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[^{11}C]PR04.MZ, a promising DAT ligand for low concentration imaging: Synthesis, efficient ^{11}C-O-methylation and initial small animal PET studies

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ABSTRACT

PR04.MZ was designed as a highly selective dopamine transporter inhibitor, derived from natural cocaine. Its binding profile indicates that [^{11}C]PR04.MZ may be suited as a PET radioligand for the non-invasive exploration of striatal and extrastriatal DAT populations. As a key feature, its structural design facilitates both, labelling with fluorine-18 at its terminally fluorinated butynyl moiety and carbon-11 at its methyl ester function. The present report concerns the efficient [^{11}C]MeI mediated synthesis of [^{11}C]PR04.MZ from an *O*-desmethyl precursor trifluoroacetic acid salt with Rb_2CO_3 in DMF in up to 95 ± 5% labelling yield. A preliminary μPET-experiment demonstrates the reversible, highly specific binding of [^{11}C]PR04.MZ in the brain of a male Sprague–Dawley rat.

Non-invasive molecular imaging is an emerging interdisciplinary field of research. Among the available techniques for the visualisation of the biochemical and physiological function of biological tissue approached by suitable imaging probes, positron emission tomography (PET) provides great potential in terms of quantification, sensitivity, temporal and lateral resolution.[1] The dopaminergic system and in particular the presynaptic dopamine transporter (DAT) represents an important biological target for the development of specific PET radioligands, due to its role in a variety of pathologies.[2]

PR04.MZ (**2**) is a highly potent (IC_{50}(hDAT) = 3 nM) DAT inhibitor based on the phenyltropane lead derived from cocaine. An inflexible, extended 4-fluorobut-2-yn-1-yl chain has been added to the tropane nitrogen. Thereby conformational flexibility has been reduced to increase selectivity among the structurally similar monoamine transporters. The mammalian DAT represents a 49% amino acid homology to the mammalian serotonin transporter (SERT) and a 67% homology to the norepinephrine transporter (NET). This modification resulted in a good selectivity profile (DAT/SERT >100, DAT/ NET ~10) compared to earlier examples of cocaine derivatives for the application as PET-radioligands.[3] PR04.MZ is currently under investigation with respect to its suitability as a PET tracer for in vivo imaging of the DAT. Although it was specifically designed as an ^{18}F-labelled radiopharmaceutical, one of its key features is the possibility to incorporate two different radiolabels. In this regard, both, ^{11}C labelling as well as ^{18}F-fluorination provides specific advantages. The shorter half-life of ^{11}C permits multiple tracer injections into the same subject on the same day.[4] Thereby, a variety of high value protocols for scientific studies, that is, test–retest, test-block or baseline-challenge, can be performed in the same subject without altering the orientation within the gantry of a PET camera. In contrast, fluorine-18 provides a more convenient half-life with regard to the total duration of the radio-synthesis, delivery to off-site PET centers and total acquisition time of clinical PET examinations.[5]

The present work is concerned with (a) the synthesis of non-radioactive **2** as reference, (b) the synthesis of the carbon-11 labelling precursor, (c) the highly efficient O-methylation of the carboxylic acid function of **3** and (d) an initial, preliminary PET-study that underlines the feasibility of exploring the DAT using [^{11}C]-**2**.

The synthetic route to PR04.MZ (**2**) and the labelling precursor (**3**) is illustrated in Scheme 1.

Commercially available cocaine hydrochloride was hydrolysed to ecgonine using dilute hydrochloric acid. Subsequent 1,2-elimination in refluxing $POCl_3$ afforded the α,β-unsaturated acid anhydroecgonine which was re-esterified under Fischer-conditions to afford the Michael-acceptor in 80% yield over three steps.[7a] The latter was subsequently exposed to a two-fold excess of *p*-tolyl magnesium bromide in CH_2Cl_2/Et_2O at low temperature. The use of *p*-tolyl lithium via transmetallation to the Gilman-cuprate was also examined. Careful protonation followed by chromatographic purification afforded 75% of the desired exo-isomer.[7b] Demethylation in refluxing dichloroethane containing 1-chloroethyl chloroformate (ACE-Cl) gave nortropane **1** in up to 90% yield. PR04.MZ (**2**) was obtained from **1** via alkylation with 4-fluoro-but-2-ynyl

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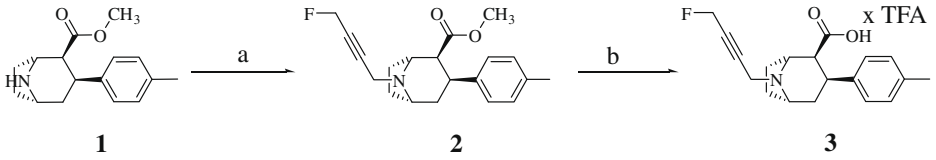


Scheme 1. Synthesis route to PR04.MZ (**2**) and labelling precursor **3**; (a) 4 fluoro-but-2-ynyl chloride; DiPEA, MeCN, 70 °C, 12 h, 95%; (b) 1 M HCl, reflux 1 d, 75%.

chloride in a yield of 95%.[3] Finally, the labelling precursor **3** was obtained by aqueous acidic hydrolysis of methyl ester **2** in refluxing dilute hydrochloric acid. HPLC-purification afforded the pure, slightly hygroscopic precursor in 60% overall yield.[8]

Several groups have reported low yields of 6–20% when employing an ammonium salt precursor for O-methylation.[6a,b] In particular, the [^{11}C]MeI methylation of a variety of carboxylic acid functions demanded liberation of the free base prior to labelling.[6a] In some cases, the highly reactive [^{11}C]methylation source [^{11}C]methyl triflate ([^{11}C]MeOTf) had to be used.[6a–c]

Analogue results were found when the initial labelling of PR04.MZ was conducted with an organic nitrogen base or common inorganic bases, such as sodium hydroxide or potassium carbonate. However, it was analysed that a system consisting of rubidium carbonate (Rb_2CO_3) as base, combined with dry *N*,*N*-dimethylformamide (DMF) as solvent, affords [^{11}C]PR04.MZ in high yield. Contrary to earlier reports, these conditions facilitate the use of readily available, high-specific-activity [^{11}C]MeI, together with a stable and insensitive 2,2,2-trifluoroacetic acid (TFA)-salt precursor. Isolation of the free base can be avoided.

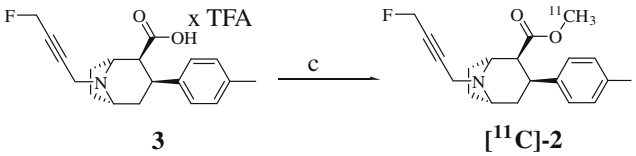
[^{11}C]Methyl iodide was obtained 14–18 min after EOB from a common GE® [^{11}C]methyl iodide module (GE box). The radioactivity was trapped in DMF at room temperature and partitioned over multiple reaction vessels to facilitate screening.[9] Appropriate labelling conditions were elucidated and verified in duplicate. Reaction of precursor **3** with [^{11}C]MeI in DMF or a 1:1 mixture of DMF and dimethylsulfoxide (DMSO) using common bases like sodium hydroxide, triethylamine, sodium or potassium carbonate or potassium hydrogen carbonate did not lead to acceptable radiochemical yields (0–7%, Table 1). Following solubility considerations, we examined rubidium carbonate and caesium carbonate as alternatives. The use of these highly soluble bases strikingly increased the labelling yields for [^{11}C]MeI methylation. Initially, 1 mg of precursor **3** was used. In a typical synthesis for biological studies, 2.04 GBq (55 ± 10 mCi) of [^{11}C]-**2** were obtained 45 min after the end of [^{11}C]CO_2-production (end of bombardment, EOB) (Scheme 2).[10]

All further reactions were carried out using system **e**, rubidium carbonate in DMF with a precursor concentration of 0.66 mg/ml. In a typical production run, base and precursor were weighed into a V-shaped borosilicate vial and 450 µl DMF were added. The vol-

Table 1
Impact of various bases on the radiochemical yield (RCY) of [^{11}C]MeI-supported methylation of precursor **3**

Entry	Base	Solvent	RCY[a] (%)
a	1 M $NaOH_{aq}$	DMF	0
b	Na_2CO_3	DMF	3 ± 1
c	K_2CO_3	DMF	7 ± 1
d	Cs_2CO_3	DMF	90 ± 8
e	Rb_2CO_3	DMF	95 ± 5
f	Rb_2CO_3	DMF/DMSO	92 ± 6

[a] Errors are given as one SEM after three independent runs.



Scheme 2. Carbon-11 labelling, (c) Rb_2CO_3 (2.1 equiv), DMF, [^{11}C]MeI, 5 min, 75 °C.

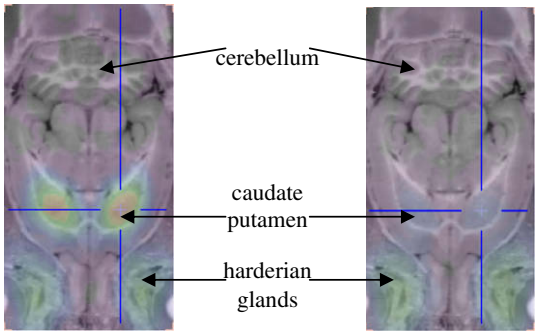


Figure 1. PET/MRI-Fusion of brain images of [^{11}C]PR04.MZ in a male Sprague–Dawley rat. Injected dose: 37 MBq, 67 GBq/µmol (1 mCi, 1.8 Ci/µmol) (left: baseline; right: block; summed images from 20 to 60 min).

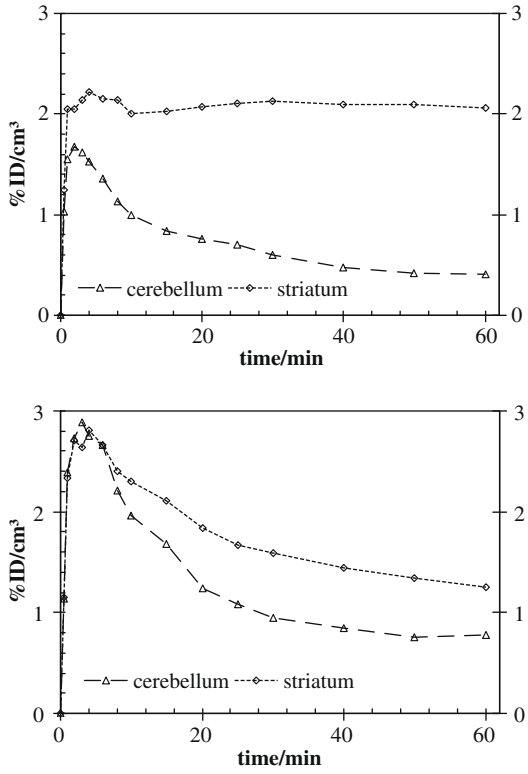


Figure 2. Time activity curve for [^{11}C]PR04.MZ in a male Sprague–Dawley rat. Injected dose: 37 MBq (1 mCi), specific activity: 67 GBq/µmol (1.8 Ci/µmol). Upper curve: baseline scan, lower curve: blocking study.

Table 2
Quantitative uptake of [^{11}C]PR04.MZ and distribution volume ratios for striatum and cerebellum, derived from the preliminary μPET-study

Aquisition time[a] (min)	Baseline			Pretreatment			Reduction[d] (%)
	%ID(striatum) (cm^3)	Striatum/cerebellum[b]	BP(striatum)[c]	%ID(striatum) (cm^3)	Striatum/cerebellum[b]	BP(striatum)[c]	
15	2.0	2.4	3.4	2.1	1.25	2.3	32
30	2.1	3.6	4.6	1.6	1.67	2.7	41
60	2.1	5.0	6.0	1.2	1.61	2.6	57

[a] Time-frame of the μPET-scan.
[b] Radioactivity–concentration ratio.
[c] Striatal binding potential from simplified reference tissue model.
[d] Effect of pretreatment to BP(striatum).

ume was increased from 300 μl to 450 μl to increase the trapping efficiency. This vial was thoroughly vortexed and connected to the [^{11}C]MeI delivery tubing. [^{11}C]MeI was directly trapped in this suspension.

Subsequently, the vial was placed in an oil bath and heated to 75 °C for 5 min. The reaction mixture was quenched with HPLC-eluent, purified by HPLC and concentrated by rotary evaporation. After evaporation to dryness, the product was dissolved in sterile sodium chloride solution and passed through a small Millipore® sterile filter (0.22 μm) into a multi-injection vial (MIV). This simple process afforded [^{11}C]-**7** in a total, non-decay corrected yield of 20% and a radiochemical purity of greater than 98% after 45 min of total synthesis duration. The specific activity of [^{11}C]PR04.MZ at this point exceeded 67 GBq/μmol (1.8 Ci/μmol).

A 300 g male adult Sprague–Dawley rat was used for a preliminary μPET-study in a CTI® Focus 120 μImager. In a test-block experiment, the animal was anaesthetised with ketamine/xylazine (90/10, 100 mg/kg), and injected with approximately 37 MBq (1 mCi) of the radiotracer, directly into the tail vene. A full-dynamic scan was performed for 60 min. Subsequently, the same animal was pretreated with 1.5 mg/kg of GBR12909, 45 min prior to the injection of a second 37 MBq dose of [^{11}C]PR04.MZ. Data acquisition was started simultaneous to the injection of tracer and continued for 60 min. Image reconstruction and corregistration was performed using the commercial image processing software PMOD (www.pmod.com). Binding potentials (BP) were calculated using the simplified reference tissue model (SRTM).

The LONI (www.loni.ucla.edu/ratdata/Rat.html) anatomical magnetic resonance imaging (MRI)-atlas of the rat brain was used for PET/MRI-fusion and the identification of brain regions. Regions of interest were drawn onto a MRI-template and copied into the co-registered μPET-datasets.

The results are shown in Figure 1. Peak uptake of 2.22% of the injected dose per cm^3 of tissue (%ID/cm^3) was achieved in the baseline scan 4 min post injection. The results clearly indicate the highly specific binding of [^{11}C]PR04.MZ to striatal DAT-binding sites in the rat brain (Fig. 2). Figure 1 further illustrates the effect of GBR12909 pretreatment in a test-block study. In comparison to the baseline scan, a significant reduction in striatal radioactivity concentration can be observed within the blocking study. In contrast, the radioactivity accumulation in the harderian glands is increased in the pretreated animal compared to the baseline scan. Furthermore, the radioactivity concentration ratios between the *striatum* and the *cerebellum* after pretreatment are significantly lower (cf. Table 2). Pre-treatment with the highly selective DAT-inhibitor leads to a significant decrease in the total activity accumulation in the basal ganglia. These findings may indicate the highly selective binding of PR04.MZ to rat dopamine transporters (rDAT).

In conclusion, an acid ammonium salt labelling precursor for [^{11}C]PR04.MZ has been prepared and successfully labelled with [^{11}C]methyl iodide in high yield. In a typical production run starting from ~6.3 GBq (~170 mCi) of [^{11}C]MeI, 2.04 GBq (55 ± 10 mCi) of injectable radiotracer were obtained after 45 min.

The value [^{11}C]PR04.MZ as a highly selective probe for the presynaptic DAT has been assessed by an preliminary rat study. These results indicate highly selective binding to rat dopamine transporters. The reversibility of the highly selective DAT-ligand was demonstrated by pre-treatment with a structurally non-analogous DAT-inhibitor (GBR12909).

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8. ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 10.09 (br s, 1H), 4.95 (d, J_{HF} = 47.5 Hz, 2H), 4.34–4.18 (m, 2H), 3.81–3.66 (m, 1H), 3..36–3.24 (m, 1H), 3.16–2.98 (m, 2H), 2.78 (dt, J = 12.5 Hz, J = 2.9 Hz), 2.39–2.29 (m, 2H), 2.27 (s, 3H), 1.98–2.18 (m, 3H), 1.96–1.84 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ in ppm: 177.0, 137.6, 134.7, 129.5, 127.1, 84.4, 78.8, 71.4, 69.1, 62.8, 62.0, 49.9, 45.8, 41.4, 34.2, 32.824.4, 24.1, 20.9, 8.5. HRMS (ESI): 316.1723 (M^+) $C_{19}H_{23}FNO_2$ requires 316.1713. HPLC: phenomenex Luna® RP18 5μ semipreparative column (10 × 250 mm, eluent: 40% MeOH in 0.05% TFA_{aq}).
9. *Base screening experiments were conducted as follows*: standard 5 ml screw-cap vials were charged with base (2 equiv), 1.4–4.6 μmol) and **3** was added (0.3–1 mg, 0.7–2.3 μmol). The solids were suspended in either DMF (0.15 ml, for reactions in pure DMF) or DMSO (0.15 ml, for reactions in a mixture of DMSO and DMF). These vials were placed in a self-made multi-vial holder and placed above an oil bath, prior to the addition of 150 μl of DMF, containing [^{11}C]MeI (37–74 MBq; 1–2 mCi). The oil bath was elevated and the vials were heated for 5 min at 75 °C. The heat-source was removed subsequently, and the reaction was immediately quenched by the addition of HPLC-eluent (1 ml). At this point, the activity inside the vial was measured and an aliquote was withdrawn to determine the radiochemical yield by HPLC and TLC. The results are summarised in Table 1.
10. HPLC-purification was performed using a Phenomenex® Luna® RP 18 semipreparative HPLC-column (dimensions 10 × 250 mm) as stationary phase. The mobile phase consisted of 36% 0.1 M ammonium formate solution (v:v) in acetonitrile. At a flowrate of 4.7 ml/min, the product eluted after a purification time of t_r ([^{11}C]-**7**) = 13 ± 1 min, in a radiochemical purity of >98%.