Synthesis and evaluation of [18F]fluoroprogestins and [18F]fluorometoprolol
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CHAPTER 5

SYNTHESIS OF N-[\(^{18}\)F]FLUOROISOPROPYL DERIVATIVES OF β-ADRENERGIC RECEPTOR LIGANDS

A method is described for the preparation of \[^{18}\)F\]fluorinated β- adrenergic receptor ligands. The key step in this route is a fluoroalkylation reaction. Two different methods are proposed and evaluated. The scope of these reactions has been tested on two model compounds. In the second part of this Chapter, the effect of fluorine substitution on the binding affinity of a β-ligand for the β-adrenoceptors is evaluated both in vitro and in vivo.

5.1 Introduction

High affinity antagonists for the β-adrenoceptor,\(^\circ\) the so-called "beta-blockers", constitute a class of compounds that are potentially very useful in a labelled form in a PET-study. As was discussed in Chapter 1, introduction of a positron emitting radionuclide in the N-alkyl-group of a beta-blocker would yield a flexible approach towards the labelling of a β₁-adrenoceptor ligand. Thus, a large number of β₁-selective ligands could be screened for their suitability as a \[^{18}\)F\]fluorinated tracer for PET.

![Chemical structures](image)

**Figure 5.1** Structure of (2S)-metoprolol 5.1, (2S)-atenolol 5.2, benzylamine 5.3 and (1R,2S)-norephedrine 5.4.

In this Chapter, two methods are described that lead to these compounds: a classical Sₘ₂ alkylation,\(^\circ\) and a reductive alkylation. The routes were tested on

\(^\circ\) *adrenoceptor* = adrenergic receptor
two model compounds: benzylamine 5.3 and norephedrine 5.4. In addition, the synthesis of a fluorinated analogue of metoprolol 5.1, a selective β₁-adrenoceptor ligand, is described. The results of an in vitro and in vivo evaluation of this ligand are described in section 5.6.

5.2 Two possible routes to [¹⁸F]fluorinated β₁-blocking compounds

The approach we used for the synthesis of the fluorine-18 labelled β-ligands consisted of a two-step sequence. Firstly, a [¹⁸F]fluoroalkyl derivative was prepared and subsequently this synthon was used for the alkylation of the desalkyl-amino group of a β-ligand. A one-step procedure was ruled out because the introduction of a leaving group in the N-isopropyl moiety, necessary for the introduction of fluorine-18, was not possible. The reaction of an amine with an 1,2-bifunctional propane derivative will yield mainly the primary substitution product, eventually leading to a N-(2-[¹⁸F]fluoro-n-propyl)amine derivative. Introduction of a leaving group by a reductive alkylation was also not successful. The reaction of 5.4 with acetol tosylate, bromoacetone and chloroacetone merely yielded the S₂,Z-product 5.5 (Scheme 5.1) instead of the condensation product.

Scheme 5.1

The concept of [¹⁸F]fluoroalkylation of amines has been used extensively for the preparation of [¹⁸F]fluorinated ligands. Some ω-substituted 1-[¹⁸F]fluoroalkanes have been prepared for this purpose, e.g. by nucleophilic substitution with [¹⁸F]fluoride of 1,ω-ditosylates and 1,ω-ditriflates. The addition of Br[¹⁸F] to 1-alkenes has also been reported and following Markovnikov's rule, this last method merely yields 1-bromo-2-[¹⁸F]fluoroalkanes. However, we needed a 2-substituted 1-[¹⁸F]fluoropropane synthon for the synthesis of a fluorine-18 labelled N-isopropyl group. At the beginning of our investigations such a compound had not been described in literature.

We investigated the potential of two synthons leading to the synthesis of N-[¹⁸F]fluoroisopropyl amines: 1) an alkylation with 1-[¹⁸F]fluoroisopropyl tosylate
5.6a, and 2) a reductive alkylation with $[^{18}F]$fluoroacetone 5.12 (Scheme 5.2). The results of the study with 5.6a are described in the next section, the reductive alkylation is discussed in section 5.5.

Scheme 5.2

5.3 Synthesis of 1-fluoroisopropyl tosylate

Starting from the commercially available (2S)-1,2-propanediol di(p-toluene-sulfonate) 5.8, several attempts have been undertaken to prepare the desired 1-fluoroisopropyl tosylate 5.6a (Scheme 5.3). However, on a preparative scale (1 eq. of fluoride) none of the reaction conditions as depicted in Table 5.1 was successful; either no reaction occurred or the elimination product 1-tosyloxy-1-propene 5.9 was isolated.

<table>
<thead>
<tr>
<th>Fluoride</th>
<th>Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Bu$_4$NF</td>
<td>CH$_3$CN, 20°C, 20 min</td>
<td>no reaction</td>
</tr>
<tr>
<td>Amberlyst A26-F</td>
<td>CH$_3$CN, 20°C, 18 h</td>
<td>no reaction</td>
</tr>
<tr>
<td>CsF</td>
<td>DMF, 20°C, 20 min</td>
<td>no reaction</td>
</tr>
<tr>
<td>CsF</td>
<td>DMSO, 60°C, 20 min</td>
<td>elimination</td>
</tr>
<tr>
<td>Kryptofix/KF</td>
<td>CH$_3$CN, 60°C, 30 min</td>
<td>elimination</td>
</tr>
</tbody>
</table>

Table 5.1 Attempted conversion of 5.8 to 5.6a.
Recently, the synthesis of 1-fluoro-2-propanol and 2-fluoro-1-propanol using a cyclic sulfate intermediate was described by Berridge et al. In our hands, the preparation of these fluoroalcohols and the subsequent tosylation of this mixture with p-toluenesulfonyl chloride in pyridine/dichloromethane resulted in a 2:1 mixture of 5.6a and its isomer 2-fluoro-1-propyl tosylate 5.6b. Without further purification, this mixture of 5.6a and 5.6b was used for the identification of the [18F]fluoro analogues 5.6a and 5.6b.

5.3.1 Synthesis of 1-[18F]fluoroisopropyl tosylate

In contrast to the attempted substitution of ditosylate 5.8 with fluoride described above, the reaction of 5.8 with [18F]fluoride proved to be successful. As depicted in Scheme 5.4, the reaction of 5.8 with Kryptofix 222/K3PO4/[18F] in acetonitrile resulted in a 3:2 mixture of 5.6a and 5.6b in 60-70% radiochemical yield (corrected for decay to beginning of synthesis, synthesis time 40 min). Both isomers 5.6a and 5.6b could be separated by HPLC (see section 5.8).
The striking difference between the synthesis of 5.6a on a preparative (millimolar) scale and the reaction on a no carrier added (n.c.a.) scale is probably due to the basic properties of fluoride. In the "cold", preparative reaction, 1 eq. of fluoride is applied, whereas the radioactive, n.c.a. reaction is performed with a minute amount of fluoride. It is tempting to assume that in this reaction, at higher concentrations of fluoride, the basic properties of fluoride prevail.

5.3.2 Alkylation with 1-[¹⁸F]fluoroisopropyl tosylate

The alkylation reaction with the [¹⁸F]fluoro(iso)propyl tosylates 5.6a,b required the addition of a considerable amount of base to the reaction mixture (10 mg K₂CO₃ in 1 ml acetonitrile). It was found that under these conditions 5.6a and 5.6b were very susceptible to elimination of H[¹⁸F]. In the synthesis of N-(1-[¹⁸F]fluoroisopropyl)benzylamine 5.10a and N-(2-[¹⁸F]fluoro-n-propyl)benzylamine 5.10b up to 80% of the radioactivity was recovered in the water layer after dichloromethane/water extraction, indicating the formation of [¹⁸F]fluoride. However, the problem of elimination could be circumvented by the in situ conversion of the tosylates 5.6a,b into the corresponding iodides 5.7a and 5.7b (Scheme 5.4) by the addition of an excess of NaI to the reaction mixture.

Thus, as depicted in Scheme 5.5, the synthesis of N-(1-[¹⁸F]fluoroisopropyl)-norephedrine 5.11a and N-(2-[¹⁸F]fluoro-n-propyl)norephedrine 5.11b from norephedrine 5.4 and 5.6a,b was carried out in acetonitrile in the presence of NaI and a Kryptofix 222/K₂CO₃ mixture as base. The radiochemical yields of 5.11a
and 5.11b were 2% and 6% (BOS), respectively. In an analogous procedure, 5.10a and 5.10b (Figure 5.2) were prepared with a radiochemical yield of 7% and 19% (BOS), respectively. In both reactions, the total synthesis time was 90 min.

![Chemical structures](image)

*Figure 5.2*

In Figure 5.3 the HPLC-chromatogram of the purification of 5.11a,b is shown. In this particular run, the synthesis of 5.11a,b was accomplished by the reaction of racemic (1R,2S and 1S,2R) norephedrine 5.4 with crude 5.6a,b (Scheme 5.5). For the preparation of 5.6a,b optically pure ditosylate (2S)-5.8 was used.

The radioactivity eluting at t<sub>r</sub> = 4 min originates from unreacted tosylates 5.6a,b and iodides 5.7a,b. Two radioactive products at t<sub>r</sub> = 12.0 and 12.9 min corresponded to "cold" authentic 5.11a. Since racemic norephedrine 5.4 was used with optically pure tosylate 5.6a, two diastereomers were expected. At a slightly longer retention time (t<sub>r</sub> = 15.7 and 18.5 min) the two diastereomers of 5.11b were observed.

The same alkylation reaction was also performed with optically pure 1R,2S-(−)-norephedrine. As could be anticipated, in this case only one diastereomer of 5.11b (t<sub>r</sub> = 15.7 min) was found. However, the reaction with (−)-norephedrine gave a HPLC-profile for 5.11a (2 peaks) visually identical with the reaction of racemic norephedrine. A likely explanation for this phenomenon is the racemisation of 1-[<sup>18</sup>F]fluoroisopropyl iodide 5.7a prior to the alkylation of norephedrine due to the presence of excess NaI in the reaction mixture (Scheme 5.4). This observation implies, that in the reaction of 5.6a with racemic (1R,2S and 1S,2R) norephedrine 5.4 as described above, actually four diastereomers of 5.11a were formed.

The two diastereomers of 5.11a which are formed can be separated by HPLC, as shown in Figure 5.3. Exchange of 5.7b with iodide does not lead to racemisation.
5.3.3 Microwave heating

The dramatic increase in reaction velocity, which is encountered when a microwave oven is used instead of conventional heating (e.g., an oil-bath), is more and more appreciated in (radio)chemistry.\textsuperscript{157} Much knowledge on the characteristics and the use of microwaves in preparative chemistry remains to be
revealed. For instance, the susceptibility of solutions to microwave induced heating is mostly related to the intrinsic polarity of the solvent.\textsuperscript{158} However, a dramatic relationship exists between the concentration of salts in solution and the energy uptake of a solution.\textsuperscript{159} Zijlstra et al.\textsuperscript{160} applied this phenomenon successfully in the [\textsuperscript{18}F]fluoroalkylation of secondary amines. We investigated the potential of microwave heating in the reaction of 5.4 with 5.6a (Scheme 5.5) in order to improve the relatively low yield.

The \textit{in situ} conversion of tosylate 5.6a to iodide 5.7a required the presence of a large excess of NaI in the reaction mixture (50 mg NaI in 1 ml acetonitrile). The microwave energy absorption of such a solution is very high and can lead to a vigorous explosion.\textsuperscript{160} Therefore, a two-step reaction sequence was applied by previously preparing and isolating 5.7a, avoiding the application of an excess of NaI in the microwave oven. Unfortunately, the use of a microwave oven in this reaction did not improve the yield significantly. As with the conventional oil-bath heating, radiochemical yields of about 2% (BOS) were found.

5.4 Reductive alkylation with fluoroacetone

In this section, the second approach towards the synthesis of a [\textsuperscript{18}F]fluoroisopropyl group is discussed. The key step in this route was a reductive alkylation with fluoroacetone. This method was also applied for the preparation of the cold reference materials 5.10a and 5.11a as depicted in Schemes 5.6 and 5.7.

\begin{center}
\includegraphics{Scheme_5.6.png}
\end{center}

\textit{Scheme 5.6}

N-(1-Fluoroisopropyl)benzylamine 5.10a was prepared from benzylamine 5.3 and fluoroacetone 5.12 in anhydrous ethanol. Without prior isolation, the
intermediate imine 5.13 was reduced with NaBH₄ to 5.10a in 50% yield (Scheme 5.6).

N-(1-Fluoroisopropyl)norephedrine 5.11a was prepared analogously to 5.10a. However, the intermediate of this reaction is an oxazolidine, instead of an imine (Scheme 5.7). Oxazolidine 5.14 could be isolated and was identified with ¹H-NMR. In contrast to the reduction of imine 5.13, we were not able to reduce oxazolidine 5.14 with NaBH₄. The reduction failed due to the fluorine substitution, since the reduction of an oxazolidine prepared from 5.4 and acetone was performed successfully with NaBH₄.¹⁶ Under more acidic conditions, the reduction of 5.14 could be accomplished with NaCNBH₃ and 5.11a was prepared as a mixture of diastereomers with a yield of 64%.

Scheme 5.7

5.4.1 Synthesis and application of [¹⁸F]fluoroacetone

The two-step reaction leading to the fluorine-18 labelled N-isopropylamines is depicted in Scheme 5.8. Both chloroacetone 5.15 and acetol tosylate 5.16 were applied as possible starting materials for the synthesis of [¹⁸F]fluoroacetone 5.12. The reaction conditions for the synthesis of 5.12 were varied as is indicated in Table 5.2. The subsequent reductive alkylations with 5.12 were without exception performed in anhydrous ethanol.

Ethanol was the solvent of choice for the cold reductive alkylation, therefore this solvent was suggested for the synthesis of 5.12. The application of DMSO and HMPA was investigated since the high boiling points of these solvents would allow the isolation of 5.12 by distillation. Beforehand, acetonitrile seemed
inappropriate since in the cold reaction acetonitrile interfered with the reduction of the oxazolidine intermediate 5.14 (Scheme 5.7). However, since acetonitrile is superior in nucleophilic substitutions with \([^{18}\text{F}]\)fluoride it was investigated in the synthesis of \([^{18}\text{F}]\)fluoroacetone 5.12.

\[
\begin{align*}
\text{CH}_3\text{CN} & \xrightarrow{K_{222}/^{18}\text{F}} \text{O} \\
\text{acetonitrile} & \xrightarrow{^{18}\text{F}} \text{O} \\
\end{align*}
\]

Scheme 5.8

<table>
<thead>
<tr>
<th>solvent</th>
<th>chloroacetone 5.15</th>
<th>acetol tosylate 5.16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.12</td>
<td>5.11a</td>
</tr>
<tr>
<td>acetonitrile</td>
<td>7</td>
<td>30</td>
</tr>
<tr>
<td>ethanol</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>DMSO</td>
<td>5</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>HMPA</td>
<td>7</td>
<td>--</td>
</tr>
</tbody>
</table>

* overall radiochemical yield (EOB).

Table 5.2 Radiochemical yield (BOS) of \([^{18}\text{F}]\)fluoroacetone 5.12 and N-(1-\([^{18}\text{F}]\)fluoroisopropyl)norephedrine 5.11a.

The yield of \([^{18}\text{F}]\)fluoroacetone 5.12 was determined by distillation of the crude reaction mixture. A large range in yields was found due to co-distillation of H\([^{18}\text{F}]\). As a consequence thereof, the radiochemical purity of 5.12 as determined by HPLC (C18-column eluted with water) was low and ranged from 30-60%. The radiochemical yields collected in Table 5.2 have been corrected for the radiochemical purity.

From Table 5.2 it can be concluded that the best results were found with acetol tosylate 5.16 as precursor for 5.12. Both DMSO and HMPA gave low yields of 5.12. Due to the protic properties of ethanol the yield of \([^{18}\text{F}]\)fluoroacetone 5.12 was relative low, but the subsequent reductive alkylation was most efficient in ethanol. The best overall radiochemical yield of 5.11a was found after the synthesis of 5.12 in acetonitrile. The reductive alkylation of 5.4 with 5.12 is
synthesis of 5.12 in acetonitrile. The reductive alkylation of 5.4 with 5.12 is somewhat hampered by the presence of co-distilled acetonitrile, but this is compensated by the high yield of 5.12. Due to the comparable volatility of [18F]fluoroacetone 5.12 and acetonitrile, the co-distillation of the solvent cannot be prevented.

N-(1-[18F]fluoroisopropyl)norephedrine 5.11a was prepared by distilling 5.12 into a solution of norephedrine, acetic acid and NaCNBH₃ in ethanol. The total synthesis time starting from K₂²⁻¹⁸F was 75 min and the overall chemical yield of 5.11a was 5% (EOB).

Recently two other groups have reported the synthesis of [18F]fluoroacetone 5.12. They also performed the synthesis of 5.12 in acetonitrile, and applied 5.12 for the synthesis of [18F]fluorocarazolol.

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In the second part of this Chapter, the potency of the N-[18F]fluoroisopropyl group as a tool in PET is evaluated. The compounds that have been selected for a biological screening were N-(1-fluoroisopropyl)norephedrine 5.11a and 1'-fluorometoprolol 5.19.

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5.5 In vitro evaluation of N-(1-fluoroisopropyl)norephedrine

Certain forms of heart failure have been associated with alterations in the sympathetic innervation of the heart. In this respect, visualisation of the reuptake system with PET can be of great interest. Some fluorine-18 and carbon-11 labelled catecholamine analogues have been prepared and screened on their ability to accumulate by the Uptake-1 mechanism in the heart. Examples of these ligands are [11C]-m-hydroxyephedrine and [18F]fluorometaraminol (Figure 5.4). The structural resemblance of 5.17 and 5.18 with the ephedrine-derivative 5.11a which was described in this Chapter, prompted us to evaluate the potential of 5.11a as a substrate for the Uptake-1 mechanism. In a qualitative study, the affinity of 5.11a for Uptake-1 was compared to the unsubstituted analogue 5.11c and norephedrine 5.4.

The evaluation of the affinity of 5.11a,c and 5.4 for Uptake-1 was performed by competition experiments with meta-[125I]iodobenzylguanidine (MIBG) for
cellular uptake and storage in human neuroblastoma SK-N-SH and rat pheochromocytoma PC12 cells. These cells possess a functional Uptake-1 system which accumulates MIBG as efficiently as the natural substrate noradrenaline. 

![Chemical structures](https://example.com/structures.png)

**Figure 5.4**

In these in vitro systems, the affinity of 5.4 for Uptake-1 was about similar to MIBG and noradrenaline, in agreement with its established indirect sympathomimetic effect on the adrenergic nerve function in man and rats. Unfortunately, compounds 5.11a and 5.11c had apparently no affinity for the catecholamine Uptake-1 system. The lack of affinity of 5.11a,c is probably due to the bulky substituents on the amine. The results indicated that neither 5.11a nor 5.11c was suitable as a tracer for the Uptake-1 mechanism. As a consequence, both compounds have not been evaluated in an in vivo model.

**5.6 Preclinical evaluation of 1'-[18F]fluorometoprolol**

The suitability of the fluoroisopropyl group as a tool in the synthesis of high affinity ligands for the β₁-adrenergic receptor was evaluated both in vitro and in vivo. In an in vitro experiment, the affinity of 1'-fluorometoprolol 5.19 for the β₁-adrenoceptors was compared to metoprolol 5.1. In an in vivo evaluation, the tissue distribution of 5.19 was studied.

The synthesis of the β-adrenoceptor ligand fluorometoprolol 5.19 proceeded similar to the synthesis of 5.11a and was prepared from desisopropylmetoprolol in 35% yield. The fluorine-18 analogue 5.19 was prepared from desisopropylmetoprolol and 1-[18F]fluoroisopropyl tosylate 5.6a. The specific activity of 5.19 was determined with UV-spectroscopy and was found to be 70-110 GBq/μmol (2000-3000 Ci/mmol) at EOS.
From the literature it is known that the S-isomer of β-adrenergic ligands is biologically the most active.\textsuperscript{169} Despite the fact that a mixture of four diastereomers of 5.19 and a racemate of 5.1 were applied in the studies described in this Chapter, a good impression was obtained on the applicability of a [\textsuperscript{18}F]fluoroisopropyl group in the labelling of β-adrenergic receptor ligands. Moreover, the difference in binding affinity between the biologically active S-isomer and the racemate of 5.1 is reported to be only a factor 2.5.\textsuperscript{170}

5.6.1 In vitro experiments with 1'-fluorometoprolol

To determine the affinity of 1'-fluorometoprolol 5.19 in comparison to its parent compound metoprolol 5.1 at β\textsubscript{1}- and β\textsubscript{2}-adrenoceptors, radioligand binding studies using (-)-[\textsuperscript{125}I]iodocyanopindolol (ICYP) were performed. These studies were carried out with β-adrenergic receptors in membranes from human SK-N-MC cells and human right atrium. The experiments were performed in the presence of ICI 118,551, a selective β\textsubscript{2}-adrenoceptor ligand, resulting in a homogeneous population of β\textsubscript{2}-adrenoceptors, or in the presence of CGP 20712A, a potent β\textsubscript{1}-adrenoceptor antagonist.

<table>
<thead>
<tr>
<th></th>
<th>5.19</th>
<th>5.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_i ) (M) (β\textsubscript{1})</td>
<td>3.5 ± 0.22 x 10\textsuperscript{-7} (n=10)</td>
<td>1.4 ± 0.23 x 10\textsuperscript{-7} (n=9)</td>
</tr>
<tr>
<td></td>
<td>( K_i ) (β\textsubscript{2})</td>
<td>1.7 ± 0.18 x 10\textsuperscript{-5} (n=10)</td>
</tr>
<tr>
<td>( K_\text{B} ) (M)</td>
<td>3.7 ± 0.43 x 10\textsuperscript{-7} (n=8)</td>
<td>8.4 ± 0.59 x 10\textsuperscript{-8} (n=7)</td>
</tr>
</tbody>
</table>

Table 5.3 \( K_i \) and \( K_\text{B} \) determination of 5.19 and 5.1.
The resulting $K_I$ values are collected in Table 5.3. Both 5.19 and 5.1 inhibited ICYP binding to the $\beta_2$-adrenoceptors. The affinity of 1'-fluorometoprolol 5.19 for both the $\beta_1$- and $\beta_2$-adrenoceptors is slightly lower than the affinity of 5.1 for these receptors. These small differences resulted in an increase of the $\beta_1/\beta_2$-selectivity ratio of 5.19 ($\beta_1/\beta_2 = 48.5$) with respect to 5.1 ($\beta_1/\beta_2 = 30.7$).

The $\beta_1$-adrenoceptor antagonistic potency of 5.19 and 5.1 was assessed on isolated electrically driven human right atria. Both 5.19 and 5.1 (in concentrations of $10^{-7}$, $10^{-6}$ and $10^{-5}$ M) did not affect basal force of contraction; on the other hand, they shifted concentration-dependently the concentration-response curve for noradrenaline-induced positive inotropic effects (mediated solely by $\beta_1$-adrenoceptor stimulation) to the right in a parallel fashion. The resulting $K_I$ values as depicted in Table 5.3 are in agreement with their $K_I$ values at the $\beta_1$-adrenoceptors.

5.6.2 In vivo evaluation of 1'-[18F]fluorometoprolol

The results of the biodistribution studies of 5.19 in male rats are summarised in Table 5.4. The distribution of 5.19 in the whole body was studied, for it is known that certain parts of the brain contain significant amounts of $\beta$-adrenergic receptors. In the heart, a low uptake of 1'-[18F]fluorometoprolol 5.19 was found (DAR = 0.67). More radioactivity was found in the lungs (DAR = 2.96), kidney (DAR = 4.57) and liver (DAR = 4.87). The high uptake in liver and kidneys can be explained by the hepatic metabolism and excretion of 5.19 and its metabolites by the kidney.

The high accumulation in the lungs has also been noted for other lipophilic $\beta$-ligands, such as propranolol. However it can be concluded from these data that the tissue distribution of 5.19 did not reflect the $\beta$-adrenoceptor density since uptake in the heart was similar to that in skeletal muscle (0.87 vs 0.54 at t = 10 min, 0.64 vs 0.62 at t = 30 min, $p = $ NS in both cases [Student T-test]) and the uptake in the heart could not be blocked by the $\beta$-antagonist propranolol (column 2).

The uptake of radioactivity in bone was low, even after 30 min. This suggests that 5.19 is not extensively defluorinated in vivo. The metabolic stability of the

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*DAR = Differential Absorption Ratio, which is calculated from the formula: $DAR = \frac{(\text{radioactivity/g tissue}) \times (\text{g body weight/total injected radioactivity})$.*
[18F]fluoride substituent in a β-fluoroethylamino moiety has also been noted by other authors and was attributed to a "β-heteroatom effect".\(^\text{174}\)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>10 min (n=4)</th>
<th>10 min* (n=2)</th>
<th>30 min (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>plasma</td>
<td>0.67 ± 0.06(^b)</td>
<td>0.62 ± 0.06</td>
<td>0.73 ± 0.08</td>
</tr>
<tr>
<td>lung</td>
<td>2.96 ± 0.62</td>
<td>2.54 ± 0.54</td>
<td>1.72 ± 0.09</td>
</tr>
<tr>
<td>heart</td>
<td>0.87 ± 0.07</td>
<td>0.77 ± 0.16</td>
<td>0.64 ± 0.08</td>
</tr>
<tr>
<td>kidney</td>
<td>4.57 ± 1.47</td>
<td>2.78 ± 0.33</td>
<td>2.18 ± 0.61</td>
</tr>
<tr>
<td>liver</td>
<td>4.87 ± 0.85</td>
<td>4.09 ± 1.04</td>
<td>3.24 ± 0.66</td>
</tr>
<tr>
<td>muscle</td>
<td>0.54 ± 0.23</td>
<td>0.34 ± 0.07</td>
<td>0.62 ± 0.01</td>
</tr>
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<td>fat</td>
<td>1.42 ± 0.21</td>
<td>0.66 ± 0.52</td>
<td>0.19 ± 0.01</td>
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<td></td>
<td>0.27 ± 0.01</td>
</tr>
<tr>
<td>septum</td>
<td>0.65 ± 0.17</td>
<td></td>
<td>0.31 ± 0.05</td>
</tr>
<tr>
<td>frontal cortex</td>
<td>0.85 ± 0.25</td>
<td></td>
<td>0.29 ± 0.04</td>
</tr>
<tr>
<td>caudal cortex</td>
<td>0.62 ± 0.05</td>
<td></td>
<td>0.32 ± 0.01</td>
</tr>
<tr>
<td>hippocampus</td>
<td>0.63 ± 0.04</td>
<td></td>
<td>0.27 ± 0.04</td>
</tr>
<tr>
<td>cerebellum</td>
<td>0.59 ± 0.06</td>
<td></td>
<td>0.27 ± 0.01</td>
</tr>
</tbody>
</table>

* rats pretreated with propranolol; \(^b\) uptake values expressed as DAR.

Table 5.4 Biodistribution of \(1'\text{-}[18F]\)fluorometoprolol \(5.19\) in rats.

The amount of radioactivity which is found in the brain is comparable to that in plasma. However, the uptake of \(5.19\) in the frontal cortex, a region rich in β\(_1\)-adrenoceptors, is not significantly higher than in the cerebellum, a region which contains mainly β\(_2\)-adrenoceptors, or the hippocampus, which has a very low β-adrenoceptor density.\(^\text{172}\) Thus, \(1'\text{-}[18F]\)fluorometoprolol \(5.19\) passes the blood-brain barrier, but its regional distribution in the CNS appears not to be related to the local concentration of β\(_1\)-adrenoceptors.

5.7 Concluding remarks and future outlooks

As was discussed in section 1.7, many beta-blockers possess a phenoxypropanolamine backbone, the amino functionality of which is often alkylated with an isopropyl- or t-butyl-group. Introduction of a positron emitting radionuclide in the N-alkyl-group would yield a flexible approach towards the labelling of a beta-
blocking agent. In this Chapter, the suitability of a $^{18}$F-fluoroisopropyl group was evaluated.

The application of a $^{18}$F-fluoroisopropyl group in the development of $\beta_1$-adrenoceptor ligands provides many opportunities for the in vivo visualisation of $\beta_1$-adrenoceptors. The introduction of a fluorine-18 label in the N-isopropyl group of metoprolol, a potent beta-blocker, was tolerated well and had only a small negative effect on the binding affinity of 1'-fluorometoprolol 5.19 to the $\beta_1$-adrenoceptor. The affinity of 5.19 for the $\beta_1$-adrenoceptor was more affected by the fluorine substitution and the $\beta_1/\beta_2$-selectivity of 5.19 increased with 50% with respect to 5.1. In the in vivo experiment, a high metabolic stability of the fluorine-18 label in the N-isopropyl group was found.

Visualisation of the $\beta_1$-adrenoceptor density of the heart with 5.19 was not possible since the $\beta_1$-affinity of 5.19 ($K_1 = 3.5 \times 10^{-7}$ M) was too low for sufficient accumulation in the heart. Nevertheless, the use of the $^{18}$F-fluoroisopropyl group remains a powerful tool for the imaging of $\beta_1$-adrenoceptors in vivo since several other beta blockers with a higher affinity for the $\beta_1$-adrenoceptor ($K_1$ in the nanomolar or subnanomolar range) can be labelled using this method. In this respect, the synthesis of $^{18}$F-fluorocarazolol ($K_1 = 0.4$ nM)$^{175}$ has recently been described.$^{83}$

The best route to fluorine-18 labelled $\beta_1$-adrenoceptor ligands proved to be reductive alkylation. With the use of $^{18}$F-fluoroacetone 5.12 as alkylating reagent (Scheme 5.8), an overall radiochemical yield of 5.11a of 5% (EOB) was obtainable. The yields of the reactions with 1-$^{18}$F-fluoroisopropyl tosylate 5.6a (Scheme 5.5) were lower (1-2% EOB). Probably, the steric hindrance at the secondary tosyl-group of 5.6a was too high to give a satisfactory yield of the alkylation reaction.

With both alkylation methods, two diastereomers of the fluorine-18 labelled $\beta$-adrenoceptor ligand are formed. The stereochemistry of a pharmaceutical is always of importance,$^{176}$ but this holds especially for visualisation techniques such as PET. The target/non-target ratio of a ligand is reduced by the presence of an isomer with a lower affinity. In addition, the establishment of a kinetic model is also complicated by the presence of multiple labelled compounds.

It is likely that the asymmetry of C-2' in the $^{18}$F-fluoroisopropyl-substituent will have a major effect on the biological properties of the ligand. Separation of the two diastereomers with HPLC is feasible, suggesting a significant difference in the physical properties of the two diastereomers (Figure 5.3). After
establishing the most appropriate configuration, the development of an asymmetric synthesis would of course be desirable.

A potentially interesting opportunity with regard to the alkylation of β-amino-alcohols is the synthesis of a [18F]fluoro-t-butyl group. This structural element is of great interest, for it facilitates the fluorine-18 labelling of several other high-affinity ligands for the β-adrenoceptor, e.g. CGP 12177 1.11. In this respect, the synthesis of a t-[11C]butyl group would be of equal importance.

Scheme 5.9

The synthesis of a [18F]fluoro-t-butyl group would require the methylation of a type of intermediate as oxazolidine 5.14 (Scheme 5.9). This reaction has been described in the literature for oxazolidines prepared from β-amino-alcohols and aromatic aldehydes.177 Few reports have been made on the alkylation of ketone acetals178 and the alkylation of a N,O-ketal has not yet been reported. Much effort will be required for the exploration of this route and the determination of its value for the synthesis of labelled ligands for PET.

5.8 Experimental part

General.- Fluoroacetone 5.12 and metoprolol 5.1 were supplied by Sigma, St. Louis, Mo, USA. Desisopropylmetoprolol was a gift from Hässle, Möln达尔, Sweden.

Microwave operation.- A microwave oven of the CEM company was used for the alkylation reactions. A 10 ml glass tube with the reaction mixture was placed in a microwave bomb. In order to prevent an explosion, the vial was sealed with a septum, which released the pressure in case it increased above 15 atm. The apparent pressure in the reaction vial was measured in a reference cell, containing the same solution as the reaction vial. The reference cell was attached to an external pressure control unit, which controls the microwave oven and maintains the pressure at a preset value of 10 atm. The reaction was run for a certain period of time after which the bomb was cooled for 15 min. Care must be taken when opening the bomb, because high pressures may develop during the microwave operation.
N-(1-Fluoroisopropyl)benzylamine 5.10a.- A mixture of 200 µl (1.8 mmol) benzylamine 5.3 and 150 µl (2.0 mmol) fluoroacetone 5.12 in 5 ml dry ethanol was heated at a temperature of 50 °C for 30 min. The reaction mixture was cooled to room temperature and 100 mg (2.6 mmol) NaBH₄ was added. After stirring for another 45 min, 20 ml water was added and the solution was extracted twice with 30 ml ether. The combined ether layers were dried over MgSO₄ and the solvent was evaporated in vacuo. The crude product was purified by bulb to bulb distillation (150 °C, 0.3 mmHg) yielding 5.10a as a colourless oil (150 mg, 49%).

1H-NMR (300 MHz) δ 1.05 (3H, d), 1.70 (1H, br s), 2.96 (1H, m), 3.77 (2H, d), 4.26 (2H, m, 2JHF = 47 Hz), 7.28 (5H, s). 13C-NMR (75 MHz) δ 15.85 (q), 50.99 (t), 51.86 (d), 86.69 (dt, 3JCF = 170 Hz), 126.70 (d), 127.76 (d), 128.17 (d), 140.01 (s). 19F-NMR (200 MHz) δ -225.17 (dt, 2JHF = 47 Hz, 3J,F = 17 Hz). Elemental Anal. C₁₅H₁₄FNNCl requires C 71.9%; H 8.4%; N 8.4%.

N-(1-Fluoroisopropyl)norephedrine 5.11a.- A mixture of 300 mg (2.0 mmol) norephedrine 5.4 and 200 µl (2.6 mmol) fluoroacetone 5.12 in 5 ml anhydrous ethanol was heated at a temperature of 50 °C for 60 min. After cooling the reaction mixture to room temperature, 300 mg (4.8 mmol) NaCNBH₄ and 1 ml water/acetic acid (v/v) were added and stirring was continued for 15 min. The resulting solution was taken up in 50 ml ether and was washed with 30 ml of a 5% Na₂CO₃-solution and 30 ml of a saturated NaCl-solution, respectively. The organic layer was dried over MgSO₄ and the solvent was evaporated in vacuo, yielding a colourless oil (350 mg, 83%) which solidified on standing. Bulb to bulb distillation (170 °C, 0.05 mmHg) yielded 5.11a as a mixture of diastereomers (270 mg, 64%).

1H-NMR (300 MHz) δ 0.84 (3H, d), 1.07-1.15 (3H, m), 2.2-2.6 (2H, br s), 3.00-3.16 (2H, m), 4.17-4.47 (2H, m, 2JHF = 47 Hz), 4.68 (1H, d), 7.33 (5H, s). 13C-NMR (75 MHz) δ 15.09 (q), 17.01 (q), 49.77 (d), 55.02 (d), 73.95 (d), 87.10 (dt, 3JCF = 171 Hz), 125.95 (d), 126.90 (d), 127.88 (d), 141.16 (s). 19F-NMR (200 MHz) δ -225.17 (dt, 2JHF = 47 Hz, 3J,F = 17 Hz). Anal. found C 68.3%; H 8.55%; N 6.7%; F 8.4%; m/z 211.136. C₁₀H₁₅NOF requires C 68.3%; H 8.6%; N 6.6%; F 9.0%; m/z 211.137.

2,4-Dimethyl-2-fluoromethyl-5-phenyl-oxazolidine 5.14.- A mixture of 300 mg (2.0 mmol) norephedrine 5.4 and 200 µl (2.6 mmol) fluoroacetone 5.12 in 5 ml anhydrous ethanol was heated at a temperature of 50 °C for 60 min. Oxazolidine 5.14 was isolated as a mixture of diastereomers after dichloromethane/water extraction and drying of the organic layer over MgSO₄.

1H-NMR (60 MHz) major component: δ 0.7 (3H, d), 1.6 (3H, d), 2.3 (1H, br s), 3.0 (1H, m), 4.3 (2H, m, 2JHF = 46 Hz), 5.1 (1H, d), 7.3 (5H, s). minor component: δ 0.7 (3H, d), 1.3 (3H, d), 2.3 (1H, br s), 3.0 (1H, m), 4.5 (2H, m, 2JHF = 46 Hz), 5.1 (1H, d), 7.3 (5H, s).

1'-Fluorometoprolol 5.19.- A mixture of 30 mg (0.14 mmol) desisopropylmetoprolol and 30 µl (0.33 mmol) fluoroacetone 5.12 in 1 ml anhydrous ethanol was heated at a
temperature of 50 °C for 30 min. The reaction mixture was cooled to room temperature and 80 mg (1.3 mmol) NaCNBH$_3$ was added followed by 0.2 ml water/acetic acid 50/50 (v/v). The mixture was stirred for 15 min and next 30 ml ether was added. The solution was washed subsequently with 30 ml of a 5% Na$_2$CO$_3$-solution and with 30 ml of a saturated NaCl-solution. The organic layer was dried over MgSO$_4$ and the solvent was evaporated in vacuo, yielding 36 mg (0.12 mmol, 87%) of an oil. Purification on a silica column eluted with dichloromethane/methanol/triethylamine 100/1/1 (v/v/v) yielded 16 mg of 5.19 as a mixture of diastereomers.

$^1$H-NMR (300 MHz) $\delta$ 1.10 (3H,d), 2.50 (2H,bs), 2.81 (2H,dd), 2.85 (1H,m), 2.98 (1H,m), 3.0 (2H,dd), 3.34 (3H,s), 3.8-4.0 (4H,m), 4.1-4.4 (2H,m, $^3$J$_{HF}$=47Hz), 6.84 (2H,d), 7.13 (2H,d). $^{13}$C-NMR (75 MHz) $\delta$ 16.06 (q), 35.17 (t), 49.10 (t), 52.90 (d), 58.52 (q), 68.42 (d), 70.20 (d), 73.68 (d), 86.57 (dt, $^3$J$_{CF}$=170Hz), 114.32 (d), 139.63 (d), 131.30 (s), 136.91 (s). Anal. (exact mass): m/z 285.174, found 285.174.

Acetol tosylate 5.16 was prepared according to the method described by Koser et al. $^{17}$

$^1$H-NMR (60 MHz) $\delta$ 2.2 (3H,s); 2.5 (3H,s); 4.5 (2H,s); 7.4 (2H,d); 7.9 (2H,d).

$^{[18F]}$Fluoroacetone 5.12.- An amount of 2 mg chloroacetone 5.15 or acetol tosylate 5.16 and K$_{222}^{[18F]}$ were dissolved in 0.5 ml acetonitrile. The reaction was carried out for 20 minutes at 60 °C and subsequently $^{[18F]}$fluoroacetone 5.12 was distilled into another flask at a temperature of 120 °C together with some acetonitrile. The radiochemical yield of crude 5.12 was 50% (BOS, synthesis time 30 min). The radiochemical purity was assayed on HPLC (Waters radial-PAK C-18 column eluted with water, flow 2 ml/min, $t_R$ = 6 min) and was found to be 30-60%.

$^{[18F]}$Fluoroisopropyl tosylate 5.6a.- An amount of 3 mg (7.8 $\mu$mol) (2S)-1,2-propanediol di(p-toluenesulfonate) 5.8 was dissolved in 0.5 ml acetonitrile containing previously prepared K$_{222}^{[18F]}$. The resulting solution was heated at 110 °C for 15 min. The reaction mixture was brought onto a silica Sep-Pak Cartridge to remove unreacted $^{[18F]}$fluoride and was eluted with 2.5 ml acetonitrile yielding the crude product. After the evaporation of the solvent, the two isomers could be separated by HPLC (Waters radial-PAK silica column (5μ, 100x8 mm), eluted with hexane/dichloromethane 60/40 (v/v), flow 2 ml/min). After 18 min, 2-$^{[18F]}$fluoro-1-propyl tosylate 5.6b (yield 34%, BOS) is eluted, 1-$^{[18F]}$fluoroisopropyl tosylate 5.6a (45%, BOS) is collected after 19 min. The total synthesis time was 45 min.

N-($^{[18F]}$Fluoroisopropyl)benzylamine 5.10a.- To a mixture of 40 mg (0.1 mmol) Kryptofix 222, 10 mg (0.07 mmol) K$_2$CO$_3$, 50 mg (0.3 mmol) NaI and 10 $\mu$l (0.1 mmol) benzylamine was added a solution of 5.6a,b in 1 ml acetonitrile. The mixture was stirred and heated in a closed vial on an oil-bath at 125 °C for 1 h. The vial was cooled and the solution was poured in 20 ml dichloromethane. The organic layer was extracted twice with 10 ml water and dried over MgSO$_4$. Evaporation of the solvent yielded the crude alkylated products 5.10a and 5.10b. The products were separated by
HPLC (Partisil column (10μ, 250×4.6 mm), eluted with hexane/methanol 97/3 (v/v), flow 2 ml/min). The desired isomer 5.10a (yield 7%, BOS) eluted after 13 min. The other radioactive product (probably 5.10b, 19%, BOS) eluted after 15 min. The total synthesis time was 90 min. The radiochemical purity of 5.10a was ascertained on reversed phase HPLC (C18-column, eluted with acetonitrile/water/ammonia 40/60/0.5 (v/v/v), flow 2 ml/min), resulting in one radioactive peak coeluting with authentic 5.10a after 9 min.

N-(l-[18F]Fluoroisopropyl)norephedrine 5.11a from 5.6a.- An amount of 10 mg (0.07 mmol) 1R,2S-(−)-norephedrine 5.4 was reacted with 5.6a,b using the same procedure as described above for 5.10a. The purification was accomplished with HPLC (Waters radial-PAK silica column (5μ, 100×8 mm), eluted with hexane/dichloromethane/methanol 60/40/1 (v/v/v), flow 2 ml/min). The alkylation with 5.6a yielded two diastereomers of 5.11a (yield 2%, BOS), tᵣ = 12.0 and 12.9 min; 1:1 ratio. The alkylation of norephedrine with 5.6b afforded one radioactive product, presumably 5.6b (5%, BOS), tᵣ = 15.7 min. The total synthesis time was 90 min. Reversed phase HPLC (C18-column, eluted with acetonitrile/water 30/70, flow 2 ml/min) of 5.6a gave one radioactive peak, coeluting with authentic 5.6a after 4 min.

**Microwave procedure.** - An amount of 50 mg NaI was added to a solution of 5.6a,b in acetonitrile and the mixture was heated at a temperature of 110 °C for 10 min. Next, crude 5.7a and 5.7b (32% of total radioactivity) was distilled onto a solution of 10 mg norephedrine and 5 mg NaHCO₃ in 1 ml acetonitrile. The reaction was run for 30 min in the CEM-microwave at a preset maximum pressure of 10 atm. The vial was cooled and the solution was poured into 20 ml dichloromethane. The organic layer was extracted twice with 10 ml water and dried over MgSO₄. Evaporation of the solvent yielded the crude alkylated products 5.11a and 5.11b. HPLC-purification was performed as described above. The total synthesis time was 60 min, the radiochemical yield of 5.11a was 1.2% (BOS).

N-(l-[18F]Fluoroisopropyl)norephedrine 5.11a via 5.12.- Freshly prepared [18F]fluoroacetone 5.12 was distilled upon a solution of 2 mg 5.4, 1 mg NaCNBH₃ and 2 μl acetic acid in 0.5 ml ethanol. After stirring at a temperature of 110 °C for 20 min, the solution was poured in water and extracted with CH₂Cl₂. The organic layer was dried over MgSO₄ and concentrated in vacuo. HPLC-purification was performed as described above. Total synthesis time was 60 min, the radiochemical yield was 30% (BOS).

1'-[18F]Fluorometoprolol 5.19 via 5.6a.- An amount of 10 mg desisopropyl-metoprolol was reacted with 5.6a in the same procedure as described for 5.11a. HPLC purification was performed on a Waters radial-PAK silica column (5μ, 100×8 mm), eluted with hexane/dichloromethane/methanol 60/40/1 (v/v/v), flow 2 ml/min). Two sets of diastereomers were separated: tᵣ = 18.6 and 19.8 min. The yield of 5.19 was 2% (BOS, synthesis time 90 min).
**In vitro evaluation of 5.19: K_7-determination.** Membranes were prepared from human right atrial and lung tissue, human lymphocytes and SK-N-MC cells and (-)-[^125]Ijodocyanopindolol (ICYP) binding was performed as described by Brown et al. The membranes were suspended in a 10 mM Tris-HCl, 154 mM NaCl-buffer pH=7.4. Binding studies were performed in the presence of 50 nM ICI 118,551 (right atrial membranes) or 300 nM CGP 20712A (lung membranes). For the drug-competition experiments, membranes were incubated with 100 pM ICYP in the presence or absence of 5.1 or 5.19 (concentrations ranging from 10^{-10} to 10^{-4} M) for 90 min at 37 °C in a total volume of 250 µl; non-specific binding was determined as binding in the presence of 1 µM (+)-CGP 12177. The drug-competition curves were analyzed by fitting the experimental data to mono- or biphasic sigmoid curves using the iterative curve fitting program InPlot (GraphPAD software, San Diego, CA). Statistical analysis was performed using the F-ratio test to measure the correctness of fit of the competition curves for either one or two sites. The resulting IC_{50} values were converted into K_7 values according to the Cheng and Prusoff-equation: K_7 = IC_{50}/([S]/K_0 + 1) where IC_{50} = concentration of antagonist that inhibited ICYP binding by 50%, [S] = concentration of ICYP in the assay (usually 100 pM), and K_0 = equilibrium dissociation constant of ICYP (25 pM at β_1-adrenoceptors and 12.5 pM at β_2-adrenoceptors).

**K_8-determination.** Strips of right atrial appendages obtained from patients without apparent heart failure undergoing coronary artery bypass grafting were mounted in a 25 ml organ bath containing a Krebs-Henseleit-solution of the following composition: 119 mM NaCl, 2.5 mM CaCl_2, 4.8 mM KCl, 1.2 mM MgSO_4, 1.2 mM KH_2PO_4, 24.9 mM NaHCO_3, 10.0 mM glucose and 0.057 mM ascorbic acid equilibrated with carbogen (95% O_2, 5% CO_2) at a temperature of 37 °C. The myocardial tissues were electrically stimulated by square wave impulses of 5 ms duration and a voltage of about 20% above threshold (3-8V, mean 4V) at a frequency of stimulation of 1.0 Hz. The developed tension of the preparations (maintained under a resting tension of 4.9 mN) was recorded via a strain gauge on a Hellige recorder. All preparations were allowed to equilibrate for at least 1 h in the Krebs-Henseleit-solution containing 5 µM phenoxybenzamine in order to block neuronal and extraneuronal uptake of catecholamines and α-adrenoceptors; this was followed by washout for at least 1 h. The cumulative concentration-response curves for noradrenaline in the presence of 5.1 or 5.19 were determined after equilibrating the preparation for 1 h. Details of the procedure have been described elsewhere. The dissociation constants were calculated according to Furchgott from the formula K_8 = [B]/(CR - 1), where [B] is the concentration of antagonist, CR is the concentration-ratio defined as the EC_{50} of noradrenaline in the presence of antagonist divided by the EC_{50} in the absence of antagonist and EC_{50} = concentration that produced 50% of maximal response.

**Tissue distribution studies of 1^{-18}F/fluorometoprolol 5.19 in rats.** Three groups of male Wistar rats (weight 180-200 g) were used for the tissue distribution studies. The rats were injected i.v. with 40 kBq (100 µCi) of 5.19 in 0.3 ml saline and were
sacrificed after 10 or 30 min (n=4). The specific activity of 5,19 was 100 GBq/μmol. One group of rats (n=2) was pretreated with 2.5 mg/kg body weight of d,l-propranolol.HCl and killed after 10 min. Plasma and tissue samples were weighed and assayed for radioactivity in a calibrated gamma counter. Tissue uptake is expressed as the DAR (Differential Absorption Ratio) and calculated from the formula: DAR = (radioactivity/g tissue) x (g body weight/total injected radioactivity).