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SYNTHESIS AND ^{11}C- RADIOLABELLING OF A TROPANE DERIVATIVE LACKING THE 2β ESTER GROUP: A POTENTIAL PET-TRACER FOR THE DOPAMINE TRANSPORTER

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SUMMARY

The synthesis and ^{11}C-radiolabelling of a new tropane analogue, 3β-(4'-chlorophenyl)-2β-(3'-phenylisoxazol-5'-yl)tropane (β-CPPIT), an inhibitor of the dopamine transporter, is reported. The desmethyl compound, 3β-(4'-chlorophenyl)-2β-(3'-phenylisoxazol-5'-yl)nortropane (5) was prepared via a six-step reaction sequence starting from cocaine. [^{11}C]-β-CPPIT was labelled by N-methylation using [^{11}C]-methyl iodide obtained from the gas phase reaction of [^{11}C]-methane with iodine in 60 ± 10 % radiochemical yield (decay corrected from [^{11}C]-methyl iodide). The overall synthesis time was on average 60 minutes at EOB (end of bombardment). The final product had a specific activity of 2000 - 2700 Ci/mmol (74 - 100 TBq/mmol) at EOS (end of synthesis) and the radiochemical purity was greater than 99%. [^{11}C]-β-CPPIT showed a logP value of 2.1 indicating that a free diffusion through the blood-brain-barrier should be possible.

KEY WORDS: Dopamine transporter, β-CPPIT, PET

INTRODUCTION

The dopamine transporters (DAT) are located presynaptically on dopaminergic nerve terminals and are responsible for the reuptake of dopamine from the synaptic cleft after secretion from presynaptic neurons [1-3]. A markedly reduced density of the transporters has been demonstrated in the basal ganglia of postmortem brains from patients with degenerative brain disorders such as Parkinson's and Alzheimer diseases [4-6].

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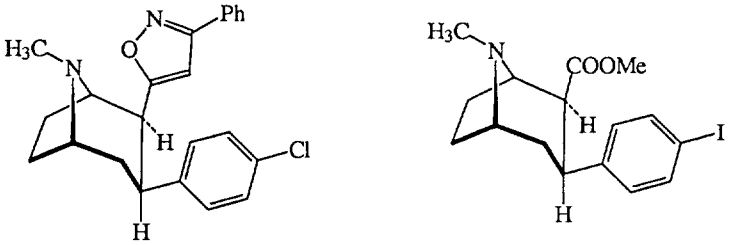
A series of cocaine analogues have been synthesized and used for the PET imaging of the DAT [7-9]. An undesired property accompanying striatal uptake of most of these cocaine analogues has been the high to moderate binding to the serotonin transporter in midbrain [8,10]. Recently, the synthesis and the in vitro biological evaluation of a new cocaine analogue, 3β-(4'-chlorophenyl)-2β-(3'-phenylisoxazol-5'-yl)tropane (β-CPPIT, Fig 1), with improved selectivity at the DAT has been published. An IC_{50} value at the DAT of 1.28 nM combined with 5-HT/DAT and NE/DAT ratios of 1891 and 393, respectively have been reported (see Table 1) [11]. Compared to β-CIT,



β-CPPIT β-CIT

Figure 1: Structures of β-CPPIT and β-CIT

(2β-carbomethoxy-3β-(4-iodophenyl)-tropane), β-CPPIT is 630-fold more selective. In contrast to existing DAT radioligands this new analogue bears the isoxazole heterocyclic group at the C-2β-position of the tropane ring and because it lacks the metabolically labile 2β ester function it is expected to be stable against the action of esterases.

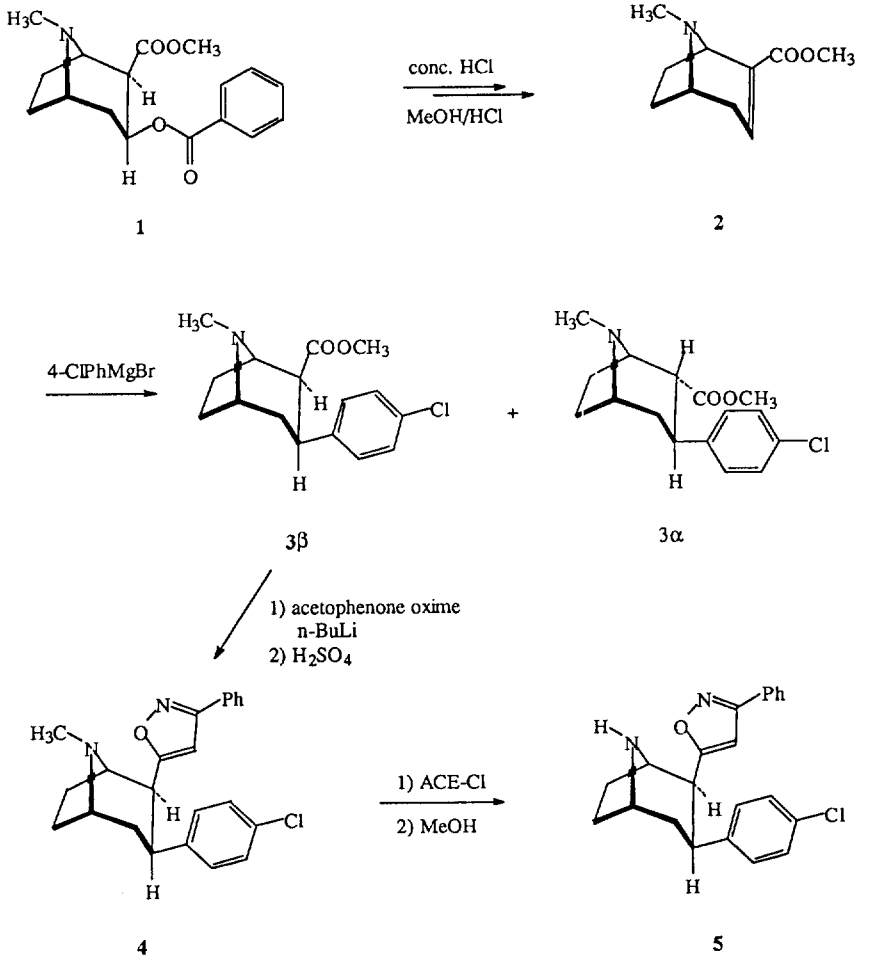
	Cocaine [12]	**β-CIT [12]**	**β-CPPIT [11]**
Dopamine (DA)	89.1 ± 4.8	1.26 ± 0.04	1.28 ± 0.18
Serotonin (5-HT)	1045 ± 89	4.21 ± 0.34	504 ± 29
Norepinephrine (NE)	3298 ± 293	36.0 ± 2.7	2420 ± 136
5-HT / DA	12	3	**1891**
NE / DA	37	29	**393**

Table 1: Cocaine derivatives and their in vitro IC_{50}-values [nM]. The numbers in parentheses are literature citations.

In this paper, we describe the synthesis of desmethyl-β-CPPIT and the radiolabelling of β-CPPIT with carbon-11 using the classical methylating agent [^{11}C]-methyl iodide.

RESULTS AND DISCUSSION

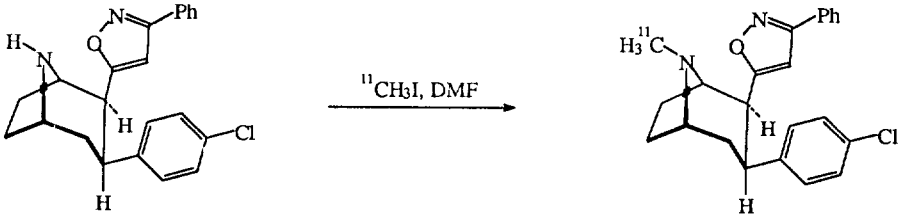
The synthetic pathway leading to β-CPPIT (4) and the desmethyl compound **5** is outlined in scheme 1. Anhydroecgonine methyl ester (2) was obtained in 75% yield by refluxing cocaine hydrochloride (1) in concentrated hydrochloric acid as reported previously [13]. The intermediate **3** was prepared according to the literature procedure [13]. The α- and β-products arising from the conjugate addition were separated by flash chromatography. The yields of the α- and β-isomers were 14 and 30% respectively, and are comparable to the reported literature yields. Unlabelled β-CPPIT (4) was obtained by treating the β-isomer of the methylester **3** with



Scheme1: Synthesis of β-CPPIT (4) and desmethyl β-CPPIT (5) starting from cocaine (1).

the dilithium salt of acetophenone oxime according to the method described by Kotian and coworkers in 54% yield [11]. The β-configuration of the C-2 substituent was assigned on the basis of the NMR coupling constant [11]. N-demethylation of β-CPPIT was accomplished by conversion to its carbamate using 1-chloroethyl chloroformate (ACE-Cl) followed by hydrolysis in methanol. Desmethyl-β-CPPIT (**5**) was obtained in 67% yield. Attempts to prepare **5** from **4** by the general procedure using 2,2,2-trichloroethyl chloroformate and zinc-acetic acid reduction failed. Presumably, the trichloroethyl carbamate formed during the reaction is sterically hindered to undergo reductive cleavage.

[^{11}C]-Methyl iodide was obtained from the gas phase reaction of [^{11}C]-methane with iodine as reported recently [14]. The radiolabelling of [^{11}C]-β-CPPIT was accomplished by N-methylation of the desmethyl precursor **5** with [^{11}C]-methyl iodide (scheme 2).



5 [^{11}C]- **4**

Scheme 2: Radiolabelling of β-CPPIT

[^{11}C]-β-CPPIT was separated from unreacted materials and radioactive impurities by semi-preparative HPLC (Figure 2, panel A) and formulated in a solution containing Polysorbatum 80 (0.1%), ethanol (10%) and 0.9% NaCl-solution (90%). The total synthesis time was on average 60 minutes (counted from EOB) and the radiochemical yield ranged between 50 and 70% (decay corrected from [^{11}C]-methyl iodide). The final product contained 0.5 - 5 µg of β-CPPIT and had a specific activity of 2000 - 2700 Ci/mmol (74 - 100 TBq/mmol) at EOS. The radiochemical purity of [^{11}C]-β-CPPIT was greater than 99% (Fig.2, panel B).

[^{13}C]-β-CPPIT synthesized and purified according to the procedure for the C-11 compound was characterized by ^{13}C-NMR and MS using positive ion mode and electrospray as interface. The ^{13}C signal at 46.7 ppm corresponded to the N-methyl group of authentic β-CPPIT. Mass spectrometry showed molecular ion peaks at m/z 379 (M+1) and 380 (M+1) for authentic β-CPPIT and ^{13}C enriched β-CPPIT, respectively.

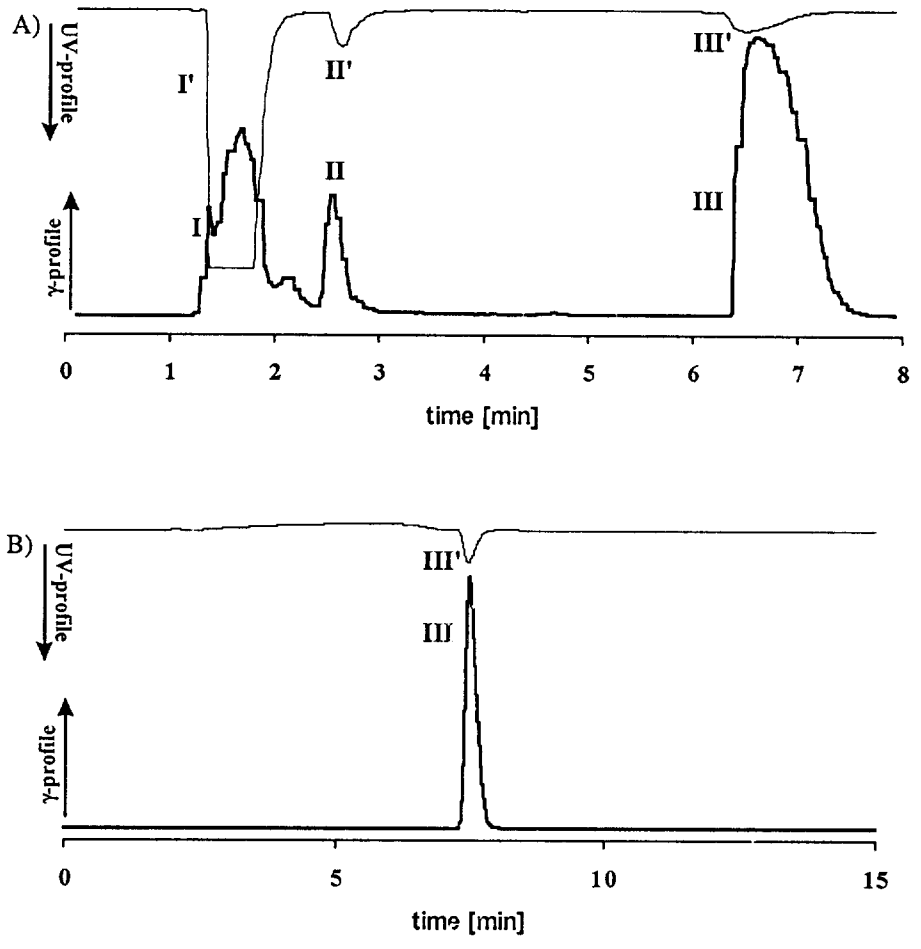


Figure 2: A) Semi-preparative HPLC of the crude reaction mixture (system A); B) Analytical HPLC of [^{11}C]-β-CPPIT (System B). I and II: unknown, I': DMF, II': precursor, III: [^{11}C]-β-CPPIT, III': β-CPPIT

The logP-value was estimated with the shake flask method described by Strijckmans and coworkers [15] and amounted to 2.1. This value indicates that β-CPPIT should be sufficiently lipophilic for free diffusion through the blood/brain barrier [16].

Preliminary biodistribution studies in mice [17] indicated that [^{11}C]-β-CPPIT has the potential of being developed as a PET radiotracer for the DAT. Details of these in vivo results will be published elsewhere.

EXPERIMENTAL

General Procedures

Diethyl ether and tetrahydrofuran were dried over sodium. The Grignard reagent, 4-chlorophenylmagnesium bromide, was obtained from Aldrich Chemie, Buchs, Switzerland. Thin layer chromatography (TLC) was performed on silica gel plates Kieselgel 60/UV_{254}, Merck) and column chromatography on silica gel (Kieselgel 60, Merck). The NMR-spectra were recorded on a Bruker AC-250 (^1H: 300 MHz; ^{13}C: 75 MHz) or on a Bruker AMX 500 (^{13}C: 125 MHz) using TMS as an internal standard and are reported in ppm (δ) downfield. Mass spectra were recorded on a Trio 2000 Spectrometer (VG Organic, UK) using positive ion mode with electrospray as interface (ES^+). Melting points were determined on a Büchi 530 apparatus (Büchi, Switzerland) and are uncorrected.

Two systems were used for isocratic HPLC separations:

System A (semi-preparative): Consisting of a Merck-Hitachi L-6000A pump, a LabSource H.S. valve 7000e with a 5 ml loop, a Merck-Hitachi L-4000A UV detector (at 254 nm), a NaI scintillation detector and a Phenomenex column, Luna, C18, (10 x 250 mm, 5μm) with 0.01M ammonium formate : CH_3CN (20 : 80) at a flow rate of 8 ml/min.

System B (analytical): Consisting of a Merck-Hitachi D-7100 pump, a 100 μl loop, a Merck-Hitachi D-7200 autosampler, a NaI scintillation detector, a Merck-Hitachi L-7400 UV detector (at 254 nm), a Phenomenex column, Luna, C18, (4.6 x 250 mm, 5 μm) with 0.1% triethylamine : CH_3CN (15 : 85) at a flow rate of 1.5 ml/min.

Anhydroecgonine methyl ester (2, Scheme 1) [13]

Cocaine hydrochloride (20.0 g, 59 mmol) was refluxed in concentrated hydrochloric acid (200 ml) for 20 hours. The reaction mixture was allowed to cool to room temperature, kept in a refrigerator at 4°C for 2 hours and the benzoic acid was removed by filtration. The filtrate was concentrated, 80 ml of methanol were added and the solvent was evaporated. The oily residue was triturated several times with portions of diethyl ether and filtered to remove traces of benzoic acid. The residue was dried at room temperature under reduced pressure for 30 minutes before adding a saturated solution of hydrogen chloride gas in methanol (250 ml). After standing for 3 days at room temperature the methanol was removed. The residue was made alkaline (pH 11-12) with 4M NaOH, extracted with diethyl ether and dried over sodium sulphate. The solvent was evaporated and the residue was distilled in vacuum (120°C, 1 mbar) to give 8.16 g (45 mmol, 75%) of the ester **2** as a yellow oil ([13]: 75%).

^1H-NMR ($CDCl_3$): 1.31 - 1.41 (m, 1 H); 1.64 - 1.72 (m, 2 H); 1.92 - 2.07 (m, 2 H); 2.19 (s, 3 H); 2.40 - 2.50 (m, 1 H); 3.08 (m, 1 H); 3.58 (s, 3 H); 3.61 - 3.63 (m, 1 H); 6.65 - 6.67 (m, 1 H).

MS: 182 (100%, $C_{10}H_{16}NO_2^+$).

2β-carbomethoxy-3β-(4'-chlorophenyl)tropane (3, Scheme 1) [13,18]

A solution of anhydroecgonine methyl ester (**2**, 3.7 g, 20 mmol) in dry diethyl ether (100 ml) was dropped to a vigorously stirred solution of 4-chlorophenylmagnesium bromide (1 M; 40 ml) in dry diethyl ether (120 ml) at -45 to -50°C (temperature of the reaction mixture). After the addition the reaction mixture was kept between -45 and -50°C for further 2 hours, cooled to -78°C and treated with a solution of trifluoroacetic acid (4.6 g, 40 mmol) in dry diethyl ether (30 ml) during 5 min. The mixture was allowed to warm to 0°C and diluted with distilled water (100 ml). The aqueous phase was acidified to pH 1 with concentrated hydrochloric acid, separated from the organic phase, made alkaline with concentrated ammonium hydroxide and extracted with diethyl ether. The combined organic phases were dried over sodium sulphate, filtered, evaporated and purified by column chromatography (diethyl ether : triethylamine 9 : 1) to give 1.8 g (30 %) of the β-isomer of the tropane **3** and 0.8 g (14 %) of the α-isomer ([13]: 15% α; 41% β).

^{1}H-NMR ($CDCl_3$): 1.64 - 1.82 (m, 4 H); 2.10 - 2.22 (m, 1 H); 2.28 (s, 3 H); 2.56 - 2.68 (m, 1 H); 2.91 - 2.96 (m, 1 H); 2.98 - 3.07 (m, 1 H); 3.42 - 3.46 (m, 1 H); 3.57 (s, 3 H); 3.59 - 3.64 (m, 1 H); 7.22 - 7.32 (m, 4 H).

MS: 318 (13%, $C_{16}H_{20}NO_2{}^{37}ClNa^+$); 316 (34%, $C_{16}H_{20}NO_2{}^{35}ClNa^+$); 296 (32%, $C_{16}H_{21}NO_2{}^{37}Cl^+$); 294 (100%, $C_{16}H_{21}NO_2{}^{35}Cl^+$).

3β-(4'-chlorophenyl)-2β-(3'-phenylisoxazol-5'-yl)tropane (β-CPPIT, 4, Scheme 1) [11]

A solution of n-butyllithium in hexane (1.6 M; 3.9 ml) was dropped under vigorous stirring at 0°C under nitrogen to a solution of acetophenone oxime (0.42 g, 3.12 mmol) in dry tetrahydrofuran (11 ml). After 1 hour stirring at 0°C a solution of the ester **3** (0.50 g, 1.71 mmol) in tetrahydrofuran (4 ml) was added slowly. The suspension was allowed to warm to room temperature and stirred for further 19 hours. The reaction mixture was poured into a stirred solution of concentrated sulphuric acid (1.4 g) in tetrahydrofuran (7 ml) and distilled water (2 ml) and refluxed for 70 minutes. After cooling to room temperature the reaction mixture was made alkaline with saturated potassium carbonate solution and extracted with methylene chloride. The combined organic phases were dried over sodium sulphate. The solvent was removed to give 0.84 g of crude isoxazole **4**. Purification by column chromatography (hexane : diethyl ether : triethylamine 80 : 18 : 2) gave 0.35 g (0.92 mmol, 54%) of the pure isoxazole **4** [11]: 50%).

^{1}H-NMR ($CDCl_3$): 1.66 - 1.78 (m, 3 H); 2.12 - 2.30 (m, 3 H); 2.31 (s, 3 H); 3.22 - 3.35 (m, 2 H); 3.35 - 3.48 (m, 2 H); 6.84 (s, 1 H); 6.94 - 6.98 (m, 2 H); 7.12 - 7.16 (m, 2 H); 7.40 - 7.47 (m, 3 H); 7.77 - 7.80 (m, 2 H).

^{13}C-NMR ($CDCl_3$, 75 MHz): 42.6 $N(CH_3)$

MS: 403 (26, $C_{23}H_{23}N_2O^{37}ClNa^+$); 401 (98, $C_{23}H_{23}N_2O^{35}ClNa^+$); 381 (33, $C_{23}H_{24}N_2O^{37}Cl^+$); 379 (100, $C_{23}H_{24}N_2O^{35}Cl^+$).

Mp.: 110 - 112°C

3β-(4'-chlorophenyl)-2β-(3'-phenylisoxazol-5'-yl)nortropane (5, Scheme 1)

1-Chloroethyl chloroformate (0.1 ml, 0.9 mmol) was added to a solution of the tropane **4** (101 mg, 0.27 mmol) in 1,2-dichloroethane (2.5 ml). The reaction mixture was refluxed at 94°C for 70 hours. The resulting suspension was concentrated under reduced pressure. The residue was dissolved in methanol (1.5 ml) and refluxed (80°C) for 5 hours. After removal of the solvent the residue was dissolved in methylene chloride (2 ml) and saturated sodium hydrogen carbonate solution (5 ml). The mixture was extracted with methylene chloride (5x10 ml). The combined organic phases were dried over sodium sulphate and concentrated under reduced pressure to give 105 mg of the crude nortropane **5**. Purification by column chromatography (hexane : diethyl ether : triethylamine 50 : 45 : 5) gave 66 mg (0.18 mmol, 67%) of the pure nortropane **5**.

^{1}H-NMR ($CDCl_3$): 1.70 - 2.40 (m, 7 H); 3.29 - 3.50 (m, 2 H); 3.74 - 3.86 (m, 2 H); 6.21 (s, 1 H); 6.98 - 7.03 (m, 2 H); 7.12 - 7.18 (m, 2 H); 7.39 - 7.44 (m, 3 H); 7.68 - 7.73 (m, 2 H).

Elemental analysis calculated for $C_{22}H_{21}N_2OCl$: C: 72.42%, H: 5.80%, N: 7.68%, O: 4.38%, Cl: 9.72%; found: C: 72.27%, H: 5.79%, N: 7.76%.

Mp.: 80 - 84°C

Radiosynthesis of [^{11}C]CH_3I

[^{11}C]CO_2 was produced via the $^{14}N(p,\alpha)$ ^{11}C reaction using the 16.5 MeV cyclotron at the University Hospital in Zurich. [^{11}C]CH_3I was prepared from [^{11}C]CO_2 in a two-step reaction sequence involving the catalytic (Ni) reduction of [^{11}C]CO_2 to [^{11}C]CH_4 and the subsequent gas phase iodination of [^{11}C]CH_4 with I_2 at 720 °C to give [^{11}C]CH_3I according to the standard procedure described in the literature [19,20]. Yields up to 50% (decay corrected from [^{11}C]CH_4) were obtained with a preparation time of approx. 12 minutes.

[^{11}C]-3β-(4'-chlorophenyl)-2β-(3'-phenylisoxazol-5'-yl)tropane ([^{11}C]-β-CPPIT, [^{11}C]-4, Scheme 2)

The nortropane **5** (0.5 mg, 1.4 μmol) was dissolved in dry dimethylformamide (300 μl). The reacti-vial containing the solution of the precursor was then positioned in a quartz lamp-heated vessel, and [^{11}C]-methyl iodide was added to the solution via a slow stream of nitrogen. Following the complete addition of the [^{11}C]-methyl iodide, the flow of nitrogen was stopped and the reacti-vial was heated to 120°C for 10 min. Purification was achieved by HPLC

(System A). After evaporation of the mobile phase, the residue was dissolved in a mixture of Polysorbatum 80 (0.1%), ethanol (10%) and 0.9% NaCl-solution (90%) and filtered through a Millipore filter (0.22 μm).

[^{13}C]-3β-(4'-chlorophenyl)-2β-(3'-phenylisoxazol-5'-yl)tropane ([^{13}C]-β-CPPIT, [^{13}C]-4)

The ^{13}C-methylation was achieved under the same reactions conditions as [^{11}C]-methylation. The product was purified by HPLC (System A) and examined by ^{13}C-NMR and mass spectrometry.

^{13}C-NMR ($CDCl_3$, 125 MHz): 42.68 ($N(CH_3)$).

MS: 380 (M+1, 100).

Lipophilicity

The lipophilicity of β-CPPIT was estimated by the shake flask method described by Strijckmans et al. [15] using a mixture of octanol and phosphate buffer (0.15 M). [^{11}C]-β-CPPIT (100 kBq) was shaken vigorously for 1 min in a test tube containing 1 ml of octanol and 1 ml of the phosphate buffer. The mixture was centrifuged for 5 min at 3000 rpm. 100 μl of each phase were taken off and the radioactivity of each sample was measured in a γ-counter. 600 μl of the octanol phase were transferred to another test tube containing 400 μl of octanol and 1 ml of phosphate buffer (0.15 M). The mixture was further shaken and centrifuged. This procedure was repeated several times. The radioactivity ratios of organic and aqueous phase (P) were calculated and the partition coefficient (log $P_{7.4}$) determined.

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