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Efficient microwave-assisted direct radiosynthesis of [^{18}F]PR04.MZ and [^{18}F]LBT999: Selective dopamine transporter ligands for quantitative molecular imaging by means of PET

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ABSTRACT

PR04.MZ 8-(4-fluoro-but-2-ynyl)-3-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2-carboxylic acid methyl ester (**1**) and LBT999 8-((*E*)-4-fluoro-but-2-enyl)-3b-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2β-carboxylic acid methyl ester (**2**) are selective dopamine reuptake inhibitors, derived from cocaine. Compounds **1** and **2** were labelled with fluorine-18 at their terminally fluorinated N-substituents employing microwave enhanced direct nucleophilic fluorination. K[^{18}F]F$^-$ Kryptofix®222 cryptate, tetrabutyl ammonium [^{18}F]fluoride and caesium [^{18}F]fluoride were compared as fluoride sources under conventional and microwave enhanced conditions. Fluorination yields were remarkably increased under microwave irradiation for all three fluoride salts. Radiochemically pure (>98%) [^{18}F]PR04.MZ (0.95–1.09 GBq, 42–135 GBq/μmol) was obtained within 34–40 min starting from 3.0 GBq [^{18}F]fluoride ion in 32–36% non-decay-corrected overall yield using K[^{18}F]F$^-$Kryptofix®222 cryptate in MeCN.

1. Introduction

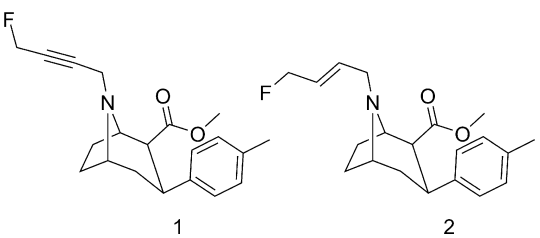
Dopaminergic pathologies are related to several neurodegenerative and psychiatric disorders.[1] As a consequence, molecular imaging of the dopaminergic signalling has attracted considerable attention.[2] In particular, the availability of the presynaptic dopamine transporter (DAT), a transmembrane protein belonging to the family of neurotransmitter–sodium symporters, can be used as an indicator on the integrity of the dopaminergic system.[2h]

Cocaine-analogue radioligands, labelled with short-lived positron emitters have shown to be the most promising lead for the development of selective probes for the DAT.[3] Although state-of-the-art in fluorine-18 labelling of tropane-analogue DAT-ligands is N-alkylation with an appropriate secondary labelling synthon, for example, 2-[^{18}F]fluoroethyl tosylate,[4a,b,5b] recent studies elucidated direct nucleophilic aliphatic fluorination routes with analogue compounds.[5a,c] Nevertheless, radio-labelling of cocaine-analogue radioligands with fluorine-18 was often complicated by (a) low N-alkylation yields, (b) poor accessibility of sp^2-carbon-bound fluorine, resulting in laborious reaction pathways, and (c) α/β-epimerisation at the C-2 position in the tropane skeleton,[5] due to the basic reaction conditions required for nucleophilic [^{18}F]F-labelling.

The present report is concerned with a reliable, microwave enhanced high yield direct fluorination method for the moderate potency tropane-analogue DAT-inhibitor LBT999 and the recently described PR04.MZ (Fig. 1).[6]

Initial direct aliphatic nucleophilic radiofluorination of PR04.MZ was performed under conventional conditions. Unfortunately, only low radiochemical yields of 19 ± 3% corrected for decay were obtained after 60–70 min radiosynthesis.[6c] Similar yields have also been reported in a simple one-step fluorine-18-labelling of LBT999, based on a chlorine-for-fluorine nucleophilic substitution.[4b] Reaction of potassium K222®cryptate [^{18}F]F$^-$ complex ([K⊂K222]$^+$ [^{18}F]F$^-$) with the chlorinated precursor at 165 °C in DMSO for 10 min followed by C-18 solid phase extraction and final semipreparative HPLC separation afforded [^{18}F]**2** in 10–16% non-decay-corrected radiochemical yield after ~65 min.

In order to increase the radiochemical yield, we considered the use of microwave enhanced conditions for nucleophilic aliphatic radiofluorination.[7] Most striking with regard to radiosynthesis employing



1 2

Figure 1. PR04.MZ (**1**) and LBT999 (**2**).

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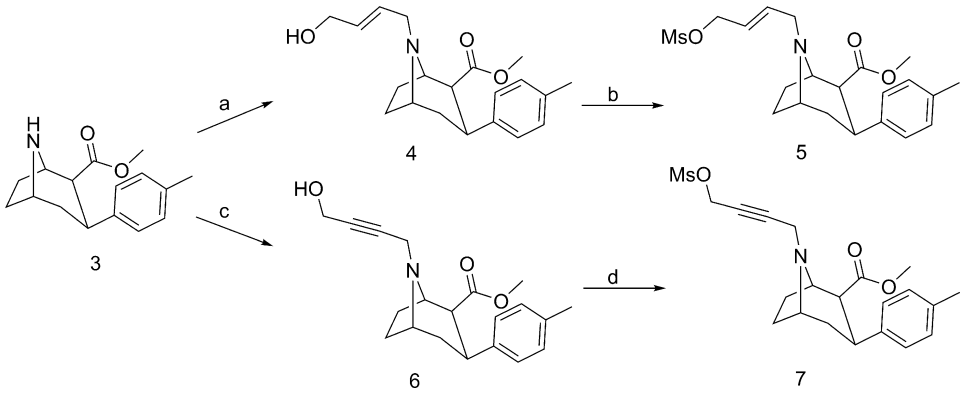


Scheme 1. Synthesis of precursors **5** and **7** for LBT999 and PR04.MZ, respectively. Reagents and conditions: (a) DiPEA, 4-hydroxybut-2-ene-1-yl bromide, MeCN; (b) Ms_2O, $Yb(OTf)_3$, CH_2Cl_2, 0 °C, 1 d; (c) DiPEA, MeCN, 4-hydroxybut-2-yne-1-yl chloride; (d) MsCl, NEt_3, CH_2Cl_2, 0 °C, 25 min.

short-lived isotopes, is the high and direct transfer of activation energy to the reactants, which mostly results in remarkably shortened reactions times.[7] Microwave-assisted synthesis has thus evolved as a powerful tool in the development of fast, chemo-selective synthetic methods.[7] Compared to conventional heating, energy transfer does not proceed gradually from the outside of the vessel to the inside. Instead, the microwave directly interacts with dipoles or charged species inside the reaction mixture. Thus, the direct energy transfer to a reaction center, a reactant or an intermediate is possible. Furthermore, the amount of activation energy, necessary for the conversion of a given molar amount of reactants can be provided within a shorter time, due to the high performance of focussed microwaves. The high energy influx facilitates different reaction pathways. In opposite to conventional conditions, thermodynamic reaction routes are preferred and kinetic side reactions are disfavoured.[7]

In general, commercially available microwave-reactors provide monomodal electromagnetic radiation ($\lambda = 12.2$ cm, $\nu = 2450$ MHz), representing the energy spectrum of molecular rotation. Therefore, it is unlikely that microwave irradiation results in cleavage of covalent bonds.[7]

2. Results and discussion

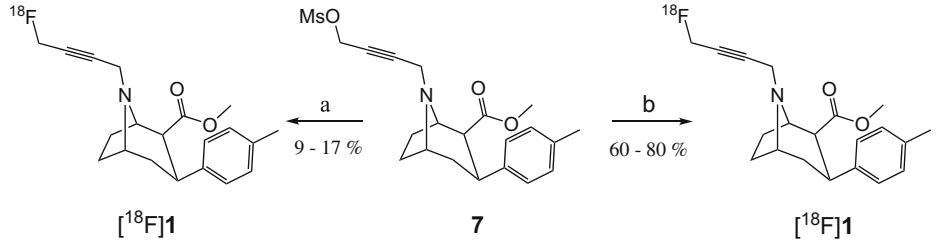
2.1. Chemistry

Reference compounds **1, 2** and nortropane **3** were synthesised from natural cocaine as published somewhere else.[8] The LBT999 labelling precursor 8-(4-methanesulfonyloxy-but-2-enyl)-3-*exo*-*p*-tolyl-8-aza-bicyclo[3.2.1] octane-2-*exo*-carboxylic acid methyl ester (**6**) was obtained from nortropane **4** via alkylation with 4-bromo-but-2-ene-1-ol in 74% yield, followed by Lewis-acid catalysed esterification with methanesulfonyl anhydride in 67% yield (Scheme 1). The labelling precursor for PR04.MZ, namely 8-(4-methanesulfonyloxy-but-2-ynyl)-3-*p*-tolyl-8-aza-icyclo[3.2.1]octane-2-carboxylic acid methyl ester (**7**), was synthesised according to Scheme 1. Compound **3** was reacted with 4-chlorobut-2-ynyl alcohol to afford **6** in 95% yield. Subsequent mesylation in dichloromethane using methanesulfonyl chloride and triethylamine afforded **7** in 94% yield.

2.2. Radiochemistry

[^{18}F]PR04.MZ ([^{18}F]**1**) was obtained by reacting **7** with [K⊂K222]$^+$[^{18}F]F$^-$, using a CEM Discover® focussed microwave reactor. The maximum pressure during the labelling reaction was 20 bar, the maximum temperature at the end of microwave irradiation was 175 °C (Scheme 2).

The optimal microwave performance was found to be 255 W (Fig. 2). Labelling precursor **7** was exposed to cyclotron-produced [^{18}F]fluoride ion as n.c.a. [K$^+$⊂K222][^{18}F]F$^-$, tetrabutyl ammonium [^{18}F]fluoride complex ([^{18}F]TBAF) or caesium [^{18}F]fluoride salt ([^{18}F]CsF), cf. Figure 4. Labelling was performed under constant microwave irradiation at 255 W for up to 45 s in a pressure-tight microwave reaction vial. The maximum pressure during the labelling reaction was 1.8×10^6 Pa. After heating for various time-periods (5 s, 10 s, 20 s, 45 s), an aliquot of the solution was analysed by TLC. A mixture of diethyl ether (Et_2O) and hexanes containing 10% of triethylamine (NEt_3) was used as mobile phase. The relative reaction yield was determined on Silica Gel 60 coated aluminium plates. The conversion to the desired fluorine-18 labelled compound was expressed as percentage of total radioactivity in the TLC lane. Volatile side products were neglected, because the head space of the vial did not contain any radioactivity. The radiochemical yield (RCY) after 45 s of heating was 60–80%. The reaction mixture was then taken into a automated syringe containing 300 μl of sterile water and directly transferred into the sample loop of a semipreparative HPLC system. Three to ten percent of the starting activity routinely remained in the microwave reaction vessel.



Scheme 2. [^{18}F]Fluorination of PR04.MZ precursor **7**; conventional conditions. Reagents and conditions: (a) 120 °C, MeCN, K_2CO_3, [K$^+$⊂K222][^{18}F]F$^-$, 3 min; (b) 175 °C, 18 bar, MeCN, K_2CO_3, [K$^+$⊂K222][^{18}F]F$^-$, 0.75 min.

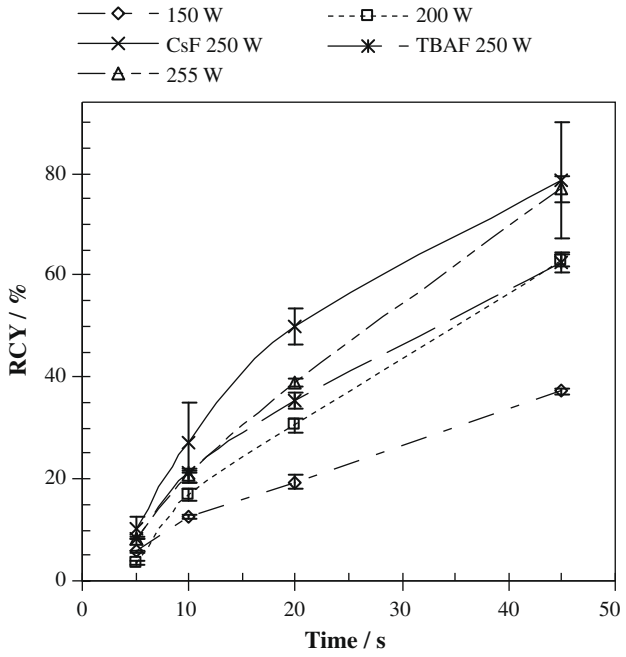


Figure 2. Labelling kinetics of [^{18}F]PR04.MZ in a CEM Discover® focussed microwave reactor (errors are ±1 S.D.: n = 6–9). Settings: T_{max} = 175 °C, pressure reaction, $p_{max} = 1.8 \times 10^6$ Pa, moderate stirring, no cooling.

Later on it was found, that the bulk of n.c.a. fluoride had been incorporated into the molecule, at this point. Prolonged heating resulted in thermal degradation to form a polar side product, cf. Figure 3. The latter eluted shortly after the HPLC inject-signal and displayed up to 20% of starting radioactivity. [^{18}F]**1** eluted after a reasonable purification time of t_R: 14.0 ± 0.5 min. The product fraction did not contain chemical impurities in concentrations above 2.4 μg/ml. In some runs, a non-significant by-product was formed which was assigned to the saponified acid of [^{18}F]PR04.MZ.

For concentration and isolation of the radiotracer, the product fraction was diluted with water and passed through a proton-loaded Merck-Lichrolut SCX strong cation exchanger. About 95% of the total radioactivity were retained on the SCX resin. Subsequently, the cartridge was washed with 3 ml of water. Sterile, isotonic PBS was used for elution of the product. A sterile filter was serially connected to the cartridge and the [^{18}F]PR04.MZ containing PBS was collected in a multi injection vial. Absorption of radioactivity on the filter pad was negligible. At this point an aliquot of the sterile filtered solution was guided into a screw-cap vial, containing 1 ml of 30% ammonium formate buffer in MeCN. This sample was used for quality control. Less than 5% of the trapped radioactivity remained on the SCX-cartridge. The radiotracer formulation was a colourless, clear solution with a pH-value of 7.3. Quality control by analytical HPLC proved, that [^{18}F]**1** was >98% radiochemically pure (**1**, t_R: 10 min). Chemical impurities were below 2.4 μg/ml. The non-radioactive labelling precursor was not detected in the tracer formulation. Specific radioactivity was 89 ± 45 GBq μmol^{-1} at end of synthesis (EOS). The radiotracer remained stable in this solution for more than 240 min.

[^{18}F]LBT999 ([^{18}F]**2**) was obtained under the same conditions in 27 ± 2% non-decay-corrected yield. The retention time for preparative purification was 8 min, and the radiochemical purity was comparable to the radiochemical purity of [^{18}F]**1**. Interestingly, the total radioactivity incorporation was remarkably lower in this case.

2.3. Comparison of fluoride sources

Initially, the microwave enhanced method was optimised using the well established potassium Kryptofix®cryptate [^{18}F]F$^-$ complex ([K$^+$⊂K222][^{18}F$^-$]). The reaction outcome, as percentage of total ^{18}F radioactivity that was converted to the desired product, was determined as a function of reaction time, precursor amount, microwave energy and temperature. During these investigations it was found, that the reaction between the [^{18}F]fluoride ion and precursors **5** and **7** proceeded very fast. Almost quantitative incorporation of the employed fluoride occurred within the first 45 s. Upon prolonged heating, the product degraded to a polar side product. The latter has also been found with thermal heating. Dollé et al. describe a similar effect within the radiosynthesis of LBT999 with prolonged heating leading to product degradation. [5a] In the present case, a maximum radioactivity incorporation of 80% was observed with [K$^+$⊂K222][^{18}F]F$^-$.

The reactions with [^{18}F]CsF and [^{18}F]TBAF were performed analogously to the conditions described above. For comparison, labelling was performed under thermal conditions with all three fluoride sources. Representative results are summarised in Figure 4.

Virtually, the use of [^{18}F]CsF gave the highest radiochemical yields. Unfortunately, [^{18}F]CsF proved to be less stable with regard to the reaction outcome than [^{18}F]TBAF and K[^{18}F]F$^-$ Krypto-

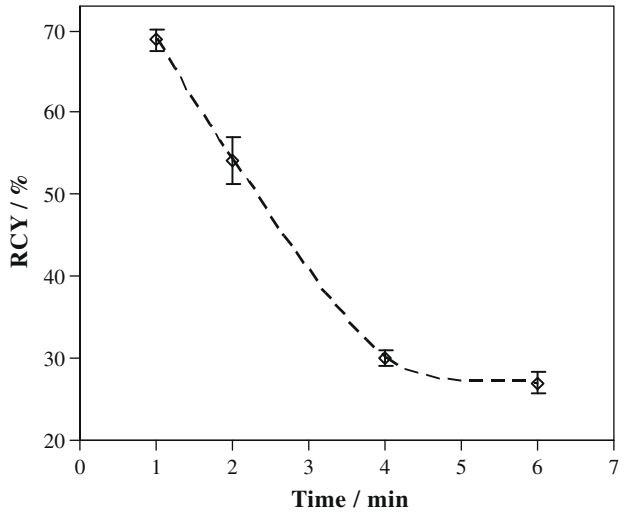


Figure 3. [^{18}F]**1** product degradation under prolonged reaction time (errors are ±1 S.D.: n = 3).

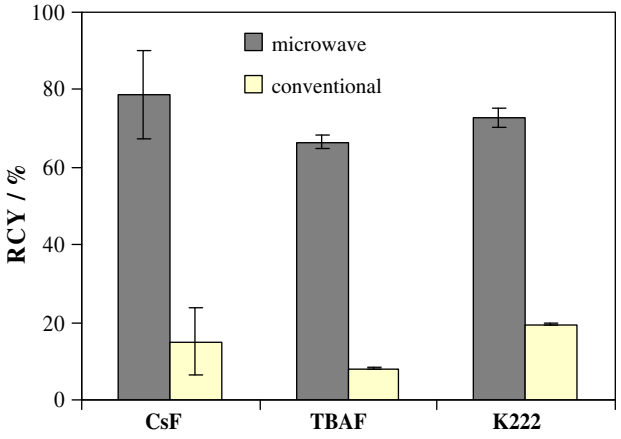


Figure 4. Comparison of maximum yields (±1 S.D.) of [^{18}F]**1** after of microwave irradiation (1 min, n = 6–9) and conventional heating (20 min, n = 4) in MeCN.

fix®222 cryptate. Thus, although the highest yields were ascribed to CsF, this salt also claimed the lowest reliability and low reproducibility.

[^{18}F]TBAF proved a better reliability, in addition the reaction worked well in low boiling THF and almost equal results were found in MeCN. However, the total reaction yields were substantially lower than with the standard [^{18}F]fluoride source K[^{18}F]F$^-$ Kryptofix®222 cryptate. For these reasons, we conclude, that K[^{18}F]F$^-$ Kryptofix®222 cryptate is the most efficient source in microwave-assisted radiofluorinations of tropane-analogue DAT ligands.

3. Conclusions

[^{18}F]PR04.MZ and [^{18}F]LBT999 have been prepared using a novel, highly efficient and time effective microwave-assisted labelling method. Radiosynthesis of [^{18}F]PR04.MZ was performed under pressure in a CEM discover® focussed microwave reactor. The use of direct molecular heating significantly increased the radiochemical yield and remarkably reduced the reaction time. [^{18}F]**1** was thus rapidly formed within the first 45 s of microwave irradiation. Interestingly, when a high microwave-intensity was maintained the product degraded. The formulated tracer was obtained in a non-decay-corrected yield of 34 ± 2% in a radiochemical purity >98% and a specific activity of 89 ± 45 GBq μmol^{-1}.

Similar conditions have been applied to synthesise [^{18}F]LBT999 [^{18}F]**2**. The yields of 27 ± 2% obtained are significantly less compared to [^{18}F]**1**, but much higher compared to the data for [^{18}F]**2** described earlier.[4b,5a]

4. Experimental

4.1. Methyl 8-((*E*)-4-hydroxybut-2-enyl)-3-*p*-tolyl-8-azabicyclo[3.2.1]octane-2-carboxylate, 5

DiPEA (129.1 mg, 1 mmol) was added to nortropane **3** (260 mg, 1 mmol) and 4-bromobut-2-enol dissolved in acetonitrile (5 ml). The reaction mixture was heated to 45 °C for 3 h. After completion of the reaction, the reaction mixture was concentrated to approximately 500 μl, which were directly transferred to a silical gel column (20 g) and eluted with MeOH/$CHCl_3$ 1:6, 79% yield. ^{1}H NMR: (300 MHz, $CDCl_3$) δ (in ppm): 7.13 (d, J = 8 Hz, 2H, ArH), 7.05 (d, J = 8 Hz, 2H, ArH), 5.78–5.57 (m, 2H), 4.1 (t, J = 5,5 Hz, 2H), 3.65 (br s, 1H), 3.47 (s, 1H, OCH_3), 3.40 (br s, 1H), 3.03–2.92 (m, 2H), 2.90–2.77 (m, 2H), 2.57 (dt, J = 12.5 Hz, J = 2.9 Hz, 1H), 2.27 (s, 3H), 2.10–1.91 (m, 2H), 1.77–1.55 (m, 3H). ^{13}C NMR: (100 MHz, CDCl3) δ (in ppm): 172.0, 139.9, 135.2, 134.4, 128.6, 127.2, 126.3, 126.0, 65.8, 62.3, 61.3, 54.9, 52.7, 50.9, 34.1, 33.8, 26.1, 25.9, 21.0. Anal. Calcd C, 72.92; H, 8.26; N, 4.25; O, 9.71. Found: C, 72.79; H, 8.03; N, 4.53. MS (FD) 329.2 $C_{20}H_{27}NO_3$ requires 329.1991.

4.2. (*E*)-4-(2-(Methoxycarbonyl)-3-*p*-tolyl-8-aza-bicyclo[3.2.1]-octan-8-yl)but-2-enyl methane sulfonate, 5

Alcohol **4** was dissolved in dichloromethane and methanesulfonyl anhydride was added. The reaction was initiated by the addition of 1 mol % of ytterbium triflate. After stirring the reaction mixture for 5 h, the reaction was terminated and the reaction mixture was concentrated in vacuo. The residue was re-dissolved in a small amount of dichloromethane and purified by flash chromatography on a silica gel column (hexanes/diethylether, 8:2, 10% triethylamine). Compound 5 (63%) was obtained as colourless crystals.

^{1}H NMR: (300 MHz, $CDCl_3$) δ (in ppm): 7.15 (d, J = 8 Hz, 2H, ArH), 7.08 (d, J = 8 Hz, 2H, ArH), 5.80–5.61 (m, 2H), 4.08 (t, J = 6 Hz, 2H), 3.66 (br s, 1H), 3.49 (s, 1H, OCH_3), 3.40 (br s, 1H), 3.03–2.92 (m, 2H), 2.90–2.77 (m, 2H), 2.89 (s, 3H, SO_2CH_3), 2.56 (dt, J = 12.5 Hz, J = 2.9 Hz, 1H), 2.28 (s, 3H), 2.10–1.90 (m, 2H), 1.78–1.55 (m, 3H). ^{13}C NMR: (100 MHz, $CDCl_3$) δ (in ppm): 172.1, 140.0, 135.2, 134.5, 128.6, 127.2, 126.2, 125.2, 65.8, 62.3, 61.3, 55.0, 52.7, 50.9, 38.1, 34.1, 33.8, 26.2, 26.0, 21.0. Anal. Calcd C, 61.89; H, 7.17; N, 3.44. Found: C, 61.86; H, 6.89; N, 3.40. MS (FD) 407.2 (100); $C_{21}H_{29}NO_5S$ requires 407.1766.

4.3. 2-*exo*-Carboxymethyl-3-(4-methylphenyl)-8–(4'-hydroxybut-2-yne-1-yl)-8-azabicyclo [3.2.1]octane, 6

Nortropane **3** (260 mg, 1 mmol) was added to 1.05 equiv of DiPEA, dissolved in acetonitrile (5 ml). 4-Hydroxybut-2-yne-1-yl chloride was added subsequently and the reaction mixture was heated to 75 °C for 13 h. After completion of the reaction the reaction mixture was concentrated to approximately 500 μl, which were directly transferred to a silical gel column (20 g) and eluted with MeOH/$CHCl_3$ 1:6, 89% yield. ^{1}H NMR: (300 MHz, $CDCl_3$) δ (in ppm): 7.16 (d, J = 8 Hz, 2H, ArH), 7.08 (d, J = 8 Hz, 2H, ArH), 4.26 (t, J = 2 Hz, 2H), 3.92 (br s, 1H), 3.53 (s, 3H, OCH3), 3.51 (br s, 1H), 3.21 (dt, J = 2 Hz, J = 16 Hz, 1H), 3.09 (dt, J = 2 Hz, J = 16 Hz, 1H), 3.02 (dt, J = 12.7 Hz, J = 5 Hz, 1H), 2.94 ('t', J = 4 Hz, 1H), 2.62 (td, J = 2.9 Hz, J = 12.5 Hz, 1H), 2.30 (s, 3H, CH3), 2.20–2.09 (m, 1H), 2.07–1.96 (m, 1H), 1.83–1.60 (m, 3H). ^{13}C NMR: (100 MHz, $CDCl_3$) d (in ppm): 172.1, 139.7, 135.3, 129.0, 128.6, 127.2, 82.8, 81.7, 62.6, 60.9, 52.8, 51.2, 50.9, 42.9, 34.1, 33.7, 25.9, 25.8, 21.0. Anal. Calcd C, 73.37; H, 7.70; N, 4.28. Found: C, 73.71; H, 7.78; N, 4.5. MS (FD) 327.2 (100); $C_{20}H_{25}NO_3$ requires 327.1834.

4.4. 2-*exo*-Carboxymethyl-3-(4-methylphenyl)-8–(4'-methanesulfonyloxybut-2-yne-1-yl)-8-azabicyclo[3,2,1]octane, 7

Alcohol **6** was added to 1.05 equiv of triethylamine dissolved in dry dichloromethane (1 ml/mmol) and cooled to 0 °C. After stirring at 0 °C for 30 min, neat methanesulfonyl chloride 1 equiv was added drop by drop without interruption. After completion of the addition the reaction mixture was stirred for **5** additional minutes when all alcohol had been consumed (TLC monitoring). The reaction was quenched by the addition of cold water (1 ml/mmol) with vigorous stirring. Subsequently, the reaction mixture was diluted with dichloromethane (1 ml/mmol). After separation of the aqueous phase, the reaction mixture was washed with 5% sodium carbonate solution, dried over potassium carbonate and concentrated in vacuo to leave a residue that was quickly chromatographed on silica gel to yield precursor **8** in 90% yield. ^{1}H NMR: (300 MHz, $CDCl_3$) δ (in ppm): 7.15 (d, J = 8 Hz, 2H, ArH), 7.08 (d, J = 8 Hz, 2H, ArH), 4.23 (t, J = 2 Hz, 2H), 3.91 (br s, 1H), 3.52 (s, 3H, OCH_3), 3.50 (br s, 1H), 3.22 (dt, J = 2 Hz, J = 16 Hz, 1H), 3.10 (dt, J = 2 Hz, J = 16 Hz, 1H), 3.02 (dt, J = 12.5 Hz, J = 5 Hz, 1H), 2.93 ('t', J = 4 Hz, 1H), 2.89 (s, 3H, SO_2CH_3), 2.61 (td, J = 2.9 Hz, J = 12.5 Hz, 1H), 2.29 (s, 3H, CH_3), 2.21–2.10 (m, 1H), 2.07–1.95 (m, 1H), 1.83–1.59 (m, 3H).^{13}C NMR: (100 MHz, $CDCl_3$) δ (in ppm): 172.1, 139.7, 135.3, 129.0, 128.6, 127.2, 82.7, 81.7, 62.5, 60.9, 52.8, 51.2, 50.9, 42.9, 37.6, 34.1, 33.7, 25.85, 25.8, 21.0. Anal. Calcd C, 62.20; H, 6.71; N, 3.45; O, 19.73; S, 7.91. Found: C, 61.9; H, 6.91; N, 3.24. MS (FD) 405.2 (100); $C_{21}H_{27}NO_5S$ requires 405.1610.

4.5. Representative protocol for microwave-assisted fluorination of tropanes

[^{18}O]H_2O containing [^{18}F]fluoride was passed through a waters accel plus light QMA strong anion exchange cartridge, preconditioned with 1 M potassium carbonate solution (10 ml) followed by sterile water (20 ml). $TBAHCO_3$ and $CsCO_3$ were used to obtain TBA[^{18}F]F and Cs[^{18}F]F. The trapped fluoride was eluted in an acetonitrile solution containing cryptand Kryptofix®222 (15 mg,

40 μmol) and 1 M potassium carbonate solution (15 μl, 15 μmol), directly into a glass micro reaction vessel (Supelco® reactivial, 5 ml). A reduced pressure of 100 mbar was applied to the tightly closed vial in an oil bath at 90 °C while a stream of nitrogen (300 ml/min) was applied. After the complete evaporation additional MeCN (1 ml) was added (two times). After evaporation of the third amount of MeCN, the nitrogen inlet was closed and full vacuum was applied to the reaction vial for 3 min. Afterwards, the dried solution was re-dissolved in MeCN (1 ml) and stirred at room temperature for 5 min.

This stock solution was transferred into a CEM pressure-reaction vial (Pyrex®) containing PR04.MZ labelling precursor (4.5 mg, 11 μmol) and an 8 mm stirring magnet. The vial was placed inside the cavity of a CEM Discover® laboratory microwave. After an irradiation with microwaves at 250 W for approximately 45 s the preset maximum pressure and temperature were reached. After cooling down of the vial for 90 s in a stream of air, the reaction mixture was taken up into a syringe containing cold water (300 μl) and directly injected into a Dionex p680 HPLC-system equipped with a Dionex UVD 170U UV-detector (λ = 254 nm) and a Raytest Gabi Star® radioactivity detector. Dionex Chromeleon software was used for UV-data analysis and Raytest Gina-star software was used for radioactivity detection. A semipreparative Phenomenex Luna RP 18 10 μ HPLC column was used as stationary phase (250 × 10 mm). Ammonium acetate buffer (0.1 M) (pH 4.7)/acetonitrile (4:6) at a flow rate of 4.7 ml/min was used as mobile phase. The product fraction was collected after a total retention time of 14 min, taken up into a syringe containing water (10 ml) and passed through a Merck Lichrolute SCX strong cation exchange cartridge (200 mg), preconditioned with 1 M hydrochloric acid (5 ml) and neutralised with water (20 ml). Subsequently, the cartridge was washed with water (3 ml). At this point usually >95% of radioactivity were trapped on the cartridge.

4.6. Formulation of [^{18}F]PR04.MZ

The radiotracer was eluted with sterile phosphate buffered saline (5 ml). A Millex®-GS 0.22 μm sterile filter ('blue') was serially connected to the cartridge, to obtain the injectable radiotracer formulation containing [^{18}F]PR04.MZ in a non-decay-corrected yield of 32–36% calculated from [^{18}F]fluoride ion starting activity. The specific activity of product [^{18}F]**1** at end of synthesis was 42–135 GBq/μmol, depending on the status of [^{18}F]fluoride ion.

4.7. Quality control of [^{18}F]PR04.MZ

The radiochemical purity and the specific radioactivity were determined using a Sykam S1100 HPLC-pump, a Berthold Flow Star LB 513 radioactivity detector and a Knauer K 2501 UV-detector (254 nm). A Phenomenex Luna RP 18 5μ (250 × 4.6 mm) analytical HPLC column was used as stationary phase at a flow rate of 1 ml/min. The mobile phase consisted of 30% 0.05 M ammonium acetate buffer at pH 4.6 in acetonitrile. The results were validated using 7 mM ammonia/acetonitrile 1:4 at 1 ml/min on the same column and by silica gel TLC: hexanes/diethylether, 4:1 containing 10% triethylamine. An aliquot of 20 μl was withdrawn from the sterile filtered formulated tracer solution and diluted with eluent. Approximately 50 kBq of this solution were injected into the HPLC. The product which was >98% radiochemically pure eluted after 10 min. The clear, colourless tracer formulation did not contain chemical impurities above 2.4 μg/ml.

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