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SYNTHESIS, *IN VITRO* PHARMACOLOGY AND RADIOSYNTHESIS OF *N*-(*CIS*-4-FLUOROMETHYLCYCLOHEXYL)-4-(1(H)-IMIDAZOL-4-YL)PIPERIDINE-1-THIOCARBONAMIDE (VUF 5000), A POTENTIAL PET LIGAND FOR THE HISTAMINE H_3 RECEPTOR.

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ABSTRACT

The synthesis of *N*-(*cis*-4-fluoromethylcyclohexyl)-4-(1(H)-imidazol-4-yl)piperidine-1-thiocarbonamide (VUF 5000) **3**, a fluorinated analogue of the potent (pA_2 value of 8.9 ± 0.1, K_i = 4.3 ± 0.9 nM) histamine H_3 receptor antagonist thioperamide **2** is described. After the establishment of the H_3 antagonistic activity of VUF 5000, pA_2 value = 9.0 ± 0.2, K_i = 2.3 ±0.5 nM, a four step synthesis for the radiolabelling of VUF 5000 with ^{18}F (half life 110 min) was developed. Within 4 hours of the end of the bombartment, [^{18}F]VUF 5000 was obtained with an average radiochemical yield of 23% (decay corrected) and a specific activity > 96.2 TBq/μmol (2.6 Ci/μmol).

Keywords : thioperamide, histamine H_3 receptor, VUF 5000, ^{18}F fluorination, *in vitro* pharmacology, radiosynthesis

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INTRODUCTION

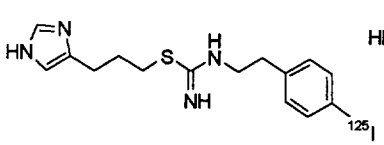
The histamine H_3 receptor, discovered in 1983 as a presynaptic autoreceptor in the central nervous system (CNS)[1], has become of great interest during the last decade. It has been shown that the H_3 receptor not only inhibits the histamine release (autoreceptor action) but also acts as a heteroreceptor, inhibiting the release of neurotransmitters like acetylcholine[2], noradrenaline[3], serotonin[4] and dopamine[5]. This regulatory effect of the H_3 receptor may be of great importance for future drug therapies for disorders of the central nervous system like Parkinson's disease (dopaminergic system), Alzheimer's disease (cholinergic system), schizophrenia, learning and memory processes and depression (serotonergic system). Agonists and antagonists of the H_3 receptor could have therapeutic benefits as has been described in several reviews on the potential of the H_3 receptor as a drug target in the central nervous system[6,7]

From the afore mentioned extensive *in vitro* research it appears that the H_3 receptor has a physiological role in the central nervous system, but the physiological and pathological role of the H_3 receptor *in vivo* is poorly understood since the *in vivo* pharmacology has only been partly described. To further elucidate the relationship between the H_3 receptor and the afore mentioned diseases in terms of the localization and the density of the H_3 receptor, a non-invasive *in vivo* method to investigate these parameters would be of great assistance. Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) are such non invasive methods and can provide the information needed, if a suitable radioligand is available.

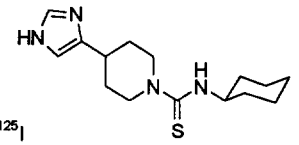
The first published radio-iodinated H_3 antagonist [^{125}I]-iodophenpropit **1**[8,9], has also been labelled with iodine-131 for biodistribution studies[10]. However this compound showed poor brain uptake and therefore the development of iodophenpropit as a potential SPECT ligand for brain imaging, after labeling with iodine-123, was not feasible[10].

Thioperamide **2** , first described by Arrang *et al*[11], is one of the best studied and characterized histamine H_3 antagonists. It has a high potency (pA_2 value of 8.9 ± 0.1[12], $K_i = 4.3 \pm 0.9$ nM[13]) and is

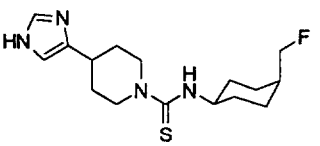
also a selective[14] H_3 antagonist. Furthermore it has been clearly shown from *ex vivo* binding studies that thioperamide penetrates the brain[15,16] after *iv* or *ip* administration, in rat and mouse. For these reasons we selected thioperamide as a template for the development of a PET ligand for the H_3 receptor. In the first instance we tried to introduce a fluorine atom at the 4-position of the cyclohexane moiety. However, this proved to be chemically rather complicated as has been described in a previous publication[17]. We now describe the synthesis, *in vitro* pharmacology and radiosynthesis of VUF 5000 **3**, a fluoromethylated analogue of thioperamide.



[^{125}I]iodophenpropit, **1**



thioperamide, **2**



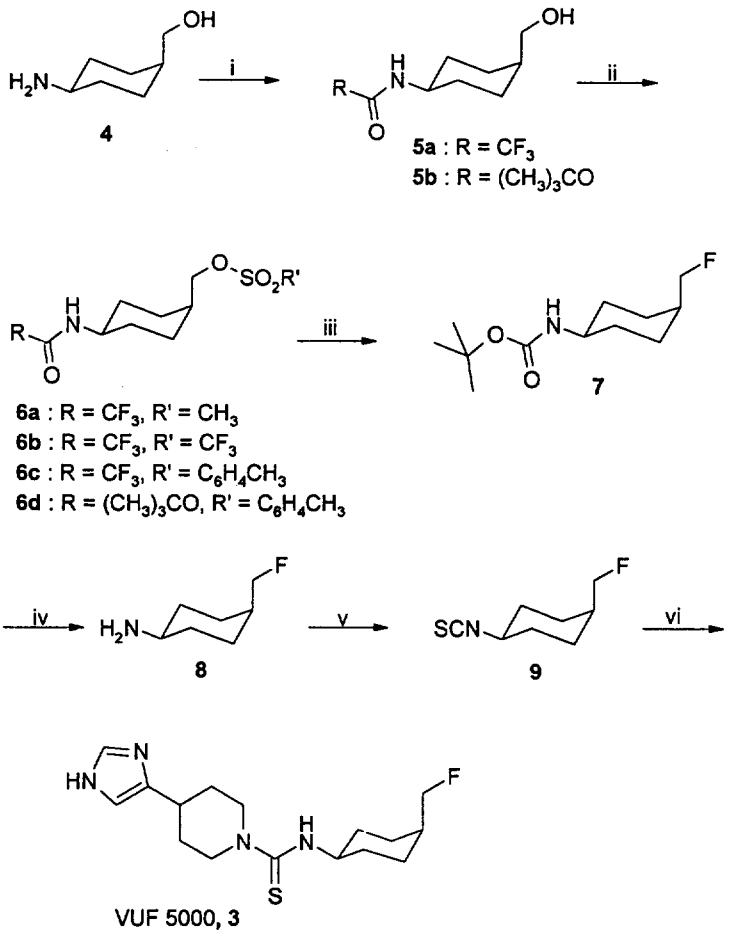
VUF 5000, **3**

RESULTS AND DISCUSSION

Chemistry

VUF 5000 **3** was synthesized according to scheme 1, starting from the readily available *cis*-(4-aminocyclohexyl)methanol[17]. Initially, the trifluoroacetyl group was used to protect the amine moiety of **4**, since this protecting group can be removed under mild conditions[18]. The hydroxy group of (*N*-trifluoroacetyl *cis*-4-aminocyclohexyl)methanol **5a** was converted into a good leaving group such as a mesylate, a tosylate or a triflate. However, attempts to fluorinate **6a,b,c** with ^{18}F in the presence of the trifluoroacetyl protecting group failed, as only a polar radioactive compound could be detected in the reaction mixture. Most likely the trifluoroacetyl group reacts with ^{18}F, after which the trifluoroacetyl group is hydrolysed under the strongly basic conditions. We therefore replaced the trifluoroacetyl group by the *t*-butoxycarbonyl (BOC) group, since this protecting group is stable under basic conditions, and can be readily removed under mild conditions. The amine moiety of **4**

was protected with di-*t*-butyldicarbonate in the presence of triethylamine in methanol and afforded **5b** in 78% yield.



Scheme 1 : Synthesis of VUF 5000.

i : di-*t*-butyldicarbonate, triethylamine, methanol, RT, 78% ; ii : p-toluenesulphonyl chloride, pyridine, RT, 62%, ; iii ; tetra-*n*-butylammonium fluoride, CH_3CN, RT, 78% ; iv : 1) chlorotrimethylsilane, KI, CH_3CN, RT, 2) methanol, RT, 51% (1+2) ; v : 1,1'-thiocarbonyl-2-1(H)pyridone, diisopropylethylamine, CH_3CN, RT, 90% ; vi : 4-[1(H)-imidazol-4-yl]piperidine, diisopropylethylamine, CH_3CN, RT, 54%.

This alcohol **5b** was transformed into the tosylate with p-toluenesulphonyl chloride in pyridine. After purification with flash column chromatography the product **6d** was recrystallized from cyclohexane, yielding analytically pure *N*-[*cis*-4-(tosyloxymethyl)cyclohexyl]-*O*-*t*-butylcarbamate in 62% yield. Subsequently, **6d** was fluorinated in 78% yield with tetra-*n*-butylammonium fluoride in dry acetonitrile at room temperature to give **7**. Deprotection of the amino group with *in situ* prepared iodotrimethylsilane in acetonitrile and subsequent treatment with methanol gave *cis*-4-fluoromethylcyclohexylamine **8**. This deprotection method was selected instead of the regular acidic hydrolysis of the BOC group, because the deprotection with iodotrimethylsilane is performed in solvents which can be easily evaporated; moreover, this reaction is fast and high yielding. Especially for the radiolabelling with ^{18}F these factors are of great importance. Care should be taken with the isolation of **8** as a free base, since it is rather volatile. Therefore **8** was converted into its fumarate before the evaporation of the solvent. This procedure yielded **8** as a fumarate salt in 51% yield.

Compound **8** was converted into the isothiocyanate **9** with 1,1'-thiocarbonyl-2(1H)-pyridone according to literature procedures[19]. Coupling of isothiocyanate to 4-(1(H)-imidazole-4-yl)piperidine, which was prepared according to Vollinga[20], in CH_3CN gave VUF 5000 in 54% yield. The free base was converted into the hemifumarate and recrystallized from acetone.

In vitro pharmacology

The histamine H_3 activity of VUF 5000 was determined using the method described by Vollinga *et al*[12]. Briefly, the pA_2 value was determined on an *in vitro* test system, based on the concentration dependent inhibitory effect of H_3 agonists on the electrically evoked contractile response (induced by endogenous acetylcholine release) of guinea pig jejunum preparations. These experiments were performed four times, each in duplicate. As agonist [R]-α-methylhistamine was used (pD_2 = 7.8 (± 0.2), N=12). The Schild slopes of the antagonist were not significantly different from unity. Results are shown in Table 1.

The binding affinity (K_i) for the H_3 receptor was determined with [^{125}I]iodophenpropit[8] on rat cerebral cortex homogenates according to the procedure described by Jansen *et al*[13]. These experiments were performed three times, each in triplicate. Results are shown in Table 1.

Table 1. *In vitro* H_3 activity of VUF 5000 and thioperamide*.

Compound	pA_2 (± SEM)	K_i (± SEM)
VUF 5000	9.0 (± 0.2)	2.3 (± 0.5) nM
thioperamide	8.9 (± 0.1)	4.3 (± 0.9) nM

* : N = 4 (pA_2) and N = 3 (K_i)

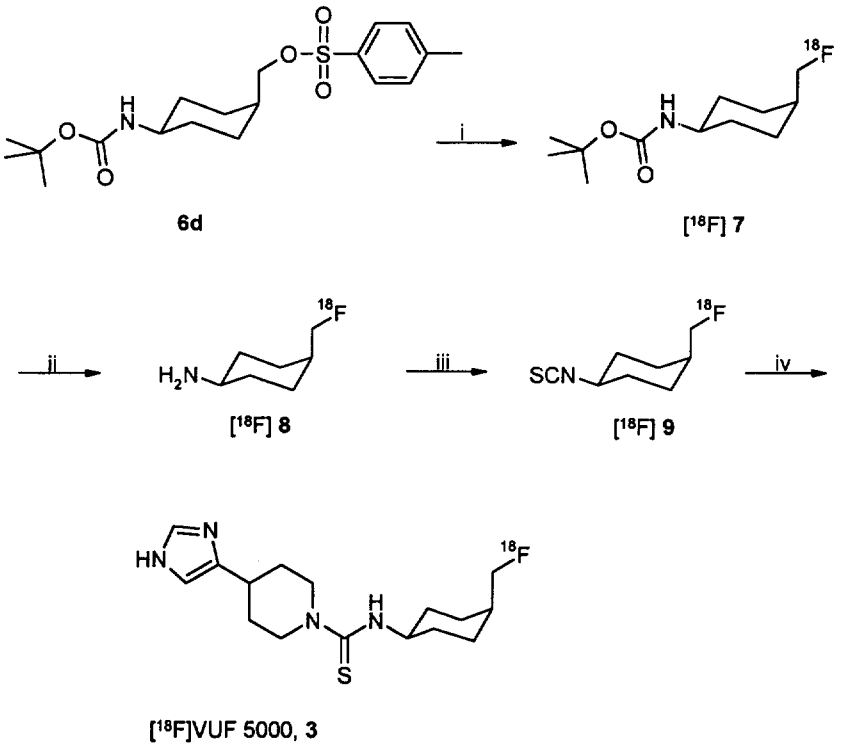
It is clear from these results that substitution of the cyclohexane moiety in thioperamide with a *cis*-fluoromethylcyclohexyl moiety does not influence the *in vitro* H_3 activity nor the affinity. We continued therefore with the radiosynthesis of [^{18}F]VUF 5000.

Radiosynthesis

[^{18}F]VUF 5000, ready for intravenous injection, was synthesized in four steps (scheme 2), without intermediate purification, within 4 hours of the end of the bombardment in an average radiochemical yield of 23% (decay corrected). The radiochemical purity was >99% (determined by HPLC) and the specific activity was >96.2 TBq/μmol (2.6 Ci/μmol) (determined by HPLC).

N-[*cis*-4-(tosyloxymethyl)cyclohexyl]-*O*-*t*-butylcarbamate **6d** was fluorinated with cyclotron produced ^{18}F and kryptofix[2.2.2] in acetonitrile in 60-86% yield in 30 minutes at 100 °C. After cooling, KI and chlorotrimethylsilane, for the *in situ* generation of iodotrimethylsilane, were added to the reaction mixture under a stream of argon. Since any unreacted ^{18}F is then converted into [^{18}F]trimethylsilylfluoride, a trap at approximately -100 °C was placed in the gas outlet to prevent the release of this volatile radioactive compound (bp 19 °C).

After 10 minutes methanol was added to the reaction mixture and stirring was continued for another 5 minutes. Subsequently the solvent was evaporated because methanol interferes with the formation of [^{18}F]**9** in the next reaction.



Scheme 2 : Synthesis of [^{18}F]VUF 5000.

i : $^{18}F^-$, kryptofix[2.2.2], K_2CO_3, CH_3CN, 100 °C, 30 min, 60-86% ; ii : 1) chlorotrimethylsilane, KI, CH_3CN, RT, 10 min, 2) methanol, RT, 5 min, 100% (1+2) ; iii : 1,1'-thiocarbonyldi-1(H)-pyridone, diisopropylethylamine, CH_3CN, 100 °C, 2 min, 80-90% ; iv : 4-(1(H)-imidazole-4-yl)piperidine, CH_3CN, 100 °C, 15 min, 50-80%.

After cooling the residue to room temperature diisopropylethylamine, 1,1'-thiocarbonyldi-2(1H)pyridone and acetonitrile were added and the reaction mixture was heated at 100 °C for 2 minutes. The reaction mixture was subsequently cooled to room temperature and 4-(1(H)-imidazole-4-yl)piperidine was added for the final step. The reaction mixture was heated at 100 °C for 15 minutes and cooled to room temperature. Then 30 ml of water was added and the solution was passed over a HPLC guard column loaded with 300 mg of RP-18 silica. The product was completely trapped and the guard column was subsequently coupled to the HPLC column and eluted. The

fractions containing [^{18}F]VUF 5000 were evaporated to dryness. The residue was dissolved in 0.2 M sodium phosphate buffer (pH 7.2) at a concentration of 1 mCi/ml and filtered over a Millex 0.22 μm filter and analyzed by HPLC. Representative HPLC chromatograms of the purification and the analysis are shown in Figure 1.

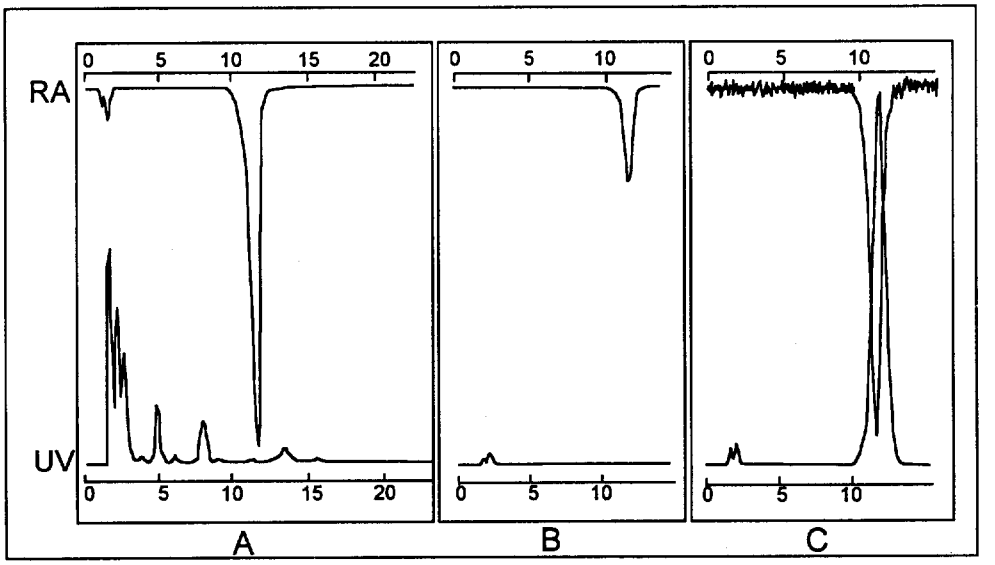


Figure 1. Representative HPLC chromatograms of the purification and analysis of [^{18}F]VUF 5000. For the HPLC conditions, see the experimental section. Panel A : purification of the reaction mixture, panel B : analysis of the isolated fraction, panel C : co-injection of a sample of the isolated fraction with carrier VUF 5000.

CONCLUSION

We have synthesized VUF 5000 **3**, established its *in vitro* histamine H_3 antagonistic activity and subsequently synthesized [^{18}F]VUF 5000, a potential PET ligand for the histamine H_3 receptor. VUF 5000 is a very good histamine H_3 antagonist, comparable to thioperamide. It can be radiolabelled in a four step radiosynthesis in 23 % radiochemical yield (decay corrected) with a specific activity of >96.2 TBq/μmol (2.6 Ci/μmol) (determined by HPLC). [^{18}F]VUF 5000 will be further investigated for use as a PET ligand.

EXPERIMENTAL

^{1}H-NMR spectra were recorded on a Bruker AC 200 (at 200.13 MHz), chemical shifts (δ) being determined relative to the solvent and converted to the TMS scale using δ = 2.50 for DMSO-d_6, 3.35 for CD_3OD and 7.26 for $CDCl_3$. ^{19}F-NMR spectra were recorded on a Bruker MSL 400 (at 376.43 MHz), with the chemical shifts being represented in ppm relative to internal $CFCl_3$. Abbreviations used in the description of the NMR spectra are : s = singlet, d = doublet, t = triplet, dd = double doublet, dt = double triplet, m = multiplet, bs = broad singulet. High resolution mass spectra were recorded on a Finnigan MAT-90 mass spectrometer operating at an ionization potential of 70 eV. Melting points were measured on a Mettler FP-5 mounted with a FP-52 microscope and are uncorrected. Flash column chromatography was performed with Baker silicagel 60. Thin layer chromatography (TLC) was performed on Merck TLC plates (silica gel 60, F_{254} , 0.25 mm), amines were detected using ninhydrine. Amides were detected using the Reindol-Hoppe coloring procedure, giving purple spots for NH- or NH_2-containing compounds. For monitoring the radiochemical reactions, the TLC plates were scanned using a MD 425s phosphorimager and quantified using the software package ImageQuant (v4.2). Elemental analyses were within 0.4% of the theoretical values. All chemicals were purchased from Aldrich, solvents were purchased from Baker or Riedel - de Haen. Acetonitrile was dried over 4Å molecular sieves and *n*-hexane was distilled before use, whilst pyridine was stored over KOH pellets. All reactions were carried out under a nitrogen atmosphere, with the radiochemical reactions being carried out under an argon atmosphere.

_N_-[*cis*-4-(hydroxymethyl)cyclohexyl]-_O_- *t*-butylcarbamate 5b.

Cis-4-aminocyclohexylmethanol **4** (3.50 g, 27.0 mmol) was added to a solution of 5.89 (27.0 mmol) of di-*t*-butyldicarbonate in 150 ml of 10% triethylamine in methanol. The reaction mixture was stirred at room temperature for 26 hours. After evaporation of the solvent under reduced pressure, the residue was taken up in 50 ml of dichloromethane and subsequently washed with 25 ml of saturated $NaHCO_3$ in water and 25 ml of water. The organic layer was separated, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was recrystallized from n-hexane to yield 4.45 g (19.4 mmol, 72%) of **5**. R_f (ethyl

acetate) = 0.70. M_p = 145 - 148 °C. ^{1}H-NMR ($CDCl_3$) : δ = 1.15-1.30 (m, 2H, cyclohexane-H3,5), 1.40 (s, 9H, *t*-butyl), 1.45-1.80 (m, 8H, cyclohexane + OH), 3.40-3.50 (m, 2H, CH_2), 3.65 (bs, 1H, cyclohexane-H4), 4.60 (bs, 0.8H, NH).

***N*-[*cis*-4-(tosyloxymethyl)cyclohexyl]-*O*- *t*-butylcarbamate 6d.**

Compound **5** (4.45 g, 19.4 mmol) and 4.06 g (21.3 mmol) of p-toluenesulphonyl chloride were dissolved in 25 ml of pyridine. The reaction mixture was stirred for 48 hr at room temperature. Then the solvent was evaporated under reduced pressure, the residue was dissolved in 50 ml of ethyl acetate and washed with 25 ml of 0.1 M HCl. The organic layer was separated, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was recrystallized from cyclohexane to yield 4.53 g (11.8 mmol, 62%) of **6**. R_f (ethyl acetate / *n*-hexane / triethylamine 5/15/1) = 0.40. M_p = 178 - 179. ^{1}H-NMR ($CDCl_3$) : δ = 1.05 - 1.30 (m, 2H, cyclohexane-H3,5), 1.41 (s, 9H, *t*-butyl), 1.45 - 1.80 (m, 7H, cyclohexane), 2.43 (s, 3H, CH_3), 3.65 (bs, 1H, cyclohexane-H4), 3.83 (d, J = 6.5 Hz, 2H, CH_2), 4.53 (bs, 0.6H, NH), 7.32 (d, J = 8.0 Hz, 2H, phenyl-H3,5), 7.76 (d, J = 8.0 Hz, 2H, phenyl-H2,6).

***N*-[*cis*-4-(fluoromethyl)cyclohexyl]-*O*- *t*-butylcarbamate 7.**

Compound **6** (1.90 g , 4.95 mmol) and 10.00 g (32.0 mmol) of tetra-*n*-butylammonium fluoride were dissolved in 150 ml of CH_3CN. The reaction mixture was stirred for 72 hr at room temperature. After evaporation of the solvent under reduced pressure, the residue was dissolved in 50 ml of dichloromethane and washed with 25 ml of a saturated $NaHCO_3$ solution in water. The organic layer was separated, dried over Na_2SO_4 and evaporated under reduced pressure. The product was isolated by flash column chromatography using ethyl acetate / *n*-hexane 1/10 as the eluent, yielding 0.90 g (3.89 mmol, 76%) of **7**. R_f (ethyl acetate / *n*-hexane 1/3) = 0.65. M_p 94- 97 °C. ^{1}H-NMR ($CDCl_3$) : δ = 1.15-1.30 (m, 2H, cyclohexane-H3,5), 1.43 (s, 9H, *t*-butyl), 1.48-1.80 (m, 7H, cyclohexane), 3.25 (bs, 1H, cyclohexane-H4), 4.23 (dd, $^2J_{FH}$ = 43 Hz, $^3J_{HH}$ = 7.8 Hz, 2H, CH_2F), 4.73 (bs, 0.7H, NH).

***cis*-4-Fluoromethylcyclohexylamine • fumarate 8.**

Compound **7** (0.87 g, 3.75 mmol) and 4.13 ml (18.75 mmol) of chlorotrimethylsilane were dissolved in 25 ml of CH_3CN. 100 mg (0.60 mmol) of KI was added and the reaction mixture was stirred for 48 hr at room temperature. Then 20 ml of methanol was added and stirring was continued for 24 hr at room temperature. After the addition of 5 ml of concentrated HCl the solvent was evaporated under reduced pressure. The residue was dissolved in 20 ml dichloromethane and washed with 10 ml of a saturated Na_2CO_3 solution in water. The organic layer was separated and dried over Na_2SO_4. Then a saturated solution of fumaric acid in diethyl ether was added dropwise until the pH was 4. The white precipitate formed was filtered off, yielding 0.44 g (1.90 mmol, 51 %) of *cis*-4-fluoromethylcyclohexylamine • 0.8fumarate **8** (determined by NMR). R_f (ethyl acetate / methanol / triethylamine 5/5/1) = 0.35. M_p = 122-126. ^{1}H-NMR (DMSO-d_6) : δ = 1.35 - 1.85 (m, 9H, cyclohexane-H), 3.10 (bs, 1H, cyclohexane-H1), 4.59 (dd, $^2J_{HF}$ = 47 Hz, $^3J_{HH}$ = 6 Hz, -CH_2F), 6.43 (s, 1.6H, fumaric acid), 8.30 (bs, 2.6H, NH_3^+).

***N*-(*cis*-4-fluoromethylcyclohexyl)-4-(1(H)-imidazol-4-yl)piperidine-1-thiocarbonamide • hemifumarate (VUF 5000) 3.**

Compound **8** (0.40 g, 1.71 mmol), 0.41 g (1.71 mmol) of 1,1'-thiocarbonyl-2(1H)pyridone and 0.60 ml (3.50 mmol) of diisopropylethylamine were dissolved in 25 ml of CH_3CN. The reaction mixture was stirred at room temperature until the reaction was complete according to TLC. Then 0.54 g (1.71 mmol) of 4-[1(H)-imidazol-4-yl]piperidine dihydrobromic acid salt and 0.60 ml (3.50 mmol) of diisopropylethylamine were added. After the reaction mixture was stirred for 18 hr at room temperature, the solvent was evaporated under reduced pressure. The residue was dissolved in 50 ml of ethyl acetate and washed with 25 ml of a saturated $NaHCO_3$ solution in water. The organic layer was separated, dried over Na_2SO_4 and evaporated under reduced pressure. The product was isolated by flash column chromatography with ethyl acetate / methanol 10/1 as the eluent. This yielded 0.32 g (0.99 mmol, 58%) *N*-(*cis*-4-fluoromethylcyclohexyl)-4-(1(H)-imidazol-4-yl)piperidine-1-thio-carbonamide. The free base was dissolved in 2 ml of methanol and to this solution a saturated solution of fumaric acid in diethyl ether was added dropwise until the pH was 4. The white precipitate was

filtered off and recrystallized from acetone to yield 359 mg (0.92 mmol, 93%) of VUF 5000 • hemifumarate **3**. R_f (ethyl acetate / methanol 5/1) = 0.30. M_p = 101.4 - 102.3 °C. ^{1}H-NMR (DMSO-d_6): δ = 1.50-1.73 (m, 10H, cyclohexane-H2,3,5,6 and piperidine-H3$_{ax}$,5$_{ax}$), 1.82 - 2.05 (m, 3H, piperidine-H3$_{eq}$,5$_{eq}$ and cyclohexane-H4), 2.70 - 2.85 (m, 1H, cyclohexane-H1), 3.11 (m, 2H, piperidine- 2$_{ax}$,6$_{ax}$), 4.42 (dd, $^2J_{FH}$ = 48 Hz, $^3J_{HH}$ = 7.0 Hz, 2H, CH_2F), 4.51 (m, 1H, piperidine-H4), 4.68 (m, 2H, piperidine-H2$_{eq}$,H6$_{eq}$), 6.45 (s, 1H, fumaric acid), 6.81 (s, 1H, imidazole-H4), 7.58 (s, 1H, imidazole-H2). ^{19}F-NMR : δ = 223.9 (dt, $^2J_{HF}$ = 47 Hz, $^3J_{HF}$ = 18 Hz, CH_2F). HRMS : 382.2087 (± 0.0008) (calculated 382.2084). Elemental analysis : found : (56.60% C, 7.21% H, 14.58% N), calculated : (56.52% C, 7.12% H, 14.65% N)

Radiosynthesis of [^{18}F]VUF 5000.

[^{18}F]Fluoride was produced by the ^{18}O(p,n)^{18}F nuclear reaction in a 1.7 ml titanium target (Nuclear Interface). Irradiation for 5 minutes with 20 μA 18 MeV protons, generated by a Philips AVF30 cyclotron, yielded around 175 mCi. The [^{18}F]fluoride was trapped on an ion exchange column containing 50 mg Biorad AG1-X8 (200-400 mesh) in the CO_3^{2-} form. After washing with of 1 ml of CH_3CN the [^{18}F]fluoride was eluted from the ion exchange column into the reaction vessel with 1 ml of a solution of 13 mg (35 μmol) of kryptofix[2.2.2] and 2.0 mg (15 μmol) of K_2CO_3 in H_2O/CH_3CN 1/9 (v/v). This solution was evaporated to dryness at 100 °C and 600 mbar. Then 1 ml of CH_3CN was added, and the solution was again evaporated under the same conditions. This was repeated twice to remove all the water from the reaction vessel. 3.8 mg (10 μmol) of **6** in 500 μl of CH_3CN was added to the residue. This mixture was sonicated for 15 minutes followed by heating for 30 minutes at 100 °C. After cooling the reaction mixture a sample for TLC (ethyl acetate / *n*-hexane 1/3, R_f of [^{18}F]**7** = 0.65) was taken and 500 μl of CH_3CN, 10 mg (60 μmol) of KI and 150 μl (700 μmol) of chlorotrimethylsilane were added. This reaction mixture was stirred for 10 minutes, under a stream of argon. The outlet of the reaction vessel was connected to a trap at -100 °C. Then 2 ml of methanol was added and stirring was continued, after 5 minutes 150 μl of concentrated HCl was added and the solution was evaporated to dryness at 100 °C and 400 mbar. Then 1 ml of CH_3CN was added and the solution was again evaporated to dryness at 100 °C

and 400 mbar. 500 µl of CH_3CN was added, a sample was taken for TLC (ethyl acetate / methanol / triethylamine 5/5/1, R_f [^{18}F]**8** = 0.30) and 3.6 mg (15 µmol) of 1,1'-thiocarbonyldi-2(1H)-pyridone and 90 µl of diisopropylethylamine were added. The reaction mixture was heated at 100 °C for 2 minutes. After cooling the reaction mixture to room temperature a sample for TLC (ethyl acetate / methanol / triethylamine 5/5/1 R_f [^{18}F]**9** = 0.90) was taken and 3.2 mg (10 µmol) of 4-[1(H)-imidazol-4-yl]piperidine was added. The reaction mixture was heated at 100 °C for 15 minutes and cooled to room temperature. A sample for TLC (ethyl acetate / methanol 1/5 R_f [^{18}F]**3** = 0.30) was taken and 30 ml of water was added. This mixture was eluted over a HPLC guard column which was loaded with 300 mg RP-18 silica to trap the product. This column was subsequently mounted on a Rheodyne HPLC injector instead of the injection loop. The mixture was injected onto a Merck Lichrospher™ RP Select B 125×4 mm (5µm) HPLC column and eluted with 0.08 M sodium phosphate buffer (pH 4) / methanol 65/35 (figure 1). Fractions containing [^{18}F]VUF 5000 were collected and evaporated to dryness at 100 °C and 400 mbar. The residue was dissolved in sodium phosphate buffer (0.2 M, pH = 7.2) at a concentration of 1 mCi/ml, filtered over a .45 µm filter and analyzed by HPLC using a Merck Lichrospher™ RP Select B 125×4 mm (5µm) HPLC column with 0.08 M sodium phosphate buffer (pH 4) / methanol 65/35 as eluent (figure 1).

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