitive binding study with $[^{125}]$-\(\alpha\)-Bgt in rat brain

$^b$ Determined in a competitive binding study with $[^{3}]$H-nicotine in rat brain

Table 3.6: Binding data for bi- and tricyclic analogues 45 and 46a-b.$^{104}$

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Another example of a synthesis of the ABE-ring system where the connectivity is not quite the same as in MLA is a study in hetisine-type diterpene alkaloids (shown in Scheme 3.10). This was designed so as to allow for the possibility of adding the C- and D-rings later on.\textsuperscript{105} Keto ester 47 was converted to pyridinediol 48.\textsuperscript{106} Alkylation with allyl bromide gave diene 49, which underwent a Cope rearrangement upon heating. After alkylating the nitrogen, the bromohydrin of the terminal olefin was formed and the alcohol protected as the THP-ether (50). Treatment with sodium hydride closed the ring by nucleophilic displacement of the bromide. Removal of the THP-ether followed by the oxidation of the resulting alcohol then gave ABE analogue 52.
The only example of the preparation of strict ABE analogues of MLA is fairly recent work by Kraus and co-workers. Starting from spirocyclic diketone 53, the silyl enol ether of the unsaturated ketone was prepared, followed by carbomethoxylation of the saturated ketone. The silyl enol ether was hydrolyzed and a Mannich reaction gave tricyclic compound 54. Ceric ammonium nitrate allowed for selective ketalization, so the AE-ring ketone could be reduced and methylated. Reduction of the ester with LiAlH₄ gave alcohol 55, which was coupled with methylsuccinimidobenzoic acid using MsCl to promote the coupling. The yield was good, but the reaction conditions caused some of
the ketal to collapse. The ketone (56a) and the ketal (56b) were formed in approximately 1:1 ratio. Biological data for these compounds has not been reported.

![Chemical structures](image)

Scheme 3.11: Synthesis of ABE analogues 56a and 56b.

3.3.4. AEF analogues

Two groups have synthesized the AEF-ring system of MLA. One group started from cyclopropyl methyl ketone (57), which was converted to cyanodiene 58 in 3 steps.\(^{109,110}\) Reduction to the aldehyde, followed by Grignard addition and then oxidation gave trienone 59. The product of a Diels-Alder reaction was
reacted with hydroxylamine to give bicyclic oxime 60. The cis-isomer was then reduced to the free amine, acetylated and then reduced to the ethylamine (61). The tricyclic AEF analogue 62 was then produced through a Mannich reaction.

Scheme 3.12: Synthesis of AEF analogue 62.

The other approach to this part of the molecule was utilized to prepare tricyclic analogues 63,111,112 and 64,113,114 but this report will focus on the synthesis of the benzoate ester analogues (64).
Figure 3.11: Structure of AEF analogues 63 and 64.

The synthesis started from penta-1,4-dien-3-ol (65). A Claisen-rearrangement followed by hydrolysis of the resulting ester gave a suitable substrate for a Diels-Alder reaction (66). Reaction of alkene with the diene gave 68, but the control of stereochemistry was not as good as would be desirable. The free alcohol was then protected and the acid converted to an aldehyde (69). Reaction with N-ethylhydroxylamine gave the nitrone, which then underwent a dipolar addition to give tricycle 70. Oxidation of the nitrogen caused the N-O bond to break and provided nitrone 71. This nitrone was reduced and amide 72 prepared. Methylation and reduction gave alcohol 73, which was then converted to (S)-methylsuccinimidoenzoate as described in Equation 3.4. Biological results for these compounds have not been reported.
3.3.5. More complex analogues

Kraus and co-workers prepared the framework for an ABDE analogue. The work was similar to that described in Section 3.3.3. Starting from compound 74 (available in two steps from ethyl cyclohexanone-2-carboxylate), allyl-substituted bicycle 75 was prepared. Ozonolysis, followed by a Wittig reaction formed a hemiketal which collapsed upon treatment with acid. Diels-Alder reaction with diene 77 formed the D-ring and deprotonation with LiHMDS effected the closure of the B-ring to form tetracyclic compound 78. This work was not taken any further, since the main purpose was to prepare the tetracyclic skeleton.

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Another group of researchers has regularly expanded on their previous work and have now prepared the ABCD-ring system suitably substituted to allow for expansion into the remaining rings. Starting from 7-t-butoxynorbornadiene (79), the CD-ring system was assembled in two steps.\textsuperscript{117}

Scheme 3.14: Synthesis of ABDE analogue 78.

Scheme 3.15: Synthesis of CD-ring system.
The addition of the next ring (see Scheme 3.16) was then effected in 9 steps.\textsuperscript{118,119} Tetracyclic intermediate 82 was formed by treatment with thionyl chloride and diazomethane, followed by heating in the presence of copper. Reduction to break open the cyclopropane-ring proceeded smoothly to give 83. The five-membered ring of 83 was carboxylated and after forming enamine 84, it was reacted with t-butyl propiolate to expand the ring into a 7-membered ring.\textsuperscript{120} Acid hydrolysis of the enamine gave enol 85. Reaction of the enol with acetic acid, acetic anhydride, and perchloric acid cleaved the two t-butyl groups off the molecule. Decarboxylation was effected by heating in toluene in the presence of $p$-toluenesulfonic acid and the double bond of the D-ring moved into conjugation with the ketone of the B-ring. This gave intermediate 86, which was then taken on for further expansion.
Scheme 3.16: Synthesis of BCD-ring system 86.

Proceeding from the BCD-ring system (86), the ketone was protected as the ketal$^{120}$ and the allylic position in ring D oxidized to give the ketone and then reduced to alcohol 87.$^{121}$ Epoxidation of both olefins was effected with trifluoroperacetic acid. The free alcohol was methylated, followed by reductive opening of one epoxide giving β-hydroxyester 88. However, the reduction also partly removed the acetate, giving approximately equal mixture of 88a and 88b. The free alcohol of 88a was oxidized to the ketone and this intermediate alkylated with benzyl acrylate to give 89. It should be noted that due to steric hindrance, only the exo-side is open to approach from the acrylate. Compound 88b was also converted to 89, by Swern oxidation, which only oxidizes the
hydroxyl-group β to the ester, alkylation with acrylate and acetylation of the free alcohol.

The benzyl ester was hydrogenated to the free acid, and the mixed anhydride prepared, followed by alkylation with the lithium salt of t-butyl trimethylsilyl malonate. The silyl-group was cleaved off, which led to spontaneous decarboxylation to give β-keto ester 90. Treatment with sodium methoxide then closed the A-ring to provide ABCD analogue 91.

Scheme 3.17: Synthesis of ABCD analogue 91.
No total synthesis of MLA has been published, and only one total synthesis of these types of alkaloids. Since it is not a total synthesis of MLA, I will not be covering that here, but it still constitutes an important step in the syntheses of these types of compounds.

3.3.6. Semi-synthesis

The first semi-synthesis of MLA was performed by acylation of lycoctonine with isatoic anhydride to form the natural compound inuline, followed by fusion with methylsuccinic anhydride to give MLA.

Four different benzoate esters of lycoctonine have also been prepared, but there is no record of the testing of biological activity of these compounds.

3.4. Conclusions

Several different types of analogues of MLA have been prepared, employing a variety of synthetic strategies. However, very few of those analogues have been tested for binding activity to nicotinic receptors. For this reason, not much is known about what substituent groups are tolerated or beneficial for binding in these types of compounds.
CHAPTER 4
SYNTHESIS AND EVALUATION OF RING E ANALOGUES OF MLA

4.1. Introduction

The original work in our research group on MLA-analogue focused on variations in the N-substitution on the piperidine ring. The results from that work indicated that varying the substitution could dramatically improve the potency of these analogues to nicotinic receptors.

Following up on that early success, additional analogues have been prepared to try to gain more information on the structure-activity relationships of these molecules (see Figure 4.1). These compounds can be classified according to the substitution pattern in the analogues prepared. Different substitution on the benzoate can be divided into analogues with varying succinimide substitution and analogues with various substituents directly on the aromatic ring. The other major category of analogues is that of N-substitution on the piperidine ring. This is where different alkyl groups were tried initially, and I have prepared various aryloxyalkyl derivatives as well as derivatives examining the effect of lengthening the chain in an arylalkyl substituent.
Figure 4.1: Overview of modifications made on ring E analogues.

Some of those analogues have shown even greater potency than previous analogues, indicating promise for future work in this area. However, all of the...
analogues prepared in our group, have been found to be non-competitive inhibitors towards \( \alpha 3\beta 4* \)-nicotinic receptors, while MLA is a competitive inhibitor. This means that the ring E analogues are not binding to the receptor at exactly the same binding site as acetylcholine and MLA. My hypothesis is that the CD-ring system of MLA may be important for competitive binding, so I have designed and prepared one analogue containing a dimethoxycyclohexyl substituent intended to mimic the D-ring of MLA. Although the connectivity of this substituent is not exactly the same as the connectivity of ring D to ring E in MLA, the flexible three-atom chain should allow it to occupy space where the D-ring might be located in MLA.

4.2. Coupling problem

During previous work on these analogues, one of the problems that came up was a poor yield in the step coupling the benzoic acid to the hydroxymethylpiperidine (see Equation 3.3). The product from the hydrogenation step had been taken on crude to the coupling step, so I decided to prepare the free base of the hydroxymethylpiperidine to see if that would be more reactive for the coupling step. However, upon purification of that product, I discovered a side-product, which was unreactive towards the coupling (see Equation 4.1). This was methylpiperidine 93, which was being formed as the hydrogenation was cleaving some of compound 12 at the benzylic-like position before reducing the pyridinium-ring. So, the reason for the poor yields in the coupling appears to
have been smaller amounts than expected of hydroxymethylpiperidine 92
present.

![Chemical structure](image)

In order to circumvent this problem, it was decided to start the process
from nipecotic acid. The ethyl ester hydrochloride (94) was prepared and then
alkylated with a suitable alkylating agent. Reduction with LiAlH₄ then gave the
hydroxymethylpiperidine (92), which can be used as a coupling partner with the
benzoic acid.

![Chemical structure](image)

Scheme 4.1: Preparation of hydroxymethylpiperidine 92.

As a test reaction, several different coupling reagents were tried for the
coupling of phenylpropanol (96) to benzoic acid 10. TBTU gave a better result

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than before, but I found that the inexpensive reagent dicyclohexylcarbodiimide (DCC) gave the best yield.

\[
\begin{align*}
\text{Coupling reagent} & \\
\text{TBTU, 78\%} & \\
\text{MsCl, 42\%} & \\
\text{TFAA, 31\%} & \\
\text{t-BuCOCl, 27\%} & \\
\text{DCC, 88\%} & \\
\end{align*}
\]

4.3. Aryl substitution

Biological results have indicated that the methyl-group on the succinimide is important for the antagonistic activity (see Table 3.3), so we decided to examine that effect in our ring E analogues. A series of analogues were prepared containing various substituents on the succinimide. This investigation was warranted as some of those analogues were among the most potent analogues we have prepared to date. We also decided to prepare a series of analogues carrying different substitution on the benzoate-ring, both substituents somewhat similar to the succinimide as well as other groups.

4.3.1. Succinimide series

It has been shown that the antagonistic activity of MLA decreases significantly when the methyl-group of the succinimide is absent, so we decided
to examine the effect of that absence in these analogues, as well as other
substitution in that position. A comparison of compounds 14c and 98 (see Figure
4.2) indicates that the methyl-group may increase the potency threefold. These
results suggested that further investigation was warranted. Khadiga Ismail
prepared analogues substituted with larger groups on the succinimide.

![Chemical structures of 14c and 98](image)

**Figure 4.2: Effect of succinimide-methyl on potency.**

In order to prepare the target analogues, a method to synthesize 3-
substituted succinic anhydrides was required. This can be accomplished by
reacting succinic anhydride (99) with t-butanol to form t-butyl monoester.\(^{125}\) The
dianion was formed and alkylated next to the ester functionality to give 2-
substituted monoesters 100. Acid hydrolysis of the t-butyl ester then gave 2-
substituted succinic acids 101 (see Scheme 4.2). This was followed by
regeneration of the anhydride to give 3-substituted succinic anhydrides \(102a-g\) (see Equation 4.3).\(^{126}\)

![Chemical reaction diagram](image)

**Scheme 4.2: Preparation of 2-substituted succinic acids.**

The final analogues were then prepared as described in section 3.3.1, by fusion of anhydrides 102 to anthranilic acid followed by coupling to the \(N\)-substituted piperidine moiety to give analogues 104 (see Scheme 4.3). It was found that larger substituents increased the activity significantly from that of the methyl-substituted compound. This led us to wonder whether adding another group onto the succinimide would improve the activity further, or whether the receptor could only accommodate one substituent. One possibility is that the substituent on the succinimide could only help with the binding when facing in one direction, and rotation around the bond between the succinimide nitrogen

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and the aromatic ring could limit the availability of the orientation that would bind better. To examine this, I prepared analogue 104h from 2, 2-dimethylsuccinic acid, where there would be a methyl-group sticking out in both directions from the succinimide. I also prepared analogue 104i from 2, 3-dimethylsuccinic acid. The starting material for that preparation was a mixture of meso- and dl-isomers, which was carried to the final product. The dl-isomer, like 104h, would always have methyl-groups on both faces of the succinimide, but the meso-isomer would have two methyl-groups on the same face of the succinimide-ring (see Equation 4.3 and Scheme 4.3). The N-phenylpropyl-derivative was used as the coupling partner in all of these analogues, since that gave the highest potency (compound 14g) from the first series of compounds.¹

![Scheme 4.3: Preparation of succinimide analogues 104a-i.](image-url)
These compounds were tested for their potency in inhibiting nAChR-stimulated catecholamine release from bovine adrenal chromaffin cells ($\alpha3\beta4^*$) and for their binding to $\alpha7$-receptors in a competitive study with $[^{125}\text{I}]-\alpha$-Bgt in rat brain (see Table 4.1).

Any substituent larger than a methyl resulted in an increase in potency towards $\alpha3\beta4^*$, and affinity for $\alpha7$ compared with compound 14g, but no obvious trend appeared from examining these results. Initially, the longer chains show greater potency, but lengthening the chain further, did not appear to make a significant difference. Neither was there a significant difference between the potency of unsaturated compounds (104d, e) and the saturated ones (104c, f, g). Adding another methyl-group results in a slight increase in potency, for both the 3, 3-dimethyl (104h) and the 3, 4-dimethyl (104i). Although there is not a large difference between those two, analogue 104i appears to be slightly more active. Possibly, the constant availability of a methyl-group is helping with the binding, but with compound 104i having higher potency, it appears as if the additional methyl-group is a bigger factor than having one on each side. These results seem to indicate that the substituents on the succinimide are not binding a specific pocket in the receptor, but rather have a non-specific interaction to promote binding, such as being inserted into a lipophilic membrane nearby. That could explain why a certain length appears to be necessary, but beyond that, there doesn't appear to be much that can be done to improve the binding.
<table>
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<th>R'</th>
<th>R''</th>
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<td>-</td>
<td>-</td>
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<td>10 nM</td>
</tr>
<tr>
<td>14g</td>
<td>Me</td>
<td>H</td>
<td>H</td>
<td>11.4 µM</td>
<td>177 µM</td>
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<tr>
<td>104a</td>
<td>Et</td>
<td>H</td>
<td>H</td>
<td>4.7 µM$^c$</td>
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<tr>
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<td>H</td>
<td>H</td>
<td>1.3 µM</td>
<td>20 µM</td>
</tr>
<tr>
<td>104h</td>
<td>Me</td>
<td>Me</td>
<td>H</td>
<td>7.3 µM$^c$</td>
<td>ND$^d$</td>
</tr>
<tr>
<td>104i</td>
<td>Me</td>
<td>Me</td>
<td>Me</td>
<td>6.1 µM$^c$</td>
<td>ND$^d$</td>
</tr>
</tbody>
</table>

$^a$ Determined by measurement of inhibition of nAChR-stimulated catecholamine release from bovine adrenal chromaffin cells, unpublished data from D. B. McKay et al.

$^b$ Determined in a competitive binding study with [$^{125}$I]-α-Bgt in rat brain, unpublished data from D. B. McKay et al.

$^c$ Preliminary data

$^d$ ND = No data available at this time

Table 4.1: Binding and function of succinimides 104a-i.
4.3.2. Benzoate series

With the great improvements in potency resulting from different substitution of the succinimide, we were interested in probing further into the effect of the substitution on the benzoate ring. We decided to use the \( N \)-sidechain that previously gave the most potent analogue (14g) as the coupling partner to the benzoates, but instead of the succinimide, I prepared analogues with phthalimide- and acetamide-substitution to examine the effect of changing that structure only slightly. We also decided to prepare two biphenyl analogues, due to a recent report, which indicates that compounds containing a biphenyl bind well to a variety of proteins.\(^{127}\) Then we added a few analogues containing smaller substituents, with different electronic properties, in the 2-position on the benzoate.

All but one of the benzoic acids were purchased. The only one which required preparation, was 2-phthalimidobenzoic acid (106), prepared from phthalic anhydride and anthranilic acid (see Equation 4.4). The analogues were then prepared by coupling the hydroxymethylpiperidine to the appropriately substituted benzoic acids to give benzoates 108a-h (see Equation 4.5).
Preliminary results from the functional study of these analogues indicate that the phthalimide-analogue (108a) and the 2-biphenyl-analogue (108c) are close in potency to the more active succinimide-analogues (see Table 4.2). Both of those analogues contain an aromatic ring, but results from the succinimide series seemed to indicate that aromaticity in the succinimide-substituent was not required for maximum potency (see Section 4.3.1). Perhaps these aromatic groups are the only ones in this series large enough to have a significant effect on the activity. To probe further the importance of aromaticity for analogue 108a,
I would suggest preparing a saturated analogue, in order to be able to compare it directly with 108a. In comparing analogues 108c and 108d, we note that the location of the substitution on the phenyl-ring appears to be somewhat important. Although the 4-phenyl analogue (108d) is quite potent, the 2-phenyl derivative is twice as potent, with the position of the phenyl-ring the only difference between the two analogues. Interesting questions would include what effect substitution on that second phenyl-ring would have, and perhaps another phenyl-ring added. Since the succinimide does not appear to be strictly required, what effect would analogues with longer alkyl chains in position 2 of the benzoate have? It is also interesting to note, that the smaller substituents are quite potent, even more so than analogue 14g, in spite of their smaller size. Information from those analogues with smaller substituents (108e-h) also indicates that there does not appear to be any difference between electron-withdrawing and electron-donating groups within these analogues.
### Compound Table

<table>
<thead>
<tr>
<th>Compound</th>
<th>α3-function (IC\textsubscript{50})\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLA</td>
<td>2.6 μM</td>
</tr>
<tr>
<td>14g</td>
<td>11.4 μM</td>
</tr>
<tr>
<td>108a</td>
<td>1.4 μM</td>
</tr>
<tr>
<td>108b</td>
<td>8.5 μM</td>
</tr>
<tr>
<td>108c</td>
<td>1.7 μM</td>
</tr>
<tr>
<td>108d</td>
<td>3.3 μM</td>
</tr>
<tr>
<td>108e</td>
<td>3.0 μM</td>
</tr>
<tr>
<td>108f</td>
<td>3.5 μM</td>
</tr>
<tr>
<td>108g</td>
<td>3.3 μM</td>
</tr>
<tr>
<td>108h</td>
<td>5.2 μM</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Determined by measurement of inhibition of nAChR-stimulated catecholamine release from bovine adrenal chromaffin cells, unpublished data from D. B. McKay et al.

Table 4.2: Preliminary data for benzoates 108a-h.

### 4.4. N-Substitution

The initial work in our group had indicated that by varying the \textit{N}-substitution on the piperidine ring, we could increase the potency significantly. We wanted to examine this further to try to find analogues with higher affinity and higher potency. I prepared \textit{N}-aryloxyethyl analogues with a variety of
substitution patterns on the aryl ring. I also prepared N-phenylalkyl analogues, lengthening the alkyl chain gradually. The biological results from those two series stimulated our interest in combining the most effective elements into a new group of analogues. This gave rise to the N-aryloxyalkyl series. Having found that all of our analogues turned out to be non-competitive antagonists, we wanted to try to figure out which part of MLA is required for competitive antagonism. To that end, I designed an analogue containing ring E and a connection to what looks like ring D, although the connectivity to ring E is different from that of MLA.

4.4.1. N-Aryloxyethyl series

First, we wanted to examine the effect of substitution on the phenyl-ring of the most active analogue, 14g. We had initially wanted to prepare direct analogues of 14g with varying substitution on the aromatic ring. However, an examination of potential synthetic routes to these compounds suggested that the synthesis would be quite long. For example, the preparation of an appropriate substrate for the alkylation of nipecotate, could be accomplished starting from substituted benzaldehydes (109) in four steps (see Equation 4.6). This is a longer synthesis than we desired, and the limited availability of appropriately substituted benzaldehydes could also present a problem with this approach.
Another possibility would be to couple a substituted bromobenzene (111) to an allyl silyl ether via a Suzuki coupling (see Equation 4.7). That would be followed by a desilylation and a tosylation, making this a three-step process. However, yields for Suzuki couplings are often modest and this is still a longer preparation than we desired. Also, the Suzuki coupling might be problematic with analogues containing additional halogens on the aromatic ring.

Considering all this, we decided to try preparing analogues of the oxa-isostere of 14g, since this could be synthesized fairly easily and a variety of substituted phenols is commercially available. In order to examine the feasibility of this substitution, we compared analogue 14g with its oxa-isostere, 14h (see Section 3.3.1 and Figure 4.3). The potency of 14h is lower than that of 14g, but it is still on the same order of magnitude. Therefore, we decided that this was a reasonable model to use for this synthesis.
4.4.1.1. Retrosynthetic analysis

The preparation of these N-aryloxyethyl analogues (112) should be possible through our standard route, but we needed a facile method to prepare compound 114 with a variety of aryl groups attached (see Scheme 4.4).
Scheme 4.4: Retrosynthesis of N-aryloxyethyl analogues 112.

This could be accomplished by a couple of different methods. I attempted to do this by reacting phenol (115) with bromoacetic acid, but that was unsuccessful. However, the reaction of phenol with allyl bromide to give allyl phenyl ether (116) was successful, and then this compound could undergo ozonolysis to give a hydroxyethyl ether, which could be converted to the tosylate and attached to the nitrogen of the piperidine ring. This was an option, but with that being a three-step process, we wanted to find a shorter route. As it turned out, phenol (115), reacted with 1,2-dibromoethane to give bromoethyl ether 117, which could be used directly as a substrate for the alkylation of the piperidine nitrogen.
4.4.1.2. Determination of target analogues

The next issue was to decide which analogues we should prepare. To aid us in that process, we used the Topliss-tree for optimizing aromatic substitution (see Figure 4.4).\textsuperscript{128} Having prepared the unsubstituted phenyl-derivative (14h) previously, we decided to prepare analogues for the next two branches in the tree and see where that would lead us. The scheme suggests that the 4-chloro analogue be prepared and depending on the result, 4-methoxy, 4-methyl or 3,4-dichloro. However, we decided to prepare several analogues at first and then compare the results. This way, regardless of the results from the 4-chloro analogue, we had the next step covered. Also, since 3-chloro was suggested as the third step in 4 out of a possible 9 scenarios, we added this analogue to the initial pool. We also decided to include 1- and 2-naphthyl. For the same reason...
as outlined in Section 4.3.2, we also included three biphenyl analogues. It
should be noted that nitro-substituted analogues could not be prepared via this
route. Aliphatic nitro groups can be reduced to the amine by lithium aluminum
hydride,\textsuperscript{129} and aromatic nitro groups are reduced more easily than aliphatic
ones, giving either the arylamine or forming a dimer with another amine.\textsuperscript{130}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4.4.png}
\caption{Topliss-tree for aromatic substitution.\textsuperscript{128}}
\end{figure}

It was determined that the 4-chloro derivative (1\textsuperscript{12}d, see Table 4.3) was
more active than the unsubstituted one, and the 3.4-dichloro derivative (1\textsuperscript{12}e)
had higher potency than 1\textsuperscript{12}d. Due to the exclusion of compounds containing
nitro-groups, I only had one more analogue to prepare to complete the series according to the continuation of the scheme (see Figure 4.5). This was 4-chloro-3-trifluoromethyl analogue 112k.

Figure 4.5: Sequence of analogues through the Topliss-tree.

4.4.1.3. Synthesis

As indicated in section 4.4.1.1, the appropriate phenols were reacted with 1,2-dibromoethane to give the aryloxyethyl bromides (114a-k) directly. These were used to alkylate ethyl nipecotate (94), to give N-substituted nipecotates 116a-k (see Scheme 4.6).
Scheme 4.6: Preparation of \(N\)-aryloxyethyl nipecotates 116a-k.

The nipecotates were then reduced in the usual fashion and the resulting hydroxymethylpiperidines (113a-k) were coupled to methylsuccinimidobenzoic acid to give benzoates 112a-k (see Scheme 4.7).

Scheme 4.7: Preparation of \(N\)-aryloxyethyl analogues 112a-k.
4.4.1.4. Evaluation

The potency of analogues 112a-k to inhibit nAChR-stimulated catecholamine release in adrenal chromaffin cells was evaluated. In spite of the unsubstituted phenoxyethyl analogue (14h) having only half the potency of 14g, some of these analogues have significantly higher potency than 14g. Most notable is the dichlorophenyl-analogue (112e). It appears that electron-withdrawing groups on the aryl ring result in an increase in the potency. The results from the biological assay of the last analogue from the Topliss-tree (112k), should complete the picture for this substitution pattern.
Table 4.3: Functional data for analogues 112a-k.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ar&lt;sup&gt;a&lt;/sup&gt;</th>
<th>α3-function (IC&lt;sub&gt;50&lt;/sub&gt;)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLA</td>
<td>-</td>
<td>2.6 μM</td>
</tr>
<tr>
<td>14g</td>
<td>-</td>
<td>11.4 μM</td>
</tr>
<tr>
<td>14h</td>
<td>Ph</td>
<td>20.2 μM</td>
</tr>
<tr>
<td>112a</td>
<td>4-MeOPh</td>
<td>6.3 μM</td>
</tr>
<tr>
<td>112b</td>
<td>2-Naphthyl</td>
<td>2.7 μM</td>
</tr>
<tr>
<td>112c</td>
<td>4-MePh</td>
<td>4.2 μM</td>
</tr>
<tr>
<td>112d</td>
<td>4-ClPh</td>
<td>3.7 μM</td>
</tr>
<tr>
<td>112e</td>
<td>3,4-Cl&lt;sub&gt;2&lt;/sub&gt;Ph</td>
<td>2.1 μM</td>
</tr>
<tr>
<td>112f</td>
<td>3-ClPh</td>
<td>4.6 μM</td>
</tr>
<tr>
<td>112g</td>
<td>1-Naphthyl</td>
<td>2.5 μM</td>
</tr>
<tr>
<td>112h</td>
<td>4-PhenylPh</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>112i</td>
<td>2-PhenylPh</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>112j</td>
<td>3-PhenylPh</td>
<td>2.0 μM</td>
</tr>
<tr>
<td>112k</td>
<td>4-Cl-3-CF&lt;sub&gt;3&lt;/sub&gt;Ph</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> See Scheme 4.7
<sup>b</sup> Determined by measurement of inhibition of nAChR-stimulated catecholamine release from bovine adrenal chromaffin cells, unpublished data from D. B. McKay et al.
<sup>c</sup> ND = No data available at this time
4.4.2. N-Phenylalkyl series

In the N-alkyl series, it was found that the N-phenylpropyl derivative had the greatest potency (see Section 3.3.1), significantly higher than the saturated alkyl-chain derivatives. We decided to examine the effect of lengthening the chain to the phenyl ring. If that ring was sticking into a pocket in the receptors to aid binding (see Figure 4.6), we wanted to know if lengthening the chain (increasing \( n \)), would cause the phenyl ring to fit better or if there was an optimum value for \( n \), beyond which the chain would be too long, the phenyl ring would not fit any longer, and the affinity would decrease.

**Figure 4.6:** Diagram of a possible binding pocket for N-side chain.
We were also interested in finding out whether aromaticity was a requirement for binding. Quite possibly, the reason for the stronger affinity of N-phenylpropyl derivative 14g could be that \(\pi\)-stacking was occurring with aromatic rings in the receptor. For that reason, we decided to include a saturated derivative (cyclohexyl, 117d) in this series (see Figure 4.7).

![Figure 4.7: Structure of analogues 117a-d.](image)

The analogues were prepared by tosylation of the corresponding alcohols, followed by alkylation of nipecotate (see Scheme 4.8).
Scheme 4.8: Preparation of N-alkylnipeocotates 120a-d.

Reduction of the ester gave hydroxymethylpiperidines 121a-d, which were then coupled to benzoic acid derivative 10, giving benzoate esters 117a-d, as described in Scheme 4.9.

Scheme 4.9: Preparation of N-phenylalkyl analogues.
<table>
<thead>
<tr>
<th>Compound</th>
<th>R&lt;sup&gt;a&lt;/sup&gt;</th>
<th>α3-function (IC&lt;sub&gt;50&lt;/sub&gt;)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>α7-binding (IC&lt;sub&gt;50&lt;/sub&gt;)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLA</td>
<td>-</td>
<td>2.6 μM</td>
<td>0.01 μM</td>
</tr>
<tr>
<td>14f</td>
<td>Ph(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>53.0 μM</td>
<td>&gt;300 μM</td>
</tr>
<tr>
<td>14g</td>
<td>Ph(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>11.4 μM</td>
<td>177 μM</td>
</tr>
<tr>
<td>117a</td>
<td>Ph(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;4&lt;/sub&gt;</td>
<td>5.3 μM</td>
<td>ND&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>117b</td>
<td>Ph(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;5&lt;/sub&gt;</td>
<td>3.2 μM</td>
<td>ND&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>117c</td>
<td>Ph(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;6&lt;/sub&gt;</td>
<td>3.4 μM</td>
<td>ND&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>117d</td>
<td>Cyclo-C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;11&lt;/sub&gt;(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>5.0 μM</td>
<td>ND&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> See Figure 4.7

<sup>b</sup> Determined by measurement of inhibition of nAChR-stimulated catecholamine release in bovine adrenal chromaffin cells, unpublished data from D. B. McKay et al.

<sup>c</sup> Determined in a competitive binding study with [125I]-α-Bgt in rat brain, unpublished data from D. B. McKay et al.

<sup>d</sup> ND = No data available at this time

Table 4.4: Data for N-phenylalkyl analogues.

As the alkyl chain separating the phenyl-ring from the piperidine nitrogen was lengthened, the potency of the analogues increased, although there was very little difference between the potency of the phenylpentyl and phenylhexyl derivatives. It appears likely, that the potency has reached a maximum for this series of compounds, but the synthesis of phenyloctyl and phenyldecyl analogues should clarify that issue. Interestingly, replacing the phenyl ring with a cyclohexyl ring (in 117d) resulted in greater potency compared with compound

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14g. This indicates that the phenyl ring is not required for activity, which makes it appear unlikely that π-stacking is the reason for increased affinity of compound 14g. One possibility is that the N-side chain of our ring E analogues is looping around and occupying space which would be occupied by the CD ring system of MLA (see Figure 4.8).

![MLA](image1)
![14g](image2)
![117d](image3)

**Figure 4.8: Possible model for N-substituent occupying space near the location of the CD-ring system of MLA.**

That could explain the increase in potency for the longer chains, since those could better accommodate the phenyl ring in such a position. If that is the case, we would also expect that the cyclohexyl ring might be a better fit than a phenyl ring, since it can adopt the chair-like conformation of ring D of MLA. This is consistent with our results so far.
4.4.3. N-Aryloxyalkyl series

When we compared the biological data for compound 14g with the data from the N-aryloxyethyl series, as well as with compounds 117b-c, we were presented with a very interesting possibility (see Figure 4.9). In going from the N-phenylpropyl derivative (14g) to the 3,4-dichlorophenoxyethyl derivative (112e), the potency increased 5-fold. If something similar were to take place with the longer chains, we could be getting analogues with potency in the nanomolar range. So, we decided to prepare a few N-aryloxybutyl and N-aryloxypentyl analogues.

![Chemical structures](image)

**14g**: $X = \text{CH}_2$, $R = \text{H}$, $IC_{50} = 11.4 \text{ M}$  
**112e**: $X = \text{O}$, $R = \text{Cl}$, $IC_{50} = 2.1 \text{ M}$  
**117b**: $X = \text{CH}_2$, $R = \text{H}$, $n = 1$, $IC_{50} = 3.2 \text{ M}$  
**117c**: $X = \text{CH}_2$, $R = \text{H}$, $n = 2$, $IC_{50} = 3.4 \text{ M}$  
**122**: $X = \text{O}$, $R = \text{Cl}$, $n = 1 \text{ or } 2$, $IC_{50} = ??$

**Figure 4.9: Comparison of carbon chains with oxa-isosteres.**

The analogues selected for synthesis were the unsubstituted phenyl, 4-methoxyphenyl as an example of an electron-donating group and 3,4-
dichlorophenyl as an example of an electron-withdrawing group, as well as being the most active compound from the N-aryloxyethyl series.

4.4.3.1. Synthesis

Having prepared the analogues of the N-aryloxyethyl series (see Section 4.4.1), and given the structural similarities between these two, I decided to try preparing these analogues via the same method. This worked very well, and in fact, the alkylation of the phenols proceeded in better yields than before, although the higher boiling point of dibromobutane and dibromopentane, compared with dibromoethane, made the purification of the bromo-intermediate a necessity. The alkylation of the nipecotate also proceeded in better yield than previously, giving nipecotates 125a-f (see Scheme 4.10).

\[
\begin{align*}
\text{HO-} & \text{Ar} & \text{Br(CH}_2\text{)}_{2+n}\text{Br} & \text{K}_2\text{CO}_3 & \text{Br} & \text{CO}_2\text{Et} \\
123a: & \text{Ar} = \text{Ph} & 71-100\% & 124a-f & n = 1, 2 & 61-82\% \\
123b: & \text{Ar} = 4-\text{MeOPh} & & & & \\
123c: & \text{Ar} = 3,4-\text{Cl}_2\text{Ph} & & & & \\
\end{align*}
\]

Scheme 4.10: Preparation of nipecotates 125a-f.

Reduction with LiAlH₄, followed by DCC-coupling then gave analogues 127a-f as before (see Scheme 4.11). No biological data is available at this time.
**Scheme 4.11: Preparation of N-aryloxyalkyl analogues 127a-f.**

4.4.4. Ring DE analogue of MLA

The results from the N-phenylalkyl series (see Section 4.4.2) raised some interesting questions. As the alkyl chain was lengthened, the potency of the analogues increased, but it appears that there are limits on the benefits of this lengthening. Perhaps that is due to the phenyl-ring fitting into a pocket in the receptor, and the longer chains allow a better fit. One possibility is that the phenyl-ring in these analogues is fitting where the D-ring of MLA fits when it binds to the receptor. This might explain why the cyclohexylpropyl derivative (117d) had double the potency of the corresponding phenyl analogue 14g (IC$_{50}$ = 5.0 µM vs. 11.4 µM, respectively). However, it doesn't explain the role of the methoxy-substituents on the D-ring in binding. In order to examine the effect of
that substitution, I decided to prepare an analogue containing what looked like
the D-ring of MLA, although the connectivity to the E-ring would be different.

![MLA and Ring DE Analogue](image)

**Figure 4.10: Structure of ring DE analogue 128.**

4.4.4.1. Retrosynthetic analysis

Using the standard procedures, I could prepare analogue 128 ($X = \text{CH}_2$), if I had access to tosylate 129 (shown in Scheme 4.12). This could possibly be prepared by manipulations of the hydroxyl-groups of commercially available cyclohexanetriol (130), followed by a Grignard reaction to introduce the three-carbon side-chain. However, although primary tosylates have been found to work quite well as substrates for Grignard reactions, secondary tosylates are generally fairly poor substrates for such reactions. In the case of the

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Grignard reaction not proceeding, a backup plan would be to prepare the oxaisostere (128, X = O). That synthesis should be fairly straightforward.

![Scheme 4.12: Retrosynthesis of DE-analogue 128.](image)

### 4.4.4.2. Synthesis

Starting off, I converted cyclohexanetriol (130) to monosilyl ether 131 according to the procedure published by Hilpert and co-workers. Exhaustive methylation then gave cis, cis-1-tert-butyl(dimethyl)silyloxy-3,5-dimethoxycyclohexane (132). Desilylation gave dimethoxycyclohexanol 133, and the tosylation of this compound then gave the desired substrate for a Grignard reaction (134).


Allyl magnesium chloride was the Grignard reagent of choice, since the olefin could then be readily converted to the terminal alcohol, which could be tosylated to give compound 129 (see Figure 4.10). The Grignard reaction was first attempted using a large excess of Grignard reagent and a catalytic amount of copper cyanide. This conversion was unsuccessful, so I increased the amount of cyanide, such that I had a 2:1 ratio between Grignard reagent and cyanide. No product was isolated from that reaction either. Finally, using a reagent commonly used in displacements of primary tosylates,131,135,136 I tried the reaction using lithium tetrachlorocuprate (Li$_2$CuCl$_4$).137 This attempt was unsuccessful as well, so I looked into preparing the oxa-analogue of 128.
cis, cis-Dimethoxycyclohexanol (133) was prepared as previously (see Schemes 4.13 and 4.14). Mitsunobu-conditions inverted the stereochemistry of the alcohol and subsequent base hydrolysis\textsuperscript{138} gave the trans, cis-dimethoxycyclohexanol (136). The alcohol was allylated to give ether 137, and ozonolysis of the alkene followed by a reductive work-up gave alcohol 138. This alcohol was then reacted with \( p \)-toluenesulfonfyl chloride to provide tosylate 129.

![Scheme 4.15: Synthesis of intermediate 129.](image)

Tosylate 129 was then used to alkylate ethyl nipecotate, giving ester 139. The ester was reduced and alcohol 140 was coupled to benzoic acid derivative 10 to give DE analogue 128 (see Scheme 4.16).
Scheme 4.16: Preparation of DE analogue 128.

I have prepared one analogue containing the E-ring of MLA as well a ring, which looks like the D-ring. Although the connectivity is slightly different, this ring might occupy the space that the D-ring of MLA would occupy during binding to the receptor. No biological data is available at this time.

4.5. Conclusions

A variety of ring E analogues of MLA have been prepared and some have been tested for inhibitory activity towards nicotinic receptors. The focus has turned towards α3-containing receptors, as that appears to be the area where these analogues would be more useful than other available nicotinic antagonists. Decisions have been taken on the direction of this project as the biological data has become available.
We tried varying the substitution on the benzoate. When we changed the substitution on the succinimide ring, we found that the larger substituents resulted in more potent analogues. However, the potency did not increase significantly by lengthening the chain beyond four carbons. Substitution directly on the benzoate resulted in a variety of analogues. The most potent were the phthalimide (108a) and the 2-phenyl analogue (108c), which are shown in Figure 4.11.

![Structures of the most potent benzoate analogues.](image)

More work has been done on the substitution on the piperidine nitrogen. That is where this project got started, and in order to expand on that part, I prepared a series of N-aryloxyethyl analogues, basing the choices of analogues on the Topliss-tree for aromatic substitution. The most potent compound was
the 3,4-dichloro analogue (112e), but the last analogue prepared (112k), has not been tested yet.

![Figure 4.12: Structures of analogues 112e and 112k.](image)

The three-carbon chain, linking the phenyl-ring with the piperidine nitrogen in compound 14g, was lengthened gradually to a six-carbon chain. The analogues with the five- and six-carbon chains were the most potent (117b and 117c, respectively, see Figure 4.13). Analogues with longer alkyl chains linking the phenyl to the nitrogen will be prepared in the future to examine whether the potency reaches a maximum.
The \( N \)-aryloxyethyl series and the \( N \)-phenylalkyl series were then combined into another series of compounds, the \( N \)-aryloxyalkyl series. Those contained butyl and pentyl chains, making them the oxaisosteres of compounds 117b and 117c. No data is currently available from this series.

The final part of my research was the design and synthesis of a ring DE analogue to try to probe whether the D-ring is significant in rendering antagonists competitive. To that end, analogue 128 was prepared, but no data is available at this time.
There is still much work that can be done to explore further possibilities for more active analogues, as well as probe the importance of different functional groups for binding to the receptor. All of the analogues, prepared by our group, that have been tested so far, have been found to be noncompetitive antagonists. In order to be able to compare the binding affinities of these ligands for different receptor subtypes and address selectivity, we need to make antagonists that are competitive in nature.
CHAPTER 5
EXPERIMENTAL

General. Chemical shifts are reported for chloroform-d solution in ppm relative to tetramethylsilane. $^1$H NMR and $^{13}$C NMR spectra were recorded at 250 MHz on a Bruker AF 250 model spectrometer or on a Bruker spectrometer with a Tecmag computer upgrade. Thin-layer chromatography was performed on pre-coated silica gel aluminum plates and visualized by UV-absorbance, staining with 5% phosphomolybdic acid (PMA) in ethanol or a solution of KMnO$_4$ in acetone. Purification was performed by flash column chromatography, using silica gel 60 (230-400 mesh), unless otherwise specified. Triethylamine and pyridine were distilled from calcium hydride and stored over potassium hydroxide. Dimethylformamide (DMF) was distilled from barium oxide and stored over 3 Å molecular sieves. Reactions, that did not contain water as a solvent, were carried out under inert (nitrogen or argon) atmosphere. Tetrahydrofuran (THF) and diethyl ether (Et$_2$O) were distilled from sodium and benzophenone just before use. Dichloromethane (CH$_2$Cl$_2$) and benzene were distilled from calcium
hydride just before use. 1,2-Dibromoethane and allyl bromide were distilled just before use. Diisopropylamine (i-Pr₂NH) was distilled under nitrogen atmosphere and stored over 3 Å molecular sieves.

![Image of molecule](image)

2-(3'-Methyl)succinimidobenzoic acid (10). Methylsuccinic anhydride (2.28 g, 20.0 mmol) was syringed into a flask and warmed to 35°C. Anthranilic acid (2.74 g, 20.0 mmol) was added in one portion and the reaction flask evacuated (~ 1 mm Hg). The reaction was heated under vacuum until all of the acid was dissolved (at 165°C), and then stirred at 145°C for 3.5 h. After cooling the reaction to rt, the mixture was chromatographed in Toluene/EtOAc/AcOH (90:5:5) giving 4.62 g (99%) of 10. ¹H NMR δ: 9.59-8.90 (bs, 1H), 8.21 (d, 1H, J = 8 Hz), 7.72 (t, 1H, J = 8 Hz), 7.56 (t, 1H, J = 8 Hz), 7.29 (d, 1H, J = 8 Hz), 3.11 (m, 2H), 2.54 (m, 1H), 1.46 (d, 3H, J = 8 Hz). ¹³C NMR δ: 180.23, 176.43, 169.95, 134.75, 133.36, 132.94, 130.30, 129.91, 126.80, 37.40, 35.54, 16.87.
Ethyl nipecotate hydrochloride (94). Ethanol (250 ml) was cooled to 0°C and AcCl (60 mmol, 4.3 ml) was added dropwise via syringe. Nipecotic acid (6.46 g, 50.0 mmol) was added in one portion, the reaction was allowed to warm to rt and then heated to reflux. After 17 h the reaction was cooled to rt and concentrated, giving 9.47 g (98%) of the ester hydrochloride (94). \(^1\)H NMR \(\delta: 9.70\) (bs, 1H), 4.20 (q, 2H, \(J = 7\) Hz), 3.53 (m, 2H), 3.10-2.70 (m, 3H), 2.30-1.80 (m, 3H), 1.61 (m, 1H), 1.28 (t, 3H, \(J = 7\) Hz). \(^13\)C NMR \(\delta: 171.25, 61.40, 44.74, 43.85, 38.42, 25.68, 21.49, 14.12\). HRMS calcd for C\(_8\)H\(_{16}\)NO\(_2\): 158.1176, found: 158.1178.

t-Butyl 2-ethylsuccinate (100a). \(^{126}\) n-BuLi (1.66 ml, 2.65 M in hexanes, 4.40 mmol) was added to a solution of diisopropylamine (0.62 ml, 4.40 mmol) in THF (4 ml) at -78°C. Mono-t-butylsuccinate \(^{125}\) (0.348 g, 2.00 mmol) was dissolved in THF (2 ml) and canulated into the reaction mixture. After stirring at 0°C for 2 h, the reaction was recooled to -78°C and freshly distilled EtI (0.22 ml, 2.80 mmol).
was added dropwise. The reaction was then allowed to warm to rt and stirred for 24 h. After quenching with water (1.2 ml) and concentrating the mixture, it was dissolved in EtOAc and washed with cold 1 M aq. HCl. The organic phase was dried with MgSO₄, filtered and concentrated. Flash chromatography using 25% EtOAc in hexanes gave 0.337 g (83%) of 100a. ¹H NMR δ: 2.65 (m, 2H), 2.42 (m, 1H), 1.61 (m, 2H), 1.42 (s, 9H), 0.92 (t, 3H, J = 8 Hz). ¹³C NMR δ: 177.03, 173.93, 80.77, 43.19, 35.27, 27.94, 24.95, 11.17.

[Chemical structure image]

2-Ethylsuccinic acid (101a). To a solution of 100a (0.141 g, 0.70 mmol) in CH₂Cl₂ (10 ml) was added trifluoroacetic acid (TFA, 1.61 ml, 20.9 mmol). After stirring at rt for 24 h, the mixture was concentrated. Flash chromatography in 40% EtOAc in hexanes gave 0.077 g (75%) of 101a. ¹H NMR δ: 11.11 (bs, 2H), 2.74 (m, 2H), 2.50 (m, 1H), 1.68 (m, 2H), 0.97 (t, 3H, J = 8 Hz). ¹³C NMR δ: 181.42, 178.66, 42.45, 35.23, 24.79, 11.32.

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3-Ethylsuccinic anhydride (102a). 2-Ethylsuccinic acid (101a, 0.075 g, 0.51 mmol) was dissolved in freshly distilled acetyl chloride (0.11 ml, 1.54 mmol) and the mixture was heated to reflux for 3 h. Cooling the reaction to rt and concentrating, gave 0.061 g (93%) of 102a. $^1$H NMR $\delta$: 3.07 (m, 2H), 2.67 (m, 1H), 2.01-1.63 (m, 2H), 1.03 (m, 3H). $^{13}$C NMR $\delta$: 173.46, 170.08, 41.91, 33.60, 23.86, 10.93.

3, 3-Dimethylsuccinic anhydride (102h). 2, 2-Dimethylsuccinic acid (0.292 g, 2.00 mmol) was dissolved in freshly distilled acetyl chloride (0.87 ml, 12.3 mmol) and the mixture was heated to reflux for 4 h. Cooling the reaction to rt and concentrating, gave 0.209 g (81%) of 102h. $^1$H NMR $\delta$: 2.79 (s, 2H), 1.42 (s, 6H). $^{13}$C NMR $\delta$: 42.82, 41.86, 25.30.
3, 4-Dimethylsuccinic anhydride (102i). 2, 3-Dimethylsuccinic acid (0.292 g, 2.00 mmol) was dissolved in freshly distilled acetyl chloride (0.87 ml, 12.3 mmol) and the mixture was heated to reflux for 4 h. Cooling the reaction to rt and concentrating, gave 0.259 g (100%) of 102i. \(^1\)H NMR \(\delta\): 2.74 (m, 2H), 1.44-1.13 (m, 6H). \(^13\)C NMR \(\delta\): 42.21, 14.15.

2-(3'-Ethyl)succinimidobenzoic acid (103a). 3-Ethylsuccinic anhydride (102a, 0.198 g, 1.54 mmol) and anthranilic acid (0.211 g, 1.54 mmol) were added to a flask and the reaction flask evacuated (down to around 1 mm Hg). The reaction was heated to 160°C under vacuum, and then allowed to cool to 145°C and stirred for 3 h. After cooling the reaction to rt, the mixture was chromatographed in Toluene/EtOAc/AcOH (90:5:5) giving 0.223 g (59%) of 103a. \(^1\)H NMR \(\delta\): 9.87 (bs, 1H), 8.16 (d, 1H, \(J = 8\) Hz), 7.67 (t, 1H, \(J = 8\) Hz), 7.51 (t, 1H, \(J = 8\) Hz), 7.24...
(d, 1H, J = 8 Hz), 2.96 (m, 2H), 2.57 (m, 1H), 2.00 (bs, 1H), 1.70 (bs, 1H), 1.05 (t, 3H, J = 7 Hz).

2-(3', 3'-Dimethyl)succinimidobenzoic acid (103h). 3, 3-Dimethylsuccinic anhydride (102h, 0.209 g, 1.63 mmol) and anthranilic acid (0.223 g, 1.63 mmol) were added to a flask and the reaction flask evacuated (down to around 1 mm Hg). The reaction was heated to 160°C under vacuum, and then allowed to cool to 145°C and stirred for 3 h. After cooling the reaction to rt, the mixture was chromatographed in Toluene/EtOAc/AcOH (90:5:5) giving 0.137 g (34%) of 103h. 1H NMR δ: 10.71 (bs, 1H), 8.17 (d, 1H, J = 8 Hz), 7.66 (t, 1H, J = 8 Hz), 7.50 (t, 1H, J = 8 Hz), 7.25 (d, 1H, J = 8 Hz), 2.72 (s, 2H), 1.41 (s, 6H). 13C NMR δ: 134.24, 132.84, 132.43, 129.81, 129.37, 126.46, 44.10, 40.46, 25.82, 25.09.
2-(3', 4'-Dimethyl)succinimidobenzoic acid (103i). 3, 4-Dimethylsuccinic anhydride (102i, 0.256 g, 2.00 mmol) and anthranilic acid (0.274 g, 2.00 mmol) were added to a flask and the reaction flask evacuated (down to around 1 mm Hg). The reaction was heated to 160°C under vacuum, and then allowed to cool to 145°C and stirred for 3 h. After cooling the reaction to rt, the mixture was chromatographed in Toluene/EtOAc/AcOH (90:5:5) giving 0.325 g (66%) of 103i.

\( ^1H \text{ NMR } \delta: 11.57 \text{ (bs, 1H), 8.15 \text{ (m, 1H), 7.65 \text{ (m, 1H), 7.49 \text{ (m, 1H), 7.24 \text{ (m, 1H), 2.59 \text{ (m, 2H), 1.39 \text{ (m, 6H).} } \text{^{13}C NMR } \delta: 134.18, 132.90, 132.37, 129.78, 129.32, 126.26, 43.67, 14.76.} } \)
$N$-(3'-Phenyl-1'-propyl)piperidinyl-3-methyl 2''-(3'''-ethyl)succinimidobenzoate (104a). DMAP (0.011 g, 0.09 mmol) was added to a solution of 103a (0.223 g, 0.90 mmol) in CH$_2$Cl$_2$ (3.0 ml). A solution of 3-hydroxymethyl-1-(3'-phenylpropyl)piperidine (0.211 g, 0.90 mmol) in CH$_2$Cl$_2$ (1.0 ml) was cannulated into the reaction mixture, which was then cooled to 0°C and DCC (0.186 g, 0.90 mmol) was added in one portion. The reaction was warmed to rt and stirred for 20 h. At that time the reaction was diluted with Et$_2$O, and the precipitate removed by filtration. Chromatography in 2% MeOH in CHCl$_3$ gave 0.277 g (66%) of 104a. $^1$H NMR $\delta$: 8.08 (d, 1H, $J = 8$ Hz), 7.63 (t, 1H, $J = 8$ Hz), 7.48 (t, 1H, $J = 8$ Hz), 7.28-7.12 (m, 6H), 4.08 (m, 2H), 3.06-2.74 (m, 4H), 2.61 (t, 2H, $J = 8$ Hz), 2.59 (m, 1H), 2.35 (t, 2H, $J = 8$ Hz), 2.13-1.54 (m, 10H), 1.05 (m, 4H). $^{13}$C NMR $\delta$: 179.13, 176.06, 164.30, 142.12, 133.32, 132.66, 131.58, 131.42, 129.79, 129.29, 128.39, 128.24, 125.68, 67.96, 58.41, 57.09, 53.98, 41.79, 35.78, 34.57, 34.38, 33.72, 28.56, 27.31, 24.68, 24.25, 10.90. HRMS calcd for C$_{28}$H$_{35}$N$_2$O$_4$:+ 463.2591, found: 463.2552.
N-(3'-Phenyl-1'-propyl)piperidinyl-3-methyl 2''-(3''', 3'''-dimethyl)succinimidobenzoate (104h). DMAP (0.007 g, 0.06 mmol) was added to a solution of 103h (0.137 g, 0.55 mmol) in CH$_2$Cl$_2$ (1.1 ml). A solution of 3-hydroxymethyl-1-(3'-phenylpropyl)piperidine (0.129 g, 0.55 mmol) in CH$_2$Cl$_2$ (1.1 ml) was canulated into the reaction mixture, which was then cooled to 0°C and DCC (0.114 g, 0.55 mmol) was added in one portion. The reaction was warmed to rt and stirred for 18 h. At that time the reaction was diluted with Et$_2$O, and the precipitate removed by filtration. Chromatography in 2% MeOH in CHCl$_3$ gave 0.216 g (85%) of 104h. $^1$H NMR $\delta$: 8.08 (m, 1H), 7.64 (m, 1H), 7.50 (m, 1H), 7.31-7.12 (m, 6H), 4.11 (m, 2H), 2.97-2.57 (m, 8H), 2.37 (m, 2H), 2.17-1.53 (m, 7H), 1.45 (bs, 6H), 1.10 (m, 1H). $^{13}$C NMR $\delta$: 157.70, 142.15, 133.25, 131.38, 129.75, 129.23, 128.38, 128.24, 127.48, 125.68, 67.92, 58.43, 57.09, 54.00, 44.10, 40.49, 35.78, 33.74, 28.53, 27.31, 26.02, 24.68.
N-(3'-Phenyl-1'-propyl)piperidinyl-3-methyl 2''-(3''', 4'''-dimethyl)succinimidobenzoate (104i). DMAP (0.016 g, 0.13 mmol) was added to a solution of 103i (0.325 g, 1.31 mmol) in CH$_2$Cl$_2$ (4.0 ml). A solution of 3-hydroxymethyl-1-(3'-phenylpropyl)piperidine (0.307 g, 1.31 mmol) in CH$_2$Cl$_2$ (1.0 ml) was cannulated into the reaction mixture, which was then cooled to 0°C and DCC (0.271 g, 1.31 mmol) was added in one portion. The reaction was warmed to rt and stirred for 18 h. At that time the reaction was diluted with Et$_2$O, and the precipitate removed by filtration. Chromatography in 2% MeOH in CHCl$_3$ gave 0.507 g (83%) of 104i. $^1$H NMR δ: 8.06 (d, 1H, J = 8 Hz), 7.63 (t, 1H, J = 8 Hz), 7.48 (t, 1H, J = 8 Hz), 7.29-7.13 (m, 6H), 4.09 (m, 2H), 2.96-2.76 (m, 2H), 2.72-2.54 (bs, 1H), 2.61 (t, 2H, J = 8 Hz), 2.36 (t, 2H, J = 8 Hz), 2.16-1.53 (m, 8H), 1.42 (d, 6H, J = 7 Hz), 1.09 (m, 1H). $^{13}$C NMR δ: 157.03, 142.07, 133.30, 131.44, 129.78, 129.20, 128.35, 128.27, 125.71, 67.86, 58.40, 57.03, 53.94, 43.52, 35.72, 33.71, 28.44, 27.25, 24.60, 14.73.

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General procedure for the preparation of salts of compounds 104a, h, i.

Benzoate (104a, h, i, 1 eq) was dissolved in a minimal amount of EtOAc, oxalic acid (1 eq) was added and stirred at rt. The mixture was concentrated to give the salt of the benzoate.

\[ \text{106} \]

2-Phthalimidobenzoic acid (106). Phthalic anhydride (0.148 g, 1.00 mmol) and anthranilic acid (0.137 g, 1.00 mmol) were added to a flask and the reaction flask evacuated (down to around 1 mm Hg). The reaction was heated to 180°C under vacuum, and then allowed to cool to 145°C and stirred for 7 h. After cooling the reaction to rt, the mixture was chromatographed in Toluene/EtOAc/AcOH (90:5:5) giving 0.116 g (43%) of 106. $^1$H NMR $\delta$: 8.16 (d, 1H, $J = 8$ Hz), 7.96-7.88 (m, 2H), 7.80-7.66 (m, 3H), 7.53 (t, 1H, $J = 8$ Hz), 7.38 (d, 1H, $J = 7$ Hz).

$^{13}$C NMR $\delta$: 134.26, 134.00, 132.31, 130.39, 129.17, 123.81.

General procedure for the preparation of benzoates 108a-h. DMAP (0.1 eq) was added to a solution of the appropriately substituted benzoic acid (1 eq) in
CH$_2$Cl$_2$ (1 M). A solution of 3-hydroxymethyl-1-(3´-phenyl(propyl)piperidine (1 eq) in CH$_2$Cl$_2$ (0.33 M) was canulated into the reaction mixture, which was then cooled to 0°C and DCC (1 eq) was added in one portion. The reaction was warmed to rt and stirred for 20 h. At that time the reaction was diluted with Et$_2$O, and the precipitate removed by filtration. Chromatography in 2% MeOH in CHCl$_3$ gave benzoates 108a-h.

\[ \text{108a} \]

**N-(3´-Phenyl-1´-propyl)piperidinyl-3-methyl 2”-phthalimidobenzoate (108a).**
The general procedure for the coupling was followed, starting with benzoic acid 11 (0.119 g, 0.45 mmol). Chromatography gave 0.125 g (58%) of 108a. $^1$H NMR $\delta$: 8.19 (d, 1H, $J = 8$ Hz), 7.98 (m, 2H), 7.81 (m, 2H), 7.74 (t, 1H, $J = 8$ Hz), 7.59 (t, 1H, $J = 8$ Hz), 7.44 (d, 1H, $J = 8$ Hz), 7.36-7.14 (m, 5H), 4.08 (m, 2H), 2.81 (m, 2H), 2.66 (t, 2H, $J = 8$ Hz), 2.34 (t, 2H, $J = 8$ Hz), 1.99-1.46 (m, 8H), 1.00 (m, 1H). $^{13}$C NMR $\delta$: 142.12, 134.21, 133.13, 132.11, 131.64, 131.56,
N-(3'-Phenyl-1'-propyl)piperidinyl-3-methyl 2''-acetamidobenzoate (108b).

The general procedure for the coupling was followed, starting with N-acetylanthranilic acid (0.101 g, 0.57 mmol). Chromatography gave 0.100 g (45%) of 108b. $^1$H NMR $\delta$: 8.76 (d, 1H, $J = 8$ Hz), 8.06 (d, 1H, $J = 8$ Hz), 7.60 (t, 1H, $J = 8$ Hz), 7.36-7.18 (m, 5H), 7.13 (t, 1H, $J = 7$ Hz), 4.26 (m, 2H), 3.06-2.86 (m, 2H), 2.69 (t, 2H, $J = 8$ Hz), 2.45 (t, 2H, $J = 7$ Hz), 2.29 (s, 3H), 2.22 (bs, 1H), 2.13-1.65 (m, 11H), 1.10-1.52 (m, 4H). $^{13}$C NMR $\delta$: 141.60, 134.61, 130.60, 128.33, 128.27, 125.73, 122.39, 120.32, 67.95, 58.37, 57.03, 53.94, 35.69, 33.68, 28.47, 27.28, 25.50, 24.95.
N-(3'-Phenyl-1'-propyl)piperidinyl-3-methyl 2''-phenylbenzoate (108c). The general procedure for the coupling was followed, starting with 2-biphenylcarboxylic acid (0.130 g, 0.66 mmol). Chromatography gave 0.219 g (80%) of 108c. $^1$H NMR δ: 7.86 (m, 1H), 7.56 (m, 1H), 7.49-7.17 (m, 12H), 3.94 (m, 2H), 2.77 (d, 1H, $J = 11$ Hz), 2.66 (m, 2H), 2.52 (d, 1H, $J = 11$ Hz), 2.30 (m, 2H), 1.91-1.25 (m, 8H), 0.81 (m, 1H). $^{13}$C NMR δ: 142.12, 141.43, 131.12, 131.06, 130.63, 129.75, 128.33, 128.24, 128.09, 127.16, 127.07, 125.68, 67.98, 58.25, 56.62, 53.86, 49.02, 35.34, 33.71, 28.47, 24.63.
*N*-(*3'-Phenyl-1'-propyl)piperidiny1-3-methyl 4'-phenylbenzoate (108d). The general procedure for the coupling was followed, starting with 4-biphenylcarboxylic acid (0.085 g, 0.43 mmol). Chromatography gave 0.144 g (81%) of 108d. $^1$H NMR δ: 8.16 (d, 2H, $J = 9$ Hz), 7.69 (m, 4H), 7.51 (m, 2H), 7.36-7.16 (m, 6H), 4.27 (m, 2H), 3.03 (d, 1H, $J = 11$ Hz), 2.90 (d, 1H, $J = 11$ Hz), 2.68 (t, 2H, $J = 8$ Hz), 2.44 (t, 2H, $J = 8$ Hz), 2.19 (m, 1H), 2.08-1.58 (m, 6H), 1.18 (m, 2H). $^{13}$C NMR δ: 151.62, 145.56, 142.12, 139.97, 130.01, 129.93, 129.00, 128.88, 128.35, 128.24, 128.06, 127.22, 126.99, 125.65, 67.68, 58.46, 57.23, 54.06, 49.08, 35.95, 33.74, 28.59, 24.77.
N-(3'-Phenyl-1'-propyl)piperidinyl-3-methyl 2''-chlorobenzoate (108e). The general procedure for the coupling was followed, starting with 2-chlorobenzoic acid (0.077 g, 0.49 mmol). Chromatography gave 0.168 g (92%) of 108e. \(^1\)H NMR \(\delta\): 7.86 (d, 1H, J = 9 Hz), 7.53-7.17 (m, 8H), 4.27 (m, 2H), 3.03 (d, 1H, J = 11 Hz), 2.87 (d, 1H, J = 11 Hz), 2.66 (t, 2H, J = 8 Hz), 2.41 (t, 2H, J = 8 Hz), 2.17 (m, 1H), 2.08-1.58 (m, 6 H), 1.49-1.09 (m, 2H). \(^{13}\)C NMR \(\delta\): 157.00, 142.12, 132.40, 131.35, 131.00, 128.33, 128.21, 126.52, 125.62, 68.35, 58.40, 57.09, 54.06, 35.81, 33.71, 28.59, 27.36, 24.71.
N-(3'-Phenyl-1'-propyl)piperidinyl-3-methyl 2''-methoxybenzoate (108f). The general procedure for the coupling was followed, starting with 2-methoxybenzoic acid (0.080 g, 0.52 mmol). Chromatography gave 0.154 g (80%) of 108f. 

$^1$H NMR δ: 7.78 (m, 1H), 7.47 (m, 1H), 7.33–7.11 (m, 5H), 6.97 (m, 2H), 4.17 (m, 2H), 3.88 (s, 3H), 2.99 (bs, 1H), 2.85 (bs, 1H), 2.63 (m, 2H), 2.39 (m, 2H), 2.21–1.57 (m, 8 H), 1.13 (m, 1H). 

$^{13}$C NMR δ: 156.80, 133.39, 131.56, 128.35, 128.24, 125.68, 120.09, 111.96, 67.57, 58.48, 57.17, 55.86, 54.12, 35.89, 33.77, 28.56, 27.36, 24.77.
N-(3'-Phenyl-1'-propyl)piperidinyl-3-methyl 2''-trifluoromethylbenzoate (108g). The general procedure for the coupling was followed, starting with 2-(trifluoromethyl)benzoic acid (0.109 g, 0.57 mmol). Chromatography gave 0.200 g (86%) of 108g. \(^1\)H NMR \(\delta\): 7.80 (m, 2H), 7.64 (m, 2H), 7.38-7.15 (m, 5H), 4.26 (m, 2H), 2.98 (bs, 1H), 2.84 (bs, 1H), 2.67 (m, 2H), 2.40 (m, 2H), 2.28-1.57 (m, 8H), 1.22 (m, 1H). \(^1\)\(^3\)C NMR \(\delta\): 156.86, 142.15, 131.67, 131.00, 130.10, 128.33, 128.18, 126.64, 126.55, 125.62, 121.13, 111.29, 68.91, 58.37, 56.85, 54.12, 35.63, 33.68, 28.56, 27.25, 24.66.
N-((3'-Phenyl-1'-propyl)piperidinyl-3-methyl 2''-fluorobenzoate (108h). The general procedure for the coupling was followed, starting with 2-fluorobenzoic acid (0.071 g, 0.51 mmol). Chromatography gave 0.089 g (49%) of 108h. H NMR δ: 8.18 (m, 2H), 7.91-7.15 (m, 7H), 4.45 (m, 2H), 3.43-1.05 (m, 15H). 13C NMR δ: 158.43, 157.03, 142.15, 134.41, 134.26, 132.02, 128.33, 128.21, 125.62, 123.90, 123.84, 116.74, 68.03, 58.40, 57.03, 54.06, 35.81, 33.71, 28.56, 27.28, 24.74.

General procedure for the preparation of salts of compounds 108a-h.
Benzoate (108a-h, 1 eq) was dissolved in a minimal amount of EtOAc, oxalic acid (1 eq) was added and stirred at rt. The mixture was concentrated to give the salt of the benzoate.

General procedure for the preparation of bromoethyl aryl ethers (114a-k).
To a solution of substituted phenol (1 eq) in acetone (1 M) was added K₂CO₃ (1 eq) in one portion and then 1,2-dibromoethane (10 eq) by syringe. The reaction
was heated to reflux for 20 h. After allowing the solvent to evaporate, water was added and the mixture was extracted with benzene (2x). The organic phase was washed with 10% aq. NaOH (3x). The organic phase was dried with MgSO₄, filtered and concentrated. The product was taken on as the crude material unless otherwise stated.

\[ \text{2-(4'-Methoxyphenyl)oxyethyl bromide (114a).} \]

The general procedure for the preparation of bromoethyl aryl ethers was followed, starting with 4-methoxyphenol (0.248 g, 2.00 mmol). Work-up gave 0.221 g (48%) of 114a. \( ^1 \text{H NMR } \delta: 6.83 \text{ (m, 4H), 4.24 \text{ (t, 2H, } J = 6 \text{ Hz), 3.89 \text{ (s, 3H), 3.61 \text{ (t, 2H, } J = 6 \text{ Hz).} } \)
\( ^{13} \text{C NMR } \delta: 154.82, 152.61, 116.51, 115.14, 69.24, 56.12, 29.70. \]

\[ \text{2-(2'-Naphthyl)oxyethyl bromide (114b).} \]

The general procedure for the preparation of bromoethyl aryl ethers was followed, starting with 2-naphthol
(0.288 g, 2.00 mmol). Chromatography in 2% EtOAc in hexanes gave 0.190 g (38%) of 114b. $^1$H NMR $\delta$: 7.80-7.10 (m, 7H), 4.43 (t, 2H, $J = 6$ Hz), 3.72 (t, 2H, $J = 6$ Hz). $^{13}$C NMR $\delta$: 130.05, 128.07, 127.18, 126.90, 124.34, 119.11, 107.57, 68.28, 29.38.

2-(4'-Methylphenyl)oxyethyl bromide (114c). The general procedure for the preparation of bromoethyl aryl ethers was followed, starting with $p$-cresol (4-methylphenol, 4-hydroxytoluene, 0.324 g, 3.00 mmol). Work-up gave 0.220 g (34%) of 114c. $^1$H NMR $\delta$: 7.10 (d, 2H, $J = 8$ Hz), 6.81 (d, 2H, $J = 8$ Hz), 4.27 (t, 2H, $J = 6$ Hz), 3.62 (t, 2H, $J = 6$ Hz), 2.30 (s, 3H). $^{13}$C NMR $\delta$: 156.39, 131.17, 130.41, 115.15, 68.48, 29.60, 20.86.

2-(4'-Chlorophenyl)oxyethyl bromide (114d). The general procedure for the preparation of bromoethyl aryl ethers was followed, starting with 4-
chlorophenol (0.386 g, 3.00 mmol). Work-up gave 0.430 g (61%) of \textbf{114d}. $^1$H NMR $\delta$: 7.25 (d, 2H, $J = 9$ Hz), 6.85 (d, 2H, $J = 9$ Hz), 4.28 (t, 2H, $J = 6$ Hz), 3.62 (t, 2H, $J = 6$ Hz). $^{13}$C NMR $\delta$: 157.12, 129.87, 126.80, 116.50, 68.60, 29.23.

\begin{center}
\includegraphics[width=0.5\textwidth]{114e}
\end{center}

\textbf{2-(3',4'-Dichlorophenyl)oxyethyl bromide (114e).} The general procedure for the preparation of bromoethyl aryl ethers was followed, starting with 3,4-dichlorophenol (0.489 g, 3.00 mmol). Work-up gave 0.706 g (87%) of \textbf{114e}. $^1$H NMR $\delta$: 7.34 (d, 1H, $J = 9$ Hz), 7.01 (d, 1H, $J = 2$ Hz), 6.79 (dd, 1H, $J = 9$ Hz and 2 Hz), 4.28 (t, 2H, $J = 6$ Hz), 3.63 (t, 2H, $J = 6$ Hz). $^{13}$C NMR $\delta$: 157.52, 133.43, 131.22, 125.21, 117.13, 115.10, 68.76, 28.91.

\begin{center}
\includegraphics[width=0.5\textwidth]{114f}
\end{center}

\textbf{2-(3'-Chlorophenyl)oxyethyl bromide (114f).} The general procedure for the preparation of bromoethyl aryl ethers was followed, starting with 3-chlorophenol (0.386 g, 3.00 mmol). Work-up gave 0.531 g (75%) of \textbf{114f}. $^1$H
NMR: δ: 7.21 (m, 1H), 6.95 (m, 2H), 6.80 (m, 1H), 4.29 (t, 2H, J = 6 Hz), 3.64 (t, 2H, J = 6 Hz). 13C NMR: δ: 159.22, 135.41, 130.69, 122.05, 115.66, 113.59, 68.44, 29.12.

2-\((1'\text{-Naphthyl})\text{oxyethyl} \) bromide (114g). The general procedure for the preparation of bromoethyl aryl ethers was followed, starting with 1-naphthol (0.433 g, 3.00 mmol). Work-up gave 0.418 g (55%) of 114g. 1H NMR: δ: 8.30 (m, 1H), 7.79 (m, 1H), 7.49 (m, 3H), 7.36 (t, 1H, J = 8 Hz), 6.81 (d, 1H, J = 8 Hz), 4.47 (t, 2H, J = 6 Hz), 3.78 (t, 2H, J = 6 Hz). 13C NMR: δ: 153.88, 134.58, 127.46, 126.57, 125.66, 125.42, 122.02, 121.03, 105.11, 68.03, 29.24.

2-\((4'\text{-Biphenyl})\text{oxyethyl} \) bromide (114h). The general procedure for the preparation of bromoethyl aryl ethers was followed, starting with 4-phenylphenol.
(4-biphenol, 0.511 g, 3.00 mmol). Work-up gave 0.443 g (53%) of 114h. \(^1H\) NMR \(\delta\): 7.69-7.31 (m, 7H), 7.00 (m, 2H), 4.34 (m, 2H), 3.68 (m, 2H). \(^13C\) NMR \(\delta\): 158.04, 141.04, 135.00, 129.14, 128.68, 127.16, 115.48, 68.39, 29.45.

2-(2’-Biphenyl)oxyethyl bromide (114i). The general procedure for the preparation of bromoethyl aryl ethers was followed, starting with 2-phenylphenol (2-biphenol, 0.511 g, 3.00 mmol). Chromatography in hexanes (increasing the polarity to 2% EtOAc in hexanes) gave 0.214 g (26%) of 114i. \(^1H\) NMR \(\delta\): 7.86 (m, 2H), 7.74-7.50 (m, 5H), 7.41-7.20 (m, 2H), 4.54 (m, 2H), 3.82 (m, 2H). \(^13C\) NMR \(\delta\): 157.38, 131.15, 129.64, 128.59, 127.95, 126.96, 121.86, 113.19, 68.56, 29.17.
2-(3'-Biphenyl)oxyethyl bromide (114j). The general procedure for the preparation of bromoethyl aryl ethers was followed, starting with 3-phenylphenol (3-biphenol, 0.511 g, 3.00 mmol). Chromatography in hexanes (increasing the polarity to 1% EtOAc in hexanes) gave 0.367 g (44%) of 114j. \(^1\)H NMR \(\delta\): 7.50-7.38 (m, 2H), 7.36-7.19 (m, 4H), 7.13-7.01 (m, 2H), 6.89-6.74 (m, 1H), 4.22 (t, 2H, \(J = 6\) Hz), 3.53 (t, 2H, \(J = 6\) Hz). \(^1\)C NMR \(\delta\): 158.43, 129.84, 128.73, 127.48, 127.13, 126.72, 120.41, 113.83, 113.42, 67.95, 29.14. HRMS calcd for C\(_{14}\)H\(_{13}\)BrO*: 276.0144, found: 276.0137.

2-(4'-Chloro-3'-trifluoromethyl)oxyethyl bromide (114k). The general procedure for the preparation of bromoethyl aryl ethers was followed, starting with 4-chloro-3-trifluoromethylphenol (0.393 g, 2.00 mmol). Work-up gave 0.539
g (53%) of 114k. \(^1\)H NMR \(\delta\): 7.41 (d, 1H, \(J = 9\) Hz), 7.23 (d, 1H, \(J = 3\) Hz), 7.01 (dd, 1H, \(J = 9\) Hz and 3 Hz), 4.31 (t, 2H, \(J = 6\) Hz), 3.66 (t, 2H, \(J = 6\) Hz). \(^{13}\)C \(\text{NMR } \delta\): 156.54, 132.47, 129.48, 120.36, 119.70, 118.77, 116.01, 114.30, 114.23, 114.15, 114.03, 68.38, 28.48. HRMS calcd for C\(_9\)H\(_7\)BrClF\(_3\)O\(^+\): 301.9315, found: 301.9327.

**General procedure for the preparation of \(N\)-aryloxyethyl nipecotates (116a-k).** Ethyl nipecotate hydrochloride (94, 1 eq) was dissolved in EtOH (1 M) and K\(_2\)CO\(_3\) (4 eq) added. Aryloxyethyl bromide (114a-k, 1.2 eq) was canulated into the reaction and heated to reflux. After 18 h, the reaction was cooled to rt, water added and the mixture was extracted with EtOAc (3x). The organic phase was washed with brine, dried with MgSO\(_4\), filtered and concentrated. Chromatography gave compounds 116a-k.

![116a](image-url)

**Ethyl \(N\)-(2'-(4''-methoxyphenyl)oxyethyl)nipecotate (116a).** The general procedure for the preparation of \(N\)-arylxyethylnipecotates was followed, starting with bromide 114a (0.221 g, 0.96 mmol). Chromatography in 40% EtOAc in
hexanes gave 0.103 g (42%) of 116a. $^1$H NMR δ: 6.82 (s, 4H), 4.12 (q, 2H, J = 7 Hz), 4.06 (t, 2H, J = 6 Hz), 3.77 (s, 3H), 3.10 (d, 1H, J = 12 Hz), 2.85 (m, 1H), 2.80 (t, 2H, J = 6 Hz), 2.59 (m, 1H), 2.30 (t, 1H, J = 11 Hz), 2.13 (t, 1H, J = 11 Hz), 1.96 (m, 1H), 1.80-1.40 (m, 3H), 1.25 (t, 3H, J = 7 Hz). $^{13}$C NMR δ: 174.52, 154.29, 153.37, 116.03, 115.02, 66.99, 60.69, 57.96, 56.29, 56.13, 54.53, 42.26, 27.22, 24.98, 14.60. HRMS calcd for C$_{17}$H$_{26}$NO$_4$: 308.1856, found: 308.1871.

![116b](image)

Ethyl N-(2'-(2''-naphthyl)oxyethyl)nipecotate (116b). The general procedure for the preparation of N-aryloxyethylnipecotates was followed, starting with bromide 114b (0.187 g, 0.745 mmol). Chromatography in 25% EtOAc in hexanes gave 0.088 g (43%) of 116b. $^1$H NMR δ: 7.74 (m, 3H), 7.52-7.13 (m, 4H), 4.23 (t, 2H, J = 6 Hz), 4.12 (q, 2H, J = 7 Hz), 3.13 (d, 1H, J = 12 Hz), 3.00-2.81 (bs, 1H), 2.90 (t, 2H, J = 6 Hz), 2.60 (m, 1H), 2.36 (t, 1H, J = 11 Hz), 2.20 (t, 1H, J = 11 Hz), 1.97 (m, 1H), 1.90-1.40 (m, 3H), 1.26 (t, 3H, J = 7 Hz). $^{13}$C NMR δ: 174.48, 157.14, 134.94, 129.74, 129.40, 128.02, 127.13, 126.73, 124.01, 119.39, 107.19, 66.30, 60.73, 57.83, 56.31, 54.57, 42.25, 27.20, 24.98, 14.61. HRMS calcd for C$_{20}$H$_{26}$NO$_3$: 328.1907, found: 328.1894.
Ethyl 1-(2′-(4′-methylphenyl)oxyethyl)nipecotate (116c). The general procedure for the preparation of N-aryloxyethylnipecotates was followed, starting with bromide 114c (0.220 g, 1.02 mmol). Chromatography in 25% EtOAc in hexanes gave 0.169 g (68%) of 116c.  

$^1$H NMR δ: 7.09 (d, 2H, $J = 8$ Hz), 6.80 (d, 2H, $J = 8$ Hz), 4.10 (m, 4H), 3.10 (d, 1H, $J = 12$ Hz), 2.85 (m, 1H), 2.81 (t, 2H, $J = 6$ Hz), 2.60 (m, 1H), 2.28 (s, 4H), 1.96 (m, 1H), 1.90-1.40 (m, 3H), 1.23 (t, 3H, $J = 7$ Hz).  

$^{13}$C NMR δ: 174.53, 157.07, 130.36, 130.25, 114.90, 66.37, 60.69, 57.90, 56.29, 54.52, 42.28, 27.22, 25.00, 20.84, 14.60. HRMS calcd for C$_{17}$H$_{26}$NO$_3$+: 292.1907, found: 292.1903.
Ethyl 1-(2'-(4''-chlorophenyl)oxyethyl)nipecotate (116d). The general procedure for the preparation of N-aryloxyethylnipecotates was followed, starting with bromide 114d (0.430 g, 1.83 mmol). Chromatography in 25% EtOAc in hexanes (increasing to 40%) gave 0.271 g (57%) of 116d. $^1$H NMR $\delta$: 7.22 (d, 2H, $J = 8$ Hz), 6.82 (d, 2H, $J = 8$ Hz), 4.10 (m, 4H, $J = 7$ Hz and 6 Hz), 3.08 (d, 1H, $J = 12$ Hz), 2.86 (m, 1H), 2.81 (t, 2H, $J = 6$ Hz), 2.58 (m, 1H), 2.31 (t, 1H, $J = 11$ Hz), 2.14 (t, 1H, $J = 11$ Hz), 1.96 (m, 1H), 1.80-1.40 (m, 3H), 1.23 (t, 3H, $J = 7$ Hz). $^{13}$C NMR $\delta$: 174.47, 157.81, 129.67, 126.03, 116.31, 66.71, 60.71, 57.75, 56.29, 54.57, 42.24, 27.17, 24.98, 14.60. HRMS calcd for C$_{16}$H$_{23}$ClNO$_3$*: 312.1361, found: 312.1351.
Ethyl 1-(2''-(3''''-dichlorophenyl)oxyethyl)nipecotate (116e). The general procedure for the preparation of \( N \)-aryloxyethylnipecotates was followed, starting with bromide 114e (0.706 g, 2.62 mmol). Chromatography in 25% EtOAc in hexanes (increasing to 40%) gave 0.411 g (54%) of 116e. \(^1\)H NMR \( \delta \): 7.32 (d, 1H, \( J = 7 \) Hz), 7.01 (d, 1H, \( J = 2 \) Hz), 6.77 (dd, 1H, \( J = 7 \) Hz and 2 Hz), 4.13 (q, 2H, \( J \approx 7 \) Hz), 4.04 (t, 2H, \( J = 6 \) Hz), 3.04 (d, 1H, \( J = 12 \) Hz), 2.85 (m, 1H), 2.80 (t, 2H, \( J = 6 \) Hz), 2.59 (m, 1H), 2.32 (t, 1H, \( J = 11 \) Hz), 2.16 (t, 1H, \( J = 11 \) Hz), 1.80-1.40 (m, 3H), 1.24 (t, 3H, \( J = 7 \) Hz). \(^1\)C NMR \( \delta \): 174.42, 158.27, 133.20, 131.01, 124.40, 116.92, 115.07, 67.03, 60.73, 57.61, 56.27, 54.56, 42.22, 27.13, 24.96, 14.60.
Ethyl 1-(2'-(3''-chlorophenyl)oxyethyl)nipecotate (116f). The general procedure for the preparation of $N$-aryloxyethylnipecotates was followed, starting with bromide 114f (0.531 g, 2.25 mmol). Chromatography in 25% EtOAc in hexanes (increasing to 40%) gave 0.129 g (22%) of 116f. $^1$H NMR $\delta$: 7.19 (t, 1H, $J = 8$ Hz), 6.95 (m, 2H), 6.79 (m, 1H), 4.09 (m, 4H), 3.07 (d, 1H, $J = 12$ Hz), 2.85 (m, 1H), 2.82 (t, 2H, $J = 6$ Hz), 2.59 (m, 1H), 2.32 (t, 1H, $J = 11$ Hz), 2.18 (t, 1H, $J = 11$ Hz), 1.98 (m, 1H), 1.80-1.40 (m, 3H), 1.26 (t, 3H, $J = 7$ Hz). $^{13}$C NMR $\delta$: 174.46, 159.95, 135.23, 130.56, 121.33, 115.46, 113.52, 66.63, 60.72, 57.69, 56.27, 54.54, 42.24, 27.16, 24.98, 14.60.
Ethyl 1-(2'-(1''-naphthyl)oxyethyl)nipecotate (116g). The general procedure for the preparation of N-aryloxyethylnipecotates was followed, starting with bromide 114g (0.418 g, 1.67 mmol). Chromatography in 25% EtOAc in hexanes gave 0.145 g (27%) of 116g. $^1$H NMR δ: 8.28 (m, 1H), 7.80 (m, 1H), 7.60-7.30 (m, 4H), 6.80 (d, 1H, $J = 8$ Hz), 4.30 (t, 2H, $J = 6$ Hz), 4.13 (q, 2H, $J = 7$ Hz), 3.19 (d, 1H, $J = 12$ Hz), 3.02 (t, 2H, $J = 6$ Hz), 2.99 (m, 1H), 2.62 (m, 1H), 2.42 (t, 1H, $J = 11$ Hz), 2.29 (t, 1H, $J = 11$ Hz), 1.98 (m, 1H), 1.80-1.40 (m, 3H), 1.23 (t, 3H, $J = 7$ Hz). $^{13}$C NMR δ: 174.48, 154.91, 134.89, 127.84, 126.78, 126.26, 126.06, 125.56, 122.46, 120.73, 105.13, 66.78, 60.77, 57.89, 56.33, 54.55, 42.29, 27.12, 25.04, 14.60. HRMS calcd for C$_{20}$H$_{26}$NO$_3$*: 328.1907, found: 328.1937.
Ethyl 1-(2'(4''-biphenyl)oxyethyl)nipecotate (116h). The general procedure for the preparation of $N$-aryloxyethylnipecotates was followed, starting with bromide 114h (0.301 g, 1.09 mmol). Chromatography in 25% EtOAc in hexanes gave 0.111 g (35%) of 116h. $^1$H NMR δ: 7.81 (m, 4H), 7.69 (m, 2H), 7.57 (m, 1H), 7.25 (d, 2H, $J$ = 9 Hz), 4.43 (m, 4H), 3.39 (d, 1H, $J$ = 11 Hz), 3.15 (m, 3H), 2.89 (m, 1H), 2.63 (t, 1H, $J$ = 11 Hz), 2.46 (t, 1H, $J$ = 11 Hz), 2.24 (m, 1H), 2.09-1.68 (m, 3H), 1.53 (t, 3H, $J$ = 7 Hz). $^{13}$C NMR δ: 158.22, 140.73, 133.86, 128.65, 128.09, 126.67, 126.61, 114.85, 65.85, 60.35, 57.41, 55.81, 54.12, 41.74, 26.78, 24.51, 14.23. HRMS calcd for $C_{22}H_{28}NO_3$: 354.2064, found: 354.2076.
**Ethyl 1-(2'-{(2'"-biphenyl)oxyethyl})nipecotate (116i).** The general procedure for the preparation of *N*-aryloxyethylnipecotates was followed, starting with bromide 114i (0.214 g, 0.77 mmol). Chromatography in 25% EtOAc in hexanes gave 0.098 g (43%) of 116i. $^1$H NMR $\delta$: 7.53 (m, 2H), 7.42-7.21 (m, 5H), 6.99 (m, 2H), 4.09 (m, 4H), 2.99 (m, 1H), 2.75 (m, 3H), 2.55 (m, 1H), 2.25 (m, 1H), 2.05 (m, 1H), 1.90 (m, 1H), 1.79-1.33 (m, 3H), 1.21 (m, 3H). $^{13}$C NMR $\delta$: 155.63, 138.46, 130.86, 129.61, 128.56, 127.77, 126.75, 121.11, 112.69, 66.52, 60.35, 57.29, 55.72, 54.00, 41.83, 26.69, 24.54, 14.23. HRMS calcd for C$_{22}$H$_{28}$NO$_3$*: 354.2064, found: 354.2076.
Ethyl 1-(2'- (3''-biphenyl)oxyethyl)nipecotate (116j). The general procedure for the preparation of N-aryloxyethylnipecotates was followed, starting with bromide 114j (0.367 g, 1.32 mmol). Chromatography in 25% EtOAc in hexanes gave 0.155 g (40%) of 116j. 1H NMR δ: 7.55 (m, 2H), 7.45-7.10 (m, 6H), 6.88 (m, 1H), 4.12 (m, 4H), 3.10 (d, 1H, J = 11 Hz), 2.86 (m, 3H), 2.60 (m, 1H), 2.33 (t, 1H, J = 11 Hz), 2.16 (t, 1H, J = 11 Hz), 1.94 (m, 1H), 1.79-1.35 (m, 3H), 1.22 (t, 3H, J = 7 Hz). 13C NMR δ: 129.78, 129.69, 128.67, 128.09, 127.42, 127.36, 127.13, 126.67, 120.09, 119.77, 114.85, 113.59, 113.54, 113.30, 65.79, 60.35, 57.44, 55.81, 54.12, 41.72, 26.75, 24.48, 14.20. HRMS calcd for C_{22}H_{28}NO_3\textsuperscript{+}: 354.2064, found: 354.2060.
Ethyl 1-(2''-(4''-chloro-3''-trifluoromethylphenyl)oxyethyl)nipecotate (116k).

The general procedure for the preparation of N-aryloxyethylnipecotates was followed, starting with bromide 114k (0.249 g, 1.29 mmol). Chromatography in 25% EtOAc in hexanes (increasing to 35%) gave 0.163 g (33%) of 116k. ^1H NMR δ: 7.38 (d, 1H, J = 10 Hz), 7.25 (m, 1H), 7.02 (m, 1H), 4.12 (m, 4H), 3.08 (d, 1H, J = 12 Hz), 2.87 (m, 1H), 2.83 (t, 2H, J = 6 Hz), 2.60 (m, 1H), 2.34 (t, 1H, J = 11 Hz), 2.18 (t, 1H, J = 11 Hz), 1.96 (m, 1H), 1.80-1.42 (m, 3H), 1.25 (t, 3H, J = 7 Hz). ^13C NMR δ: 174.00, 157.23, 132.24, 129.13, 124.78, 118.69, 114.23, 114.15, 66.64, 60.35, 57.20, 55.88, 54.18, 41.79, 26.69, 24.52, 14.16.

General procedure for the reduction to form alcohols 113a-k. LiAlH₄ (200 mol%) was suspended in THF (2 M) and cooled to 0°C. A solution of the ethyl ester (116a-k, 1 eq) in THF (0.5 M) was canulated into the reaction and warmed to rt. The mixture was stirred for 24 h, and then cooled to 0°C and diluted with Et₂O. For each mmol used of LiAlH₄, 40 µl of H₂O were added and the mixture was stirred for 15 mins. Then 40 µl/(mmol LiAlH₄) of 15% aqueous NaOH were
added, stirred for 15 mins and finally, 100 μl(/mmol LiAlH₄) of H₂O were added. The precipitate was removed by filtration and washed with Et₂O. The final products (113a-k) were not purified, since all of them looked very good by crude NMR.

3-Hydroxymethyl-N-(2'-((4'’-methoxyphenyl)oxyethyl)piperidine (113a). The general procedure for the reduction of the ester was followed, starting with nipecotate 116a (0.093 g, 0.30 mmol). Work-up gave 0.083 g (>100%) of 113a. ¹H NMR δ: 6.82 (s, 4H), 4.03 (t, 2H, J = 6 Hz), 3.78 (s, 3H), 3.61 (m, 2H), 2.92 (d, 1H, J = 12 Hz), 2.77 (t, 3H, J = 6 Hz), 2.20 (m, 3H), 1.69 (m, 4H), 1.21 (m, 1H). ¹³C NMR δ: 154.30, 153.36, 116.03, 115.04, 67.60, 66.88, 58.31, 58.20, 56.13, 55.15, 38.32, 27.81, 25.07. HRMS calcd for C₁₅H₂₄NO₃⁺: 266.1751, found: 266.1773.
3-Hydroxymethyl-N-(2''-(2''-naphthyl)oxyethyl)piperidine (113b). The general procedure for the reduction of the esters was followed, starting with nipecotate 116b (0.088 g, 0.27 mmol). Work-up gave 0.068 g (89%) of 113b. $^1$H NMR δ: 7.73 (m, 3H), 7.50-7.10 (m, 4H), 4.20 (t, 2H, $J = 6$ Hz), 3.53 (m, 2H), 3.16 (bs, 1H), 2.99 (d, 1H, $J = 12$ Hz), 2.90-2.77 (m, 1H), 2.83 (t, 2H, $J = 6$ Hz), 2.24 (m, 1H), 2.09 (m, 1H), 1.71 (m, 4H), 1.09 (m, 1H). $^{13}$C NMR δ: 157.13, 134.95, 129.78, 129.42, 128.03, 127.16, 126.75, 124.03, 119.39, 107.21, 67.20, 66.08, 58.36, 58.06, 55.15, 38.53, 27.72, 25.08.

3-Hydroxymethyl-N-(2''-(4''-methylphenyl)oxyethyl)piperidine (113c). The general procedure for the reduction of the esters was followed, starting with nipecotate 116c (0.169 g, 0.58 mmol). Work-up gave 0.138 g (95%) of 113c. $^1$H
NMR δ: 7.10 (d, 2H, J = 9 Hz), 6.80 (d, 2H, J = 9 Hz), 4.06 (t, 2H, J = 6 Hz), 3.60 (m, 2H), 2.92 (d, 1H, J = 12 Hz), 2.82-2.69 (m, 1H), 2.80 (t, 2H, J = 6 Hz), 2.28 (s, 4H), 2.18 (t, 1H, J = 10 Hz), 2.10-1.50 (m, 5H), 1.17 (m, 1H). 13C NMR δ: 157.06, 130.38, 130.26, 114.90, 67.62, 66.26, 58.30, 58.15, 55.13, 38.33, 27.82, 25.08, 20.84.

3-Hydroxymethyl-N-(2′-(4″-chlorophenyl)oxyethyl)piperidine (113d). The general procedure for the reduction of the esters was followed, starting with nipecotate 116d (0.271 g, 0.87 mmol). Work-up gave 0.236 g (>100%) of 113d. 1H NMR δ: 7.22 (d, 2H, J = 9 Hz), 6.83 (d, 2H, J = 9 Hz), 4.08 (t, 2H, J = 6 Hz), 3.59 (m, 2H), 2.94 (d, 1H, J = 12 Hz), 2.84-2.70 (m, 1H), 2.79 (t, 2H, J = 6 Hz), 2.30-2.10 (m, 3H), 1.90-1.50 (m, 4H), 1.15 (m, 1H). 13C NMR δ: 157.80, 129.68, 126.04, 116.31, 67.49, 66.54, 58.34, 57.99, 55.16, 38.38, 27.76, 25.07. HRMS calcd for C14H21ClNO2+: 270.1255, found: 270.1254.
3-Hydroxymethyl-N-(2′-(3″,4″-dichlorophenyl)oxyethyl)piperidine (113e).

The general procedure for the reduction of the esters was followed, starting with nipecotate 116e (0.411 g, 1.19 mmol). Work-up gave 0.347 g (96%) of 113e. $^1$H NMR $\delta$: 7.34 (d, 1H, $J = 7$ Hz), 7.03 (d, 1H, $J = 2$ Hz), 6.77 (dd, 1H, $J = 7$ Hz and 2 Hz), 4.09 (t, 2H, $J = 6$ Hz), 3.60 (m, 2H), 2.93 (d, 1H, $J = 12$ Hz), 2.82-2.65 (m, 1H), 2.78 (t, 2H, $J = 6$ Hz), 2.26 (m, 2H), 2.10 (t, 1H, $J = 11$ Hz), 1.90-1.50 (m, 4H), 1.13 (m, 1H). $^{13}$C NMR $\delta$: 158.24, 133.20, 131.03, 124.40, 116.88, 115.08, 67.40, 66.81, 58.32, 57.86, 55.14, 38.40, 27.70, 25.06. HRMS calcd for C$_{14}$H$_{20}$Cl$_2$NO$_2$$: 304.0866, found: 304.0870.

3-Hydroxymethyl-N-(2′-(3″-chlorophenyl)oxyethyl)piperidine (113f). The general procedure for the reduction of the esters was followed, starting with
nipecotate 116f (0.129 g, 0.41 mmol). Work-up gave 0.104 g (93%) of 113f. $^1$H NMR $\delta$: 7.21 (t, 1H, $J = 8$ Hz), 6.94 (m, 2H), 6.81 (m, 1H), 4.10 (t, 2H, $J = 6$ Hz), 3.62 (m, 2H), 2.97 (d, 1H, $J = 12$ Hz), 2.84-2.70 (m, 1H), 2.80 (t, 2H, $J = 6$ Hz), 2.20 (m, 3H), 1.90-1.50 (m, 4H), 1.14 (m, 1H). $^{13}$C NMR $\delta$: 159.94, 135.26, 130.61, 121.38, 115.46, 113.56, 67.52, 66.44, 58.34, 57.97, 55.15, 38.39, 27.77, 25.09.

3-Hydroxymethyl-N-(2'-(1''-naphthyl)oxyethyl)piperidine (113g). The general procedure for the reduction of the esters was followed, starting with nipecotate 116g (0.145 g, 0.44 mmol). Work-up gave 0.118 g (94%) of 113g. $^1$H NMR $\delta$: 8.23 (m, 1H), 7.78 (m, 1H), 7.50-7.30 (m, 4H), 6.80 (d, 1H, $J = 7$ Hz), 4.31 (t, 2H, $J = 6$ Hz), 3.61 (m, 2H), 3.00 (m, 1H), 2.95 (t, 2H, $J = 6$ Hz), 2.82 (m, 1H), 2.39 (t, 1H, $J = 11$ Hz), 2.26 (t, 1H, $J = 11$ Hz), 2.10-1.50 (m, 5H), 1.18 (m, 1H). $^{13}$C NMR $\delta$: 154.97, 134.93, 127.85, 126.79, 126.27, 126.11, 125.59, 122.47, 120.73, 105.18, 67.63, 66.75, 58.43, 58.18, 55.18, 38.44, 27.81, 25.19.
3-Hydroxymethyl-N-(2’-(4”-biphenyl)oxyethyl)piperidine (113h). The general procedure for the reduction of the esters was followed, starting with nipecotate 116h (0.095 g, 0.27 mmol). Work-up gave 0.073 g (87%) of 113h. $^1$H NMR δ: 7.74-6.66 (m, 9H), 4.03 (m, 2H), 3.45 (m, 2H), 3.03-0.56 (m, 12H). $^{13}$C NMR δ: 158.22, 133.80, 128.65, 128.06, 126.64, 126.58, 114.82, 66.84, 65.73, 57.93, 57.67, 54.70, 38.08, 27.31, 24.66.

3-Hydroxymethyl-N-(2’-(2”-biphenyl)oxyethyl)piperidine (113i). The general procedure for the reduction of the esters was followed, starting with nipecotate 116i (0.092 g, 0.26 mmol). Work-up gave 0.082 g (>100%) of 113i. $^1$H NMR δ: 7.53 (m, 2H), 7.42-7.22 (m, 5H), 6.99 (m, 2H), 4.09 (t, 2H, $J = 6$ Hz), 3.46 (m,
2H), 2.81 (d, 1H, J = 11 Hz), 2.72 (t, 2H, J = 6 Hz), 2.66 (m, 1H), 2.14 (m, 1H), 2.01 (m, 3H), 1.80-1.45 (m, 3H), \(^{13}\)C NMR 8: 155.60, 130.80, 129.64, 128.56, 127.74, 126.69, 121.02, 112.52, 66.93, 66.64, 57.73, 57.55, 54.64, 38.08, 27.16, 24.71. HRMS calcd for C\(_{20}\)H\(_{26}\)NO\(_2\): 312.1958, found: 312.1957.

3-Hydroxymethyl-N-(2'-(3''-biphenyl)oxyethyl)piperidine (113j). The general procedure for the reduction of the esters was followed, starting with nipecotate 116j (0.144 g, 0.41 mmol). Work-up gave 0.136 g (>100%) of 113j. \(^1\)H NMR 8: 7.55 (m, 2H), 7.46-7.10 (m, 6H), 6.90 (m, 1H), 4.16 (t, 2H, J = 6 Hz), 3.58 (m, 2H), 2.95 (d, 1H, J = 11 Hz), 2.81 (t, 2H, J = 6 Hz), 2.77 (m, 1H), 2.29 (t, 1H, J = 10 Hz), 2.16 (t, 1H, J = 10 Hz), 1.91-1.52 (m, 5H). \(^{13}\)C NMR 8: 151.99, 136.80, 135.23, 129.69, 128.67, 128.09, 127.36, 127.13, 119.77, 114.85, 113.59, 113.30, 113.24, 67.04, 65.68, 57.84, 57.70, 54.70, 37.87, 27.28, 24.60.
3-Hydroxymethyl-N-(2'-(4''-chloro-3'')-trifluoromethylphenyl)oxyethyl)piperidine (113k). The general procedure for the reduction of the esters was followed, starting with nipecotate 116k (0.162 g, 0.43 mmol). Work-up gave 0.134 g (93%) of 113k. $^1$H NMR $\delta$: 7.36 (d, 1H, $J = 9$ Hz), 7.21 (d, 1H, $J = 3$ Hz), 6.98 (dd, 1H, $J = 9$ Hz and 3 Hz), 4.08 (t, 2H, $J = 6$ Hz), 3.53 (m, 2H), 2.96 (d, 1H, $J = 11$ Hz), 2.81 (m, 1H), 2.77 (t, 2H, $J = 6$ Hz), 2.20 (t, 1H, $J = 11$ Hz), 2.04 (t, 1H, $J = 11$ Hz), 1.87-1.50 (m, 5H). $^{13}$C NMR $\delta$: 157.16, 132.20, 124.74, 120.40, 118.65, 114.15, 114.07, 113.99, 66.60, 66.36, 57.94, 57.40, 54.68, 38.14, 27.20, 24.60.

General procedure for coupling alcohols 113a-k to the acid (10) to form benzoates 112a-k. DMAP (0.1 eq) was added to a solution of 2-(3'-methyl)succinimidobenzoic acid (10, 1 eq) in CH$_2$Cl$_2$ (1 M). A solution of alcohol 113a-k (1 eq) in CH$_2$Cl$_2$ (0.33 M) was canulated into the reaction mixture, which was then cooled to 0°C and DCC (1 eq) was added in one portion. The reaction was warmed to rt and stirred for 20 h. At that time, the reaction was diluted with
Et₂O, and the precipitate removed by filtration. Chromatography (in 2% MeOH in CHCl₃ unless otherwise stated) gave 112a-k in fairly good yields.

**112a**

\[ \text{A/-(2"-(4"-Methoxyphenyl)oxyethyl)piperidinyl-3"-methyl 2""-(3""-methyl)succinimidobenzoate (112a).} \]

The general procedure for the coupling was followed, starting with hydroxymethylpiperidine 113a (0.080 g, 0.30 mmol). Chromatography in 3% MeOH/CHCl₃ gave 0.118 g (81%) of 112a. \(^1\)H NMR δ: 8.07 (d, 1H, J = 8 Hz), 7.63 (t, 1H, J = 8 Hz), 7.43 (t, 1H, J = 8 Hz), 7.25 (d, 1H, J = 8 Hz), 6.82 (m, 4H), 4.05 (m, 4H), 3.75 (s, 3H), 3.02 (m, 3H), 2.89 (d, 1H, J = 12 Hz), 2.78 (t, 2H, J = 6 Hz), 2.53 (m, 1H), 2.30-1.90 (m, 3H), 1.69 (m, 4H), 1.43 (bs, 3H), 1.09 (m, 1H). \(^13\)C NMR δ: 180.26, 176.31, 164.74, 154.26, 153.39, 133.75, 133.16, 131.94, 130.20, 129.72, 127.78, 116.03, 115.03, 68.30, 67.09, 58.16, 57.87, 56.11, 54.94, 37.39, 36.21, 35.69, 27.47, 25.09, 16.88.

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N-(2''-(2'-Naphthyl)oxyethyl)piperidinyl-3-methyl 2'''-(3'''-methyl)succinimidobenzoate (112b). The general procedure for the coupling was followed, starting with hydroxymethylpiperidine 113b (0.068 g, 0.24 mmol). Chromatography gave 0.072 g (60%) of 112b. $^1$H NMR $\delta$: 8.03 (d, 1H, $J = 8$ Hz), 7.70 (m, 3H), 7.59 (t, 1H, $J = 8$ Hz), 7.46-7.10 (m, 6H), 4.25 (t, 2H, $J = 6$ Hz), 4.10 (m, 2H), 3.07 (m, 4H), 2.89 (t, 2H, $J = 6$ Hz), 2.52 (m, 1H), 2.13 (m, 3H), 1.70 (m, 4H), 1.42 (bs, 3H), 1.10 (m, 1H). $^{13}$C NMR $\delta$: 180.27, 176.31, 164.76, 157.14, 134.96, 133.76, 133.15, 131.93, 130.19, 129.75, 129.71, 129.40, 128.02, 127.74, 127.18, 126.72, 124.00, 119.41, 107.23, 68.25, 66.39, 57.99, 57.81, 54.96, 37.40, 36.19, 35.72, 30.10, 27.43, 25.07, 16.74.
\( N-(2'-(4''-\text{Methylphenyl})\text{oxyethyl})\text{piperidinyl}-3-\text{methyl} \ 2'''-(3'''-\text{methyl})\text{succinimidobenzoate} \ (112c) \). The general procedure for the coupling was followed, starting with hydroxymethylpiperidine 113c (0.138 g, 0.55 mmol). Chromatography gave 0.227 g (88\%) of 112c. \(^1\)H NMR \( \delta \): 8.10 (d, 1H, \( J = 7 \text{ Hz} \)), 7.66 (t, 1H, \( J = 7 \text{ Hz} \)), 7.45 (t, 1H, \( J = 7 \text{ Hz} \)), 7.23 (d, 1H, \( J = 7 \text{ Hz} \)), 7.08 (d, 2H, \( J = 8 \text{ Hz} \)), 6.80 (d, 2H, \( J = 8 \text{ Hz} \)), 4.10 (m, 4H), 3.01 (m, 4H), 2.80 (t, 2H, \( J = 6 \text{ Hz} \)), 2.54 (m, 1H), 2.28 (s, 3H), 2.20-1.60 (m, 7H), 1.48 (bs, 3H), 1.08 (m, 1H). \(^{13}\)C NMR \( \delta \): 180.31, 176.34, 164.71, 157.05, 133.78, 133.17, 132.00, 130.33, 130.27, 130.20, 129.75, 127.73, 114.89, 68.30, 66.40, 58.08, 57.84, 54.89, 37.40, 36.18, 35.75, 27.46, 25.08, 20.87, 16.75.

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N-(2''-(4''-Chlorophenyl)oxyethyl)piperidinyl-3-methyl 2''''-(3''''''-methyl)succinimidozenzoate (112d). The general procedure for the coupling was followed, starting with hydroxymethylpiperidine 113d (0.235 g, 0.87 mmol). Chromatography gave 0.365 g (87%) of 112d. $^1$H NMR $\delta$: 8.05 (d, 1H, $J = 8$ Hz), 7.63 (t, 1H, $J = 8$ Hz), 7.48 (t, 1H, $J = 8$ Hz), 7.21 (m, 3H), 6.82 (d, 2H, $J = 9$ Hz), 4.06 (m, 4H), 2.99 (m, 4H), 2.80 (t, 2H, $J = 6$ Hz), 2.51 (m, 1H), 2.04 (m, 3H), 1.72 (m, 4H), 1.46 (bs, 3H), 1.10 (m, 1H). $^{13}$C NMR $\delta$: 180.27, 176.27, 164.74, 157.79, 133.80, 133.16, 131.87, 130.21, 129.71, 129.67, 127.72, 126.00, 116.32, 68.20, 66.74, 57.90, 57.77, 54.98, 37.40, 36.15, 35.70, 27.38, 25.02, 16.75.
N-(2''-(3'', 4''-Dichlorophenyl)oxyethyl)piperidinyl-3-methyl 2''''-(3'''''-
 methyl)succinimidobenzoate (112e). The general procedure for the coupling
was followed, starting with hydroxymethylpiperidine 113e (0.347 g, 1.14 mmol).
Chromatography gave 0.466 g (79%) of 112e. $^1$H NMR δ: 8.08 (d, 1H, $J = 7$ Hz),
7.63 (t, 1H, $J = 7$ Hz), 7.45 (t, 1H, $J = 7$ Hz), 7.26 (m, 2H), 7.00 (d, 1H, $J = 2$ Hz),
6.73 (dd, 1H, $J = 7$ Hz and 2 Hz), 4.02 (m, 4H), 3.00 (m, 4H), 2.78 (t, 2H, $J = 6$
Hz), 2.54 (m, 1H), 2.03 (m, 3H), 1.69 (m, 4H), 1.43 (bs, 3H), 1.12 (m, 1H). $^{13}$C
NMR δ: 158.28, 133.85, 133.15, 131.88, 131.03, 130.23, 129.74, 124.32,
116.86, 115.12, 68.21, 67.10, 57.80, 55.00, 37.40, 36.20, 27.38, 25.07.
N-(2′-(3′′-Chlorophenyl)oxyethyl)piperidinyl-3-methyl 2″′-(3″′′-methyl)succinimidobenzoate (112f). The general procedure for the coupling was followed, starting with hydroxymethylpiperidine 113f (0.104 g, 0.39 mmol). Chromatography gave 0.145 g (78%) of 112f. $^1$H NMR δ: 8.06 (d, 1H, $J = 8$ Hz), 7.63 (t, 1H, $J = 8$ Hz), 7.47 (t, 1H, $J = 8$ Hz), 7.20 (m, 2H), 6.92 (m, 2H), 6.80 (d, 1H, $J = 8$ Hz), 4.11 (m, 4H), 3.01 (m, 4H), 2.81 (t, 2H, $J = 6$ Hz), 2.53 (m, 1H), 2.06 (m, 3H), 1.72 (m, 4H), 1.49 (bs, 3H), 1.10 (m, 1H). $^{13}$C NMR δ: 179.92, 159.54, 134.82, 133.42, 132.75, 131.44, 130.19, 129.82, 129.36, 127.33, 120.93, 115.04, 113.16, 67.84, 66.27, 57.48, 54.53, 37.01, 35.78, 35.26, 27.00, 24.65.
112g

N-(2'-(1''-Naphthyl)oxyethyl)piperidinyl-3-methyl 2'''-(3''''-methyl)succinimidobenzoate (112g). The general procedure for the coupling was followed, starting with hydroxymethylpiperidine 113g (0.118 g, 0.42 mmol). Chromatography gave 0.196 g (94%) of 112g. \(^{1}H\) NMR \(\delta\): 8.20 (d, 1H, \(J = 7\) Hz), 7.98 (d, 1H, \(J = 8\) Hz), 7.77 (m, 1H), 7.60 (dt, 1H, \(J = 8\) Hz and 2 Hz), 7.49-7.30 (m, 5H), 7.25-7.18 (m, 2H), 6.81 (d, 1H, \(J = 7\) Hz), 4.34 (t, 2H, \(J = 5\) Hz), 4.12 (m, 2H), 3.23-2.95 (m, 4H), 2.65-2.07 (m, 3H), 1.98-1.52 (m, 4H), 1.50-1.01 (m, 6H), 0.85 (m, 1H). \(^{13}C\) NMR \(\delta\): 133.39, 129.69, 129.29, 127.45, 125.82, 125.30, 121.78, 120.55, 104.92, 70.27, 67.39, 57.44, 57.09, 37.00, 35.37, 31.29, 29.69, 16.13.
N-(2′-(4′′-Biphenyl)oxyethyl)piperidinyl-3-methyl 2′′′-(3′′′-methyl)succinimidobenzoate (112h). The general procedure for the coupling was followed, starting with hydroxymethylpiperidine 113h (0.073 g, 0.23 mmol). Chromatography gave 0.115 g (93%) of 112h. $^1$H NMR 8: 8.05 (d, 1H, $J = 8$ Hz), 7.68-7.18 (m, 10H), 6.96 (d, 2H, $J = 8$ Hz), 4.13 (m, 4H), 3.18-2.79 (m, 6H), 2.52 (m, 1H), 2.08 (m, 2H), 1.86-1.58 (m, 3H), 1.45 (bs, 3H), 1.09 (m, 1H). $^{13}$C NMR 8: 158.25, 133.36, 129.75, 129.32, 128.65, 128.06, 126.67, 126.61, 114.88, 67.83, 66.02, 57.61, 57.41, 54.53, 42.18, 37.00, 35.75, 27.01, 24.66, 16.30.
N-(2′-(2″-Biphenyl)oxyethyl)piperidinyl-3-methyl 2‴-(3‴″-methyl)succinimidobenzoate (112i). The general procedure for the coupling was followed, starting with hydroxymethylpiperidine 113i (0.066 g, 0.21 mmol). Chromatography gave 0.082 g (74%) of 112i. \( ^1H \) NMR \( \delta \): 8.04 (d, 1H, \( J = 7 \) Hz), 7.63 (t, 1H, \( J = 7 \) Hz), 7.57-7.19 (m, 9H), 7.00 (m, 2H), 4.04 (m, 4H), 3.06 (m, 2H), 2.88 (d, 1H, \( J = 10 \) Hz), 2.77 (m, 1H), 2.72 (m, 2H), 2.51 (m, 1H), 2.09-1.81 (m, 4H), 1.77-1.36 (m, 5H), 1.01 (m, 1H). \( ^{13}C \) NMR \( \delta \): 155.63, 138.40, 133.27, 131.41, 130.95, 130.74, 129.72, 129.55, 129.23, 128.47, 127.71, 126.72, 120.93, 112.55, 67.83, 66.67, 57.44, 57.32, 54.29, 36.94, 35.81, 35.19, 26.96, 24.71, 16.27.
$N$-(2'-(3'''-Biphenyl)oxyethyl)piperidinyl-3-methyl 2''''-(3''''''-methyl)succinimidobenzoate (112j). The general procedure for the coupling was followed, starting with hydroxymethylpiperidine 113j (0.127 g, 0.41 mmol). Chromatography gave 0.165 g (77%) of 112j. $^1$H NMR $\delta$: 7.88 (m, 1H), 7.40 (m, 3H), 7.30-6.92 (m, 8H), 6.73 (m, 1H), 3.96 (m, 4H), 2.97-2.59 (m, 6H), 2.33 (m, 1H), 1.88 (m, 3H), 1.54 (m, 3H), 1.26 (bs, 3H), 0.90 (m, 1H). $^{13}$C NMR $\delta$: 158.20, 142.53, 140.87, 133.22, 131.32, 129.61, 129.20, 128.59, 127.98, 127.25, 127.02, 126.55, 119.56, 114.79, 113.48, 113.24, 67.77, 65.94, 57.58, 57.38, 54.47, 36.88, 35.72, 35.14, 29.60, 24.63, 16.24.
N-(2′-(4″-Chloro-3″-trifluoromethylphenyl)oxyethyl)piperidinyl-3-methyl 2‴-(3‴-methyl)succinimidobenzoate (112k). The general procedure for the coupling was followed, starting with hydroxymethylpiperidine 113k (0.134 g, 0.40 mmol). Chromatography gave 0.212 g (96%) of 112k. ¹H NMR δ: 8.06 (d, 1H, J = 8 Hz), 7.65 (t, 1H, J = 8 Hz), 7.46 (t, 1H, J = 8 Hz), 7.33 (d, 1H, J = 9 Hz), 7.24 (m, 2H), 6.98 (dd, 1H, J = 9 Hz and 3 Hz), 4.09 (m, 4H), 3.16-2.83 (m, 4H), 2.78 (t, 2H, J = 6 Hz), 2.53 (m, 1H), 2.20-1.91 (m, 3H), 1.81-1.57 (m, 3H), 1.45 (bs, 3H), 1.09 (m, 1H). ¹³C NMR δ: 179.79, 175.71, 164.22, 157.20, 133.36, 132.66, 132.20, 131.30, 129.75, 129.21, 129.09, 128.59, 124.74, 120.40, 118.73, 114.11, 114.03, 113.91, 113.84, 67.68, 66.67, 57.32, 54.53, 48.94, 36.90, 35.70, 35.27, 26.89, 24.56, 16.21. HRMS calcd for C₂₇H₂₉ClF₃N₂O₅⁺: 553.1712, found: 553.1686.

General procedure for preparing salts of compounds 112a-k. Benzoate (112a-k, 1 eq) was dissolved in a minimal amount of EtOAc, the corresponding
acid (oxalic acid or benzoic acid, 1 eq) was added and stirred at rt. The mixture was concentrated to give the salt of the benzoate.

**General procedure for the preparation of tosylates 119a-d.** DMAP (0.11 eq) was added to a solution of alcohol (1 eq) in CH$_2$Cl$_2$ (0.83 M) and the mixture was cooled to 0°C. Et$_3$N (1.5 eq) was then added via syringe, followed by TsCl (1.1 eq) in one portion. The reaction was warmed to rt and stirred for 20 h. The work-up consisted of adding 1 M HCl, extracting the aq. phase with CH$_2$Cl$_2$, washing the organic phase with sat. aq. NaHCO$_3$ and then with brine. The organic phase was dried with MgSO$_4$, filtered and concentrated. Flash chromatography was performed in 2% EtOAc in hexanes (increasing to 5%) unless otherwise stated.

![Structure](119a)

**4-Phenyl-1-butyl tosylate (119a).** The general procedure for the preparation of tosylates was followed, starting with 4-phenyl-1-butanol (1.50 g, 10.0 mmol). Chromatography gave 2.442 g (80%) of 119a. $^1$H NMR δ: 7.91 (d, 2H, $J = 8$ Hz), 7.46 (d, 2H, $J = 8$ Hz), 7.43-7.20 (m, 5H), 4.16 (t, 2H, $J = 7$ Hz), 2.69 (t, 2H, $J = 7$ Hz), 2.57 (s, 3H), 1.78 (m, 4H). $^{13}$C NMR δ: 145.10, 141.96, 133.51, 130.24, 128.75, 128.28, 126.32, 70.80, 35.48, 28.73, 27.48, 22.04.
5-Phenyl-1-pentyl tosylate (119b). The general procedure for the preparation of tosylates was followed, starting with 5-phenyl-1-pentanol (0.329 g, 2.00 mmol). Chromatography gave 0.521 g (82%) of 119b. \(^1\)H NMR \(\delta\): 7.81 (d, 2H, \(J = 11\) Hz), 7.40-7.00 (m, 7H), 4.01 (t, 2H, \(J = 8\) Hz), 2.55 (t, 2H, \(J = 9\) Hz), 2.42 (s, 3H), 1.80-1.30 (m, 6H). \(^13\)C NMR \(\delta\): 145.03, 142.55, 133.65, 130.20, 128.73, 128.69, 128.27, 126.16, 70.86, 36.05, 31.11, 29.10, 25.39, 22.01.

6-Phenyl-1-hexyl tosylate (119c). The general procedure for the preparation of tosylates was followed, starting with 6-phenyl-1-hexanol (0.357 g, 2.00 mmol). Chromatography gave 0.553 g (83%) of 119c. \(^1\)H NMR \(\delta\): 7.80 (d, 2H, \(J = 9\) Hz), 7.40-7.00 (m, 7H), 4.00 (t, 2H, \(J = 8\) Hz), 2.55 (t, 2H, \(J = 9\) Hz), 2.43 (s, 3H), 1.60 (m, 4H), 1.30 (m, 4H). \(^13\)C NMR \(\delta\): 145.01, 142.88, 133.70, 130.19, 128.73, 128.66, 128.27, 126.07, 70.99, 36.14, 31.55, 29.15, 28.94, 25.62, 22.01.
3-Cyclohexyl-1-propyl tosylate (119d). The general procedure for the preparation of tosylates was followed, starting with 3-cyclohexyl-1-propanol (0.427 g, 3.00 mmol). Chromatography in 2% EtOAc/Hexanes gave 0.840 g (94%) of 119d. \(^1\)H NMR δ: 7.82 (d, 2H, J = 11 Hz), 7.35 (d, 2H, J = 11 Hz), 4.02 (t, 2H, J = 9 Hz), 2.43 (s, 3H), 1.63 (m, 7H), 1.11 (m, 6H), 0.81 (m, 2H). \(^{13}\)C NMR δ: 144.98, 133.72, 130.16, 128.28, 71.43, 37.40, 33.49, 33.24, 26.92, 26.61, 22.00.

**General procedure for the preparation of N-alkyl nipecotates (120a-d).** Ethyl nipecotate hydrochloride (94, 1 eq) was dissolved in EtOH (1 M) and K\(_2\)CO\(_3\) (4 eq) added. Alkyl tosylate (119a-d, 1.2 eq) was canulated into the reaction and heated to reflux. After 18 h, the reaction was cooled down to rt, water added and the mixture extracted with EtOAc (3x). The organic phase was washed with brine and then dried with MgSO\(_4\), filtered and concentrated. Chromatography in 25% EtOAc/Hexanes (unless otherwise stated) gave compounds 120a-d.
Ethyl N-(4'-phenyl-1'-butyl)nipecotate (120a). The general procedure for the preparation of N-alkyl nipecotates was followed, starting from tosylate 119a (0.365 g, 1.20 mmol). Chromatography in 1% EtOAc/Hexanes (increasing to 40%) gave 0.153 g (53%) of 120a. $^1$H NMR $\delta$: 7.20 (m, 5H), 4.12 (q, 2H, $J = 7$ Hz), 3.00 (d, 1H, $J = 12$ Hz), 2.79 (d, 1H, $J = 12$ Hz), 2.62 (m, 3H), 2.36 (t, 2H, $J = 7$ Hz), 2.10 (t, 1H, $J = 11$ Hz), 1.95 (m, 2H), 1.58 (m, 7H), 1.25 (t, 3H, $J = 7$ Hz). $^{13}$C NMR $\delta$: 173.50, 142.13, 128.47, 128.32, 125.79, 60.54, 58.37, 54.68, 53.36, 40.94, 35.62, 29.18, 26.73, 25.49, 23.82, 14.16. HRMS calcd for C$_{18}$H$_{28}$N: 290.2115, found: 290.2100.

Ethyl N-(5'-phenyl-1'-pentyl)nipecotate (120b). The general procedure for the preparation of N-alkyl nipecotates was followed, starting with tosylate 119b (0.521 g, 1.64 mmol). Chromatography gave 0.237 g (57%) of 120b. $^1$H NMR $\delta$:
7.20 (m, 5H), 4.13 (q, 2H, J = 7 Hz), 3.00 (d, 1H, J = 12 Hz), 2.77 (d, 1H, J = 12 Hz), 2.59 (m, 3H), 2.32 (t, 2H, J = 8 Hz), 2.09 (t, 1H, J = 11 Hz), 1.93 (m, 2H), 1.80-1.30 (m, 9H), 1.23 (t, 3H, J = 7 Hz). $^{13}$C NMR $\delta$: 174.71, 143.09, 128.77, 128.63, 126.00, 60.64, 59.35, 55.94, 54.26, 42.37, 36.29, 31.77, 27.67, 27.50, 27.16, 25.07, 14.61. HRMS calcd for $\text{C}_{19}\text{H}_{30}\text{NO}_2$: 304.2271, found: 304.2264.

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Ethyl N-(3'-cyclohexyl-1'-propyl)nipecotate (120d). The general procedure for the preparation of N-alkyl nipecotates was followed, starting with tosylate 119d (2 eq, 0.840 g, 2.83 mmol). Chromatography gave 0.378 g (95%) of 120d. $^1$H NMR δ: 4.11 (q, 2H, $J = 7$ Hz), 3.00 (d, 1H, $J = 12$ Hz), 2.79 (d, 1H, $J = 12$ Hz), 2.54 (m, 1H), 2.30 (t, 2H, $J = 8$ Hz), 2.09 (t, 1H, $J = 11$ Hz), 1.93 (m, 2H), 1.76-1.30 (m, 10H), 1.24 (t, 3H, $J = 7$ Hz), 1.18 (m, 6H), 0.86 (m, 2H). $^{13}$C NMR δ: 174.20, 60.31, 59.34, 55.34, 53.75, 41.75, 37.64, 35.27, 33.37, 27.04, 26.69, 26.38, 24.52, 24.02, 14.19. HRMS calcd for $C_{17}H_{32}NO_2^+$: 282.2428, found: 282.2449.

General procedure for ester reduction to form alcohols 121a-d. LiAlH$_4$ (200 mol%) was suspended in THF (2 M) and cooled to 0°C. The ethyl ester (120a-d, 1 eq) in THF (0.5 M) was canulated into the reaction and warmed to rt. After stirring for 24 h, the mixture was cooled to 0°C, and diluted with Et$_2$O. For each mmol used of LiAlH$_4$, 40 μl H$_2$O were added and the mixture was stirred for 15 mins. Then, 40 μl/(mmol LiAlH$_4$) of 15% aqueous NaOH were added, stirred for 15 mins and finally, 100 μl/(mmol LiAlH$_4$) of H$_2$O were added. The precipitate was removed by filtration and washed with Et$_2$O. No purification was deemed necessary, since all of the products looked very good by crude NMR.
3-Hydroxymethyl-N-(4'-phenyl-1'-butyl)piperidine (121a). The general procedure for the ester reduction was followed, starting with nipecotate 120a (0.153 g, 0.53 mmol). That gave 0.116 g (89%) of 121a. \(^1\)H NMR \(\delta\): 7.22 (m, 5H), 3.60 (m, 2H), 2.82 (d, 1H, \(J = 10\) Hz), 2.73-2.42 (m, 1H), 2.60 (t, 2H, \(J = 7\) Hz), 2.42 (t, 2H, \(J = 7\) Hz), 2.16 (m, 2H), 1.90-1.50 (m, 7H), 1.18 (m, 2H). \(^{13}\)C NMR \(\delta\): 142.86, 128.78, 128.66, 126.06, 67.79, 59.48, 58.08, 54.77, 38.15, 36.22, 29.83, 28.17, 26.88, 25.05.

3-Hydroxymethyl-N-(5'-phenyl-1'-pentyl)piperidine (121b). The general procedure for the ester reduction was followed, starting with nipecotate 120b (0.237 g, 0.78 mmol). That gave 0.205 g (100%) of 121b. \(^1\)H NMR \(\delta\): 7.22 (m, 5H), 3.60 (m, 2H), 2.79 (d, 1H, \(J = 12\) Hz), 2.59 (m, 3H), 2.10 (m, 5H), 1.90-1.10 (m, 11H). \(^{13}\)C NMR \(\delta\): 143.08, 128.78, 128.63, 125.99, 67.96, 59.59, 58.07, 183.
3-Hydroxymethyl-N-(6'-phenyl-1'-hexyl)piperidine (121c). The general procedure for the ester reduction was followed, starting with nipecotate 120c (0.305 g, 0.96 mmol). That gave 0.262 g (99%) of 121c. $^1$H NMR $\delta$: 7.23 (m, 5H), 3.60 (m, 2H), 2.81 (d, 1H, $J = 12$ Hz), 2.71-2.52 (m, 1H), 2.59 (t, 2H, $J = 7$ Hz), 2.30 (t, 2H, $J = 8$ Hz), 2.10 (m, 2H), 1.90-1.10 (m, 13H). $^{13}$C NMR $\delta$: 143.19, 128.79, 128.62, 125.97, 67.94, 59.69, 58.11, 54.80, 38.11, 36.30, 31.79, 29.61, 28.26, 27.89, 27.21, 25.08. HRMS calcd for $C_{18}H_{30}NO^+$: 276.2322, found: 276.2313.

3-Hydroxymethyl-N-(3'-cyclohexyl-1'-propyl)piperidine (121d). The general procedure for the ester reduction was followed, starting with nipecotate 120d.
(0.378 g, 1.35 mmol). That gave 0.313 g (97%) of 121d. \(^1\)H NMR \(\delta\): 3.61 (m, 2H), 2.75 (m, 3H), 2.28 (t, 2H, \(J=7\) Hz), 2.17 (t, 1H, \(J=11\) Hz), 2.04 (t, 1H, \(J=11\) Hz), 1.90-1.40 (m, 11H), 1.20 (m, 7H), 0.90 (m, 2H). \(^{13}\)C NMR \(\delta\): 67.85, 60.06, 58.06, 54.79, 38.13, 38.05, 35.75, 33.77, 28.23, 27.08, 26.79, 25.06, 24.55. HRMS calcd for C\(_{15}\)H\(_{30}\)NO+: 240.2322, found: 240.2327.

**General procedure for coupling the alcohols (121a-d) to the acid (10) to form benzoates 117a-d.** DMAP (0.1 eq) was added to a solution of 2-(3'-methyl)succinimidoanthranilic acid (10, 1 eq) in CH\(_2\)Cl\(_2\) (1 M). A solution of alcohol 121a-d (1 eq) in CH\(_2\)Cl\(_2\) (0.33 M) was cannulated into the reaction mixture, which was then cooled to 0°C and DCC (1 eq) was added in one portion. The reaction was warmed to rt and stirred for 20 h. At that time the reaction was diluted with Et\(_2\)O, and the precipitate removed by filtration. Chromatography gave 117a-d in fairly good yields.
N-(4'-Phenyl-1'-butyl)piperidinyl-3-methyl 2”-(3’”-methyl)succinimidobenzoate (117a). The general procedure for the coupling was followed, starting with hydroxymethylpiperidine 121a (0.116 g, 0.47 mmol). Chromatography in 3% MeOH/CHCl₃ gave 0.142 g (66%) of 117a. ¹H NMR δ: 8.10 (d, 1H, J = 8 Hz), 7.64 (t, 1H, J = 8 Hz), 7.51 (t, 1H, J = 8 Hz), 7.34-7.04 (m, 6H), 4.13 (m, 2H), 3.10 (m, 4H), 2.70 (m, 3H), 2.38 (t, 2H, J = 8 Hz), 2.10-0.80 (m, 14H). ¹³C NMR δ: 142.52, 133.36, 131.47, 129.78, 129.29, 128.35, 128.21, 125.62, 68.00, 59.01, 57.20, 54.06, 37.00, 35.84, 35.37, 35.25, 29.46, 27.33, 26.52, 24.71, 16.33.
N-(5'-Phenyl-1'-pentyl)piperidinyl-3-methyl 2''-(3'''-methyl)succinimidobenzoate (117b). The general procedure for the coupling was followed, starting with hydroxymethylpiperidine 121b (0.204 g, 0.78 mmol). Chromatography in 2% MeOH/CHCl₃ gave 0.299 g (80%) of 117b. H NMR δ: 8.12 (d, 1H, J = 7 Hz), 7.63 (t, 1H, J = 7 Hz), 7.46 (t, 1H, J = 7 Hz), 7.19 (m, 6H), 4.10 (m, 2H), 3.01 (m, 4H), 2.73-2.45 (bs, 1H), 2.62 (t, 2H, J = 7 Hz), 2.32 (t, 2H, J = 7 Hz), 2.10-1.20 (m, 16H). C NMR δ: 180.26, 176.30, 164.80, 143.09, 133.78, 133.21, 132.00, 130.22, 129.73, 128.79, 128.63, 127.81, 126.00, 68.45, 59.55, 57.60, 54.49, 37.41, 36.29, 36.22, 35.70, 31.78, 30.09, 27.77, 27.69, 27.12, 25.12, 16.89.
N-(6'-Phenyl-1'-hexyl)piperidinyl-3-methyl 2''-(3''''-methyl)succinimidobenzoate (117c). The general procedure for the coupling was followed, starting with hydroxymethylpiperidine 121c (0.262 g, 0.95 mmol). Chromatography in 2% MeOH/CHCl₃ gave 0.350 g (75%) of 117c. ¹H NMR δ: 8.10 (d, 1H, J = 8 Hz), 7.63 (t, 1H, J = 8 Hz), 7.49 (t, 1H, J = 8 Hz), 7.20 (m, 6H), 4.09 (m, 2H), 3.00 (m, 4H), 2.67-2.42 (bs, 1H), 2.61 (t, 2H, J = 7 Hz), 2.32 (t, 2H, J = 8 Hz), 2.20-1.30 (m, 18H). ¹³C NMR δ: 180.26, 143.20, 133.78, 131.98, 130.22, 129.72, 128.79, 128.61, 127.78, 125.96, 68.43, 59.61, 57.56, 54.46, 37.41, 36.30, 36.21, 35.66, 31.80, 29.61, 27.88, 27.76, 27.15, 25.10.
N-(3'-Cyclohexyl-1'-propyl)piperidinyl-3-methyl 2''-(3'''-methyl)succinimidobenzoate (117d). The general procedure for the coupling was followed, starting with hydroxymethylpiperidine 121d (0.313 g, 1.31 mmol). Chromatography in 3% MeOH/CHCl₃ gave 0.467 g (79%) of 117d. ¹H NMR δ: 8.07 (d, 1H, J = 8 Hz), 7.64 (t, 1H, J = 8 Hz), 7.51 (t, 1H, J = 8 Hz), 7.25 (d, 1H, J = 8 Hz), 4.09 (m, 2H), 2.96 (m, 4H), 2.55 (m, 1H), 2.30 (t, 2H, J = 8 Hz), 1.98 (m, 2H), 1.80-1.40 (m, 14H), 1.30-0.80 (m, 9H). ¹³C NMR δ: 180.24, 176.27, 164.77, 133.76, 133.18, 131.98, 130.22, 129.70, 127.77, 68.46, 60.00, 57.57, 54.49, 38.05, 37.41, 36.23, 35.75, 33.78, 27.79, 27.09, 26.79, 25.14, 24.58, 16.73. HRMS calcd for C₂₇H₃₉N₂O₄⁺: 455.2904, found: 455.2909.

General procedure for preparing salts of compounds 117a-d. Benzoate (117a-d, 1 eq) was dissolved in a minimal amount of EtOAc, the corresponding acid (oxalic acid or benzoic acid, 1 eq) was added and stirred at rt. The mixture was concentrated to give the salt of the benzoate.
General procedure for preparing bromobutyl aryl ethers (124a-c). To a solution of the appropriate phenol (1 eq) in acetone (1 M), was added K$_2$CO$_3$ (1 eq) in one portion and then 1,4-dibromobutane (10 eq) by syringe. The reaction was heated to reflux for 20 h. After allowing the solvent to evaporate, water was added and the mixture was extracted with EtOAc (2x). The organic phase was washed with 10% aq. NaOH (3x). Dried with MgSO$_4$, filtered and concentrated. Flash chromatography in hexanes (increasing the polarity to 4% EtOAc in hexanes) was performed unless otherwise stated.

1-Bromo-4-phenoxybutane (124a). The general procedure for the preparation of bromobutyl aryl ethers was followed, starting with phenol (0.282 g, 3.00 mmol). Chromatography in hexanes gave 0.668 g (97%) of 124a. $^1$H NMR $\delta$: 7.37-7.21 (m, 2H), 7.01-6.83 (m, 3H), 3.98 (t, 2H, $J = 6$ Hz), 3.48 (t, 2H, $J = 6$ Hz), 2.07 (m, 2H), 1.94 (m, 2H). $^{13}$C NMR $\delta$: 158.79, 129.44, 120.67, 114.42, 66.64, 33.45, 29.45, 27.90.
1-Bromo-4-(4'-methoxy)phenoxybutane (124b). The general procedure for the preparation of bromobutyl aryl ethers was followed, starting with 4-methoxyphenol (0.372 g, 3.00 mmol). Chromatography gave 0.652 g (84%) of 124b. \( ^1H \text{NMR} \delta: 6.81 \text{ (s, 4H), 3.93 (t, 2H, } J = 6 \text{ Hz), 3.75 (s, 3H), 3.48 (t, 2H, } J = 6 \text{ Hz), 2.04 (m, 2H), 1.92 (m, 2H).} \) \( ^{13} \text{C NMR} \delta: 153.82, 152.96, 115.35, 114.61, 67.41, 55.69, 33.49, 29.45, 27.97. \) HRMS calcd for C\(_{11}\)H\(_{15}\)BrO\(_2\)Na\(^+\): 281.0148, found: 281.0139.

1-Bromo-4-(3', 4'-dichloro)phenoxybutane (124c). The general procedure for the preparation of bromobutyl aryl ethers was followed, starting with 3, 4-dichlorophenol (0.489 g, 3.00 mmol). Chromatography gave 0.752 g (84%) of 124c. \( ^1H \text{NMR} \delta: 7.29 \text{ (d, 1H, } J = 9 \text{ Hz), 6.96 (d, 1H, } J = 3 \text{ Hz), 6.72 (dd, 1H, } J = 9 \text{ Hz and } 3 \text{ Hz), 3.94 (t, 2H, } J = 6 \text{ Hz), 3.46 (t, 2H, } J = 6 \text{ Hz), 2.12-1.85 (m, 4H).} \) \( ^{13} \text{C NMR} \delta: 157.85, 132.78, 130.61, 123.89, 116.24, 114.46, 67.41, 33.18, 29.26, 27.66. \) HRMS calcd for C\(_{10}\)H\(_{11}\)Cl\(_2\)BrO\(^\cdot\) 295.9365, found: 295.9347.
General procedure for preparing bromopentyl aryl ethers (124d-f). To a solution of the appropriate phenol (1 eq) in acetone (1 M), was added K$_2$CO$_3$ (1 eq) in one portion and then 1,5-dibromopentane (10 eq) by syringe. The reaction was heated to reflux for 20 h. After allowing the solvent to evaporate, water was added and the mixture was extracted with EtOAc (2x). The organic phase was washed with 10% aq. NaOH (3x). Dried with MgSO$_4$, filtered and concentrated. Flash chromatography in hexanes (increasing the polarity to 4% EtOAc in hexanes) was performed unless otherwise stated.

1-Bromo-5-phenoxy pentane (124d). The general procedure for the preparation of bromopentyl aryl ethers was followed, starting with phenol (0.282 g, 3.00 mmol). Chromatography in hexanes gave 0.732 g (100%) of 124d. $^1$H NMR $\delta$: 7.34-7.22 (m, 2H), 7.00-6.84 (m, 3H), 3.96 (t, 2H, $J = 7$ Hz), 3.43 (t, 2H, $J = 7$ Hz), 1.94 (m, 2H), 1.82 (m, 2H), 1.64 (m, 2H). $^{13}$C NMR $\delta$: 158.90, 129.40, 120.59, 114.42, 67.37, 33.56, 32.48, 28.44, 24.83. HRMS calcd for C$_{11}$H$_{15}$BrO$^-$: 242.0301, found: 242.0315.

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1-Bromo-5-(4'-methoxy)phenoxy pentane (124e). The general procedure for the preparation of bromopentyl aryl ethers was followed, starting with 4-methoxyphenol (0.372 g, 3.00 mmol). Chromatography gave 0.580 g (71%) of 124e. $^1$H NMR $\delta$: 6.82 (s, 4H), 3.90 (t, 2H, $J = 6$ Hz), 3.75 (s, 3H), 3.42 (t, 2H, $J = 7$ Hz), 1.92 (m, 2H), 1.78 (m, 2H), 1.60 (m, 2H). $^{13}$C NMR $\delta$: 153.74, 153.08, 115.39, 114.61, 68.19, 55.69, 33.60, 32.48, 28.52, 24.83. HRMS calcd for C$_{12}$H$_{17}$BrO$_2$Na$: 295.0304$, found: 295.0319.

1-Bromo-5-(3', 4'-dichloro)phenoxy pentane (124f). The general procedure for the preparation of bromopentyl aryl ethers was followed, starting with 3, 4-dichlorophenol (0.489 g, 3.00 mmol). Chromatography gave 0.744 g (79%) of 124f. $^1$H NMR $\delta$: 7.28 (d, 1H, $J = 9$ Hz), 6.96 (d, 1H, $J = 3$ Hz), 6.72 (dd, 1H, $J = 9$ Hz and 3 Hz), 3.91 (t, 2H, $J = 7$ Hz), 3.42 (t, 2H, $J = 7$ Hz), 2.01-1.72 (m, 4H), 1.60 (m, 2H). $^{13}$C NMR $\delta$: 167.09, 132.74, 130.61, 123.77, 116.24, 114.50,
General procedure for preparing N-phenoxyalkylnipecotates (125a-f). Ethyl nipecotate hydrochloride (94, 1eq) was dissolved in EtOH (1.5 M) and K$_2$CO$_3$ (4 eq) was added. Appropriately substituted phenoxyalkyl bromide 124a-f (1.2 eq) in EtOH (1.85 M) was canulated into the reaction and heated to reflux. After 20 h, the reaction was cooled to rt, water added and the mixture was extracted with EtOAc (3x). The organic phase was washed with brine, dried with MgSO$_4$, filtered and concentrated. Nipecotates 125a-f were purified by flash chromatography in 25% EtOAc in hexanes (increasing to 40%) unless otherwise stated.

Ethyl 1-(4'-phenoxy)butylnipecotate (125a). The general procedure for the preparation of N-phenoxyalkylnipecotates was followed, starting with bromobutane 124a (0.630 g, 2.75 mmol). Chromatography gave 0.423 g (61%)
of 125a. $^1$H NMR $\delta$: 7.35-7.20 (m, 2H), 7.00-6.83 (m, 3H), 4.11 (q, 2H, $J = 7$ Hz), 3.95 (t, 2H, $J = 6$ Hz), 2.97 (d, 1H, $J = 11$ Hz), 2.75 (d, 1H, $J = 12$ Hz), 2.52 (m, 1H), 2.38 (t, 2H, $J = 7$ Hz), 2.13 (t, 1H, $J = 11$ Hz), 2.05-1.86 (m, 2H), 1.86-1.38 (m, 7H), 1.23 (t, 3H, $J = 7$ Hz). $^{13}$C NMR $\delta$: 174.24, 158.98, 129.36, 120.44, 114.42, 67.57, 60.23, 58.45, 55.46, 53.79, 41.91, 27.28, 27.00, 24.64, 23.43, 14.19. HRMS calcd for C$_{10}$H$_{18}$NO$_4$+: 306.2064, found: 306.2062.

Ethyl 1-(4'-4''-methoxy)phenoxy)butylnipecotate (125b). The general procedure for the preparation of N-phenoxyalkynipecotates was followed, starting with bromobutane 124b (0.638 g, 2.46 mmol). Chromatography in 25% EtOAc in hexanes gave 0.509 g (74%) of 125b. $^1$H NMR $\delta$: 6.82 (s, 4H), 4.10 (q, 2H, $J = 7$ Hz), 3.90 (t, 2H, $J = 7$ Hz), 3.75 (s, 3H), 2.97 (d, 1H, $J = 12$ Hz), 2.75 (d, 1H, $J = 11$ Hz), 2.37 (t, 2H, $J = 7$ Hz), 2.12 (t, 1H, $J = 11$ Hz), 2.04-1.86 (m, 2H), 1.86-1.38 (m, 7H), 1.23 (t, 3H, $J = 7$ Hz). $^{13}$C NMR $\delta$: 174.24, 153.62, 153.16, 115.35, 114.57, 68.34, 60.23, 58.48, 55.69, 55.42, 53.75, 41.91, 27.35, 27.00, 24.60, 23.39, 14.16. HRMS calcd for C$_{19}$H$_{30}$NO$_4$+: 336.2169, found: 336.2175.
Ethyl 1-(4′-(3″, 4″-dichloro)phenoxy)butylnipecotate (125c). The general procedure for the preparation of N-phenoxyalkyiNipecotates was followed, starting with bromobutane 124c (0.672 g, 2.25 mmol). Chromatography gave 0.574 g (82%) of 125c. \( ^1H \text{NMR } \delta: 7.26 (d, 1H, J = 9 \text{ Hz}), 6.93 (d, 1H, J = 3 \text{ Hz}), 6.70 (dd, 1H, J = 9 \text{ Hz and } 3 \text{ Hz}), 4.09 (q, 2H, J = 7 \text{ Hz}), 3.89 (t, 2H, J = 7 \text{ Hz}), 2.94 (d, 1H, J = 11 \text{ Hz}), 2.72 (d, 1H, J = 11 \text{ Hz}), 2.51 (m, 1H), 2.35 (t, 2H, J = 7 \text{ Hz}), 2.11 (t, 1H, J = 11 \text{ Hz}), 2.04-1.83 (m, 2H), 1.82-1.30 (m, 7H), 1.21 (t, 3H, J = 7 \text{ Hz}). \) \( ^{13}C \text{NMR } \delta: 174.16, 158.05, 132.70, 130.53, 123.58, 116.20, 114.50, 68.27, 60.23, 58.29, 55.38, 53.75, 41.83, 26.97, 24.56, 23.24, 14.16. \) HRMS calcd for \( \text{C}_{18}\text{H}_{26}\text{Cl}_{2}\text{NO}_3^+ \): 374.1284, found: 374.1272.
Ethyl 1-(5'-phenoxy)pentylnipecotate (125d). The general procedure for the preparation of N-phenoxyalkynipecotates was followed, starting with bromopentane 124d (0.674 g, 2.77 mmol). Chromatography in 25% EtOAc in hexanes gave 0.553 g (75%) of 125d. ¹H NMR δ: 7.28 (m, 2H), 6.97-6.87 (m, 3H), 4.14 (q, 2H, J = 7 Hz), 3.96 (t, 2H, J = 7 Hz), 3.00 (d, 1H, J = 11 Hz), 2.78 (d, 1H, J = 11 Hz), 2.53 (m, 1H), 2.37 (t, 2H, J = 7 Hz), 2.13 (t, 1H, J = 11 Hz), 2.02-1.86 (m, 2H), 1.86-1.65 (m, 3H), 1.65-1.37 (m, 6H), 1.26 (t, 3H, J = 7 Hz). ¹³C NMR δ: 174.27, 159.02, 129.36, 120.44, 114.42, 67.65, 60.23, 58.80, 55.50, 53.83, 41.91, 29.18, 27.04, 26.62, 24.64, 24.05, 14.19. HRMS calcd for C₁₉H₃₀NO₃⁺: 320.2220, found: 320.2231.
Ethyl 1-(5’-(4”-methoxy)phenoxy)butylnipecotate (125e). The general procedure for the preparation of N-phenoxyalkynipecotates was followed, starting with bromopentane 124e (0.530 g, 1.94 mmol). Chromatography gave 0.443 g (78%) of 125e. \( ^1H \) NMR \( \delta \): 6.82 (s, 4H), 4.10 (q, 2H, \( J = 7 \) Hz), 3.88 (t, 2H, \( J = 7 \) Hz), 3.74 (s, 3H), 2.97 (d, 1H, \( J = 11 \) Hz), 2.74 (d, 1H, \( J = 11 \) Hz), 2.52 (m, 1H), 2.33 (t, 2H, \( J = 7 \) Hz), 2.09 (t, 1H, \( J = 11 \) Hz), 2.00-1.85 (m, 2H), 1.82-1.67 (m, 3H), 1.62-1.37 (m, 6H), 1.22 (t, 3H, \( J = 7 \) Hz). \( ^13C \) NMR \( \delta \): 174.27, 153.62, 153.20, 115.35, 114.57, 68.42, 60.23, 58.80, 55.69, 55.50, 53.83, 41.91, 29.26, 27.04, 26.62, 24.64, 24.05, 14.16. HRMS calcd for C\(_{20}\)H\(_{32}\)NO\(_4^+\): 350.2326, found: 350.2337.
Ethyl 1-(5'-(3''', 4'''-dichlorophenoxy)butynipecotate (125f). The general procedure for the preparation of \(N\)-phenoxyalkynipecotates was followed, starting with bromopentane 124f (0.700 g, 2.24 mmol). Chromatography gave 0.570 g (79%) of 125f. \(^1H\) NMR \(\delta:\) 7.27 (d, 1H, \(J = 9\) Hz), 6.94 (d, 1H, \(J = 3\) Hz), 6.71 (dd, 1H, \(J = 9\) Hz and 3 Hz), 4.10 (q, 2H, \(J = 7\) Hz), 3.90 (t, 2H, \(J = 6\) Hz), 2.94 (d, 1H, \(J = 11\) Hz), 2.72 (d, 1H, \(J = 11\) Hz), 2.52 (m, 1H), 2.36 (t, 2H, \(J = 7\) Hz), 2.10 (t, 1H, \(J = 11\) Hz), 2.05-1.84 (m, 2H), 1.84-1.32 (m, 9H), 1.22 (t, 3H, \(J = 7\) Hz). \(^{13}C\) NMR \(\delta:\) 174.20, 158.09, 132.70, 130.53, 123.58, 116.20, 114.50, 68.31, 60.23, 58.29, 55.42, 53.79, 41.87, 26.97, 24.60, 23.24, 14.16.

General procedure for preparing hydroxymethylpiperidines (126a-f). Li\(\text{AlH}_4\) (200 mol%) was suspended in THF (2 M) and cooled to 0 °C. A solution of nipecotate 125a-f (1 eq) in THF (0.5 M) was canulated into the reaction and warmed to rt. The mixture was stirred for 20 h, and then cooled to 0 °C and diluted with \(\text{Et}_2\text{O}\). For each mmol used of Li\(\text{AlH}_4\), 40 \(\mu\)l of \(\text{H}_2\text{O}\) were added and the mixture was stirred for 15 mins. Then 40 \(\mu\)l/(mmol Li\(\text{AlH}_4\)) of 15% aqueous
NaOH were added, stirred for 15 mins and finally, 100 µl/(mmol LiAlH₄) of H₂O were added. The precipitate was removed by filtration and washed with Et₂O. Concentrating the filtrate gave 1-substituted 3-hydroxymethylpiperidines 126a-f, which did not require purification.

![Image of 126a](image)

3-Hydroxymethyl-1-(4'-phenoxy)butylpiperidine (126a). The general procedure for the reduction of the ester was followed, starting with nipecotate 125a (0.392 g, 1.28 mmol). Work-up gave 0.343 g (>100%) of 126a. ¹H NMR δ: 7.25 (m, 2H), 6.89 (m, 3H), 3.95 (t, 2H, J = 7 Hz), 3.57 (m, 2H), 2.81 (d, 1H, J = 11 Hz), 2.64 (m, 1H), 2.36 (t, 2H, J = 7 Hz), 2.14 (t, 1H, J = 11 Hz), 2.01 (t, 1H, J = 11 Hz), 1.88-1.50 (m, 9H). ¹³C NMR δ: 158.98, 129.36, 120.47, 114.42, 67.53, 67.30, 58.80, 57.55, 54.33, 37.80, 27.70, 27.31, 24.64, 23.43. HRMS calcd for C₁₆H₂₆NO₂⁺: 264.1958, found: 264.1958.
3-Hydroxymethyl-1-(4′-(4″-methoxy)phenoxy)butylpiperidine (126b). The general procedure for the reduction of the ester was followed, starting with nipecotate 125b (0.477 g, 1.42 mmol). Work-up gave 0.425 g (>100%) of 126b. 

$^1$H NMR δ: 6.80 (s, 4H), 3.89 (t, 2H, $J = 6$ Hz), 3.75 (s, 3H), 3.54 (m, 2H), 2.83 (d, 1H, $J = 11$ Hz), 2.63 (m, 1H), 2.34 (t, 2H, $J = 7$ Hz), 2.15-1.89 (m, 2H), 1.85-1.47 (m, 9H). $^{13}$C NMR δ: 153.62, 153.12, 115.35, 114.57, 68.31, 67.02, 58.83, 57.55, 55.69, 54.33, 37.95, 27.66, 27.43, 24.64, 23.36. HRMS calcd for $C_{17}H_{28}NO_3^+$: 294.2064, found: 294.2067.

3-Hydroxymethyl-1-(4′-(3″, 4″-dichloro)phenoxy)butylpiperidine (126c). The general procedure for the reduction of the ester was followed, starting with nipecotate 125c (0.408 g, 1.09 mmol). Work-up gave 0.350 g (97%) of 126c. 

$^1$H
NMR δ: 7.30 (d, 1H, J = 9 Hz), 6.98 (d, 1H, J = 3 Hz), 6.74 (dd, 1H, J = 9 Hz and 3 Hz), 3.93 (t, 2H, J = 6 Hz), 3.60 (m, 2H), 2.84 (d, 1H, J = 11 Hz), 2.65 (m, 1H), 2.37 (t, 2H, J = 7 Hz), 2.14 (t, 1H, J = 10 Hz), 2.01 (t, 1H, J = 10 Hz), 1.89-1.50 (m, 9H). 13C NMR δ: 158.05, 132.70, 130.57, 116.20, 114.54, 68.27, 67.18, 58.64, 57.55, 54.33, 37.83, 27.66, 27.08, 24.64, 23.28. HRMS calcd for C16H24Cl2NO2+: 332.1179, found: 332.1176.

3-Hydroxymethyl-1-(5'-phenoxy)pentylpiperidine (126d). The general procedure for the reduction of the ester was followed, starting with nipecotate 125d (0.540 g, 1.69 mmol). Work-up gave 0.467 g (100%) of 126d. 1H NMR δ: 7.29 (m, 2H), 6.90 (m, 3H), 3.93 (t, 2H, J = 7 Hz), 3.61 (m, 2H), 2.78 (d, 1H, J = 11 Hz), 2.60 (m, 1H), 2.31 (t, 2H, J = 8 Hz), 2.15 (t, 1H, J = 10 Hz), 2.03 (t, 1H, J = 10 Hz), 1.89-1.39 (m, 11H). 13C NMR δ: 159.06, 129.40, 120.47, 114.46, 67.65, 67.49, 59.11, 57.71, 54.41, 37.72, 29.22, 27.82, 26.65, 24.68, 24.13. HRMS calcd for C17H28NO2+: 278.2115, found: 278.2114.
3-Hydroxymethyl-1-(5'- (4''-methoxy)phenoxy)pentylpiperidine (126e). The general procedure for the reduction of the ester was followed, starting with nipecotate 125e (0.409 g, 1.17 mmol). Work-up gave 0.359 g (100%) of 126e. 

$^1$H NMR δ: 6.80 (s, 4H), 3.88 (t, 2H, $J = 7$ Hz), 3.76 (s, 3H), 3.58 (m, 2H), 2.80 (d, 1H, $J = 11$ Hz), 2.61 (m, 1H), 2.30 (t, 2H, $J = 7$ Hz), 2.12 (m, 1H), 1.99 (m, 1H), 1.84-1.36 (m, 11H). $^{13}$C NMR δ: 153.62, 153.16, 115.35, 114.57, 68.42, 67.33, 59.11, 57.67, 55.69, 54.37, 37.76, 29.26, 27.74, 26.62, 24.68, 24.09. 

HRMS calcd for C$_{16}$H$_{30}$NO$_3$: 308.2220, found: 308.2227.

3-Hydroxymethyl-1-(5'- (3'', 4''-dichloro)phenoxy)pentylpiperidine (126f). 

The general procedure for the reduction of the ester was followed, starting with nipecotate 125f (0.542 g, 1.40 mmol). Work-up gave 0.520 g (>100%) of 126f.
$^1$H NMR $\delta$: 7.29 (d, 1H, $J = 9$ Hz), 6.96 (d, 1H, $J = 3$ Hz), 6.73 (dd, 1H, $J = 9$ Hz and 3 Hz), 3.90 (t, 2H, $J = 7$ Hz), 3.56 (m, 2H), 3.02 (bs, 1H), 2.86 (d, 1H, $J = 11$ Hz), 2.65 (d, 1H, $J = 11$ Hz), 2.32 (t, 2H, $J = 7$ Hz), 2.11 (t, 1H, $J = 10$ Hz), 1.96 (t, 1H, $J = 10$ Hz), 1.89-1.36 (m, 11H). $^{13}$C NMR $\delta$: 158.09, 132.70, 130.53, 123.62, 116.24, 114.50, 68.34, 67.02, 58.99, 57.63, 54.33, 37.87, 28.91, 27.66, 26.46, 24.64, 23.98. HRMS calcd for C$_{17}$H$_{26}$Cl$_2$NO$_2$: 346.1335, found: 346.1312.

**General procedure for preparing methylsuccinimidobenzoates (127a-f).**

DMAP (0.1 eq) was added to a solution of 2-(3'-methylsuccinimido)benzoic acid (10, 1 eq) in CH$_2$Cl$_2$ (1 M). A solution of 3-hydroxymethylpiperidine 126a-f (1 eq) in CH$_2$Cl$_2$ (0.33 M) was canulated into the reaction mixture, which was then cooled to 0 °C and DCC (1 eq) was added in one portion. The reaction was warmed to rt and stirred for 20 h. At that time, the reaction was diluted with Et$_2$O and the precipitate removed by filtration. Chromatography (in 2% MeOH in CHCl$_3$ unless otherwise stated) gave benzoates 127a-f.
1-(4'-Phenoxy)butylpiperidin-3-yl 2"-(3"'-methylsuccinimido)benzoate (127a). The general procedure for the coupling was followed, starting with hydroxymethylpiperidine 126a (0.316 g, 1.20 mmol). Chromatography gave 0.422 g (74%) of 127a. \(^{1}H\) NMR \(\delta\): 8.09 (d, 1H, \(J = 8\) Hz), 7.63 (t, 1H, \(J = 8\) Hz), 7.48 (t, 1H, \(J = 8\) Hz), 7.24 (m, 3H), 6.89 (m, 3H), 4.10 (m, 2H), 3.95 (t, 2H, \(J = 7\) Hz), 3.20-2.77 (m, 4H), 2.55 (m, 1H), 2.38 (t, 2H, \(J = 7\) Hz), 2.15-1.53 (m, 10H), 1.44 (bs, 3H). \(^{13}C\) NMR \(\delta\): 179.86, 176.02, 164.26, 158.90, 133.36, 132.66, 131.61, 131.46, 129.75, 129.32, 127.27, 120.40, 114.38, 67.96, 67.49, 58.64, 57.09, 53.94, 36.90, 35.78, 35.31, 35.16, 27.28, 24.68, 23.39, 16.25. HRMS calcd for C\(_{29}\)H\(_{35}\)N\(_2\)O\(_5\)^+: 479.2540, found: 479.2517.
1-(4"-(4"'-Methoxy)phenoxy)butylpiperidinyl-3-methyl 2""-((3""')-methylsuccinimido)benzoate (127b). The general procedure for the coupling was followed, starting with hydroxymethylpiperidine 126b (0.380 g, 1.30 mmol). Chromatography gave 0.503 g (76%) of 127b. $^1$H NMR δ: 8.11 (d, 1H, $J = 8$ Hz), 7.64 (t, 1H, $J = 8$ Hz), 7.50 (t, 1H, $J = 8$ Hz), 7.25 (d, 1H, $J = 8$ Hz), 6.83 (s, 4H), 4.12 (m, 2H), 3.92 (t, 2H, $J = 7$ Hz), 3.76 (s, 3H), 3.20-2.97 (m, 2H), 2.91 (d, 1H, $J = 11$ Hz), 2.81 (d, 1H, $J = 11$ Hz), 2.65-2.30 (m, 1H), 2.38 (t, 2H, $J = 7$ Hz), 2.15-1.53 (m, 10H), 1.44 (bs, 3H). $^{13}$C NMR δ: 179.75, 175.79, 164.18, 153.55, 153.08, 133.25, 131.38, 129.71, 129.21, 127.27, 115.27, 114.50, 68.27, 67.92, 58.64, 57.05, 55.61, 53.90, 36.86, 35.74, 35.12, 27.31, 27.24, 24.64, 23.36, 16.21. HRMS calcd for C$_{29}$H$_{37}$N$_2$O$_5^+$: 509.2646, found: 509.2668.
1-(4''-(3'', 4''-Dichloro)phenoxy)butylpiperidinyl-3-methyl 2''''-(3''''-methylsuccinimido)benzoate (127c). The general procedure for the coupling was followed, starting with hydroxymethylpiperidine 126c (0.324 g, 0.98 mmol). Chromatography gave 0.463 g (87%) of 127c. $^1$H NMR $\delta$: 8.09 (d, 1H, $J = 8$ Hz), 7.63 (t, 1H, $J = 8$ Hz), 7.47 (t, 1H, $J = 8$ Hz), 7.29-7.21 (m, 2H), 6.95 (d, 1H, $J = 3$ Hz), 6.71 (dd, 1H, $J = 9$ Hz and 3 Hz), 4.10 (m, 2H), 3.90 (t, 2H, $J = 7$ Hz), 3.20-2.95 (m, 2H), 2.95-2.72 (m, 2H), 2.50 (m, 1H), 2.36 (t, 2H, $J = 7$ Hz), 2.14-1.36 (m, 14H). $^{13}$C NMR $\delta$: 179.63, 175.71, 164.14, 157.93, 133.21, 132.59, 132.47, 131.34, 131.27, 130.41, 129.64, 129.09, 127.19, 123.35, 116.05, 114.38, 68.15, 67.72, 58.33, 56.85, 53.87, 36.79, 35.62, 35.00, 27.08, 26.85, 24.48, 23.12, 16.14. HRMS calcd for C$_{26}$H$_{33}$Cl$_2$N$_2$O$_5$+: 547.1761, found: 547.1746.
1-(5'-Phenoxy)pentylpiperidinyl-3-methyl 2''-(3'''-methylsuccinimido)benzoate (127d). The general procedure for the coupling was followed, starting with hydroxymethylpiperidine 126d (0.457 g, 1.65 mmol). Chromatography gave 0.533 g (66%) of 127d. \( ^1\text{H NMR} \delta: 8.11 \ (d, \ 1\text{H}, \ J = 8 \text{ Hz}), 7.65 \ (t, \ 1\text{H}, \ J = 8 \text{ Hz}), 7.51 \ (t, \ 1\text{H}, \ J = 8 \text{ Hz}), 7.34-7.20 \ (m, \ 3\text{H}), 6.98-6.85 \ (m, \ 3\text{H}), 4.12 \ (m, \ 2\text{H}), 3.95 \ (t, \ 2\text{H}, \ J = 7 \text{ Hz}), 3.20-2.80 \ (m, \ 4\text{H}), 2.54 \ (m, \ 1\text{H}), 2.39 \ (t, \ 2\text{H}, \ J = 7 \text{ Hz}), 2.20-1.40 \ (m, \ 16\text{H}). \) \( ^{13}\text{C NMR} \delta: 179.83, 175.90, 164.30, 158.98, 133.32, 132.70, 131.42, 129.75, 129.32, 127.27, 120.40, 114.38, 67.88, 67.57, 58.91, 57.01, 53.94, 36.90, 35.62, 29.10, 27.20, 26.38, 24.52, 24.02, 16.52. \) HRMS calcd for C\(_{29}\)H\(_{37}\)N\(_2\)O\(_5\): 493.2697, found: 493.2675.
1-(5′-(4′-Methoxy)phenoxy)pentylpiperidinyl-3-methyl 2′′′-(3′′′-methylsuccinimido)benzoate (127e). The general procedure for the coupling was followed, starting with hydroxymethylpiperidine 126e (0.321 g, 1.04 mmol). Chromatography gave 0.421 g (77%) of 127e. 1H NMR δ: 8.11 (d, 1H, J = 8 Hz), 7.67 (t, 1H, J = 8 Hz), 7.52 (t, 1H, J = 8 Hz), 7.25 (d, 1H, J = 8 Hz), 6.83 (s, 4H), 4.11 (m, 2H), 3.91 (t, 2H, J = 7 Hz), 3.78 (s, 3H), 3.20-2.78 (m, 4H), 2.54 (m, 1H), 2.35 (t, 2H, J = 7 Hz), 2.16-1.88 (m, 2H), 1.88-1.40 (m, 14H). 13C NMR δ:
1-(5"-(3", 4"-Dichloro)phenoxy)pentylpiperidinyl-3-methyl 2""-(3""""-methylsuccinimido)benzoate (127f). The general procedure for the coupling was followed, starting with hydroxymethylpiperidine 126f (0.498 g, 1.44 mmol). Chromatography gave 0.674 g (84%) of 127f. $^1$H NMR δ: 8.11 (d, 1H, $J$ = 8 Hz), 7.66 (t, 1H, $J$ = 8 Hz), 7.51 (t, 1H, $J$ = 8 Hz), 7.34-7.23 (m, 2H), 6.98 (d, 1H, $J$ = 3 Hz), 6.73 (dd, 1H, $J$ = 9 Hz and 3 Hz), 4.12 (m, 2H), 3.91 (t, 2H, $J$ = 7 Hz), 3.20-2.99 (m, 2H), 2.99-2.78 (m, 2H), 2.55 (m, 1H), 2.35 (t, 2H, $J$ = 7 Hz), 2.15-1.38 (m, 16H). $^{13}$C NMR δ: 179.83, 175.79, 164.26, 158.13, 133.36, 132.70, 131.46, 130.57, 129.79, 129.29, 123.58, 116.20, 114.54, 68.38, 67.96, 58.95, 57.16, 54.10, 36.98, 35.82, 35.19, 28.95, 27.31, 26.58, 24.68, 23.94, 16.29. HRMS calcd for C$_{29}$H$_{35}$Cl$_2$N$_2$O$_5$: 561.1918, found 561.1919.
General procedure for preparing salts of compounds 127a-f. The benzoate (127a-f, 1 eq) was dissolved in a minimal amount of EtOAc, oxalic acid (1 eq) was added and stirred at rt. The mixture was concentrated to give the oxalate salt.

\[ \text{cis, cis-3, 5-Dihydroxy-1-tert-butyldimethylsilyloxy-cyclohexane (131).} \]

cis, cis-1, 3, 5-Cyclohexanetriol dihydrate (0.169 g, 1.02 mmol) azeotroped with benzene until the water had been removed (determined by weighing). Dry 1, 3, 5-cyclohexanetriol was suspended in 2.7 ml THF, and tert-butyldimethylsilyl chloride (0.174 g, 1.15 mmol) and Et$_3$N (0.16 ml, 1.12 mmol) added. NaH (0.044 g, 60% in mineral oil, 1.11 mmol) was added in one portion and the mixture stirred at rt for 2 h. Warmed to 50 °C for 3 h and then stirred at rt overnight. Reaction was cooled to 0 °C, filtered and washed with cold Et$_2$O. The filtrate was concentrated and then suspended in hexanes. The solid was filtered off and washed with hexanes. This gave 0.170 g (68%) of cis, cis-3, 5-dihydroxy-1-tert-butyldimethylsilyloxy-cyclohexane (131). $^1$H NMR δ: 3.79 (m, 3H), 2.12-1.96 (m, 211
cis, cis-3, 5-Dimethoxy-1-tert-butyldimethylsilyloxy cyclohexane (132). NaH (0.127 g, 60% in mineral oil, 3.18 mmol) was rinsed with hexanes and suspended in 2.0 ml THF. cis, cis-3, 5-Dihydroxy-1-tert-butyldimethylsilyloxy cyclohexane (0.357 g, 1.45 mmol) in 4.0 ml THF was added and then freshly distilled Mel (0.45 ml, 7.24 mmol). The mixture was stirred at rt for 23 h, and then NaH (0.046 g, 60% in mineral oil, 1.16 mmol) was added. Stirred until the reaction was complete by TLC (25 h). The reaction was quenched with saturated aq. NH₄Cl and extracted with EtOAc (3x). The organic phase was washed with H₂O and brine. Dried with MgSO₄, filtered and concentrated. Flash chromatography in 3% EtOAc in hexanes (increasing to 5%) gave 0.385 g (97%) of cis, cis-3, 5-dimethoxy-1-tert-butyldimethylsilyloxy cyclohexane (132). ¹H NMR δ: 3.57 (m, 1H), 3.37 (s, 6H), 3.13 (m, 2H), 2.42 (m, 1H), 2.22 (m, 2H), 1.36-1.00 (m, 3H), 0.90 (s, 9H), 0.07 (s, 6H). ¹³C NMR δ: 74.59, 66.67, 55.92, 41.41, 36.86, 25.80, 18.12, -4.67. HRMS calcd for C₁₄H₃₀O₃SiNa⁺: 297.1856, found: 297.1877.
cis, cis-3, 5-Dimethoxycyclohexan-1-ol (133). cis, cis-3, 5-Dimethoxy-1-tert-butyldimethylsilyloxycyclohexane (1.045 g, 3.81 mmol) dissolved in 7.6 ml of AcOH/THF/H$_2$O (3:1:1) and stirred at rt for 48 h. The reaction mixture was concentrated and chromatographed in 50% EtOAc in hexanes to give 0.383 g (63%) of cis, cis-3, 5-dimethoxycyclohexan-1-ol (133). $^1$H NMR δ: 3.52 (m, 1H), 3.35 (s, 6H), 3.20 (m, 2H), 2.32 (m, 3H), 2.13 (s, 1H), 1.37-1.13 (m, 3H). $^{13}$C NMR δ: 74.55, 65.98, 56.04, 40.36, 36.63. HRMS calcd for C$_8$H$_{16}$O$_3$Na$: 183.0992$, found: 183.0991.

trans, cis-3, 5-Dimethoxy-1-cyclohexyl benzoate (135). Ph$_3$P (1.169 g, 4.46 mmol) and benzoic acid (0.544 g, 4.46 mmol) added to a solution of cis, cis-3, 5-dimethoxycyclohexan-1-ol (133, 0.357 g, 2.23 mmol) in 2.5 ml of THF. Diethyl azodicarboxylate (DEAD, 0.776 g, 4.46 mmol) was added dropwise over 5 minutes and the reaction mixture was stirred at rt for 18 h. The reaction mixture was...
was concentrated and 20% EtOAc in hexanes was added to precipitate most of the triphenylphosphine oxide formed. The suspension was filtered through Celite, concentrated and chromatographed in 10% EtOAc in hexanes (increasing to 15%) to give 0.548 g (93%) of \textit{trans}, cis-3, 5-dimethoxy-1-cyclohexyl benzoate (135). \textit{\textsuperscript{1}H NMR} δ: 8.02 (m, 2H), 7.65-7.42 (m, 3H), 5.59 (m, 1H), 3.60 (m, 2H), 3.39 (s, 6H), 2.61 (m, 1H), 2.38 (m, 2H), 1.50 (m, 2H), 1.25 (m, 1H). \textit{\textsuperscript{13}C NMR} δ: 165.39, 133.01, 129.48, 128.39, 128.16, 74.05, 69.74, 56.04, 37.37, 35.47. HRMS calcd for C\textsubscript{15}H\textsubscript{20}O\textsubscript{3}Na\textsuperscript{+}: 287.1254, found: 287.1250.

\textit{trans, cis-3, 5-Dimethoxycyclohexan-1-ol} (136). Dowex 1x8-100 resin (2.23 g wet weight, 7.8 mmol)\textsuperscript{138} was added to a solution of \textit{trans}, cis-3, 5-dimethoxy-1-cyclohexyl benzoate (0.524 g, 1.98 mmol) in 6.6 ml of MeOH/THF (3:1) and stirred at rt for 48 h. The reaction mixture was filtered to remove the resin, concentrated and chromatographed in 25% EtOAc in hexanes (increasing to 40%) to give 0.295 g (93%) of \textit{trans}, cis-3, 5-dimethoxycyclohexan-1-ol (136). \textit{\textsuperscript{1}H NMR} δ: 4.33 (m, 1H), 3.58 (m, 2H), 3.36 (s, 6H), 2.49 (m, 1H), 2.12 (m, 2H), 1.55 (s, 1H), 1.40 (m, 2H), 1.17 (m, 1H). \textit{\textsuperscript{13}C NMR} δ: 73.89, 66.25, 56.00, 38.30, 37.33. HRMS calcd for C\textsubscript{8}H\textsubscript{16}O\textsubscript{3}Na\textsuperscript{+}: 183.0992, found: 183.1003.
trans, cis-3, 5-Dimethoxycyclohexyl allyl ether (137). To a solution of 136 (0.258 g, 1.61 mmol) in THF (5 ml) was added NaH (95%, 0.045 g, 1.77 mmol). Freshly distilled allyl bromide (0.35 ml, 4.02 mmol) was added and stirred at rt for 72 h. NaH was added after 19 h (0.020 g, 0.80 mmol), 44 h (0.020 g, 0.80 mmol), and 67 h (0.040 g, 1.61 mmol), due to the reaction being found to be incomplete at those times. The reaction was quenched with saturated aqueous NH₄Cl and the mixture extracted with EtOAc (3x). The organic phase was washed with H₂O and brine, dried with MgSO₄, filtered and concentrated. Chromatography in 2% EtOAc in hexanes (increasing to 5%) gave 0.154 g (84%) of trans, cis-3, 5-dimethoxycyclohexyl allyl ether (137). ¹H NMR δ: 5.90 (m, 1H), 5.33-5.13 (m, 2H), 3.98 (m, 2H), 3.87 (m, 1H), 3.50 (m, 2H), 3.36 (s, 6H), 2.50 (m, 1H), 2.25 (m, 2H), 1.32-1.06 (m, 3H). ¹³C NMR δ: 135.11, 116.48, 74.05, 72.81, 69.28, 55.92, 37.21, 35.39. HRMS calcd for C₁₁H₂₀O₃Na⁺: 223.1305, found: 223.1292.
trans, cis-3, 5-Dimethoxycyclohexyl 2'-hydroxyethyl ether (138). Ozone was bubbled through a solution of 137 (0.210 g, 1.05 mmol) in CH₂Cl₂ at -78°C for 5 mins (when the solution turned blue). Argon was bubbled through for 5 mins to remove excess ozone and then NaBH₄ (0.040 g, 1.05 mmol) was added. After stirring for 1 h, more NaBH₄ (0.079 g, 2.10 mmol) was added and the mixture warmed to rt. Upon the addition of MeOH (1.0 ml), H₂ gas evolved and all the NaBH₄ went into solution. After stirring for 3 h, the reaction was quenched by the addition of 1 M HCl. The mixture was extracted with CH₂Cl₂ (2x) and the organic phase dried with MgSO₄, filtered and concentrated. This gave 0.202 g (94%) of 138. ¹H NMR δ: 3.88 (m, 1H), 3.73 (t, 2H, J = 5 Hz), 3.54 (t, 2H, J = 5 Hz), 3.51-3.41 (m, 2H), 3.37 (s, 6H), 2.49 (m, 1H), 2.25 (m, 2H), 1.46-1.09 (m, 3H). ¹³C NMR δ: 73.93, 69.39, 66.25, 62.02, 55.96, 37.06, 35.31. HRMS calcd for C₁₀H₂₀O₄Na⁺: 227.1254, found: 227.1267.
2-(trans, cis-3', 5'-Dimethoxycyclohexyl)oxyethyl tosylate (129). DMAP (0.011 g, 0.09 mmol) was added to a solution of 138 (0.140 g, 0.69 mmol) in CH₂Cl₂ (1.0 ml). After cooling to 0°C, Et₃N (0.14 ml, 1.03 mmol) was added and then p-toluenesulfonyl chloride (0.170 g, 0.89 mmol). The reaction was warmed to rt and stirred for 20 h. After adding 1 M HCl, the mixture was extracted with CH₂Cl₂ (2x) and the organic phase washed with H₂O and brine, dried with MgSO₄, filtered and concentrated. Chromatography in 2% EtOAc in hexanes (increasing to 40%) gave 0.140 g (57%) of tosylate 129. ¹H NMR δ: 7.81 (d, 2H, J = 9 Hz), 7.36 (d, 2H, J = 9 Hz), 4.14 (t, 2H, J = 5 Hz), 3.78 (m, 1H), 3.60 (t, 2H, J = 5 Hz), 3.45-3.30 (m, 2H), 3.34 (s, 6H), 2.46 (s, 3H), 2.44 (m, 1H), 2.15 (m, 2H), 1.29-1.02 (m, 3H). ¹³C NMR δ: 144.77, 129.79, 127.85, 74.01, 73.74, 69.39, 65.78, 55.88, 37.10, 35.12, 21.61. HRMS calcd for C₁₇H₂₆O₆SNa⁺: 381.1342, found: 381.1333.
Ethyl 1-(2'-(trans, cis-3", 5"-dimethoxycyclohexyl)oxyethyl)nipecotate (139). Ethyl nipecotate (0.047 g, 0.30 mmol) was dissolved in EtOH (0.5 ml) and K₂CO₃ (0.125 g, 0.90 mmol) added. A solution of 129 (0.108 g, 0.30 mmol) in EtOH (1.0 ml) was cannulated into the mixture and heated to reflux. After 20 h, the reaction was cooled to rt and diluted with H₂O. The mixture was extracted with EtOAc (3x), and the organic phase was washed with brine, dried with MgSO₄, filtered and concentrated. Chromatography in EtOAc gave 0.060 g (58%) of 139. ¹H NMR δ: 4.14 (q, 2H, J = 7 Hz), 3.81 (m, 1H), 3.56 (t, 2H, J = 6 Hz), 3.52-3.39 (m, 2H), 3.36 (s, 6H), 3.06 (d, 1H, J = 11 Hz), 2.81 (d, 1H, J = 11 Hz), 2.59 (t, 2H, J = 6 Hz), 2.56-2.43 (m, 3H), 2.32-1.81 (m, 5H), 1.79-1.36 (m, 5H), 1.27 (m, 3H). ¹³C NMR δ: 174.12, 74.09, 73.74, 66.60, 60.31, 58.25, 55.92, 54.22, 41.91, 37.21, 35.35, 26.81, 24.64, 14.23. HRMS calcd for C₁₈H₃₄NO₅⁺: 344.2432, found: 344.2438.

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3-Hydroxymethyl-1-(2'-trans, cis-3'', 5''-dimethoxycyclohexyl)oxyethyl)piperidine (140). LiAlH₄ (0.005 g, 0.12 mmol) was suspended in THF (0.5 ml) and cooled to 0 °C. A solution of nipecotate 139 (0.020 g, 0.06 mmol) in THF (1.0 ml) was cannulated into the reaction and warmed to rt. The mixture was stirred for 20 h, and then cooled to 0 °C and diluted with Et₂O. Then, 3 μl of H₂O were added and the mixture was stirred for 15 mins, before adding another 3 μl of 15% aqueous NaOH. The mixture was stirred for 15 mins and finally, 8 μl of H₂O were added. The precipitate was removed by filtration and washed with Et₂O. Concentrating the filtrate gave 0.017 g (93%) of 3-hydroxymethylpiperidine 140, which was taken on to the next step without further purification. ¹H NMR δ: 3.82 (m, 1H), 3.69 (m, 2H), 3.57 (t, 2H, J = 7 Hz), 3.48 (m, 2H), 3.37 (s, 6H), 2.85 (d, 1H, J = 11 Hz), 2.66 (m, 1H), 2.57 (t, 2H, J = 7 Hz), 2.49 (m, 1H), 2.24 (m, 3H), 2.04 (m, 2H), 1.46-1.09 (m, 7H). ¹³C NMR δ: 73.74, 67.41, 66.33, 62.79, 58.45, 57.94, 55.92, 54.84, 37.56, 37.17, 35.39, 29.68, 27.47.
1-(2'-(trans, cis-3'', 5''-Dimethoxycyclohexyl)oxyethyl)piperidinyl-3-methyl 2''''-(3''''-methylsuccinimido)benzoate (128). DMAP (0.001 g, 0.01 mmol) was added to a solution of 2-(3'-methylsuccinimido)benzoic acid (10, 0.013 g, 0.06 mmol) in \( \text{CH}_2\text{Cl}_2 \) (1.0 ml). A solution of 3-hydroxymethylpiperidine 140 (0.017 g, 0.06 mmol) in \( \text{CH}_2\text{Cl}_2 \) (1.0 ml) was canulated into the reaction mixture, which was then cooled to 0 °C and DCC (0.011 g, 0.06 mmol) was added in one portion. The reaction was warmed to rt and stirred for 20 h. At that time, the reaction was diluted with \( \text{Et}_2\text{O} \) and the precipitate removed by filtration. Chromatography in 5% MeOH in \( \text{CHCl}_3 \) gave 0.006 g (23%) of benzoate 128. \( ^1\text{H NMR} \delta: \) 8.12 (d, 1H, \( J = 8 \text{ Hz} \)), 7.68 (t, 1H, \( J = 8 \text{ Hz} \)), 7.54 (t, 1H, \( J = 8 \text{ Hz} \)), 7.26 (m, 1H), 4.16-3.99 (m, 2H), 3.82 (m, 1H), 3.62-3.39 (m, 4H), 3.35 (s, 6H), 3.10 (m, 2H), 2.92 (m, 1H), 2.58 (m, 2H), 2.48 (m, 2H), 2.23 (m, 2H), 2.14-1.89 (m, 4H), 1.77-1.55 (m, 4H), 1.52-1.02 (m, 6H).
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