Enone prodrugs of catecholamines
Venhuis, Bastiaan Johan

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2002

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Chapter 5

(−)-GMC6650, a highly potent orally active
dopaminergic prodrug

ABSTRACT

A trans-benzo[g]quinoline derived enone prodrug (5.16) was synthesized in six steps. (−)-GMC6650 (5.16a) was inactive in vitro at DA D₁, D₂, D₃, and D₄ receptors, but was pharmacologically active in striatal microdialysis experiments, where 5.16a showed to be highly potent after administration po and sc.¹ Instead, (+)-GMC6650 (5.16b) was inactive in both in vitro and in vivo experiments. Dose response studies showed 5.16a to be pharmacologically active at 3.0 nmol kg⁻¹ po and 1.0 nmol kg⁻¹ sc. Maximum effect was observed for 10 nmol kg⁻¹ po and sc. Compound 5.16a when administered sc, has a more potent profile in microdialysis than one of the most potent members of the aporphine family, N-propylaporphine.¹ In a preliminary study in the Ungerstedt model for Parkinson’s disease, 5.16a was able to alleviate Parkinsonian symptoms at 20 nmol kg⁻¹ sc showing increased potency over PD217015 (2.3a) and also a slower onset of action.²


Chapter 5

5.1 INTRODUCTION

In Chapters 2 and 3, syntheses of prodrugs of dopaminergic agents were described in relation to their possible application in the treatment of Parkinson’s disease. These prodrugs have enone structures and induced potent dopaminergic behavior at low doses (0.1 mg kg\(^{-1}\) po) in the Ungerstedt rat model of Parkinson’s disease. The bioactivation of the N,N-di-n-propyl substituted 2.3 was found to be enantioselective for the (S)-enantiomer 2.3a that was converted to (S)-5,6-di-hydroxy-2-(N,N-di-n-propylamino)tetralin ((S)-5,6-di-OH-DPAT, (S)-5.1). Analogs with other N,N-dialkyl substituents are thought to be also bioactivated to their corresponding catecholamines. The nature of the N,N-dialkyl substituents possibly also have an effect on the bioactivation process.

![Scheme 5.1](image)

**Scheme 5.1** Hypothesized bioactivation of enone prodrugs. Enone prodrug (5.1), hydroxylated metabolite (5.2), catecholamine (5.3).

Bioactivation of an enone prodrug to its corresponding catecholamine was hypothesized to proceed through an intermediate metabolite that was discovered in brain tissue of rats treated with 2.3 and 3.5 (Scheme 5.1). The first step in the bioactivation process was suggested to be a hydroxylation of the \(\alpha'\)-keto position, also a known process in steroid metabolism. Subsequent oxidation and rearrangement (double keto-enol tautomerization) in a second step would result in the formation of the catecholamine. \(\alpha'\)-Hydroxylation and subsequent oxidation, possibly describe the general pathway of bioactivation of enone prodrugs.

In general, the (S)-6-dialkylamino-3,4,5,6,7,8-hexahydro-2H-naphthalen-1-one structure (5.1), could be considered to be a ‘template’ for bioactivation of enones to their corresponding catecholamines (Figure 5.1). There is an interesting resemblance between this template and the pharmacophore of \(\alpha\)-rotameric DA agonists (5.2). The catecholamines, that are the anticipated pharmacologically active metabolites of the prodrugs in Chapters 2 and 3 all fit pharmacophore...
5.2. Therefore, it was interesting to synthesize other potential enone prodrugs of catecholamines that also fitted pharmacophore 5.2, yet were not of a 2-aminotetralin structure. Pharmacological evaluation of these new enones could provide further insight in the structural requirements for the bioactivation of enones.

\[
\begin{align*}
\text{R}' & \quad \text{R} \\
\text{5.1} & \quad \text{5.2}
\end{align*}
\]

**Figure 5.1** Suggested template for bioactivation (5.1), and the α-rotameric form of the DA agonist pharmacophore (5.2).

Literature describes a number of potent α-rotameric DA agonists whose enone counterparts structurally strongly resemble 5.1.5-8 (R)-Aporphines (5.3 and 5.4), and especially (R)-apomorphine (see Chapter 1), are used in the clinic to treat Parkinson’s disease and usually is only administered in later stages of the condition when other therapies fail (Figure 5.2). Difficulty in administration and numerous adverse effects, connected with the poor kinetics of the compounds, limit the application of these drugs.7,9,10

\[
\begin{align*}
\text{R} & \quad \text{5.3} \quad \text{R} = \text{Me} \\
\text{5.4} \quad \text{R} = \text{n-Pr}
\end{align*}
\]

**Figure 5.2** Structures of some potent α-rotameric DA agonists. (R)-apomorphine (5.3); (R)-N-n-propylaporphine (5.4); 6,7-di-OH-PBGQ (5.5); CV 205-502, (quinagolide, 5.6).

(4aR,10aS)-N-n-propyl-6,7-di-OH-1,2,3,4,4a,5,10,10a-octahydro-benzo[g]quinoline (6,7-di-OH-PBGQ, 5.5) is a known highly potent centrally acting mixed DA D₁/D₂ agonist.5,8 Its
phenolic analog, the benzo[g]quinoline derivative CV 205-502 is a directly acting DA D2 agonist, and is on the market as an anti-prolactine agent (Quinagolide), with a long elimination half-life. CV 205-502 was also found to be efficacious in the treatment of Parkinson’s disease.

In the enone prodrugs of general structure 5.1, the N,N-dialkylamine substituent may rotate freely around the CH–N bond. In the bioactivation process, it may assume any conformation rotationally possible. Rigidification of the general structure 5.1 to a three-ring system, gives an enone that is more restricted to a flat conformation what might affect the bioactivation process. Therefore, we set out to synthesize and pharmacologically evaluate the potential enone prodrug of 5.5.

5.2 CHEMISTRY

5.2.1 Benzo[g]quinoline derived enone prodrug

3-(4-Methoxyphenyl)-acrylic acid (5.7) was hydrogenated in the presence of a palladium catalyst, to the corresponding propionic acid derivative (5.8, Scheme 5.2). Subsequent treatment with, respectively, thionyl chloride and n-propylamine gave amide 5.9. Reduction of 5.9 with LiAlH4 in refluxing tetrahydrofuran gave the secondary amine 5.10. All of these reaction were straightforward and carried out under standard conditions and gave high yields (>98%).

Scheme 5.2 Reagents and conditions: a) H2, 10% Pd/C, EtOH, 45°C, 3h; b) i) SOCl2, CH2Cl2, RT, 1h; ii) n-PrNH2, 5%-NaOH/H2O, CH2Cl2, RT, 1h; c) LiAlH4, THF, reflux, 12h.
Crucial in the total synthesis the construction of a fused heterocyclic ring system. This was achieved by performing a Birch reduction of the aromatic ring in **5.10** using Li/NH$_3$ (l). Upon acidic hydrolysis the enol ether was converted to the ketone and the double bond shifted to give an $\alpha,\beta$-unsaturated ketone intermediate **5.13** (Scheme 5.3). The amine then, acting as a nucleophile added to the double bond linking the nitrogen to the $\beta$ position.

![Scheme 5.3](image)

**Scheme 5.3** *a) Li/NH$_3$ (l), THF, t-BuOH, $-60^\circ$C* *b) H$^+$/H$_2$O.*

Cyclization of N-n-propylated amine **5.13** gave a diastereomeric mixture of the *trans* and *cis* isomer in an 85:15 ratio as determined by GC analysis in 85% yield. $^1$H-NMR confirmed the major product to have a *trans* configuration ($J_{4a-8a} = 11$ Hz). However, no efforts were made to separate the diastereomers at this stage because addition and elimination of the amine over the double bond proved reversible in the last two reaction steps. This discovery was supported by reports on the epimerization of **5.14a** and **5.14b** under acidic conditions (Scheme 5.4).

![Scheme 5.4](image)

**Scheme 5.4** *Reversible elimination and addition almost solely gives the trans-isomer.*
Compounds \textbf{5.14a} and \textbf{5.14b} were synthesized by a strategy different from ours, and a cis/trans mixture was isolated. Heating the cis/trans mixture of \textbf{5.14a} in strong acid resulted in the selective formation of the trans isomer. After heating \textbf{5.14b} in strong acid, also a small percentage of the cis-isomer was also isolated. It is interesting to note that this intramolecular cyclization does not seem to occur in the case of reduced 4-methoxy-phenylethylamines.\textsuperscript{17}

Construction of the third ring starts by introducing a C\textsubscript{4} moiety by a Wittig reaction (Scheme 5.5).\textsuperscript{18} Four Wittig adducts were identified by GC/MS, consistent with the presence of two chiral centres and the formation of an either E or Z substituted double bond. This mixture of diastereomers containing \~10\% triphenylphosphine oxide, was used without purification for the final ring closure in polyphosphoric acid (PPA) at 100\textdegree C.\textsuperscript{18}

\begin{center}
\begin{tikzpicture}
\node[anchor=west] at (0,0) {5.14};
\node[anchor=west] at (2.25,0) {5.15};
\node[anchor=west] at (4.5,0) {5.16};
\node[anchor=west] at (6.75,0) {5.17};
\draw[->] (0,0) -- (0,-0.25); \draw[->] (2.25,0) -- (2.25,-0.25); \draw[->] (4.5,0) -- (4.5,-0.25);
\end{tikzpicture}
\end{center}

\textbf{Scheme 5.5} Synthesis of GMC6650 (\textbf{5.16}), GMC6651 (\textbf{5.17}). Reagents and conditions: a) Br\textsuperscript{−} (Ph)\textsubscript{3}P+: (CH\textsubscript{2})\textsubscript{3}CO\textsubscript{2}Et, t-BuONa, DMF, 0\textdegree C, 24h; b) PPA, 100\textdegree C, 3h.

The \textit{trans} isomer of the desired compound was formed with a selectivity of over 95\% when allowing PPA to hydrolyze during work-up at 80\textdegree C for 1 h. Lower hydrolysis temperatures gave a less favorable cis/trans ratio. Upon purification on a silica column it was found that the cis-isomer was partially converted to the energetically favored trans-isomer. The same transformation was observed after heating a cis/trans mixture in a 4N HCl solution. The reversible de-amination and re-amination as described for the fused two-ring systems evidently also works for this three-ring system and is likely to proceed in a similar fashion as for compounds \textbf{5.14a} and \textbf{5.14b} (Scheme 5.6).\textsuperscript{16} These characteristics of the ring system allowed for the high yield isolation of the desired \textit{trans}-isomer.
(--)-GMC6650, a highly potent orally active dopaminergic prodrug

Scheme 5.6 Reversible de-amination and re-amination favoring the trans-isomer.

Reaction conditions dictate product formation in this final reaction step and small changes in reaction conditions and work-up procedure change cyclization direction and stereoisomer ratio. For instance, an increase in reaction temperature in the PPA cyclization to 115°C led to extensive formation (30%) of an unstable by-product. GC/MS analysis showed it to have the same molecular mass but different fragmentation characteristics to those of GMC6650 and GMC6651. It was concluded that the most likely structure would be that of 5.18 (identified by GC/MS only, Scheme 5.7). Increase in reaction temperature of only 15°C makes the molecule overcome the steric hindrance induced by the heterocyclic ring and its n-propyl substituent.

Scheme 5.7 Increased reaction temperature gives by-product 5.18.

5.2.2 Diels-Alder route to 5.16

Another synthesis route was investigated to obtain the benzo[g]quinoline prodrug 5.16 in a short total synthesis. According to literature, 5.20 was prepared in one step from 5.19 and amine 5.22 was prepared in one step from 5.21 (Scheme 5.8).19-21
Scheme 5.8 Syntheses of (A) 3-ethynyl-cyclohexen-1-one (5.19) and (B) 1-(N-n-propylamine)-4-pentene (5.22). Reagents and conditions: a) CH≡CMgBr, THF, RT, 20h; b) H⁺/H₂O, RT, 15 min; c) n-propylamine, diethyl ether, reflux, 12h.

By mixing the two compounds, an instantaneous reaction occurred to give intermediate 5.23 (Scheme 5.9). An intramolecular Diels-Alder reaction of this compound would give a cis or trans product depending on an exo or endo reaction fashion (5.24). Since the cis isomer of 5.16 was readily isomerized to the trans isomer, an exo or endo reaction fashion is not relevant. The double bond was thought to isomerize readily to the energetically most favoured position on the ring junction to give the desired α,β-unsaturated enone 5.16. One example of a highly similar intramolecular Diels-Alder reaction, also followed by a rearrangement of the remaining double bond has been reported. After heating 5.22 under pressure for 72h, some (~2%) of the intermediate had reacted to give 5.16. Higher temperature or a suitable catalyst may be required to make this route of interest for preparation of 5.16 on a large scale.

5.2.3 Resolution of the enantiomers of 5.16

The enantiomers of racemic GMC6650 (5.16) were separated by semi-preparative HPLC, using a chiral column. (−)-GMC6650 is referred to as 5.16a and (+)-GMC6650 as 5.16b. Both enantiomers were isolated in a high optical purity as determined by chiral (ee > 99.8%).
(--)-GMC6650, a highly potent orally active dopaminergic prodrug

\[ \text{Scheme 5.9} \] Diels-Alder route to GMC6650 (5.16). Reagents and conditions: 

a) \( \text{CH}=\text{CMgBr}, \text{THF, RT, 2h} \);

b) \text{n-propylamine, diethyl ether, reflux, 12h} ;

c) \text{1,2-DCB, pressure, 190\textdegree C, 72h}.

5.3 PHARMACOLOGY

5.3.1 Receptor binding

Compounds (--)-GMC6650 (5.16a) and (+)-GMC6650 (5.16b) were tested for \textit{in vitro} binding at the DA D\textsubscript{1}, D\textsubscript{2}, D\textsubscript{3} and D\textsubscript{4} receptor. The displacement of the radioligand was measured at a fixed concentration of the test compound (50 or 100 nM). Estimated was whether this concentration of the test compound was above or below the \text{IC}_{50} of the radioligand. When at the tested concentration the displacement of the radioligand is below the \text{IC}_{50}, the test compound is considered not active at the receptor.

5.3.2 \textit{In vivo} pharmacology

The \textit{in vivo} activity of GMC6650 (5.16), (--)-GMC6650 (5.16a) and (+)-GMC6650 (5.16b) was measured by striatal microdialysis experiments.\textsuperscript{1} If the enones were converted to DA agonists that enter the striatum and bind to the presynaptic DA receptors, the normal DA release of the striatal dopaminergic neurons would decrease.\textsuperscript{24} Measuring the change in DA
release in the striatum as a function of time reflects onset of action, potency and duration of action of the test compound. The normal DA release before administration of the test compound is set to 100% and the change in output is expressed in relation to this percentage. The striatum is rich in DA D₁ and DA D₂ receptors and is a brain area of interest for the treatment of Parkinson’s disease. Compounds effective in this presynaptic model are likely to be good candidates for treatment of Parkinson’s disease. The compounds were tested both po and sc.

To treat Parkinson’s disease a DA agonist needs to stimulate the post-synaptic receptors. Therefore a preliminary study in the Ungerstedt model for Parkinson’s disease was performed.² The pharmacological effect of one dose, administered sc, was studied. See chapter 2 for a detailed description of the Ungerstedt model.

5.4 RESULTS AND DISCUSSION

5.4.1 *In vitro* pharmacology

Newly synthesized compounds (−)-GMC6650 (5.16a) and (+)-GMC6650 (5.16b), at the tested concentrations were not able to displace 50% of the radioligand used for the receptors tested (Table 5.1). Therefore, under these test conditions, the IC₅₀ of both the enantiomers for the DA D₁ and DA D₂ receptors is >100 nM and for the DA D₃ and DA D₄ receptors it is >50 nM. These results indicate that compounds 5.16a and 5.16b can be considered inactive *in vitro*.

<table>
<thead>
<tr>
<th></th>
<th>DA D₁ (SCH23390)²</th>
<th>DA D₂ (Spiperone)²</th>
<th>DA D₃ (Spiperone)²</th>
<th>DA D₄ (Nemonapride)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.16a</td>
<td>100/ -1%</td>
<td>100/ 15%</td>
<td>50/ 23%</td>
<td>50/ -4%</td>
</tr>
<tr>
<td>5.16b</td>
<td>100/  7%</td>
<td>100/  3%</td>
<td>50/ 28%</td>
<td>50/ -19%</td>
</tr>
</tbody>
</table>

²Tritiated radioligand used.

Table 5.1 *In vitro* affinity of 5.16a and 5.16b for several DA receptor subtypes. Values: (concentration test compound (nM)/ % displacement of the radioligand)
5.4.2 Striatal microdialysis studies on GMC6650 (5.16)

Racemic GMC6650 (5.16) was administered to male Wistar rats sc or po at three different doses (10, 30 and 100 nmol kg⁻¹). Results show a significant decrease in striatal DA release at all doses tested after both sc and po administration (Figure 5.3).

**Figure 5.3** Effects on striatal DA release in freely moving rats after sc or po administration of 5.16. The results are the mean ± SEM of data obtained from 4 rats. Injection at t = 0 min.
The 10, 30 and 100 nmol kg\(^{-1}\) doses \textit{sc} and \textit{po} induced significantly decreased DA levels 45 min post-administration (p < 0.05). DA levels remained significantly different from basal levels for the rest of the duration of the experiment (p < 0.05). The exception to this was the 30 nmol kg\(^{-1}\), dose \textit{po} that was significantly different from basal DA level until 300 min post-administration (p < 0.05). However, the effect of 30 nmol kg\(^{-1}\) \textit{po} does not significantly differ from that of 10 nmol kg\(^{-1}\) up until 330 min. \textbf{5.16}, administered \textit{sc} or \textit{po} at 100 nmol kg\(^{-1}\) induced a maximum effect possible (about 20% of basal levels). The racemic enone \textbf{5.16}, evidently induces a pronounced and long-lasting pharmacological effect \textit{in vivo}.

For the highest dose tested, no differences in effect of \textbf{5.16} after its administration \textit{sc} or \textit{po} were observed. In both cases, the maximum effect possible was reached meaning that they can not actually be compared. There is no statistically significant difference in the effect between administration \textit{sc} and \textit{po} of \textbf{5.16} at 10 nmol kg\(^{-1}\). However, this is the case for \textbf{5.16} dosed at a dose of 30 nmol kg\(^{-1}\). During the experiments, \textbf{5.16} also induced post-synaptic activity like sniffing, rearing, and licking (see also Chapter 2), which is consistent with the effects of a centrally acting DA agonist.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.4.png}
\caption{Effects on striatal DA release in freely moving rats after \textit{sc} administration of \textbf{5.16}. The results are the mean ± SEM of data obtained from 4 rats. Injection at \textit{t} = 0 min.}
\end{figure}
5.4.3 Striatal microdialysis studies on (+)-GMC6650 (5.16b)

Optically pure (+)-GMC6650 (5.16b, ee >99.8%) was administered to male Wistar rats \textit{sc} at 1000 nmol kg\textsuperscript{-1}. Results from a striatal microdialysis study show no significant effect on the striatal DA levels (Figure 5.4). No DA agonist related behavioral effects were observed. Enantiomer 5.16b therefore is not pharmacologically active at the DA receptors in the CNS at this high dose.

5.4.4 Striatal microdialysis studies on (−)-GMC6650 (5.16a)

Optically pure (−)-GMC6650 (5.16a, ee >99.8%) was administered to male Wistar rats \textit{sc} or \textit{po} at several doses (1, 3 and 10 nmol kg\textsuperscript{-1}). Results from a striatal microdialysis study show a significant effect for all doses tested (Figure 5.5). A dose response study on 5.16a administered \textit{sc} shows an increasing pharmacological effect upon higher dosing. At 10 nmol kg\textsuperscript{-1} \textit{sc}, a significant effect was observed from 45 min post-administration until the end of the experiment (p < 0.05).

For 3 nmol kg\textsuperscript{-1} \textit{sc} this was from 60 min post-administration until 315 min post-administration (p < 0.05). After that time point DA levels quickly return to basal levels. At the lowest dose tested, 1 nmol kg\textsuperscript{-1} \textit{sc}, a significant decrease was observed 75 min post-administration, which lasted until 205 min post-administration (p < 0.05).

Administration of 3 nmol kg\textsuperscript{-1} or 10 nmol kg\textsuperscript{-1} of 5.16a \textit{po} also induced a significant decrease in DA basal levels (Figure 5.6). A significant effect was observed for 3 nmol kg\textsuperscript{-1} \textit{po}, 45 min post-administration, what lasted until 270 min post-administration (p < 0.05). For 10 nmol kg\textsuperscript{-1} \textit{po} already 30 min post-administration a significant decrease was observed. This effect lasted for the length of the experiment (p < 0.05).

Comparing the pharmacological effects of the oral and subcutaneous administration routes leads to the observation that these effects are highly similar. For doses of 3 nmol kg\textsuperscript{-1} and 10 nmol kg\textsuperscript{-1} of 5.16a the effects are not statistically different. Behavioral (post-synaptic) effects were observed for 3 nmol kg\textsuperscript{-1} \textit{sc} and for 10 nmol kg\textsuperscript{-1} \textit{sc} and \textit{po}. Lower doses tested were only able to stimulate the more sensitive pre-synaptic receptors.
Figure 5.5 Effects on striatal DA release in freely moving rats after sc administration of 5.16a. The results are the mean ± SEM of data obtained from 4 rats. Injection at $t = 0$ min.

Figure 5.6 Effects on striatal DA release in freely moving rats after po administration of 5.16a. The results are the mean ± SEM of data obtained from 4 rats. Injection at $t = 0$ min.

Literature describes striatal microdialysis studies on some aporphines carried out under the same conditions as our experiments. The aporphine family is considered to incorporate the most potent DA agonists known. Yet, compound 5.16a displays a more potent pharmacological effect after administration po. Comparison of the pharmacological effects after administration sc
shows high similarity for the doses of 3 nmol kg\(^{-1}\) \textit{sc} and for 10 nmol kg\(^{-1}\) \textit{sc}. However, at 1 nmol kg\(^{-1}\) \textit{sc}, compound \textbf{5.16a} is able to induce a significant pharmacological effect whereas the most potent aporphine, ((R)-(−)-N-n-propyl-apomorphine, \textbf{5.4}) is unable to do so. In addition, compound \textbf{5.16a} is more potent in striatal microdialysis experiments than compound \textbf{2.3a}.\cite{333}

### 5.4.5 \textbf{5.16a in the Ungerstedt model for Parkinson’s disease}

A preliminary study of the pharmacological effect of \textbf{5.16a} was conducted in the Ungerstedt rat model for Parkinson’s disease, a post-synaptic model. A dose of 20 nmol kg\(^{-1}\) was administered \textit{sc} and the effect was monitored for 4h (Figure 5.7).

The pharmacological effect of \textbf{5.16a} was noticeable 60 min post-injection when 2 out of 4 rats responded to treatment. At the second time point of measurement all rats were behaviorally active and the effect was significant. After 4h, the pharmacological effect was still significant (p < 0.05). Though this study had a preliminary character, it is evident that \textbf{5.16a} is active in the Ungerstedt model for Parkinson’s disease. Compound \textbf{5.16a} displays a potent and lengthy pharmacological effect at this low dose \textit{sc}. Interestingly, the onset of action is slower than that for \textbf{2.3a} and its analogs (Chapter 2 and 3). Compound \textbf{2.3a} induces a maximal pharmacological effect 60 min post-administration, while for \textbf{5.16a} this is at least after 120 min (no data was collected between 120 min and 240 min).

Assuming that compound \textbf{5.16a} is bioactivated to (4aR,10aS)-N-n-propyl-6,7-di-OH-1,2,3,4,4a, 5,10,10a-octahydro-benzo[g]quinoline (\textbf{5.5}, Figure 5.2), the bioactivation of this flat and rigid molecule could proceed at a different rate than the bioactivation of compound \textbf{2.3a} and its analogs.

### 5.5 CONCLUSIONS

Compound 5.16a is a prodrug of a highly potent DA agonist with a long lasting effect, both after administration \textit{sc} and \textit{po}. Analogously to compound 2.3a and its analogs (Chapter 2 and 3), compound 5.16a is inactive \textit{in vitro} but is bioactivated \textit{in vivo}. It is assumed that compound 5.16a follows the same path of bioactivation as compound 2.3a and is converted to (−)-trans-N-n-propyl-6,7-di-OH-2,3,4,4a,5,10,10a-octahydro-benzo[g]quinoline (\textbf{5.5}).
Figure 5.7 Pharmacological effect of 20 nmol kg⁻¹ of 5.16a in the Ungerstedt model for Parkinson’s disease expressed as full contralateral rotations per 15 min at 7 points in time over 8h. Each point is the mean ± s.e.m., for 4 determinations.

5.6 EXPERIMENTAL SECTION

5.6.1 Chemistry

General remarks. Melting points were determined in open glass capillaries on an Electrothermal digital melting-point apparatus and are uncorrected. ¹H and ¹³C NMR spectra were recorded at 200 MHz and 50.3 MHz, respectively, on a Varian Gemini 200 spectrometer. The splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Chemical shifts are given in δ units (ppm) and are relative to the solvent. Coupling constants are given in Hertz (Hz). The spectra recorded were consistent with the proposed structures. IR spectra were obtained on a ATI-Mattson spectrometer. Electronic ionization (EI) mass spectra were obtained on a Unicam 610-Automass 150 GC-MS system. Elemental analyses were performed by the Microanalytical Department of the University of Groningen and were within ± 0.4 % of the theoretical values, except where noted. All chemicals used were commercially available (Aldrich or Acros) and were used without further purification.
3-(4-Methoxyphenyl)-propionic acid n-propyl amide (5.9) 3-(4-Methoxyphenyl)-acrylic acid (8.8 g, 49 mmol) was dissolved in ethanol (200 mL) and a catalytic amount of 10% Pd/C (100 mg) was added. After shaking for 3h under a H₂ atmosphere (3 atm) for 3h at RT, the mixture was filtered over Celite® and evaporated. The residue was refluxed in dichloromethane (200 mL) with thionyl chloride (6.6 mL, 90 mmol) for 1h. The volatiles were evaporated and the resulting oil was dissolved in dichloromethane (100 mL). This was added to a vigorously stirred mixture of 5% aqueous NaOH (200 mL), dichloromethane (100 mL) and n-propylamine (3.0 mL, 71 mmol). After stirring for 1h the layers were separated and the aqueous layer was extracted with dichloromethane (3 x 50 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL) and was dried over MgSO₄. Evaporation of the solvent gave the amide in quantitative yield (10.7 g, 49 mmol, 100%). IR (neat) cm⁻¹ 3300, 2961; 1734, 1642; MS (EI) m/z 221 (M⁺).

N-(3-(4-Methoxyphenyl)-propyl)-N-propyl amine (5.10) To a stirred mixture of LiAlH₄ (8.0 g, 200 mmol) in tetrahydrofuran (100 mL) was added dropwise a solution of 3-(4-methoxyphenyl)-propionic acid n-propyl amide (10.7 g, 49 mmol) in tetrahydrofuran (100 mL). After refluxing for 12h the mixture was cooled to 50°C and excess hydride was destroyed by careful addition of water (10 mL), 5% aqueous NaOH (40 mL) and water (20 mL) allowing reflux conditions. The hot slurry was filtered and the white precipitate was washed thoroughly with ethanol. Volatiles were evaporated and the resulting oil dissolved in ethyl acetate (50 mL) what was extracted with 0.5 N aqueous HCl (4 x 50 mL). The acidic phase was made alkaline (pH = 9) by addition of 30% aqueous NaOH and extracted with ethyl acetate (4 x 50 mL). The organic layers were combined, washed with brine, dried (MgSO₄) and evaporated to dryness to give an oil that partially crystallized in diethyl ether as the hydrochloride salt. Recrystallization from acetone/diethyl ether gave white flaky crystalline material. Total yield (as free base): 9.9 g, 48 mmol, 98%, mp 176-177°C. IR (neat) cm⁻¹ 2960, 2772, 1611, 1514; ¹H-NMR (CDCl₃) δ 9.46 (br s, 1H), 7.16 (d, 2H), 6.90 (d, 2H), 3.72 (s, 3H), 2.82 (br s, 4H), 2.59 (t, 2H), 2.15 (p, 2H), 1.83 (h, 2H), 0.89 (t, 3H) ppm; ¹³C-NMR (CDCl₃) δ 156.6, 130.3, 127.7, 112.4, 53.7, 47.9, 45.66, 30.3, 25.9, 17.8, 9.7 ppm; MS (EI) m/z 207 (M⁺); Anal. (C₁₃H₂₁NO·HCl) C, H and N.

N-n-Propyl-trans-7-keto-1,2,3,4,4a,5,8,8a-octahydro-[6H]-quinoline (5.14) N-(3-(4-methoxyphenyl)-propyl)-N-propyl amine (6.15 g, 31.45 mmol) was dissolved in THF (60 mL),
t-BuOH (4.65 g, 5.93 mL, 62.89 mmol). The mixture was cooled to -60°C and liquid NH₃ (60 mL) was introduced. Then Li metal (1.70 g, 0.24 mol) was gradually added in small portions and the blue mixture was stirred at -60°C for 4 h. The color was discharged by addition of a MeOH/aqueous NH₄Cl (sat) solution (1:1, 20 mL) and the cooling bath removed. After NH₃ had evaporated the pH of the slurry was adjusted to 1 by addition of concentrated hydrochloric acid and stirred for 24 h. Then the mixture was basified to pH 10 (30% NaOH, T < 15°C) and solid NaCl was introduced until the organic layer separated. The aqueous solution was extracted with dichloromethane (8 x 50 mL) and the combined organic layers were washed with brine and dried over MgSO₄. Evaporation yielded a red oil that was purified by column chromatography (silica, dichloromethane/ethanol, 20:1) to yield a colorless oil (4.69 g, 24.05 mmol, 76%). A sample was converted to the hydrochloride for analysis, mp 148-150°C. IR (KBr) 2950, 2384, 1711, 1464 cm⁻¹; ¹H-NMR (CDCl₃) δ 3.10 (dt, 1H, J = 3.91 Hz, 9.52 Hz), 1.23-1.80 (m, 7H), 1.93-2.72 (m, 10H), 0.84 (t, 3H) ppm; ¹³C-NMR (CDCl₃) δ 210.4, 59.5, 54.3, 46.3, 36.6, 36.0, 33.7, 26.8, 23.6, 22.7, 18.0, 10.3 ppm; MS (EI) m/z 195 (M⁺); Anal. (C₁₂H₂₁NO·HCl) C, H and N.

N-n-Propyl-trans-2,3,4,4a,5,7,8,9,10,10a-decahydrobenzo[g]quinolin-6-one (5.16) and N-n-Propyl-cis-2,3,4,4a,5,7,8,9,10,10a-decahydrobenzo[g]quinolin-6-one (5.17) To a cooled (0°C) suspension of KOtBu (2.5 g, 25.6 mmol) in dry dimethylformamide (4 mL) flushed with N₂ was added dropwise a solution of (3-ethoxycarbonylpropyl)-triphenylphosphonium bromide (12.9 g, 28.2 mmol) in dry, N₂ flushed dimethylformamide (25 mL). When the addition is complete the mixture was stirred at 0°C for 30 min. Then a solution of trans-N-propyl-7-keto-1,2,3,4,4a,5,8,8a-octahydro-[6H]-quinoline (2.5 g, 12.8 mmol) in dry, N₂ flushed dimethylformamide (25 mL) was added dropwise at 0°C. After stirring at 0°C for 4 h the temperature was allowed to rise to RT and stirring was continued overnight. Water (50 mL) was added and the mixture was filtered through Celite (2 g). The filtrate was extracted with hexane (5 x 25 mL). The combined organic layers were dried (MgSO₄), filtered and evaporated to give a beige solid (9.1 g).

The solid was dissolved in dichloromethane (10 mL) and was added to PPA (40 g) at 100°C while stirring. After 4 h stirring at that temperature the reaction mixture was allowed to cool to about 80°C when crushed ice (50 g) was introduced. Stirring was continued at that temperature for 1 h and then the solution was allowed to cool to RT. Concentrated ammonia was added until pH = 8 and then the solution was extracted with dichloromethane (6 x 100 mL). The
combined organic layers were dried (MgSO\textsubscript{4}), filtered and evaporated. The residue was purified by column chromatography (silica, dichloromethane/methanol, gradient) and the products were subsequently converted to the hydrochloric salt and recrystallized from diethyl ether/ethanol.

5.16 (trans isomer) Yield 0.61 g, 2.2 mmol (67%), mp 235°C. IR (KBr) 2928, 2592, 1668, 1457, 1394 cm\(^{-1}\); \(^1\)H-NMR 500MHz (CDCl\textsubscript{3}) \(\delta\) 3.06 (d, 1H, J = 11.2Hz), 2.72-2.78 (dt, 1H), 2.15-2.55 (m, 10H), 1.51-1.99 (m, 9H), 1.01-1.10 (dq, 1H), 0.89 (t, 3H) ppm; \(^{13}\)C-NMR 200MHz (CDCl\textsubscript{3}) \(\delta\) 197.0, 152.6, 129.8, 59.6, 53.6, 51.2, 36.1, 35.2, 34.9, 29.3, 29.4, 28.1, 23.2, 20.8, 15.8, 10.4 ppm; MS (EI) \(m/z\) 249 (M\(^+\)); Anal. (C\textsubscript{16}H\textsubscript{25}NO\textsubscript{·}HCl) C, H and N.

5.17 (Cis isomer) Yield 0.07 g, 0.3 mmol (6%). IR (KBr) 2928, 2592, 1668, 1457, 1394 cm\(^{-1}\); \(^1\)H-NMR 500MHz (CDCl\textsubscript{3}) \(\delta\) 3.20 (t, 1H, J= 11Hz), 2.75 (d, 1H), 2.00-2.58 (m, 12H) 1.82-2.00 (m, 2H), 1.52-1.79 (m, 4H), 1.38 (d, 1H), 1.22-1.29 (dq, 1H), 0.90 (t, 3H) ppm; \(^{13}\)C-NMR (CDCl\textsubscript{3}) \(\delta\) 197.3, 151.1, 128.7, 54.8, 53.5, 45.1, 36.3, 31.0, 29.7, 26.3, 24.0, 23.3, 22.6, 20.9, 18.0, 10.3 ppm; MS (EI) \(m/z\) 249 (M\(^+\)); Anal. (C\textsubscript{16}H\textsubscript{25}NO\textsubscript{·}HCl) C, H and N.

Resolution 5.16 A 5 mg mL\(^{-1}\) solution of racemic GMC6650 in hexane / isopropanol (4/1 (v/v)) was injected into a HPLC system using a Water 510 HPLC pump fitted with a 500 \(\mu\)L loop and a Chiralpack AD semi-preparative column (250 x 10 mm). Mobile phase was a mixture produced by an ISCO Model 2360 Gradient Programmer and consisted of 98 % hexane (containing 0.1% (w/w) triethylamine) and 2% isopropanol / hexane (1/1 (w/w)). Flow of the mobile phase was 4.0 mL min\(^{-1}\). The separate enantiomers were detected by a Water 486 Millipore Tunable Absorbance Detector (\(\lambda = 254\) nm, AUFS = 2.0) and were recorded on paper using a Kipp & Zonen flatbed recorder (chart speed 5 mm min\(^{-1}\), \(\alpha = 1.33\); \(k_1 = 2.16\); \(k_2 = 2.88\)). Fractions were collected by hand. After evaporation of the mobile phase the optical rotation of the two fractions was determined using a Perkin Elmer 241 Polarimeter. First eluting fraction: \([\alpha]_d^20 = +185^\circ\) (c = 0.08, methanol). Second eluting fraction: \([\alpha]_d^20 = -214^\circ\) (c = 0.07, methanol). Both enantiomers were analyzed for their purity using the same HPLC system but now fitted with a Chiralpack AD analytical column (250 x 4.6 mm) and a 20 \(\mu\)L loop (e.e. = >99.9% for both enantiomers). Both enantiomers were converted to their corresponding maleate salts and were recrystallized from ethanol / diethylether. Melting points: (–)-5.16a-Maleate mp: 192°C, (+)-5.16b-Maleate mp: 186°C.

3-Ethynyl-2-cyclohexen-1-one (5.20)\(^{19}\) To a solution of 0.5 N ethynyl-magnesium bromide in tetrahydrofuran (100 mL) was added under N\(_2\) and stirring 3-ethoxy-2-cyclohexen-1-
one (3.75 g, 26.8 mmol) in tetrahydrofuran (12.5 mL). The mixture was stirred at RT for 20h when it was acidified with 1N HCl (200 mL). After stirring for 15 min, the acidic phase was extracted with dichloromethane (5 x 50 mL). The combined organic extracts were washed with water (2 x 50 mL) and dried (MgSO₄). Evaporation of the solvent gave an oil that was purified by column chromatography (silica, ethylacetate/hexane 1:9) to yield 3-ethynyl-2-cyclohexen-1-one (5.19) as a yellow oil, 2.71 g, 22.6 mmol, (84%). Analysis were according to literature data.

1-(N-n-Propylamine)-4-pentene (5.22)²¹ 5-bromo-1-pentene (25 g, 0.17 mol) was added to n-propylamine (150 mL, >10 eq) and this was refluxed for 90h. Excess n-propylamine was evaporated and the residue was triturated with diethylether (200 mL). The white solid was filtered, washed thoroughly with diethylether and dried under vacuum to give 20 g of the hydrobromide salt. The salt was suspended in dichloromethane (50 mL) and excess 5% NaOH solution was added until pH = 9. The organic layer was separated and the water layer was extracted with dichloromethane (4 x 25 mL). The combined organic layers were dried (Na₂SO₄) and evaporated. The resulting oil was distilled and 12.8 g (59%) of the product was collected at 152-155°C (lit. 155°C).¹ H-NMR (CDCl₃) δ 4.87-5.67 (m, 1H), 4.87-5.03 (m, 2H), 2.48-2.60 (m, 4H), 2.02 (dq, 2H), 1.37-1.62 (4H), 1.24 (H), 0.87 (t, 3H) ppm; ¹³C-NMR (CDCl₃) δ 210.4, 59.5, 54.3, 46.3, 36.6, 36.0, 33.7, 26.8, 23.6, 22.7, 18.0, 10.3 ppm.

Diels-Alder route to 5.16 A solution of 3-ethynyl-2-cyclohexen-1-one (5.20) (1.80 g, 15.0 mmol) in 1,2-dichloro-benzene (50 mL) was added to a solution of 1-propylamine-4-pentene (5.21) in 1,2-dichloro-benzene (50 mL). The solution was stirred for 30 min at RT and then heated under pressure for 72 h at 190°C. After cooling, the mixture was poured in 4N HCl (400 mL) and this was stirred at rt for 2 h. The acidic layer was separated and extracted with diethylether (2x 50 mL). Then the aqueous layer was made alkaline (pH = 8) with concentrated ammonia and was extracted with dichloromethane (5 x 50 mL). The combined organic layers were washed with brine (50 mL) and dried (MgSO₄). Analysis by GC-MS showed the presence of about 2% of 5.16.
5.6.2 Pharmacology.

**General remarks.** Racemic 5.16 was tested as a hydrochloride salt, optically pure compounds were tested as maleate salts. The *in vitro* binding affinity experiments were performed at Lundbeck AC/S, Copenhagen, Denmark. Microdialysis studies were performed at the laboratory animal unit of the Rijksuniversiteit Groningen, The Netherlands. The effects of the compounds in the Ungerstedt model for Parkinson’s disease was studied at the University of Uppsala, Sweden.

**Receptor binding studies.**

- **D₁ binding.** By this method the inhibition of drugs of the binding of [³H]-SCH 23390 (0.20 nM, Kᵩ 0.45 nM) to dopamine D₁ receptors in membranes from rat corpus striatum is determined *in vitro*. Method and results are described by Hyttel and Larsen.²⁵,²⁶

- **D₂ binding.** By this method the inhibition of drugs of the binding of [³H]-Spiperone (0.50 nM, Kᵩ 0.20 nM) to dopamine D₂ receptors in membranes from rat corpus striatum is determined *in vitro*. Method and results are described by Hyttel and Larsen.²⁷,²⁸

- **D₃ binding.** By this method the inhibition by drugs of the binding of [³H]-Spiperone (0.3 nM, Kᵩ 0.45 nM) to membranes of human cloned dopamine D₃ receptors expressed in CHO-cells is determined *in vitro*. Method modified from R.G. MacKenzie et al.²⁹ CHO-cells expressing the human cloned D₃ dopamine receptor are harvested and the cell suspension centrifuged at 1000 rpm for 7 min at 4°C. The supernatant is frozen. At the day of experiment the cell pellet is thawed at room temperature and diluted in assay buffer (25 Mm TRIS-HCl pH 7.4 + 6.0 mM MgCl₂ + 1.0 mM EDTA) to the desired concentration. 50 µL Displacer (10 µM Haloperidol, test compound or assay buffer) and 230 µL buffer is added to a 96 well deep plate. Then 50 µL 0.3 nM [³H]-spiperone is added. The reaction is initiated by addition of 670 µL membrane suspension (test concentration 26 µg protein/670 µL). Packard GF/C unifilter (96 well) is pretreated with 0.1% PEI-solution 10-15 min before filtration. After 60 min. of incubation at 25°C the reaction is terminated by filtration at Tomtec unifilter. The filters are washed twice with ice cold assay buffer. The filters are dried for 1.5 hours at 50°C, 35 µL scintillation liquid is added and bound radioactivity is counted in Wallac Tri-Lux scintillation counters.

- **D₄ binding.** By this method the inhibition of drugs of the binding of [³H]-Nemonapride (0.50 nM, Kᵩ 0.20 nM) to cloned human dopamine D₄ receptors is determined *in vitro*. Method and results are described by Meier et al.³⁰
**Microdialysis.** Male Wistar rats (from CDL, Groningen, The Netherlands) weighing 280-320 g were used for microdialysis experiments. The rats were housed in plexiglas cages, eight animals in each cage, with free access to water and food. The cages were placed in a room with controlled environmental conditions (21 °C; humidity 60-65%; lights on at 8 a.m. and off at 8 p.m.). The animals were housed at least one week after arrival prior to surgery. Animal procedures were conducted in accordance with guidelines published in the NIH Guide for the Care and Use of Laboratory Animals and all protocols were approved by the Groningen University Institutional Animal Care and Use Committee.

On-line brain microdialysis in freely moving animals was performed as described previously ¹. Data were converted into percentage of basal levels. The basal levels were determined from four consecutive samples (less than 20% variation), and set at 100%. During 360 min after administration of the compound the dopamine release was measured. The measurements were processed using Microsoft Excel and GraphPad Prism for Windows (GraphPad Inc.). Microdialysis data were compared using one-way analysis of variance (ANOVA) for repeated measurements, followed by Dunnett’s Method post-hoc test. A significance level of 0.05 was applied.

**The Ungerstedt model for Parkinson’s disease.** Contralateral turning experiments were essentially according to the original reference by Ungerstedt and Arbuthnott. ⁴⁶ Briefly, rats were lesioned in right medial forebrain bundle (P4.8mm, L1.1mm, V-8.2mm from bregma) with 8 mg / 4 mL of 6-hydroxydopamine HBr in saline with ascorbic acid 1 mg/ml added. After 3 weeks recovery, completeness of lesion was assessed with apomorphine 50 mg kg⁻¹ sc. Only animals rotating more than 100 turns in an hour were used in subsequent experiments. Rats were removed from home cages in morning, weighed, dosed and placed into harnesses in rotarot apparatus. Rats sit in stainless steel, flat bottomed, hemispheric bowls. The number of full contralateral rotations per 15 min, was scored by a trained observer. Data is presented as full rotations in contralateral directions.

### 5.7 REFERENCES


