Synthesis and evaluation of [18F]fluoroprogestins and [18F]fluorometoprolol
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CHAPTER 4

APPROACHES TO THE SYNTHESIS OF 6α-[18F]FLUORO-PROGESTINS

In this Chapter, the chemistry carried out to synthesize 6α-[18F]fluorinated steroids is presented. Two major routes leading to this class of compounds have been investigated, namely a nucleophilic substitution and the opening of an epoxide. In addition, the possibility of [18F]fluorination via a transformation of the 3-keto-4-ene entity of progesterone was investigated.

4.1 Introduction

Steroids with structural variations at the C-6 position have received much attention throughout the years. Substituents that have been introduced in this position are, for example, methyl,\textsuperscript{51} chloride\textsuperscript{124} and fluoride.\textsuperscript{125} Many of these substituted steroids have been screened for their biological properties, and in some cases an enhanced biological activity with respect to the unsubstituted steroid was observed. Of particular interest to the present investigations was the increased affinity of 6α-fluoroprogesterone 4.1 for the progesterone receptor (PR).\textsuperscript{57} The group of Katzenellenbogen\textsuperscript{175} has evaluated a number of 6-fluoroprogestins of type 4.3 for their \textit{in vivo} binding characteristics by using \textsuperscript{3}H-labelled analogues. They suggested that these might be useful as [18F]fluorinated tracers for PET.

![Chemical structures](image)

\textit{Figure 4.1} Possible [18F]fluorinated tracers for PET.
In this Chapter, the approaches to the synthesis of a 6α-[18F]fluoro-substituted progestin are described. In the addition to the interest in the synthesis of 4.1, the introduction of a fluorine-18 label at C-6 would allow the synthesis of the class of fluorine-18 labelled high affinity progestins like 4.2.

4.2 Radiochemical approaches towards 6α-[18F]fluoroprogesterone

A great deal of effort has been invested in the synthesis of fluorinated steroids. Throughout the years, the use of HF and F₂ in organic chemistry has been supplanted by easier-to-handle reagents as BF₃-etherate. Despite the superior results of these reagents in the synthesis of fluorinated compounds, these methods are of limited use in the preparation of [18F]fluorinated ligands. The main reason why these reagents are rarely applied in radiochemistry is brought about by their dramatic effect on the radiochemical yield and the specific activity of the [18F]fluorinated products (see Chapter 2).

\[ X = \text{Br} \]
\[ X = \text{OMs} \]
\[ X = \text{OTs} \]
\[ X = \text{OTf} \]

Scheme 4.1

The preference for avoiding carrier added reagents for the synthesis of 4.1 led us to focus our attention primarily on a nucleophilic substitution with no carrier added (n.c.a.) [18F]fluoride (route i, Scheme 4.1). Obviously, problems can be anticipated using a $S_n2$ reaction for the synthesis of 4.1, because of the secondary
C-6 carbon atom and the susceptibility of this position towards elimination. However, dramatic differences in the nucleophilicity and reactivity of fluoride have been found between a reaction on a preparative (millimolar) scale and on a n.c.a. scale. A striking example of this phenomenon is reported in Chapter 5. We felt that the dramatic gain in specific activity of a synthesis using a n.c.a. S_N2-reaction justified the exploration of this route for the synthesis of 6-[^{18}F]fluorinated steroids.

The second approach (route ii) was based on reports in the literature concerning the conversion of α-chloroketones into thiophenol-ethers\textsuperscript{129} and the synthesis of 6-oxo-steroids\textsuperscript{130}. Both types of reaction have in common that [^{18}F]fluoride would attack a vinylic carbon atom avoiding a direct nucleophilic substitution of a leaving group. These experiments are described in section 4.4.

Despite the expected low specific activity and radiochemical yield of the reaction, the opening of an epoxide with [^{18}F]fluoride was also considered (route iii). We attempted to perform this reaction with a minimum of catalyst, or even on a n.c.a. basis (section 4.5).

4.3 Nucleophilic substitution

4.3.1 6α-Fluoroprogesterone

The compounds that were screened as substrates in nucleophilic substitutions with [^{18}F]fluoride were 6β-bromoprogesterone 4.4a and the sulfonic esters of 6β-hydroxyprogesterone: mesylate 4.4b, tosylate 4.4c and triflate 4.4d.

\begin{center}
\textit{Scheme 4.2}
\end{center}

\[4.4a\]
**Bromine substitution.** - 6β-Bromoprogesterone 4.4a was prepared by the reaction of progesterone and NBS in CCl₄ in 30% yield. Several sources of fluoride were applied in the substitution reaction. The reactions were carried out with the phase transfer catalysts (PTC) Amberlyst A-26, TBAF and Kryptofix 222/[^18F]fluoride (K₂₂₂/[¹⁸F]) or as a heterogeneous reaction with KF or AgF (Table 4.1).

<table>
<thead>
<tr>
<th>fluoride</th>
<th>solvent</th>
<th>temp °C</th>
<th>time (h)</th>
<th>result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amb. A-26</td>
<td>ether</td>
<td>35</td>
<td>24</td>
<td>elimination</td>
</tr>
<tr>
<td>TBAF</td>
<td>CH₂OH</td>
<td>20</td>
<td>48</td>
<td>no reaction</td>
</tr>
<tr>
<td>TBAF</td>
<td>CH₂CN</td>
<td>20</td>
<td>2</td>
<td>elimination</td>
</tr>
<tr>
<td>TBAF</td>
<td>CH₂CN/CH₂OH</td>
<td>20</td>
<td>72</td>
<td>no reaction</td>
</tr>
<tr>
<td>TBAF</td>
<td>DMSO</td>
<td>20</td>
<td>15</td>
<td>elimination</td>
</tr>
<tr>
<td>KF</td>
<td>glycol/CH₂CN 1/1</td>
<td>80</td>
<td>2</td>
<td>elimination</td>
</tr>
<tr>
<td>KF</td>
<td>glycol/CH₂CN 1/1</td>
<td>20</td>
<td>48</td>
<td>no reaction</td>
</tr>
<tr>
<td>AgF</td>
<td>CH₂CN/CH₂O 1/1</td>
<td>100</td>
<td>2</td>
<td>no reaction</td>
</tr>
<tr>
<td>AgF</td>
<td>CH₂CN</td>
<td>75</td>
<td>3</td>
<td>no reaction</td>
</tr>
<tr>
<td>K₂₂₂/[¹⁸F]</td>
<td>CH₂CN</td>
<td>70</td>
<td>0.5</td>
<td>no product</td>
</tr>
</tbody>
</table>

**Table 4.1 Conversion of 4.4a to 4.1.**

As can be seen from Table 4.1, none of the investigated experimental conditions resulted in the formation of the desired 4.1. Either no reaction occurred or elimination of HBr, instead of substitution of bromide by fluoride, resulted in the formation of pregna-4,6-diene-3,20-dione 4.7 (Scheme 4.2). The tendency towards elimination of HBr is enhanced in 4.4a, because a double bond is created in conjugation with the α,β-unsaturated ketone. In the experiment with K₂₂₂/[¹⁸F] no distinction could be made between the possibility that H[^¹⁸F] was eliminated from 4.1, or that [¹⁸F]fluoride failed to react, since in both cases only [¹⁸F]fluoride is recovered.

**Sulfonic ester substitution.** - The potential of the sulfonic esters 4.4b,c,d as possible substrates for fluoride substitution was investigated. Of these three sulfonic esters, only 6β-mesyloxyprogesterone 4.4b could be obtained. Compound 4.4b was prepared from 6β-hydroxyprogesterone 4.8 and methanesulfonyl chloride. It was impossible to synthesize the corresponding tosylate 4.4c using p-toluenesulfonyl chloride. The reaction of 4.4a with silver tosylate also failed. The 6β-triflate 4.4d, if formed at all, was too unstable to be isolated.
Substitution experiments of $4.4b$ with K$_{222}/^{18}$F in acetonitrile did not reveal the formation of the desired $6\alpha-[^{18}\text{F}]$fluoroprogesterone $4.1$. The radioactivity that was retained after the reaction was only soluble in water, indicative of the presence of $[^{18}\text{F}]$fluoride.

4.3.2 $6\alpha$-Fluoromethylprogesterone

The synthesis of a $6\alpha$-fluoromethyl derivative was considered as an alternative for the direct introduction of fluorine in the steroid skeleton at the $6\alpha$-position. From literature it is known that a methyl substituent is tolerated well at the $6\alpha$-position of the steroid skeleton.$^{51,133}$ We felt that introducing fluorine in the $6\alpha$-methyl substituent would not grossly affect the biological properties of the steroid.

The introduction of $[^{18}\text{F}]$fluoride in the $6\alpha$- methyl group could be conducted by a nucleophilic substitution on a more accessible primary carbon atom, which has an increased reactivity towards nucleophilic substitution. Indeed, $6\alpha$-fluoromethyl-4,5-dihydroprogesterone was prepared by Nussbaum et al.$^{134}$ by the reaction of $6\alpha$-iodomethyl-4,5-dihydroprogesterone with silver fluoride in acetonitrile/water. A considerable amount of elimination product was formed, despite the fact that the steroid lacked a 4,5-double bond. This underlines the sensitivity of the C-6 position towards elimination. The performance of this reaction with n.c.a. $[^{18}\text{F}]$fluoride in an aqueous solution is not possible, because the presence of water in the reaction mixture will diminish the nucleophilicity of $[^{18}\text{F}]$fluoride.
Scheme 4.4

<table>
<thead>
<tr>
<th>Fluoride</th>
<th>solvent</th>
<th>temp °C</th>
<th>time (h)</th>
<th>result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amb. A-26</td>
<td>ether</td>
<td>30</td>
<td>48</td>
<td>elimination</td>
</tr>
<tr>
<td>Amb. A-26</td>
<td>CH₃CN</td>
<td>20</td>
<td>24</td>
<td>no reaction</td>
</tr>
<tr>
<td>Amb. A-26</td>
<td>CH₂Cl₂</td>
<td>20</td>
<td>24</td>
<td>no reaction</td>
</tr>
<tr>
<td>CsF</td>
<td>CH₃CN</td>
<td>20</td>
<td>72</td>
<td>elimination</td>
</tr>
<tr>
<td>TBAF</td>
<td>CH₃CN</td>
<td>20</td>
<td>24</td>
<td>elimination</td>
</tr>
<tr>
<td>KF</td>
<td>CH₃CN</td>
<td>70</td>
<td>3</td>
<td>elimination</td>
</tr>
</tbody>
</table>

Table 4.2 Conversion of 4.9 to 4.10.

We used 6-tosyloxyethylprogesterone 4.9 as substrate for the [¹⁸F]fluoride substitution. Compound 4.9 was prepared from 6-hydroxymethylprogesterone¹³⁵ and p-toluenesulfonyl chloride. Several fluorinating agents were used in the substitution reaction, leading either to the recovery of 4.9 or to the elimination product 4.11 (Table 4.2). The formation of a double bond in conjugation with the α,β-unsaturated ketone is apparently very favourable and elimination of p-toluenesulfonic acid is preferred above substitution of the tosylate.
4.4 Alternative routes to 6-fluoro steroids

In this section three reactions are given that in a modified way could effectuate the synthesis of \(^{18}\text{F}\)fluoro-steroids. These reactions have in common that \(^{18}\text{F}\)fluoride would attack a vinylic carbon atom without a direct substitution of a leaving group.

\[\text{Scheme 4.5}\]

An example of the nucleophilic attack of fluoride at an sp\(^2\)-carbon atom is the synthesis of 6β-fluoroandrostenedione 4.13, as described by Mann and Pietrzak.\(^{136}\) The reaction was carried out with Olah's reagent (pyridine/30-70\% HF). The proposed mechanism is depicted in Scheme 4.5. The first step is a conversion of the 3-keto-4-ene structure of 4.12 to a 3,5-dienol structure. The hydroxy group at C-2 is protonated and acts as a leaving group as fluoride attacks C-6. The 3,4-double bond shifts to Δ\(^2\), after which the 3-keto-4-ene structure is restored. Compound 4.13 was also prepared by a direct substitution of 6β-bromoandrostenedione with Olah's reagent.\(^{136}\)

\[\text{Scheme 4.6}\]

Another interesting approach was described by Reese and Sanders,\(^{129}\) who applied a tosylhydrazone derivative for the substitution of chloride in α-
chloroketones by thiophenol (Scheme 4.6). α-Chlorocyclohexanone was converted to tosylhydrazone 4.14 by reaction of the ketone with p-toluenesulfonhydrazide. After elimination of HCl, a formal addition of thiophenol takes place. The ketone functionality was regenerated with BF₃·Et₂O in aqueous acetone. A possible disadvantage of using this method with steroids is that α,β-unsaturated hydrazones are reported to be susceptible to rearrangements during hydrolysis.¹³⁷

A third example of this type of reaction is the aqueous oxidation of 3-keto-4-ene steroids as described by Jasiczak.¹³⁰ The proposed mechanism of the reaction of androstenedione 4.18 with 2,3,5-triphenyl-tetrazolium chloride (TTC) 4.19 is shown in Scheme 4.7. Initial deprotonation at C-6 followed by the addition of the enolate anion to 4.19 results in the formation of adduct 4.20. Attack of base (OH⁻) at C-6 cleaves the N-O bond and restores the 3-keto-4-ene entity, forming 6-hydroxyandrostenedione as an intermediate. A repetition of this reaction sequence resulted in the formation of androst-4-ene-3,6,17-trione 4.21.

Of the above-mentioned examples, we evaluated the methods based on a derivation of the 3-keto-4-ene entity¹²⁹,¹³⁰ in the synthesis of a 6-[^1⁸F]fluoro steroid. This choice was made, for it is possible to implement these methods for a large number of 3-keto-4-ene steroids, without the necessity of a large synthetic
programme. In addition, with both methods it should be possible to carry out the reaction with n.c.a. $[^{18}\text{F}]$fluoride.

Tosylhydrazone. In our experiments with $p$-toluenesulfonylhydrazide, testosterone 4.23, containing only one keto-functionality, was used as a model compound (Scheme 4.8).

Scheme 4.8

The synthesis of the corresponding 3-diazo-diene-derivative of testosterone 4.26 was investigated by two different routes. Either tosylhydrazone 4.24 was brominated at C-6 with phenyl trimethylammonium tribromide (PTAB) or NBS leading to 4.26; or 4.23 was brominated and converted to 4.26 by the reaction with $p$-toluenesulfonylhydrazide (Scheme 4.8). Unfortunately, neither pathway was successful for the synthesis of 4.26. The bromination of 4.24 failed to give the desired product 4.26, and the conversion of 4.25 to 4.26 was also unsuccessful. A possible explanation for the failure of these attempted syntheses of 4.26 remains unknown. Obviously, no experiments with $[^{18}\text{F}]$fluoride could be carried out.

Tetrazolium chloride. In contrast to the aqueous oxidation of androstenedione 4.18 with 4.19, the reaction with $[^{18}\text{F}]$fluoride had to be carried out under anhydrous conditions. We investigated the fluorination of both 4.18 and progesterone 4.27.
The reactions were performed under a nitrogen or argon atmosphere. The enolate anion of the steroid was prepared under various conditions (Table 4.3). After the addition of fluoride and 4.19 to the solution the reaction mixture developed a purple colour, indicative of the formation of 4.22. However, under none of the investigated conditions could fluorinated product be detected, nor was evidence found that the steroid had been oxidized. Probably, 4.19 reacts directly with base forming the coloured formazan 4.22 instead of reacting with the enolate anion.
4.5 Opening of 5α,6α-epoxide with fluoride

The fact that we were unable to synthesize 6α-[¹⁸F]fluoroprogesterone 4.1 by a nucleophilic substitution forced us to reevaluate the use of epoxides for introducing [¹⁸F]fluorine into the steroid molecule (route iii, Scheme 4.1).

![Scheme 4.10](image)

The synthesis of 4.1 has been described previously by Bowers et al. Briefly, the synthesis entails a shift of the Δ⁴ double bond of progesterone to the Δ⁵ position; the 3- and 20-ketone positions are protected as ketals and subsequently the Δ⁵ double bond is oxidized by permonophtalic acid to a mixture of 5α,6α- and 5β,6β-epoxides. The desired α-epoxide 4.6 could be isolated in 38% yield by crystallisation from acetone. The reaction of 4.6 with BF₃-etherate yielded fluorohydrin 4.28, which was converted in acetic acid/HCl to 4.1. This product was used as a reference compound in the radioactive experiments described in this Chapter.

We found that the way in which the hydrolysis/elimination was carried out had a remarkable effect on the stereochemistry of C-6. Stirring crude 4.28 in a previously prepared solution of HCl in acetic acid yielded predominantly 6β-[¹⁸F]fluoroprogesterone 4.1b, whereas stirring 4.28 in acetic acid and conducting
HCl through the solution yielded the desired 6α-epimer 4.1α. The synthesis of 4.1β has also been described by Bowers et al.14¹ who prepared 4.1β under alkaline conditions from 6β-fluoropregnane-3,5α-diol-20-one 3-acetate.

Apart from the epimerisation of C-6, the stereochemistry at C-17 was also affected by the acidic conditions. Of both 4.1α and 4.1β about 10% was found to be 6-[¹⁸F]fluoro-17-epiprogesterone.

<table>
<thead>
<tr>
<th>coupling</th>
<th>4.1α</th>
<th>4.1β</th>
</tr>
</thead>
<tbody>
<tr>
<td>²J₆H,₆F</td>
<td>48.0</td>
<td>48.7</td>
</tr>
<tr>
<td>³J₆H,7αH</td>
<td>12.4</td>
<td>2.9</td>
</tr>
<tr>
<td>³J₆H,7βH</td>
<td>6.2</td>
<td>2.6</td>
</tr>
<tr>
<td>⁴J₄H,₆F</td>
<td>-</td>
<td>5.1</td>
</tr>
<tr>
<td>⁴J₄H,6H</td>
<td>2.2</td>
<td>-</td>
</tr>
</tbody>
</table>

*Table 4.4 Spin-spin coupling (Hz) of H₄, H₆ and H₇ in 4.1.*

The isomers 4.1α and 4.1β were identified by the characteristic splitting patterns of H₆ in the ¹H-NMR spectra. The coupling constants of H₆ with its neighbouring protons and fluoride are collected in Table 4.4. It is known that the axial-axial coupling of neighbouring protons is larger than an axial-equatorial coupling (Karplus relationship). Thus, in 4.1α, with the proton in the axial (β) position, the coupling is larger than in 4.1β. The same patterns were found for 6β-fluoroandrostenedione by Wittstruck et al.14² Additional proof for the structure of 4.1β was the long range coupling of 6β-fluorine with the angular 19-methyl group (J = 2 Hz).14³

**4.5.1 Reaction of 5α,6α-epoxide with [¹⁸F]fluoride**

*No carrier added [¹⁸F]fluoride.* - The successful reaction of epoxides with hydrogen fluoride has been described in several papers.14⁴ These reactions were carried out either in water or with anhydrous hydrogen fluoride.12⁷ However, with a few exceptions14⁵ almost no reactions with n.c.a. H[¹⁸F] have been reported, since the reactivity of anhydrous n.c.a. H[¹⁸F] is seriously decreased due to the tendency of [¹⁸F]fluoride to stick to all kinds of material (see Chapter 2). Nevertheless, we attempted to apply anhydrous n.c.a. H[¹⁸F] in the synthesis of 4.1.
Anhydrous n.c.a. $\text{H}^{[18F]}$ can be prepared by the $^{20}\text{Ne}(d,\alpha)^{18}\text{F}$ nuclear reaction (Chapter 2). This type of $\text{H}^{[18F]}$-target was not available in our institute, so anhydrous $\text{H}^{[18F]}$ had to be prepared in a different way. Shane and Winchell\textsuperscript{146} prepared anhydrous $\text{H}^{[18F]}$ by the addition of concentrated sulfuric acid to $[^{18}\text{O}]\text{water}$ containing $[^{18}\text{F}]\text{fluoride}$. $\text{H}^{[18F]}$ can be isolated from the acidified solution by distillation or extraction. We found the efficiency of the isolation of $\text{H}^{[18F]}$ by extraction with chloroform/methanol (9/1) to be higher than the recovery by distillation ($150\,^\circ\text{C}$): 23\% and 13\%, respectively. The presence of $\text{H}^{[18F]}$ was established by the evaporation of the extracted mixture of $\text{CHCl}_3/\text{CH}_3\text{OH}/\text{H}^{[18F]}$, resulting in a loss of 97\% of the solubilised radioactivity.

Due to the higher transfer efficiency, extraction of $\text{H}^{[18F]}$ was preferred in the experiments with 4.6. The reaction of 4.6 with anhydrous $\text{H}^{[18F]}$ was carried out at room temperature for 1 h. Subsequently, the reaction mixture was extracted with water removing more than 95\% of the radioactivity. The residual radioactivity was examined on TLC (silica, $\text{CH}_3\text{CN}$), but revealed no presence of labelled product.

The reaction of 4.6 with $[^{18}\text{F}]\text{fluoride}$ in acetonitrile was examined either by conventional heating on an oil-bath or by a microwave oven. Both reactions were performed both in the presence and absence of acetic acid or a monobasic phosphate as proton donor (Table 4.5). The addition of potassium iodide served to increase the susceptibility of the reaction mixture to microwave heating\textsuperscript{160}. However, with any reaction labelled product was found and only unreacted $[^{18}\text{F}]\text{fluoride}$ was recovered.

<table>
<thead>
<tr>
<th>heating</th>
<th>conditions</th>
<th>time (min)</th>
<th>result</th>
</tr>
</thead>
<tbody>
<tr>
<td>microwave, 600W</td>
<td>5 mg $\text{NH}_4\cdot\text{KHPO}_4$</td>
<td>4x2</td>
<td>No reaction</td>
</tr>
<tr>
<td>oil-bath, 50 $^\circ\text{C}$</td>
<td>5 mg $\text{NH}_4\cdot\text{KHPO}_4$</td>
<td>60</td>
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</tr>
<tr>
<td>microwave, 600W</td>
<td>12 mg KI</td>
<td>5x2</td>
<td>,,</td>
</tr>
<tr>
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<td>4x2</td>
<td>,,</td>
</tr>
<tr>
<td>microwave, 600W</td>
<td>acetic acid, $[^{18}\text{F}]_{aq}$</td>
<td>5x2</td>
<td>,,</td>
</tr>
<tr>
<td>microwave, 600W</td>
<td>acetic acid/pyridine, $[^{18}\text{F}]_{aq}$</td>
<td>5x2</td>
<td>,,</td>
</tr>
</tbody>
</table>

Table 4.5 Reactions of 4.6 with $K_{222}[^{18}\text{F}]$ in $\text{CH}_3\text{CN}$.

*Carrier added $[^{18}\text{F}]\text{fluoride}$. - Since it was impossible to synthesize n.c.a. 4.1, the synthesis of 4.1 was investigated with carrier added $[^{18}\text{F}]\text{fluoride}$ in a similar
manner as was described by Bowers et al. The reaction of $^{18}\text{F} \text{BF}_3 \text{Et}_2\text{O}$ with \ref{4.6} was investigated with several stoichiometric ratios of fluoride and epoxide. The amount of BF$_3$Et$_2$O in the procedure was minimised in order to increase the specific activity of \ref{4.1}.

The $^{18}\text{F}$fluoride was dried by three successive evaporations with benzene. As has been stated in Chapter 2, drying of $^{18}\text{F}$fluoride in the absence of a PTC, will reduce the resolubility. We anticipated, however, that with the addition of carrier fluoride, i.e. BF$_3$Et$_2$O, an exchange reaction would facilitate the solubilisation of $^{18}\text{F}$fluoride. Indeed, after 1 hour in an ultrasonic bath, a resolubilisation of $^{18}\text{F}$fluoride was found ranging from 50-60%.

\begin{align*}
\text{Scheme 4.11}
\end{align*}

\begin{table}[h]
\centering
\begin{tabular}{lllll}
\hline
\textbf{catalyst} & \textbf{$\mu\text{mol}$} & \textbf{R$^a$} & \textbf{reaction time (h)} & \textbf{yield$^b$} \\
& & & \textbf{4.28} & \textbf{4.1} \\
\hline
BF$_3$Et$_2$O & 1600 & -- & 18 & 5 & 64$^c$ \\
BF$_3$Et$_2$O & 0.5 & 57 & 1 & 1 & 25 \\
BCl$_3$ & 12 & 20 & 1 & 1 & 0 \\
BBr$_3$ & 20 & 83 & 1 & 1 & 0 \\
\hline
\end{tabular}
\caption{Reaction of $^{18}\text{F}$fluoride in ether/benzene (1/1) with \ref{4.6}.}
\end{table}

a Resolubility of $^{18}\text{F}$fluoride (%); b radiochemical yield based on resolubilised $^{18}\text{F}$fluoride and corrected for decay (EOB); c yield of cold reaction.

In contrast to the reported reaction time of 18 hours, the reaction with $^{18}\text{F}$fluoride was found to be complete after 1 hour. The best result in our experiments was found after the addition of 0.5 $\mu\text{mol}$ BF$_3$Et$_2$O to the reaction.
mixture and resulted in a 25% radiochemical yield of $4.1$ (EOB). The specific activity of $4.1$ in this run, as determined by UV-spectroscopy, was estimated to be 4 MBq/μmol (100 Ci/mol, EOS). This result is about a factor 10,000 below the required specific activity for successful steroid receptor studies.$^{14}$

Reduction of the amount of BF$_3$.Et,O to such a level that an acceptable specific activity could be obtained, resulted in a dramatic decrease of the radiochemical yield. We investigated the possibility of using other halogenated boranes like BBr$_3$ and BCl$_3$ as catalysts for the reaction, but these experiments resulted in a poor radiochemical yield.

### 4.6 Concluding remarks

In this Chapter, our efforts to prepare 6α-[18F]fluoroprogesterone $4.1$ are described. These investigations were intended to lead to a fluorine-18 labelled, high affinity progestin. This new class of tracers could be an alternative for the 21-[18F]fluorinated steroids as [18F]FENP (described as $3.4b$ in Chapter 3) which have until now failed to visualise progesterone receptors in vivo.$^{32,123}$

As has been described in detail, we were not able to prepare $4.1$ via a $S_n2$-reaction. This route was initially the method of choice, for it facilitates the synthesis of [18F]fluorinated tracers with high specific activity. Probably, the problems with synthesizing $4.1$ using a nucleophilic approach arise from the reactivity of the γ-position relative to the α,β-unsaturated ketone and the basicity of the employed reaction conditions. This combination resulted in elimination rather than substitution of the leaving group.

The carrier added opening of the 5α,6α-epoxide $4.6$ was successful for the synthesis of $4.1$ (Scheme 4:11). We prepared $4.1$ with a radiochemical yield of 25% (EOB) and a specific activity of 4 MBq/μmol (100 Ci/mol, EOS). Unfortunately, this specific activity is not sufficient for the performance of in vivo receptor studies. In order to improve the specific activity of $4.1$ the suitability of two other Lewis catalysts (BBr$_3$ and BCl$_3$) was investigated, but these reactions resulted in poor radiochemical yields. Other catalysts have not yet been applied in the synthesis of $4.1$. However, many related reactions have been described in the literature concerning the opening of epoxides and the synthesis of halohydrins. Among these are reactions of epoxides with triphenylphosphine,$^{147}$ dimethylboron bromide$^{148}$ and B-bromo-bis-(dimethylamino)borane.$^{149}$ The potential of
these and other catalysts remains to be screened to find a suitable catalyst for this promising reaction, which does not affect the specific activity or inactivates [\(^{18}\)F]fluoride.

4.7 Experimental part

*General.* See also section 3.6 for general remarks. KF was dried in an oven at 50 °C. THF was distilled immediately before use from sodium or LiAlH₄.

*Microwave operation.* A Parr microwave digestion bomb containing a 10 ml borosilicate tube sealed with a Schott GL 14 cap was placed in a fixed position in a domestic Miele M 686 microwave oven. The reaction mixture was heated during 2 min at 600 W, after which the bomb was cooled to room temperature and the cap was replaced. This sequence was repeated 4-5 times. Care must be taken when opening the bomb, because high pressures may develop during the microwave operation.

4.7.1 6α-Fluoroprogesterone 4.1: nucleophilic substitution

6β-Bromoprogesterone\(^{131}\) 4.4a: reaction with Amberlyst A-26.- An amount of 50 mg (0.13 mmol) 4.4a was dissolved in a minimum amount of CH₂Cl₂ and 3 ml ether. An amount of 36 mg (1 eq.) Amberlyst A-26 was added and the stirred solution was refluxed for 24 h. The resin was filtered off and the solvent was evaporated in vacuo. \(^1\)H-NMR analysis revealed that the elimination product pregna-4,6-diene-3,20-dione 4.7 was formed. \(^1\)H-NMR (60 MHz) δ 0.7 (3H,s), 1.5 (3H,s), 2.1 (3H,s), 0.6-2.6 (16H,m), 5.6 (1H,s), 6.1 (2H,s). UV (CHCl₃): 281.3 nm.

reaction with TBAF.- Two equivalents of dried TBAF were added to a solution of 50 mg (0.13 mmol) 4.4a (see Table 4.1). After the reaction was complete, the solvent was evaporated in vacuo and the residu was dissolved in 2 ml ether and washed with 3 portions of 2 ml water. The organic layer was dried over MgSO₄, and the solvent was evaporated in vacuo.

reaction with AgF.- To a solution of 50 mg (0.08 mmol) 4.4a in 3 ml solvent (Table 4.1), 50 mg (0.39 mmol) AgF was added. The resulting suspension was stirred under reflux for 2-3 h, next the silver salts were filtered off and 5 ml ether and 5 ml water were added. The ether layer was washed three times with 2 ml water, dried over MgSO₄ and concentrated in vacuo.

6β-Mesypropdgerosterone 4.4b.- To a solution of 50 mg (0.15 mmol) 6β-hydroxy-progesterone\(^{132}\) 4.8 and 0.25 ml triethylamine in 3 ml CH₂Cl₂, 50 µl (0.6 mmol) methanesulfonyl chloride was added. The mixture was stirred for 1 h at 0 °C and 2 h at room temperature. The solution was extracted with a dilute HCl-solution, washed with water and dried over MgSO₄. After evaporation of the solvent, crude 4.4b was purified
on a silica column. Elution of the column with \( \text{CH}_2\text{Cl}_2 \), removing excess methane-sulfonyl chloride, followed by \( \text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH} \) 97/3 (v/v) yielded 15 mg (0.05 mmol, 24%) of the 6b-mesylate 4.4b.

\(^1\text{H}-\text{NMR (60 MHz)} \) \( \delta \) 0.7 (3H,s), 1.3 (3H,s), 2.1 (3H,s), 0.8-2.7 (18H,m), 3.0 (3H,s), 6.0 (1H,s).

reaction of 4.4a,b with \[^{18}\text{F}\]fluoride.- An amount of 5 mg (0.01 mmol) 4.4a or 4.4b was added to a solution of \( \text{K}_{222/19}^{[18}\text{F} \) in 0.5 ml \( \text{CH}_3\text{CN} \) and the mixture was stirred at 50 °C for 0.5-3 h. The solution was cooled to room temperature and the mixture was eluted over a silica Sep-pak with 3 ml \( \text{CH}_3\text{CN} \). Unreacted \[^{18}\text{F}\]fluoride was retained on the Sep-pak, the presence of 4.1 in the eluent could not be detected.

4.7.2 6α-Fluoromethylprogesterone 4.10: nucleophilic substitution

6-Tosyloxymethylprogesterone 4.9.- To a cooled solution of 200 mg (0.58 mmol) 6-hydroxymethylprogesterone,\(^{135} \) 3 ml triethylamine and a catalytic amount of DMAP in 30 ml \( \text{CH}_2\text{Cl}_2 \), 1 g (4.9 mmol) \( p \)-toluenesulfonyl chloride was added in small portions. After stirring for 1 h at 0 °C, the stirring was continued for 4 h at room temperature. After removal of the solvent, the residue was taken up in \( \text{CH}_2\text{Cl}_2 \) and purified on a silica column. The excess of \( p \)-toluenesulfonyl chloride was eluted with \( \text{CH}_2\text{Cl}_2 \), the product eluted with dichloromethane/methanol 98/2 (v/v) as a mixture of 6α/6β-isomers. The yield of 4.9 was 46 mg (0.09 mmol, 15%).

\(^1\text{H}-\text{NMR (60 MHz)} \) 4.9α \( \delta \) 0.6 (3H,s), 1.0 (3H,s), 2.0 (3H,s), 2.4 (3H,s), 1.0-2.0 (19H,m), 4.0 (1H,s), 5.7 (1H,s), 7.3 (2H,d), 7.7 (2H,d). 4.9β \( \delta \) 0.6 (3H,s), 1.1 (3H,s), 2.0 (3H,s), 2.4 (3H,s), 1.0-2.0 (19H,m), 4.0 (1H,s), 5.4 (1H,s), 7.3 (2H,d), 7.7 (2H,d).

reaction with Amberlyst A-26.- To a solution of 50 mg (0.09 mmol) 4.9, 120 mg (4 eq. F) Amberlyst A-26 was added. After the reaction, the ion-exchange resin was filtered off and the solvent was evaporated. For details and results see Table 4.2.

reaction with cesium fluoride.- An amount of 50 mg (0.3 mmol) CsF was added to a solution of 30 mg (0.06 mmol) 4.9 in 4 ml \( \text{CH}_3\text{CN} \). The mixture was stirred for 72 h at room temperature after which the solvent was evaporated.

reaction with tetrabutylammonium fluoride (TBAF).- Two equivalents of anhydrous TBAF (45 mg, 0.16 mmol) were added to a solution of 45 mg (0.08 mmol) 4.9 in 2 ml \( \text{CH}_3\text{CN} \). After stirring the mixture for 24 h at room temperature the solvent was evaporated and the residu was taken up in 5 ml \( \text{CH}_2\text{Cl}_2 \). The excess TBAF was removed by three extractions with 2 ml water. The organic layer was dried over MgSO\(_4\) and concentrated \textit{in vacuo}. 57
4.7.3 Vinylic reactions

6-Bromotestosterone 4.25.- A solution of 1.3 g (3.5 mmol) PTAB in 10 ml THF was added to a solution of 1 g (3.5 mmol) testosterone 4.23 in 25 ml THF, cooled in ice. After the addition was complete, the mixture was stirred for 30 min. The precipitate was filtered off and the solution was concentrated in vacuo, yielding 1.0 g (2.8 mmol, 80%) of a mixture of 6α- and 6β-bromotestosterone 4.25α,β.

1H-NMR (60 MHz) 4.25α δ 0.8 (3H,s), 1.2 (3H,s), 1.0-2.8 (17H,m), 3.7 (2H,m), 5.0 (1H,m), 6.4 (1H,s). 4.25β δ 0.8 (3H,s), 1.5 (3H,s), 1.0-2.8 (17H,m), 3.7 (2H,m), 5.0 (1H,m), 5.8 (1H,s).

6-Bromotestosterone-3-tosylhydrazone 4.26 via 4.25.- A solution of 500 mg (1.4 mmol) 4.25 and 260 mg (1.4 mmol) p-toluenesulfonhydrazide in 10 ml methanol containing one drop of concentrated sulfuric acid was refluxed for 1 h. The solution was cooled and poured in water. The mixture was extracted twice with 50 ml CH₂Cl₂ and the combined organic layers were dried over MgSO₄. The solvent was evaporated in vacuo, yielding an unidentified mixture of compounds.

Testosterone-3-tosylhydrazone 4.24.- A solution of 1 g (3.5 mmol) 4.23 and 1 g (5.4 mmol) p-toluenesulfonhydrazide in 10 ml methanol containing a drop of concentrated sulfuric acid was refluxed for 1.5 h. The reaction mixture was cooled, 50 ml CH₂Cl₂ was added and the solution was extracted twice with 25 ml of a saturated NaHCO₃-solution and twice with 25 ml water. After drying over MgSO₄, the solvent was evaporated in vacuo, yielding 1.2 g (2.7 mmol, 80%) 4.23 as a mixture of isomers.

1H-NMR (60 MHz) major component: δ 0.7 (3H,s); 0.95 (3H,s), 2.4 (3H,s), 0.8-2.4 (20H,m), 3.6 (1H,t), 5.8 (1H,s), 7.2 (2H,d), 7.8 (2H,d). minor component: δ 0.7 (3H,s); 1.00 (3H,s), 2.4 (3H,s), 0.8-2.4 (20H,m), 3.6 (1H,t), 6.0 (1H,s), 7.2 (2H,d), 7.8 (2H,d).

6-Bromotestosterone-3-tosylhydrazone 4.26 via 4.24.- An amount of 1.2 g (2.7 mmol) 4.24 was dissolved in 20 ml THF. The solution was cooled in ice and 1.2 g (3.2 mmol) PTAB dissolved in 10 ml THF was added at such a rate, that the orange colour of the PTAB slowly disappeared. After stirring for another 30 min, the mixture was filtered and the solvent was evaporated in vacuo. 1H-NMR did not reveal the presence of 4.26.

6-Fluoroandrostenedione 4.13 via reaction with 4.19 (TTC) and Amberlyst A-26.- A solution of 20 mg (0.36 mmol) KOH and 100 mg (0.35 mmol) androstenedione 4.18 in 10 ml ethanol was stirred for 15 min. Next an amount of 100 mg (1 eq. F) Amberlyst A-26 and 120 mg (0.36 mmol) 4.19 were subsequently added and the purple mixture was stirred for another 30 min. After addition of 25 ml 10% HCl and 100 ml CH₂Cl₂, the organic layer was separated and washed twice with 25 ml water. After drying over MgSO₄, the solvent was evaporated in vacuo. Column chromatography was performed on a silica column eluted with CH₂Cl₂, yielding an unidentified mixture of compounds.
**6α-[¹⁸F]Fluoroprogesterone via reaction with 4.19 (TTC) and K₂²³F.** - A solution of 15 mg (0.05 mmol) progesterone 4.27 and 3 mg (0.03 mmol) tBuOK in 2 ml absolute ethanol was stirred for 20 min under an argon atmosphere. Subsequently K₂²³F and 15 mg (0.05 mmol) 4.19 were added and the mixture was stirred for 30 min. Next, the mixture was poured into 25 ml of 3% HCl and extracted with CH₂Cl₂. The organic layer was washed with water and dried over MgSO₄. HPLC (5μ silica, hexane/dichloromethane/isopropanol 80/20/1 (v/v/v), 2 ml/min) did not reveal the presence of 4.1.

**4.7.4 6α-Fluoroprogesterone 4.1: epoxide-opening**

**6α-Fluoroprogesterone 4.1.** - To a solution of 200 mg (0.47 mmol) of progest-5α,6α-epoxy-3,20-diketal 4.6 in 20 ml ether/benzene 50/50 (v/v), 0.2 ml (1.6 mmol) BF₃-etherate was added carefully. The solution was stirred for 18 h at room temperature. The mixture was poured into 20 ml water and the organic layer was separated and washed with respectively 2x 10 ml 5% Na₂CO₃, 3x 10 ml water and 20 ml of a saturated NaCl-solution. After drying over MgSO₄, the solvent was evaporated, yielding 150 mg of 4.28.

The crude fluorohydrin 4.28 was dissolved in 15 ml glacial acetic acid and dry HCl-gas was bubbled through the solution for 1 h. After standing for another 4 h, 20 ml water was added and the solution was extracted with 30 ml ethyl acetate. The organic layer was extracted subsequently with 30 ml water and with a 5% Na₂CO₃-solution to neutrality. After drying over MgSO₄, the solvent was evaporated and the crude product 4.1 was purified on a silica column, eluted with toluene/ethyl acetate 80/20 (v/v), yielding 100 mg (0.30 mmol, 64%) of 4.1. An analytically pure sample of 4.1 was obtained using preparative HPLC on a Chrompack silica column, eluted with hexane/chloroform/isopropanol 80/20/0.2 (v/v/v).

**¹H-NMR (300 MHz) δ 0.62 (3H,s), 1.14 (3H,s), 2.08 (3H,s), 1.1-2.6 (18H,m), 5.08 (1H,dm, J_HF=48Hz), 6.04 (1H,s).** ¹⁹F-NMR (200 MHz) δ -183.47 (d, J_F=46Hz). **¹³C-NMR (75 MHz) δ 13.23 (q), 18.05 (q), 20.87 (t), 22.81 (t), 24.30 (t), 29.58 (s), 31.42 (d), 33.30 (q), 33.66 (t), 36.25 (t), 38.22 (t), 38.32 (t), 43.82 (s), 53.40 (d), 55.57 (d), 63.28 (d), 88.07 (dd, J_CF=186Hz), 119.74 (d), 165.67 (s), 198.48 (s), 206.64 (s).

6β-Fluoroprogesterone 4.1β. - 4.16 was prepared analogously to 4.1α but the elimination step was performed by stirring for 1 h in a previously prepared solution of HCl in acetic acid.

**¹H-NMR (300 MHz) δ 0.69 (3H,s), 1.30 (3H,d, J=1.5Hz), 2.13 (3H,s), 1.0-2.6 (18H,m), 4.99 (1H,dm, J_HF=49Hz), 5.88 (1H,d, J_CF=5Hz).** ¹⁹F-NMR (200 MHz) δ -165.63 (dt, J_HF=49Hz, J_CF=10Hz). **¹³C-NMR (75 MHz, characteristic signals) δ 13.29 (q), 18.36 (q), 29.96 (q), 93.22 (dd, J_CF=167Hz), 128.44 (dd, J_CF=10Hz).

**reaction of 4.6 with [¹⁸F]BF₃.Et₂O.** - [¹⁸F]Water containing [¹⁸F]fluoride was evaporated and the remaining [¹⁸F] was carefully dried by three successive evaporations with 0.3 ml benzene. A volume of 0.6 ml ether/benzene (1/1) was added to the dry [¹⁸F]fluoride.
followed by 0.5 µmol BF₃·Et₂O (25 µl of a solution of 24 µl BF₃·Et₂O in 10 ml ether) and 1 mg (2 µmol) 4.6. The mixture was sonicated for 1 h at room temperature. The solution was separated from undissolved [¹⁸F]fluoride and the solvent was evaporated. The residu was dissolved in acetic acid and HCl was conducted through the solution for 1 h. Next, the solution was dissolved in 50 ml CH₂Cl₂ and extracted with 30 ml water and 30 ml of a 5% NaHCO₃-solution. The organic layer was dried over MgSO₄ and concentrated in vacuo. HPLC-purification (5µ silica, hexane/dichloromethane/isopropanol 80/20/1 (v/v/v), 3 ml/min) yielded 25% (EOB) of 4.1 (10% 4.1β tₚ=8.0 min; 90% 4.1α tₚ=15.2 min). The total synthesis time was 170 min.

Preparation of anhydrous hydrogen[¹⁸F]fluoride: extraction.- A mixture of [¹⁸F]fluoride, 0.5 ml H₂SO₄ and 1.0 ml chloroform/methanol 9/1 (v/v) was left in an ultrasonic bath for 15 min. The upper organic layer was separated, yielding 42% of the initial total radioactivity. After drying over Na₂SO₄, 23% was found in the organic layer.

Preparation of anhydrous hydrogen[¹⁸F]fluoride: distillation.- To a vessel containing [¹⁸F]fluoride, 0.5 ml H₂SO₄ was added and the vessel was heated under a helium flow at a temperature of 150 °C. The gasflow was led through a Na₂SO₄-column into a 5% solution of NaHCO₃ which retained 13% of the initial activity. The remainder of the activity was found in the Na₂SO₄-column (15%) and the distillation vial (60%).

reaction of 4.6 with H[¹⁸F].- An amount of 2 mg 4.6 was added to a solution of H[¹⁸F] in 0.5 ml CHCl₃/CH₃OH 9/1 (v/v) and stirred at ambient temperature for 1 h. The solution was diluted with 10 ml CHCl₃ and washed with 5 ml water. The organic layer was dried over MgSO₄ and the solvent was evaporated. Of the residu, 3% (EOB) dissolved in CH₂Cl₂, but TLC analysis (silica, CH₃CN) revealed only radioactivity at Rf=0, indicative of [¹⁸F]fluoride.

reaction of 4.6 with K₂₂₂[¹⁸F] in microwave oven.- A solution of salt (Table 4.5), 10 mg 4.6 and K₂₂₂[¹⁸F] in 1 ml acetonitrile was heated at 600 W in the microwave oven for 4-5 intervals of 2 min. After cooling, the reaction mixture was dissolved in dichloromethane and extracted with water. The organic layer was dried over MgSO₄ and the solvent was evaporated in vacuo.

reaction of 4.6 with [¹⁸F]fluoride in microwave oven.- [¹⁸O]Water containing [¹⁸F]fluoride was evaporated next to dryness and 1 ml acetic acid or 1 ml acetic acid/pyridine 9/1 (v/v) containing 10 mg of 4.6 was added to the radioactivity. The reaction was run for 5x2 min at 600 W. After cooling, the reaction mixture was dissolved in 20 ml dichloromethane and extracted subsequently with 10 ml water and 10 ml of a 5% Na₂CO₃-solution. The organic layer was dried over MgSO₄ and the solvent was evaporated in vacuo.