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Radiosynthesis of (±)-(2-((4-(2-[^{18}F]fluoro-ethoxy)phenyl)bis(4-methoxy-phenyl) methoxy)ethylpiperidine-3-carboxylic acid: a potential GAT-3 PET ligand to study GABAergic neuro-transmission *in vivo*

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Summary

A dysfunction of GABAergic neurotransmission is related to diseases such as epilepsy, Huntington-disease and Parkinson-syndrome. A new ^{18}F-fluorine labelled GABA transporter ligand for the GABA-transporter subtype GAT-3 was developed which may allow the *in vivo* visualisation of GABAergic neurotransmission. The precursors ethyl (2-(4-hydroxyphenyl)bis (4-methoxyphenyl)-methoxy)ethyl)-piperidine-3-carboxylate and ethyl(2-((4-(2-tosylethoxy)phenyl)-bis(4-methoxyphenyl)-methoxy) ethyl)piperidine-3-carboxylate were synthesised and labelled by the use of 2-[^{18}F]fluoroethyltosylate or [^{18}F]fluoride. Subsequent cleavage of the ester moiety gave the final product (±)-(2-((4-(2-[^{18}F]fluoroethoxy)phenyl)bis(4-methoxy-phenyl)methoxy)ethyl)-piperidine-3-carboxylic acid in a decay corrected yield of 33–36%. Preliminary biodistribution kinetics were determined with BALB/c mice *ex vivo* for brain, liver, kidney, spleen, blood and bone. (2-((4-(2-[^{18}F]fluoroethoxy)-phenyl)bis(4-methoxyphenyl)methoxy)-ethyl) piperidine-3-carboxylic acid showed a maximum brain uptake after 1 h p.i. of about 0.3% ID/g. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: GABA; GABA transporter ligand; [^{18}F]fluoroethyl-derivatives

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Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system.[1] For most neurotransmitters including GABA, neurotransmission is terminated by rapid uptake of neurotransmitters via specific, high-affinity transporters located in the presynaptic terminal and/or surrounding glial cells.[2] Efforts have been made to examine the inhibitory neurotransmission by directly radiolabelling GABA with carbon-11.[3] These attempts were unsuccessful because GABA does not permeate the blood–brain barrier. GABA derivatives such as [^{11}C] methoxy-norchloroprogabidic acid[4] were not able to visualize GABA receptors *in vivo*. Furthermore, the *in vivo* evaluation of the tritiated $GABA_A$/Benzodiazepine receptor ligands [methyl-^{3}H]L655,708 and [ethyl-^{3}H]RY80 as putative PET ligands showed that both were not suitable candidates for further development.[5] Alternatively, a promising target for PET might be found in the GABA transporter ligands.

The approach to utilise neurotransmitter transporters located at the presynaptic receptor membrane has been demonstrated for other CNS receptor systems. For the dopaminergic system in particular, there are labelled dopamine transporter (DAT) ligands available. Individual PET tracers have been in use for quantifying the presynaptic synthesis of dopamine as well as for the binding of dopamine and dopamine derivatives at D_2-like receptors which are located at the postsynaptic membrane. Still, DAT-ligands provide valuable additional tools for quantifying dopaminergic function *in vivo*.[6–11]

GABA is synthesised *in vivo* via enzymatic decarboxylation of glutamate, stored in vesicles and finally released into the synaptic cleft where it interacts with $GABA_A$ and $GABA_B$ receptors. Its action is terminated by high-affinity transporters. GABA transporters (GAT) can be inhibited by pharmacologic agents, increasing the level of neurotransmitter in the synapse and thus enhancing GABAergic transmission. Up to now five subtypes of GABA transporter have been reported, three GABA transporters, GAT-1 to GAT-3, a GABA/betain transporter, BGT-1 and the structurally diverse vesicular GABA transporter, vGAT. The isoforms have been sequenced and cloned.[20, 21]

Several potential radioligands for GABA uptake sites have been synthesised,[6–9] most of them derivatives of nipecotic acid. However, pharmacological evaluation of these ligands was either not included or restricted to the characterisation of GABA uptake inhibition which not

necessarily is synonymous with the ligands' affinity to GABA transporters. 1-[4-[4-[^{11}C]methoxyphenyl)-4-phenyl-but-3-enyl]-piperidine-3-carboxylic acid[9] and l-[3,3-bis-(4-[^{18}F]trifluoromethylphenyl)-propyl]piperidine-3-carboxylic acid[6] are very similar in structure to 1-(4,4-diphenyl-but-3-enyl)piperidine-3-carboxylic acid (SK&F 89976A) and 1-[3,3-bis-(4-trifluoromethylphenyl)propyl]1,2,5,6-tetrahydro-pyridine-3-carboxylic acid (CI-966) which were evaluated for their subtype specificity.[18] Both are potent GAT-1 inhibitors (IC_{50} values for inhibition of [^{3}H]GABA uptake of 0.13 and 0.26 μM) compared to GAT-3 inhibition (1990 and 333 μM, respectively).[18] Until now, no information is available about the analogous binding characteristics of the ^{11}C- and ^{18}F-labelled derivatives of SK&F 89976A[9] and CI-966.[6] However, ^{11}C-methylation of SK&F 89976A[9] or the exchange of the guvacine structure element with nipecotic acid in the case of CI-966[6] for example, not necessarily influence the subtype specificity of the ligands. Thus, the PET ligands proposed so far for GABA uptake sites bind predominantly to the human GABA transporter subtype 1.

The regional distribution of the GABA transporter subtypes varies significantly.[15,16,17,20] GAT-1 and GAT-2 are widespread in the human brain whereas GAT-3 is more concentrated in the hypothalamus and midline thalamus, making the latter sub-type an interesting target for PET. In order to develop a regionally specific PET ligand, the GAT-3 system thus seems to be more promising than GAT-1 or GAT-2.

A variety of lipophilic transporter ligands for all subtypes of GABA-transporters have been synthesised. Starting from one of these studies, Dhar and co-workers synthesized triaryl-substituted nipecotic acid derivatives with high affinity and selectivity for the GAT-3 subtype.[18] The GAT-3 ligand 1-[2,2,2-tris-(4-methoxy-phenyl)propyl]piperidine-3-carboxylic acid (SNAP 5114) with an IC_{50} value of 10 μM for the inhibition of [^{3}H]GABA uptake[18] has the highest potency of all evaluated compounds and was therefore selected as a lead compound for the development of ^{18}F- and eventually ^{11}C-labelled derivatives for potential applications using PET. The ^{18}F-fluorine labelled derivative (±)-(2-((4-(2-[^{18}F]fluoroethoxy)phenyl)-bis(4-methoxyphenyl)methoxy) ethyl)piperidin-3-carboxylic acid ([^{18}F]fluoroethyl-SNAP-5114) (**1**) (Figure 1) was considered to be a candidate for measuring the dynamic changes of GABA concentration in the synaptic cleft.

If the labelled GABA transporter ligand reached the transporter and the binding was reversible, stimulation of GABA release into the synaptic cleft would lead to a dissociation of the GAT-3/GAT-3-ligand

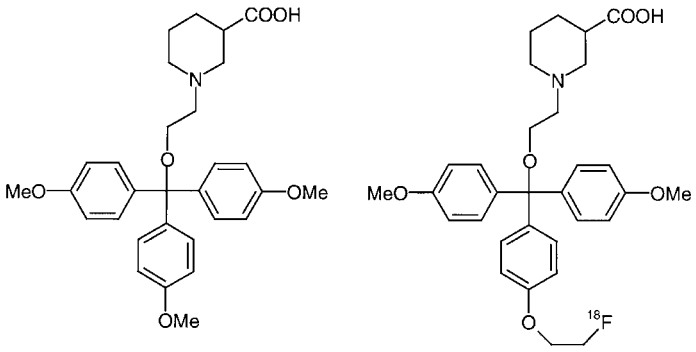


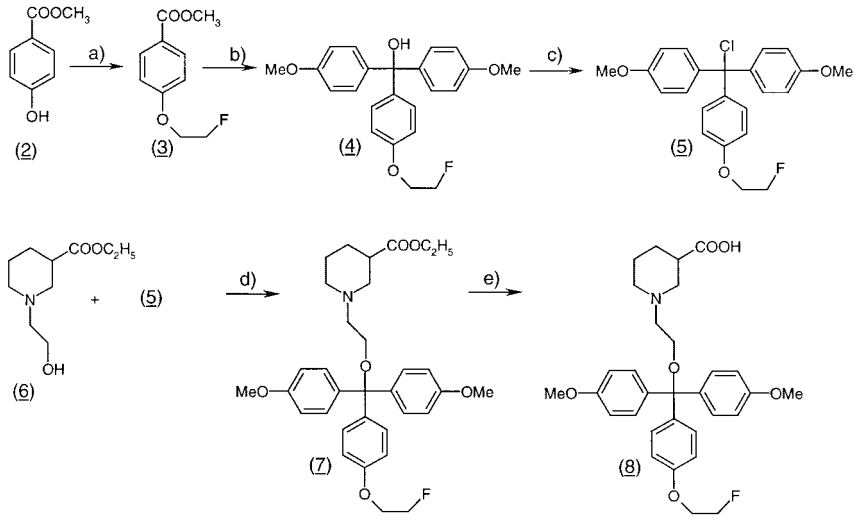
Figure 1. Structures of the original compound SNAP-5114 and the fluoroethylated derivative [^{18}F]fluoroethyl-SNAP-5114 (1)

complex. This process could possibly be visualized with PET as an indirect measure of the GABA concentration in the synaptic cleft, being useful to indicate illness-related changes in GABAergic neurotransmission.

Results and discussions

It was the strategy of these syntheses to label the compound SNAP-5114 with [^{18}F]fluorine in a simple way avoiding too many reaction steps. Consequently, the two precursors ethyl(2-((4-(2-tosylethoxy)phenyl)-bis(4-methoxyphenyl)methoxy)-ethyl) piperidine-3-carboxylate (**15**) and (2-(4-hydroxyphenyl)bis(4-methoxyphenyl)-methoxy)ethyl)piperidine-3-carboxylate (**16**) were synthesized. (**16**) comprises a phenolic hydroxy function and could – with regard to the original structure of the GAT-3 ligand SNAP 5114 – be labelled with 2-[^{18}F]fluoroethyltosylate[21] in two steps. Compound (**15**) could be labelled directly with [^{18}F]fluoride with subsequent cleavage of the nipecotic ester moiety. The precursor synthesis of (**16**) was published recently.[22] The synthesis of the standard compound (**8**) was performed in a similar manner (Scheme 1).

The synthesis of (**15**) could be achieved by carrying out the following reaction steps: methyl 4-hydroxybenzoate (**2**) was first reacted with 2-bromoethanol and subsequently with 3,4-dihydro-2H pyran to obtain methyl 4-(2-(2-oxanyloxy)ethoxy) benzoate (***10***). This compound was then coupled with 4-methoxyphenyl magnesium bromide and reacted

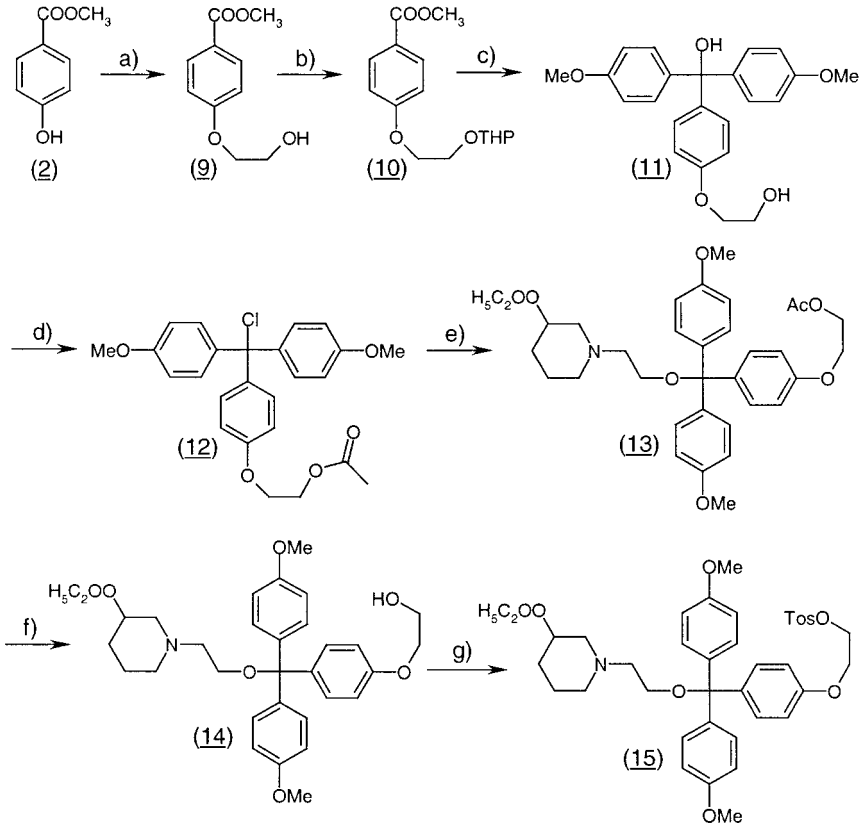


Scheme 1. (a) methyl 4-hydroxybenzoate, 1-bromo-2-fluoroethane, MeOH; (b) 4-methoxyphenyl magnesium bromide, THF; (c) acetylchloride; (d) (6), (5), DMAP, THF; (e) LiOH (1 N), methanol

with acetyl chloride to yield 2-(4-(bis(4-methoxyphenyl)chloro-methyl)-phenoxy)ethyl acetate (**12**). Ethyl (2-hydroxyethyl)piperidine-3-carboxylate (**6**) was reacted with (**12**) in THF. The acetyl group was removed with sodium methanolate in methanol to yield (**14**). Finally, the hydroxy moiety was tosylated with tosyl chloride to yield compound (**15**) in an overall yield of 2.6% (Scheme 2).

The final radiolabelling with 2-[^{18}F]fluoroethyltosylate was performed in DMSO as a solvent at a temperature of 80°C within 5 min yielding the desired product ethyl (2-((4-(2-[^{18}F]fluoroethoxy)phenyl)bis(4-methoxyphenyl)methoxy)ethyl)piperidine-3-carboxylate (**17**) in a decay corrected radiochemical yield of 37–40% (Scheme 3). Purification of the crude product was accomplished by HPLC. Subsequent quantitative cleavage of the remaining ethyl ester moiety within 5 min gave the final product [^{18}F]fluoroethyl-SNAP-5114 (**1**) in 33–36% radiochemical yield (decay corrected). The total preparation time till a solution for the biodistribution studies was obtained was approximately 50 min.

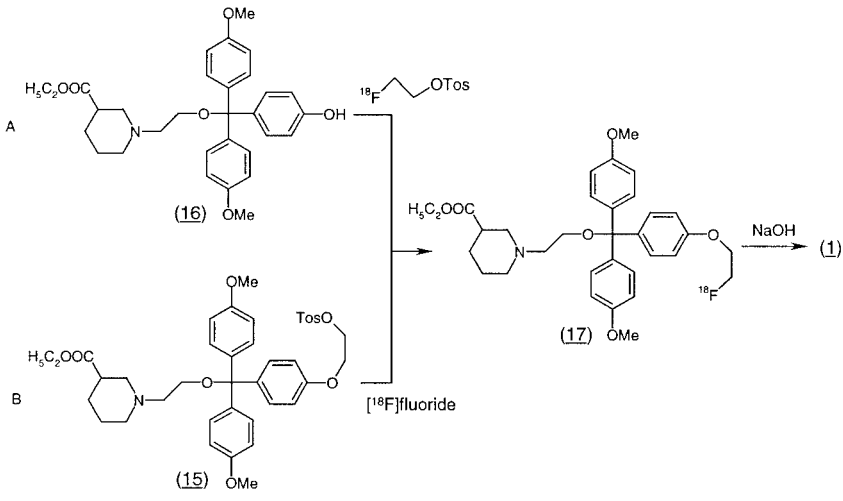
Alternatively, a direct labelling approach was performed. The radiolabelling of (**15**) with [^{18}F]fluoride was carried out in acetonitrile at a temperature of 80°C within 40 min to yield the ^{18}F-fluorinated product in 21% radiochemical yield (decay corrected) (Scheme 3). Further work-up was accomplished as described above. The total



Scheme 2. (a) 2-bromoethanol, (b) 3,4-dihydro-2H-pyran, (c) 4-methoxyphenyl magnesium bromide, H_2SO_4, (d) acetyl chloride, (e) ethyl (2-hydroxyethyl)piperidine-3-carboxylate, (f) sodium methanolate, methanol, (g) 4-toluenesulfonyl chloride

preparation time was $\geqslant$70 min and the total decay corrected radiochemical yield of (**1**) was 19%.

The *in vitro* stability of (**1**) was measured 24 h after the radiosynthesis with radio TLC and showed 95% of the radioligand to be intact. The maximum brain uptake of (±)-(2-((4-(2-[^{18}F]fluoroethoxy)phenyl)bis(4-methoxyphenyl)methoxy)ethyl)-piperidin-3-carboxylic acid in BALB/c mice was 0.3% ID/g and was reached at 1 h p.i. Excretion was primarily via the urine (20% ID/g after 60 min). The kidneys showed an increasing uptake of 3–5% ID/g radioactivity within 60 min. The liver uptake was $<$5% after 60 min. These results suggest that further *in vitro* comparative studies of the cold standard compound (**8**) against the original tritiated GAT-3 ligand [^{3}H]SNAP-5114 are warranted. For that



Scheme 3. Radioactive labelling of (16) with 2-[^{18}F]fluoroethyltosylate (A) and of (15) with [^{18}F]fluoride (B) to yield (17) followed by subsequent ester hydrolysis to (1)

purpose the tritium labelled original SNAP-5114 was synthesised.[20] The *in vitro* evaluation of fluoroethylated derivative of SNAP-5114 (**8**) is under investigation.

Experimental

1-Bromo-2-fluoroethane (Lancaster), ethyl piperidine-3-carboxylate (Fluka), 4-bromo anisole (Fluka), methyl 4-hydroxy benzoate (Fluka), acetyl chloride (Merck), dimethylaminopyridine (DMAP) (Merck) and Kryptofix 2.2.2. (Merck) were purchased from the companies shown in brackets. All other reagents were of analytical grade and were used without further purification. Solid-phase columns were purchased from Merck (Lichrolut EN). Analytical thin layer chromatography (TLC) were performed using plates from Merck (Silicagel 60 F_{254}). High-performance liquid chromatography (HPLC) was performed with a Sycam S1100 system, UV detection was obtained using a UV detector Sycam S3200. NMR spectra were recorded using a Bruker 200-MHz-FT–NMR spectrometer AC 200. Chemical shifts are quoted in δ (ppm) downfield from tetramethylsilane (TMS) as an internal standard. IR spectra were recorded using a Perkin Elmer FT–IR spectrometer 1760X with KBr presslings. MS spectra were obtained on a Finnigan MAT90

spectrometer. Detection of radioactivity was performed using an instant imager (Packard Canberra) for radio-TLC and detection of radio-HPLC was performed using a NaI-radiodetector (Canberra Packard). The HPLC-system was used isocratically for the purification of the ester protected ^{18}F-fluorinated tracer. [^{18}F]Fluoride as produced via the ^{18}O(p,n)^{18}F reaction was purchased. Purification of the labelling precursor 2-[^{18}F]fluoroethyltosylate was accomplished using an HPLC column (Lichrosphere RP18-EC5, 250 × 10 mm). Purification of the labelled compound ethyl (2-((4-(2-[^{18}F]fluoroethoxy)phenyl)bis(4-methoxyphenyl)methoxy)ethyl)piperidine-3-carboxylate was achieved using a HPLC column (Lichrosphere PRP 1-10, 250 × 4.6 mm).

The preliminary animal experiments were carried out in compliance with the German legal regulation of animal experimentation. About 1.5 MBq each of (**1**) were injected intraveneously in the tail vein of eight BALB/c mice and the biodistribution of radioactivity was measured *ex vivo* for brain, liver, kidney, muscle, suprarenal body, colon, small intestine, stomach and heart at 5, 10, 25 and 60 min p.i. Detection of the radioactivity in organ tissue was carried out using a NaI(TI)-detector.

*Ethyl(2-hydroxyethyl)piperidine-3-carboxylate (**6**)*

To a solution of ethyl piperidine-3-carboxylate (2.48 ml, 16 mmol) in 1,4-dioxane (20 ml) were subsequently added 2-bromo ethanol (1.13 g, 16 mmol), potassium carbonate (6.63 g, 48 mmol) and a small amount of sodium iodide. The mixture was refluxed overnight, concentrated under reduced pressure, extracted with ethyl acetate (30 ml), filtered and dried. The organic layer was evaporated under reduced pressure. The product (**6**) was obtained as a yellow oil after separation using column chromatography (2:1, ethylacetate/hexane) (2.7 g, 13.3 mmol, 83%) according to a published procedure.[4]

^{1}H-NMR (200 MHz, $CDCl_3$): (3H, m, 1.2 ppm), (8H, m, 1.5–2.6 ppm), (2H, t, 2.6 ppm), (IH, m, 2.7–2.76 ppm), (lH, m, 2.85–2.9 ppm), (2H, t, 3.6 ppm), (2H, q, 4.1 ppm).

Elemental Analysis ($C_{10}H_{19}NO_3$) C, H, N.

*Methyl 4-(2-fluoroethoxy)benzoate (**3**)*

To a sodium methanolate solution (sodium: 0.46 g (0.02 mol), methanol (30 ml)) methyl 4-hydroxybenzoate (**2**) (3 g, 0.02 mol) and l-bromo-2-fluoroethane (2.54 g, 0.02 mol) were added. The mixture was refluxed

under exclusion of moisture for 5 h. The reaction mixture was passed into water and the precipitating product was collected, washed with water and dried under vacuum. The yield of methyl 4-(2-fluoroethoxy) benzoate (**3**) was 69% (2.7 g, 0.013 mol).

^{1}H-NMR ($CDCl_3$): (3H, s, 4.0 ppm), (1H, m, 4.1 ppm), (1H, m, 4.3 ppm), (1H, m, 4.6 ppm), (1H, m, 4.9 ppm), (4H, m, 6.9–8 ppm).

IR: *v*(CO): 1210, *v*(C–F): 1180–1325, *v*(CO): 1730.

*(4-(2-Fluoroethoxy)phenyl)bis(4-methoxyphenyl)methanol (**4**)*

To a Grignard solution of 4-methoxyphenyl magnesium bromide (0.024 mol) in THF a solution of methyl 4-(2-fluoroethoxy)benzoate (**3**) (2.3 g, 0.012 mol) in THF was slowly added under stirring. The mixture was kept at 4°C over night, hydrolysed with saturated ammonia chloride solution, extracted with ether and the organic layer was dried over sodium sulphate. The solvent was evaporated under reduced pressure. The crude product was recrystallised from acetic acid/water to yield (4-(2-fluoroethoxy)phenyl)bis(4-methoxyphenyl)methanol(**4**) (4.2 g, 0.01 mol, 95%).

^{1}H-NMR ($CDCl_3$): (6H, s, 3.8 ppm), (1H, m, 4.1 ppm), (1H, m, 4.3 ppm), (1H, m, 4.6 ppm), (1H, m, 4.9 ppm), (12H, m, 6.5–7.5 ppm).

IR: *v*(C_t–OH): 1000–1200, *v*(C–F): 1180–1325, *v*(Ph–O–CH_3): 2830.

*(4-(2-Fluoroethoxy)phenyl)bis(4-methoxyphenyl)chloride (**5**)*

To (4-(2-fluoroethoxy)phenyl)bis(4-methoxyphenyl)methanol (**4**) (4.2 g, 0.01 mol) was added acetyl chloride (50 ml) and the mixture was refluxed for 2 h. After removing the excess of acetyl chloride under reduced pressure, product (**5**) was quantitatively obtained as a red solid.

^{1}H-NMR ($CDCl_3$): (6H, s, 3.8 ppm), (1H, m, 4.1 ppm), (1H, m, 4.3 ppm), (1H, m, 4.6 ppm), (1H, m, 4.9 ppm), (12H, m, 6.5–7.5 ppm).

IR: *v*(C–Cl): 560–630, *v*(C–F): 1180–1325, *v*(Ph–O–CH_3): 2830.

*Ethyl(2-((4-(2-fluoroethoxy)phenyl)bis(4-methoxyphenyl)methoxy) ethyl)-piperidine-3-carboxylate (**7**)*

To a solution of ethyl (2-hydroxyethyl)piperidine-3-carboxylate (**6**) (1.63 g, 8.1 mmol) in dry THF (10 ml) were subsequently added triethylamine (2 ml, 18.6 mmol), 2-dimethylaminopyridine (DMAP) (98 mg) and (4-(2-fluoroethoxy)phenyl)bis(4-methoxyphenyl)chloride

(**5**) (3.7 g, 10.5 mmol) and stirred over night at room temperature. The solution was passed into a mixture of dichloromethane (50 ml) and water (10 ml). The organic layer was dried, filtered and evaporated. The crude reaction product was purified using flash-chromatography (ethylacetate/hexane/triethylamine 40/60/+0.1%) on silica gel and (**7**) was obtained as a yellow oil with a yield of 35%.

^{1}H-NMR ($CDCl_3$): (3H, t, 1.24 ppm), (5H, m, 1.45–2.0 ppm), (1H, t, 2.2 ppm), (1H, m, 2.5–2.55 ppm), (2H, t, 3.2 ppm), (6H, s, 3.7 ppm), (2H, q, 4.0 ppm), (1H, m, 4.1 ppm), (1H, m, 4.3 ppm), (1H, m, 4.6 ppm), (1H, m, 4.9 ppm), (12H, m, 7.3–7.9 ppm).

Elemental Analysis ($C_{33}H_{40}FNO_6$) C, H, N.

*(2-((4-(Fluoroethoxy)phenyl)bis(4-methoxyphenyl)methoxy)ethyl) piperidine-3-carboxylic acid (**8**)*

To a solution of ethyl (2-((4-(2-fluoroethoxy)phenyl)bis(4-methoxyphenyl)-methoxy)ethyl)piperidine-3-carboxylate (**7**) (0.61 g, 1.35 mmol) in ethanol (6 ml) LiOH (1N) (2 ml, 2.7 mmol) was added and stirred at room temperature for 18 h. The reaction mixture was adjusted to pH 6 using a sodium hydrogen phosphate solution (5%) and passed into mixture of dichloromethane/water (60 ml/10 ml). The organic layer was dried over sodium sulphate, filtered and separated from the solvent to yield (**8**) as a foamy solid (0.46 g, 0.9 mmol, 72%).

^{1}H-NMR ($CDCl_3$): (5H, m, 1.45–2.0 ppm), (1H, t, 2.2 ppm), (1H, m, 2.5–2.55 ppm), (2H, t, 3.2 ppm), (6H, s, 3.7 ppm), (1H, m, 4.1 ppm), (1H, m, 4.3 ppm), (1H, m, 4.6 ppm), (1H, m, 4.9 ppm), (12H, m, 7.3–7.9 ppm).

MS (FD): m/z (% relative intensity) 537.3 (100.0, $[M]^+$), 491,2 (6.63, $[M\text{-}C_2H_4F]^+$).

*Methyl 4-(2-hydroxyethoxy)benzoate (**9**)*

To a solution of methyl (4-hydroxy)benzoate sodium salt (3.8 g, 22.19 mmol prepared as described for (**3**)) in methanol (20 ml) were added 2-bromo ethanol (2.72 g, 22 mmol) and a small amount of sodium iodide. This mixture was stirred at 60°C for 18 h, the precipitating sodium bromide was removed by suction, the remaining solution was evaporated to dryness and the crude product was purified via flash-chromatography on silica gel (ethylacetate/*n*-hexane 1:1). The product

methyl 4-(2-hydroxyethoxy)benzoate (**9**) was obtained as a white solid (1.5 g, 7.6 mmol, 35%).

^{1}H-NMR ($CDCl_3$): (2H, t, 2.7 ppm), (2H, t, 3.3 ppm), (3H, s, 3.8 ppm), (4H, m, 6.2–8.0 ppm).

MS (FD): *m/z* (% relative intensity) 181.231 (100.0, [M + 1]).

*Methyl 4-(2-(2-oxanyloxy)ethoxy)benzoate (**10**)*

To a solution of (**9**) (1.5 g, 7.6 mmol) and 3,4-dihydro-2H pyrane (2.5 g, 30 mmol) in dichloromethane (ca 5 ml) few drops of iodo trimethylsilane were added and stirred at 25°C for 30 min (TLC control). The mixture was evaporated to dryness and purified via flash-chromatography on silica gel (ethylacetate/hexane, 70:30) to yield (**10**) (1.95 g, 6.9 mmol, 90%).

^{1}H-NMR ($CDCl_3$): (6H, m, 1.4–1.8 ppm), (2H, t, 2.7 ppm), (2H, t, 3.3 ppm), (1H, m, 3.5 ppm), (3H, s, 3.8 ppm), (1H, m, 3.8 ppm), (1H, m, 4.3 ppm), (4H, m, 6.2–8.0 ppm).

2-*(4-(bis(4-methoxyphenyl)hydroxymethyl)phenoxy)ethan-1-ol (**11**)*

To a Grignard solution of 4-methoxyphenyl magnesium bromide (0.014 mol) was added a solution of (**10**) (1.7 g, 6 mmol) in THF (10 ml) under vigorous stirring within 10 min. The solution was kept at 4°C over night and was hydrolysed with 1 N H_2SO_4, extracted with ether, dried over sodium sulphate and the organic layer was evaporated under reduced pressure. The crude product was recrystallised from acetic acid/water to yield the product (**11**) as red crystals (1.8 g, 4.9 mmol, 85%).

^{1}H-NMR ($CDCl_3$): (2H, t, 2.7 ppm), (2H, t, 3.3 ppm), (6H, s, 3.8 ppm), (12H, m, 6.5–7.5 ppm).

MS (FD): *m/z* (% relative intensity) 440.8 (100.0, [M +]), 442.5 (15.6, $[M + 2]^+$).

2-*(4-(bis(4-methoxyphenyl)chloromethyl)phenoxy)ethyl acetate (**12**)*

2-(4-(Hydroxy bis(4-methoxyphenyl)methyl)phenyl)ethanol (**11**) (1.8 g, 4.9 mmol) were added to acetyl chloride (30 ml) and refluxed for 2 h. The acetylchloride was removed under reduced pressure and the crude product was recrystallised from acetic acid/water (2.0 g, 4.75 mmol, 98%).

^{1}H-NMR ($CDCl_3$): (3H, s, 2.1), (2H, t, 2.7 ppm), (2H, t, 3.3 ppm), (6H, s, 3.8 ppm), (12H, m, 6.3–7.3 ppm).

IR ν(1/cm): ν(C–Cl): 560–630, ν(CO): 1210, ν(CO): 1730, ν(Ph–O–CH_3): 2830.

*2-(4-(bis(4-methoxyphenyl)(2-(3-(ethoxycarbonyl)piperidyl)ethoxy)-methyl)-phenoxy)ethyl acetate (**13**)*

To a solution of ethyl (2-hydroxyethyl)piperidine-3-carboxylate (**6**) (0.8 g, 4 mmol) in dry THF (5 ml) were added subsequently triethylamine (1 ml, 9.3 mmol), 4-dimethyl aminopyridine (50 mg) and 2-(4-(bis(4-methoxyphenyl)chloromethyl)-phenoxy)ethyl acetate (**12**) (1.72 g, 4 mmol). The reaction mixture was stirred overnight at room temperature and passed into a dichloro methane/water (5:1) mixture (50 ml). The organic layer was dried over sodium sulphate, filtered, concentrated in vacuum and purified via flash-chromatography (ethylacetate/*n*-hexane/triethylamine, 1:1:0.1%) to yield the product (**13**) as a yellow oil (0.55 g, 0.88 mmol, 22%).

^{1}H-NMR ($CDCl_3$): (3H, t, 1.2 ppm), (11H, m, 1.3–3.0 ppm), (3H, s, 2.1), (2H, t, 2.6 ppm), (2H, t, 2.7 ppm), (2H, t, 3.1 ppm), (2H, t, 3.3 ppm), (6H, s, 3.7 ppm), (6H, s, 3.8 ppm), (2H, q, 4.0 ppm), (12H, m, 6.5–7.5 ppm).

Elemental Analysis ($C_{33}H_{43}NO_8$) C, H, N.

*Ethyl(2-((4-(2-hydroxyethoxy)phenyl)bis(4-methoxyphenyl)methoxy)-ethyl)piperidine-3-carboxylate (**14**)*

2-(4-(Bis(4-methoxyphenyl)(2-(3-(ethoxycarbonyl)piperidyl)ethoxy)methyl)-phenoxy)ethyl acetate (**13**) (0.4 g, 0.64 mmol) was dissolved in methanol and the pH value was adjusted to 8.5 with a 5% sodium methanolate solution in methanol (indicator: wet pH paper). This mixture was stirred overnight, water was added slowly, the precipitating solid was filtered off and dried in vacuum to yield ethyl (2-((4-(2-hydroxyethoxy)phenyl)bis(4-methoxyphenyl)methoxy)ethyl)piperidine-3-carboxylate (**14**) as a yellow solid (0.28 g, 0.5 mmol, 78%).

^{1}H-NMR ($CDCl_3$):(3H, t, 1.2 ppm), (11H, m, 1.3–3.0), (2H, t, 2.6 ppm), (2H, t, 2.7 ppm), (2H, t, 3.1 ppm) (6H, s, 3.7 ppm), (6H, s, 3.8), (2H, q, 4.0 ppm), (12H, m, 6.5–7.5 ppm).

IR ν(1/cm): ν(OH): 1010–1080, ν(OH): 3650.

*Ethyl(2-((4-(2-tosylethoxy)phenyl)bis(4-methoxyphenyl)methoxy)-ethyl)piperidine-3-carboxylate (**15**)*

To a solution of *p*-toluene sulphonyl chloride (95 mg, 0.5 mmol) and ethyl (2-((4-(2-hydroxyethoxy)phenyl)bis(4-methoxyphenyl)methoxy)ethyl)piperidine-3-carboxylate (**14**) (0.2 g, 0.36 mmol) in chloroform at 0°C, pyridine (79 mg, 1 mmol) was added under stirring. The mixture was stirred for 1 h and concentrated in vacuum. The crude product was purified via chromatography (silica gel Si 60) (ethyl acetate/*n*-hexane/triethylamine, 30/70/0.1%) to yield the product (**15**) as a yellow oil (0.15 g, 0.2 mmol, 58%).

^{1}H-NMR ($CDCl_3$): (4H, m, 7.3–7.7 ppm), (12H, m, 6.8–7.3 ppm), (2H, t, 3.8 ppm), (3H, s, 2.4 ppm), (2H, t, 2.2 ppm), (3H, t, 1.1–1.3 ppm), (11H, m, 1.4–2.7 ppm), (4H, dt, 3–3.2 ppm), (6H, s, 3.7 ppm), (2H, q, 4.1 ppm).

MS (FD): m/z (% relative intensity) 545.7 (100.0, $[M^+–C_7H_7SO_3]^+$), 717.8 (2.0 $[M+l]^+$).

Elemental Analysis ($C_{40}H_{47}NO_9S$) C, H, N.

2-[^{18}F]fluoroethyltosylate

No-carrier-added (NCA) aqueous [^{18}F]fluoride (540–1100 MBq) prepared by the $^{18}O(p,n)^{18}F$ nuclear reaction on an enriched water (95%) target was added to a solution of K_2CO_3 (15 µl (1 N))/Kryptofix 2.2.2. (10–15 mg) in CH_3CN (800 µl). The water was removed using a stream of nitrogen at 80°C and coevaporated to dryness with CH_3CN (2×1 ml).

To the dried Kryptofix 2.2.2./[^{18}F]fluoride (380–750 MBq) complex in acetonitrile (1 ml) ethyleneglycol-1,2-ditosylate (8–10 mg, 20–25 µmol) was added and heated under stirring in a sealed vial for 3 min. Purification of the crude product was achieved using HPLC (acetonitrile/water, 50:50, flow rate: 5 ml/mm t_r: 8 min). After diluting the HPLC fraction containing the 2-[^{18}F]fluoroethyltosylate with water, the product was loaded on a 18C-Sepac column, dried with nitrogen gas and eluated with 1 ml of tempered (40–50°C) DMF to yield a product activity of 300–600 MBq 2-[^{18}F]fluoroethyltosylate.

*Ethyl(2-((4-(2-[^{18}F]fluoroethoxy)phenyl)bis(4-methoxyphenyl)methoxy)-ethyl)piperidine-3-carboxylate (**17**) via 2-[^{18}F]fluoroethyltosylate*

To ethyl (2-(4-hydroxyphenyl)bis(4-methoxyphenyl)methoxy)ethyl)piperidine-3-carboxylate (**16**) (8 mg, 0.0154 mol) dissolved in DMF

(0.5 ml), a sodium hydroxide solution (5 N, 2.5 µl) was added and tempered for at least 5 min at 80°C. A solution of 2-[^{18}F]fluoroethyltosylate (300–600 MBq) in DMF (300–500 µl) was added and stirred in a sealed reaction vessel at 80°C for 5 min. The product was purified with HPLC (methanol/water, 80:20 + 0.1% triethylamine, flow rate: 4 ml/min, t_r: 12.3 min). After diluting the HPLC fraction containing the product with water, it was loaded on a solid-phase column (LiChrolut EN, Merck), dried with nitrogen and eluated with 1 ml methanol to yield 200–450 MBq of the product (**17**). HPLC analysis showed a radiochemical purity of > 99%.

*(2-((4-([^{18}F]fluoroethoxy)phenyl)bis(4-methoxyphenyl)methoxy)ethyl)-piperidine-3-carboxylic acid (**1**)*

To the methanolic solution containing 200–450 MBq (**17**), a NaOH solution (1.25 N, 200 µl) was added and stirred at 80°C for 5 min. The solution was then diluted with a five fold excess of water and passed over a solid-phase column (Li-Chrolut EN, Merck), dried with nitrogen, eluated with 1 ml ethanol and diluted with physiological saline solution (10 ml) (180–400 MBq). HPLC analysis (methanol/water, 80 : 20 + 0.1% triethylamine, flow rate: 4 ml/min, t_r: 4.1 min) showed that the radiochemical purity was > 99%. The specific activity of (**1**) was between 0.3 and 0.5 Ci/µmol as determined via a UV-calibration curve.

*Ethyl(2-((4-(2-[^{18}F]fluoroethoxy)phenyl)bis(4-methoxyphenyl)methoxy)-ethyl)piperidine-3-carboxylate (**17**) via [^{18}F]fluoride and ester cleavage to (**1**)*

No-carrier-added (NCA) aqueous [^{18}F]fluoride (280–1100 MBq) prepared by the $^{18}O(p,n)^{18}F$ nuclear reaction on an enriched water (95%) target was added to a solution of K_2CO_3 (15 µl (1 N))/Kryptofix 2.2.2. (10–15 mg) in CH_3CN (800 µl). The water was removed using a stream of nitrogen at 80°C and coevaporated to dryness with CH_3CN (2 × 1 ml).

To the dried Kryptofix 2.2.2/[^{18}F]fluoride complex (200–800 MBq) in acetonitrile (0.5 ml) (**15**) (15 mg, 0.02 mmol) in acetonitrile (0.5 ml) was added and heated at 80°C for 40 min. The product was purified with HPLC (methanol/water, 80:20 + 0.1% triethylamine, flow rate: 4 ml/mm, t_r: 12.3 min). After diluting the HPLC fraction containing the product with water, it was loaded on a solid-phase column (Li-Chrolut

EN, Merck), dried with nitrogen and eluated with 1 ml methanol to yield 60–240 MBq of product. HPLC analysis indicated a radiochemical purity of >99%. Ester hydrolysis of (**17**) was achieved as described above to yield (**1**) (54–215 MBq) in a decay corrected yield of 19% with a radiochemical purity >99% (HPLC). The specific activity of (**1**) was determined via a UV-calibration curve and found to be between 0.3 and 0.5 Ci/μmol.

Conclusion

The radiosynthesis of a potential GAT-3-PET radioligand (±)-(2-((4-([^{18}F]fluoroethoxy)phenyl)bis(4-methoxyphenyl)methoxy)ethyl)piperidine-3-carboxylic acid (**1**) for the human GABA transporter subtype GAT-3 was carried out in an overall decay corrected radiochemical yield of 70% in the case of 2-[^{18}F]fluoroethyltosylate as the labelling precursor. The direct labelling approach has been proven to be less efficient for two reasons. Firstly, the overall yield of the precursor synthesis of (**15**) was only 2.6% in comparison to 27% in the case of precursor synthesis (**16**) and secondly, the labelling with [^{18}F]fluoride could be achieved with 32% radiochemical yield after 40 min only (decay corrected).

By labelling the precursor (**16**) with 2-[^{18}F]fluoroethyltosylate, (**1**) could be obtained in an injectable ethanolic physiological saline solution which was stable up to 95% for 24 h. Preliminary biodistribution studies indicated that the uptake of the radioactivity was 0.3% ID/g in mice brain which requires further comparisons of (**1**) with the tritiated original GAT-3 ligand [^{3}H]SNAP-5114.

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