Synthesis and evaluation of [18F]fluoroprogestins and [18F]fluorometoprolol
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CHAPTER 6

EPILOGUE

6.1 Starting points

In this thesis, the syntheses of two classes of receptor binding ligands (progestins and \( \beta \)-adrenoceptor ligands) for application in Positron Emission Tomography (PET) are described. PET is a technique which enables the visualisation of physiological processes in the human body. In this respect, PET is complementary to imaging techniques as CT or MRI, that visualise the structure of the human body rather than the biochemistry. In PET, short lived radionuclides (\( t_\alpha < 2 \) h) are used that decay by positron emission (\( \beta^+ \)-decay). This particular form of radioactive decay allows a quantitative reconstruction of the distribution of the radioactive compound inside the human body. The radionuclide fluorine-18 was chosen for our studies because the relative long half-life of 110 min allows the performance of studies in a time frame of 2-3 h. Especially with steroid receptor experiments, such a time frame is required for optimum target/non-target ratios.

6.2 \[^{18}\text{F}]\text{Fluoro-progestins}

The visualisation of progesterone receptors is of interest since a relation exists between the receptor density of a tumor and the response to endocrine therapy.\(^{24}\) We investigated two positions in the progesterone molecule for the introduction of fluorine-18. One attempt was directed towards C-21 in the acetyl side chain of the molecule (Scheme 6.1), the other objective was the introduction of a fluorine-18 substituent at C-6a (Scheme 6.2). These two positions allowed the screening of two different classes of progestins, either based on progesterone or on 17\( \alpha \)-alkylated derivatives of androstenedione.

\( 21-[^{18}\text{F}]\text{Fluoro-progestins}. \) - The synthesis of the 21-\[^{18}\text{F}]\text{fluoro-progestins} was performed by a nucleophilic substitution of a tosylate leaving group by \[^{18}\text{F}]\text{fluoride}. Using this approach, four structurally related progestins were prepared: 21-\[^{18}\text{F}]\text{fluoro-16\( \alpha \)-methyl-19-norprogesterone (\[^{18}\text{F}]\text{FMNP}) 3.4a, 21-}\[^{18}\text{F}]\text{fluoro-16\( \alpha \)-ethyl-19-norprogesterone (\[^{18}\text{F}]\text{FENP}) 3.4b, 21-}\[^{18}\text{F}]\text{fluoro-16\( \alpha \)-methylprogesterone (\[^{18}\text{F}]\text{FMP}) 3.4c and 21-}\[^{18}\text{F}]\text{fluoro-16\( \alpha \)-ethylprogesterone (\[^{18}\text{F}]\text{FEP}) 3.4d. The radiochemical yield of 3.4a,b,c,d was 10\% (EOB).
average specific activity was 250 GBq/μmol (7,000 Ci/mmol, EOS). The yield as well as the specific activity of the products were sufficient for the performance of in vivo receptor studies. Two of these ligands, 3.4a and 3.4b respectively, were screened in an in vivo tissue distribution study in immature, female Wistar-rats, showing a highly selective and specific uptake in the target organ (i.e. the uterus). These results suggested that both compounds were potential tracers for the in vivo evaluation of PR. However, patient studies with 3.4b failed to visualise a breast tumor that was known to contain both ER and PR. Accumulation of [18F]FENP 3.4b in a meningioma was found in 2 out of 6 cases, but the uptake of 3.4b seemed not to be correlated to the PR-density.52

Scheme 6.1

6α-[18F]Fluoroprogesterone.- The failure of [18F]FENP 3.4b in the visualisation of the PR-density of the tumors, prompted us to evaluate the 6α-position as an alternative for C-21. The synthesis of 6α-[18F]fluoroprogesterone 4.1 was investigated by three different approaches: i) a nucleophilic substitution, ii) a transformation of the 3-keto-4-ene entity of progesterone and iii) the opening of an epoxide.

The route using a Sn2-reaction was initially the method of choice, for it facilitates the synthesis of [18F]fluorinated tracers with high specific activity. The introduction of [18F]fluorine in a 6-methyl group was also investigated and was attempted by substitution of the corresponding tosylate. However, these reactions
resulted either in the recovery of starting material or in the elimination of the leaving group. The problems with synthesizing 4.1 using a nucleophilic approach were probably due to the $\gamma$-position of the leaving group relative to the $\alpha,\beta$-unsaturated ketone and the basicity of the employed reaction conditions. This combination resulted in elimination rather than substitution of the leaving group.

The second approach was based on a conversion of the 3-keto-4-ene entity of progesterone to a 3,5-dien-3-ol derivative. In this reaction, $[^{18}\text{F}]$fluoride would attack a vinylic carbon atom, avoiding a direct nucleophilic substitution of a leaving group. However, this approach did not lead to the successful synthesis of $6\alpha-[^{18}\text{F}]$fluorinated steroids.

![Scheme 6.2](image)

**Scheme 6.2**

The synthesis of 4.1 was successfully accomplished by making a concession with respect to the specific activity of 4.1. $6\alpha-[^{18}\text{F}]$Fluoroprogesterone 4.1 was prepared by the BF$_3$.Et$_2$O-catalysed opening of the 5$\alpha,6\alpha$-epoxide 4.6 (Scheme 6.2) with a yield of 25% (EOB) and a specific activity of 5 GBq/μmol (100 Ci/mol, EOS). Since the specific activity of the carrier added product was not sufficient for the performance of *in vivo* receptor studies, 4.1 has not been screened in a biological study.

An alternative catalyst for the synthesis of 4.1 has not yet been found. However, in the literature many catalysts have been described for the opening of epoxides and the synthesis of haloxydrins, e.g. triphenylphosphine$^{147}$ and mono-bromo-boranes.$^{148,149}$ The potential of these and other catalysts remains to be screened in order to fully evaluate the scope of the C-6 position in the labelling of steroids for PET.
6.3 $[^{18}\text{F}]$fluorinated $\beta_1$-adrenoceptor ligands

The second objective of the investigations described in this thesis, was directed towards the synthesis of a $[^{18}\text{F}]$fluorinated ligand for the *in vivo* visualisation of $\beta_1$-adrenoceptors. This receptor density is of interest, since in various forms of cardiac diseases the $\beta_1$-adrenoceptor density is known to be decreased.\(^{62}\)

Two types of $[^{18}\text{F}]$fluoroalkylation were applied for this purpose, i) with 1-$[^{18}\text{F}]$fluoroisopropyl tosylate 5.6a and ii) with $[^{18}\text{F}]$fluoroacetone 5.12. Both synthons were prepared in 40 min with a yield of 45% (5.6a) and 20% (5.12) at BOS. Noteworthy was the extremely low radiochemical purity of $[^{18}\text{F}]$fluoroacetone (30-60%). However, in the subsequent alkylation reaction of a primary amino functionality the results with 5.12 were superior to 5.6a (30% and 2% BOS, respectively), resulting in an overall radiochemical yield (EOB) of 5% (5.12) and 1% (5.6a). Both methods yielded sufficient quantities of fluorine-18 labelled ligands for the performance of *in vivo* receptor studies.

Scheme 6.3

The potential of a $[^{18}\text{F}]$fluoroisopropyl group as a tag in $\beta_1$-adrenoceptor ligands was studied with (+)-1'-$[^{18}\text{F}]$fluorometoprolol 5.19, the $[^{18}\text{F}]$fluorinated analogue of metoprolol (a $\beta_1$-selective beta-blocker). $[^{18}\text{F}]$Fluoroisopropyl tosylate 5.6a was applied for the synthesis of 5.19, the specific activity of the final product was 100 GBq/µmol (3,000 Ci/mmol).

1'-Fluorometoprolol 5.19 showed in two *in vitro* assays a similar affinity at $\beta_1$-adrenoceptors (about 0.3 µM) as metoprolol but with a slightly higher $\beta_1/\beta_2$-
adrenoceptor selectivity ratio (48.6 vs. 30.7). However, the \emph{in vivo} experiments with 5.19 showed almost no receptor-mediated uptake in the heart, probably because the affinity of (fluoro)metoprolol for the β₁-adrenoceptors is too low for successful imaging. Nevertheless, the \emph{in vitro} experiments suggested that the fluoroisopropyl group is a suitable structural element for the synthesis of \[^{18}\text{F}\]fluorinated β₁-adrenoceptor binding ligands. Moreover, the uptake of radioactivity in bone in the \emph{in vivo} studies was low, suggesting a promising metabolic stability of the fluorine-18 label. Work is in progress by us and others\textsuperscript{83} to synthesize fluorine-18 labelled ligands with a higher affinity for the β₁-adrenoceptors.