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Research Article

Gas phase production of [^{11}C]methyl iodide-d_3. Synthesis and biological evaluation of *S*-[*N*-methyl-^{11}C]citalopram and deuterated analogues

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Summary

Three ^{11}C-labelled tracers for the serotonin reuptake site, *S*-[*N*-methyl-^{11}C]citalopram (**[^{11}C]-4**), *S*-[*N*-methyl-d_3-^{11}C]citalopram (**[^{11}C]-12**), and *S*-[*N*-methyl-^{11}C]citalopram-α,α-d_2 (**[^{11}C]-13**) were synthesized and the distribution of radioactivity after injection of radioligand was examined *ex vivo* in rats. The deuterated analogue of (*S*)-desmethylcitalopram, (*S*)-1-(4-fluorophenyl)-1-(3-methylamino-[3-d_2]-propyl)-1,3-dihydro-isobenzofuran-5-carbonitrile (**11**), was synthesized in a multi-step synthesis from escitalopram (**4**) and used as precursor in the synthesis of **[^{11}C]-13**. In analogy with the reported gas phase synthesis of [^{11}C]methyl iodide the first gas phase synthesis of [^{11}C]Methyl iodide-d_3 is reported. The ^{1}H/^{2}H kinetic isotope effect related to the synthesized compounds were investigated in *ex vivo* rat studies, where the brain regions of interest to cerebellum ratios of the tracers **[^{11}C]-4**, **[^{11}C]-12** and **[^{11}C]-13** were compared. The *ex vivo* data indicated no significant differences in binding in any of the investigated brain regions after injection of the three tracers.

Key Words: deuterium; specific radioactivity; serotonin reuptake sites

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Introduction

In the last decades a range of selective serotonin reuptake inhibitors (SSRIs), which have proven to be highly efficient in the treatment of major depression and other psychiatric disorders, have been developed.[1] Several of the marketed SSRIs have been radiolabelled with positron-emitting isotopes such as carbon-11 ($t_{1/2} = 20.3$ min) or fluorine-18 ($t_{1/2} = 109.7$ min) and investigated as potential positron emission tomography (PET) tracers for measurements of the serotonin transporters (SERTs) in the primate brain. Fluvoxamine (**1**),[2,3] sertraline (**2**),[4] citalopram (**3**)[5–7] and escitalopram (**4**)[8] have been labelled with carbon-11, whereas fluoxetine (**5**) has been labelled with carbon-11[9] as well as fluorine-18,[10] Figure 1.

None of these marketed SSRIs do, however, exhibit appropriate *in vivo* properties as PET ligands, mostly due to insufficient target to background ratios.[11–13] Recently, diarylsulfides such as [^{11}C]DASB[14,15] and [^{11}C]MADAM[16] have shown promising results as PET tracers for the SERT in humans.[17,18]

Escitalopram (**4**), the therapeutically active enantiomer of citalopram (**3**),[19] displays high affinity for the SERT and very high selectivity over the dopamine transporter (DAT) and the noradrenaline transporter (NAT).[19,20] The log *P* value of escitalopram (**4**) is 3.6.[21] Recent studies of SERT tracers such as [^{11}C]DASB and other diaryl sulfides indicate that a log *P* value in the range of 2–3 is optimal in order to obtain a sufficient BBB penetration as well as low non-specific binding.[22] Based on these observations the log *P* value of escitalopram (**4**) could be argued to be in the upper end of the optimal range for a PET ligand. However, despite a thalamus to cerebellum ratio in rats of 1.8 60 min after injection of *S*-[*N*-methyl-^{11}C]citalopram (**[^{11}C]-4**) only a very

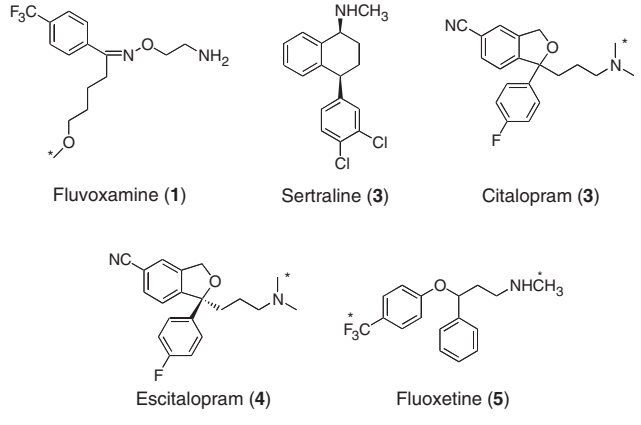
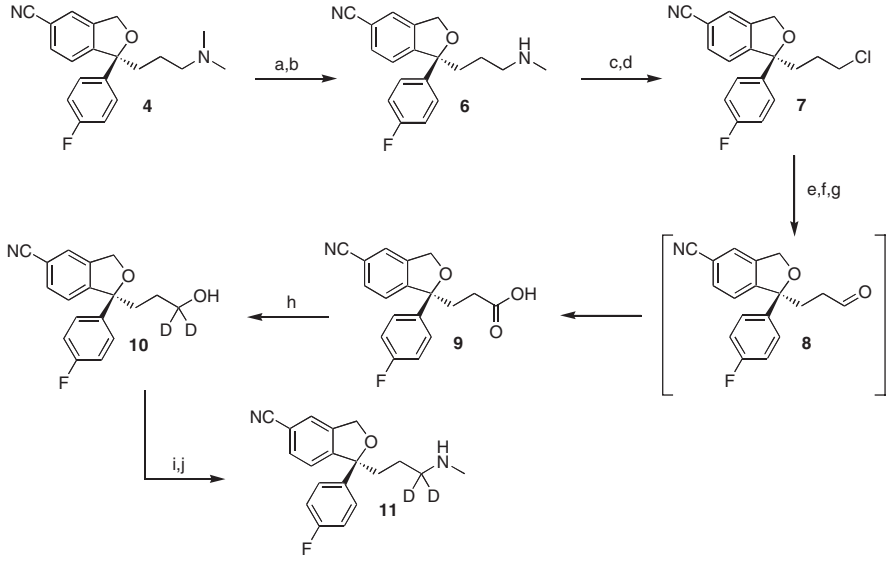


Figure 1. SSRIs currently available on the marked as antidepressant drugs. The * indicates the position of the label

low target to background ratio was found in human volunteers studied with PET.[8]

The major metabolite found in patients treated with citalopram (**3**) is desmethylcitalopram,[23] which has a 10-fold lower affinity for the SERT as compared to citalopram (**3**).[19] In one study where a single dose of 20 mg citalopram infusion was given to healthy males, the only detectable metabolite in plasma after 140 min was the propanoic acid derivative (the racemic form of compound **9**, Scheme 1).[24] The enzymes cytochrome P450 and monoamine oxidase (MAO) have been identified as responsible for the *in vivo* metabolism of citalopram (**3**).[25–28] Metabolism of citalopram will therefore also lead to formation of secondary hydrophilic metabolites such as formaldehyde and dimethylamine. Accordingly, in a PET study with *S*-[*N*-methyl-^{11}C]citalopram (**[^{11}C]-4**), radiolabelled metabolites like *S*-[*N*-methyl-^{11}C]desmethylcitalopram, [^{11}C]formaldehyde and/or [^{11}C]dimethylamine could be formed. It can be speculated that [^{11}C]formaldehyde and [^{11}C]dimethylamine due to their limited lipophilicity do not really leave the brain within the time window of a PET study and might therefore result in non-specific binding.[29–31] There are, to the best of our knowledge, no reports on the *in vivo* metabolism of citalopram (**3**) applied in tracer doses, which could be different from the pharmacokinetic data obtained after treatment with pharmacological doses.



Reaction conditions: a) $ClCOOCH_2CCl_3$, toluene, reflux, 3h, b) Zn, KH_2PO_4, THF, reflux, 2h, 70% (11% of **7** was formed). c) $BrCH_2COOEt$, K_2CO_3, reflux, 3h, d) ClCOOEt, toluene, reflux, 3h, 52% (from **4** the overall yield of **7** amounts to 47%). e) NaI, acetone, reflux, 18h, f) $NaHCO_3$, DMSO, 120°C, 20 min., g) NaOH, $AgNO_3$, 0°C, 30 min., 36%. h) $NaBD_4$, $BF_3{\cdot}Et_2O$, THF, 0°C-rt, 18h, 76%. i) MsCl, pyridine, 0°C-rt, 1h, j) H_2NMe in THF (2M), 80°C, 18h, 78%.

Scheme 1. Synthesis of the deuterated *S*-desmethyl citalopram derivative 11

We hypothesized that deuterated analogues of escitalopram labelled with carbon-11 could delay the *in vivo* metabolism in the brain and thereby reduce formation of radiolabelled metabolites. This would potentially lead to increased target to background ratios if the deuterium labels were placed in a position where the proton abstraction by MAO or cytochrome P450 takes place in a rate limiting step. $^{1}H/^{2}H$ Kinetic isotope effects (KIE) have previously been observed in *ex vivo* rat studies and in PET studies of MAO activity in the brain when for example [^{11}C]dimethylphenetylamine and [^{11}C]L-deprenyl were compared with the corresponding deuterated counterparts (α-hydrogens substituted with deuterium).[32–34]

In this paper, we report the synthesis of *S*-desmethylcitalopram-α,α-d_2 (**11**) and the radiosynthesis of *S*-[*N*-methyl -^{11}C]citalopram (**[^{11}C]-4**), *S*-[*N*-methyl-d_3-^{11}C]citalopram (**[^{11}C]-12**) and *S*-[*N*-methyl-^{11}C]citalopram-α,α-d_2 (**[^{11}C]-13**), respectively. Comparative *ex vivo* rat studies of **[^{11}C]-4**, **[^{11}C]-12** and **[^{11}C]-13** were performed in order to evaluate the binding properties of the deuterated radioligands for the SERT.

Results and discussion

Chemistry

The synthesis of the precursors, *S*-desmethylcitalopram (**6**) and deuterium labelled *S*-desmethylcitalopram **11**, were initiated from readily available escitalopram (**4**) in order to obtain the target molecules as pure enantiomers, Scheme 1.

Treatment of escitalopram (**4**) with 2,2,2-trichloroethyl chloroformate followed by reduction with zinc dust gave *S*-desmethylcitalopram (**6**) and the chloro derivative **7** in 70 and 11% yield, respectively. Treatment of *S*-desmethylcitalopram (**6**) with ethyl bromoacetate followed by ethyl chloroformate yielded **7** in 52% yield. Thus, the combined overall yield of **7** was 47% calculated from the amount of escitalopram (**4**). The chloro derivative **7** was converted to the carboxylic acid derivative **9** in a three-step procedure. Compound **7** was first converted to the corresponding iodide in a Finkelstein reaction[35] followed by oxidation with dimethyl sulfoxide[36,37] to yield aldehyde **8**. The aldehyde **8** was further oxidized with a mixture of silver nitrate and sodium hydroxide[38] to the carboxylic acid derivative **9** in 36% overall yield.

A key step in the synthesis of **11** was the selective reduction of **9** with a deuterated reagent in order to introduce two deuterium atoms in the α-position relative to the hydroxy group in the phthalane side chain. For this purpose, deuterated diborane was freshly prepared by reaction of boron trifluoride diethyl etherate and sodium borodeuteride.[39–41] The suspension was slowly added to the carboxylic acid derivative **9** in dry tetrahydrofuran. The reduction preceded smoothly giving the deuterium labelled alcohol **10** in 76%

yield with complete incorporation of deuterium. Interestingly, it was noted that the order of addition of the deuterated diborane reagent effected the extent of deuterium incorporated in **10**. Thus, adding sodium borodeuteride to the carboxylic acid derivative **9** followed by addition of the boron trifluoride diethyl etherate resulted in formation of the alcohol **10** with 4–5% incorporation of hydrogen in the side chain.

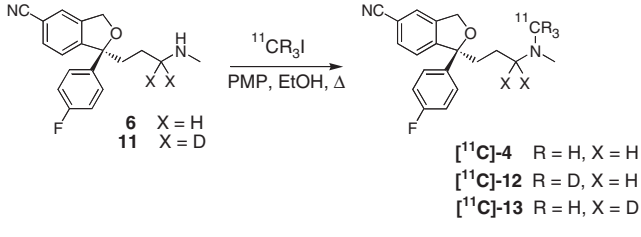
Treatment of the deuterium labelled alcohol **10** with methylsulfonyl chloride in pyridine followed by amination with methylamine in THF gave the desired product **11** in 78% yield.

The optical purity of **11** was determined by chiral HPLC and was >99% as in the starting material. ^{1}H NMR confirmed the incorporation of deuterium in **11** due to the absence of signals at δ 2.85 ppm corresponding to the deuterated positions. LC–MS analysis further confirmed the introduction of two deuterium atoms, since *S*-desmethylcitalopram (**6**) and its deuterated counterpart **11** showed identical retention times on HPLC and the molecular ion of **11** was increased by 2 mass units compared to compound **6**.

Radiochemistry

Synthesis of [^{11}C]methyl iodide-d_3 in a gas phase reaction has not previously been described. [^{11}C]Methyl iodide-d_3 was obtained in analogy with the reported procedure for gas phase synthesis of [^{11}C]methyl iodide.[42] Bombarding a gas target containing a mixture of N_2 and D_2 (95/5) with 16 MeV protons produced [^{11}C]methane-d_4. [^{11}C]Methane-d_4 was in several cycles passed through a quartz tube at 720°C containing I_2 vapor and [^{11}C]methyl iodide-d_3 was synthesized. By changing the target gas from N_2/D_2 to N_2/H_2 [^{11}C]methyl iodide was produced as described above.[42] The radiosynthesis of *S*-[*N*-methyl-^{11}C]citalopram (**[^{11}C]-4**), *S*-[*N*-methyl-d_3-^{11}C]citalopram (**[^{11}C]-12**) and *S*-[*N*-methyl-^{11}C]citalopram-α,α-d_2 (**[^{11}C]-13**), Scheme 2, were performed in ethanol and purified on a normal phase semi-preparative HPLC column.[7]

By applying normal phase HPLC purification the ^{11}C-labelled product eluted before the *S*-desmethyl precursor and contamination of the product



Scheme 2. Radiosynthesis of *S*-[*N*-methyl-^{11}C]citalopram ([^{11}C]-4), *S*-[*N*-methyl-d_3-^{11}C]citalopram ([^{11}C]-12), and *S*-[*N*-methyl-^{11}C]citalopram-α,α-d_2 ([^{11}C]-13)

with the precursor was thereby avoided. In addition, the synthesis time was minimized due to quick evaporation of the low boiling organic solvents. After evaporation of the solvents the residue was formulated in 2% ethanol. The radiochemical yield of the final product was 30–40% (decay corrected radiochemical yield based on the amount of trapped [^{11}C]methylation agent). By reverse phase analytical HPLC, the identity of the product was confirmed by co-elution with an authentic sample of citalopram (**3**). The radiochemical purity was determined to be $>98\%$.

The specific radioactivity of the purified products (synthesis time was 45 min) was found to be in the range of 37–130 GBq/μmol at the end of synthesis. Increasing the beam current and the irradiation time might increase the specific radioactivity. The incorporation of the deuterium label in *S*-[*N*-methyl-d_3-^{11}C]citalopram (**[^{11}C]-12**) was confirmed by GC–MS and LC–MS analysis. After decay mass spectra with molecular ions at m/z: 327[M^+] and 328[$M^+ + 1$] corresponding to the carbon-12 analog of **[^{11}C]-12** was obtained. The difference of 3 mass units (the molecular weight of citalopram is 324) corresponds to the incorporation of three deuterium molecules in the CD_3 unit. No compound with a molecular ion at m/z: 325[$M^+ + 1$] corresponding to citalopram (**3**) could be identified.

Ex vivo studies

S-[*N*-Methyl-^{11}C]citalopram (**[^{11}C]-4**), *S*-[*N*-methyl-d_3-^{11}C]citalopram (**[^{11}C]-12**) or *S*-[*N*-methyl-^{11}C]citalopram-α,α-d_2 (**[^{11}C]-13**) were injected into the tail vein of the rats. Each rat received 5–25 MBq of the ligand in question with a specific radioactivity ranging from 10 to 18 GBq/μmol, at the time of injection. With the given specific radioactivity and assuming an SERT density in rat thalamus of about 11 fmol/mg protein (B. Elfving, unpublished data) the occupancy in the rat thalamus was calculated to constitute $\sim 0.2\%$ and can therefore be considered to be negligible. The optimal thalamus to cerebellum ratio for carbon-11 labelled escitalopram has previously been shown to be reached 60 min after tracer injection.[7,8] At this time point the rats were decapitated and the brains were dissected, weighed and the radioactive content of the dissected regions was measured. Ratios between the radioactive content of the regions of interest (ROI) and the reference region, cerebellum, was determined (Figure 2). Cerebellum was used as reference region due to the low density of SERTs.[43] Pretreatment with paroxetine resulted in thalamus to cerebellum ratios of approximately 1.2, indicating that the majority of the specific binding could be blocked (data not shown).

As seen in Figure 2, there were no significant differences between the ROI to cerebellum ratios for **[^{11}C]-4**, **[^{11}C]-12** and **[^{11}C]-13** in any of the investigated regions.

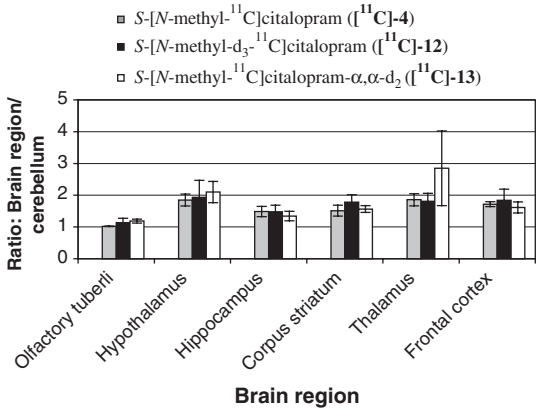


Figure 2. Ratios between six ROIs and cerebellum (mean ± S.D., $n = 3$) 60 min after tracer injection

The absence of a $^{1}H/^{2}H$ KIE could indicate that the tracers were not metabolized in the brain. It can, however, not be ruled out that brain metabolism of carbon-11 labelled escitalopram by cytochrome P450 or MAO actually takes place leading to formation of non-specific radiolabelled metabolites. This would be the case if the *in vivo* metabolism of **[^{11}C]-12** and **[^{11}C]-13** only to a minor extent was affected by the deuterium substitutions.

In conclusion, we have developed an efficient procedure to produce [^{11}C]methyl iodide-d_3 with high specific radioactivity (up to 130 GBq/μmol) in a gas phase reaction. The gas phase methylation system can produce either [^{11}C]methyl iodide-d_3 or [^{11}C]methyl iodide by changing the target gas. *S*-[*N*-methyl-^{11}C]citalopram (**[^{11}C]-4**), *S*-[*N*-methyl-d_3-^{11}C]citalopram (**[^{11}C]-12**) and *S*-[*N*-methyl-^{11}C]citalopram-α,α-d_2 (**[^{11}C]-13**) were synthesized in high specific radioactivity and evaluated in *ex vivo* rat studies. No significant differences between the ROI to cerebellum ratios for **[^{11}C]-4**, **[^{11}C]-12** and **[^{11}C]-13** were found in any of the investigated regions. The substitution of deuterium did thus not have any significant effect on the ROI to cerebellum ratios. Whether the rate of brain metabolism was largely unaffected by the deuterium substitutions or brain metabolism by cytochrome P450 and MAO is minimal is, however, not clear. A more direct way of addressing this question would be by conducting direct measurements of the labelled metabolite content in the rat brain.

Experimental

General

Chemicals were purchased from commercial suppliers and used without further purification. THF was distilled under N_2 from sodium-benzophenone.

Purifications were performed by flash chromatography (silica gel, 230–400 mesh). NMR spectra were obtained on a Bruker 500 instrument operating for ^{1}H at 500 MHz and for ^{13}C at 125 MHz. Chemical shifts are reported in ppm using tetramethylsilane (TMS) as internal standard. Coupling constants are given in Hz and coupling patterns are abbreviated as s = singlet, d = doublet, t = triplet, q = quintet and m = multiplet. Melting points were determined with a Büchi B-540 apparatus and are uncorrected.

Gas chromatograms and mass spectra were obtained on a Varian Saturn 2000 GC/MS spectrometer equipped with a phenomenex 'Zebran 2B-5' (L: 15 m, i.d. 0.25 mm) column. Column temperature started at 60°C and was linearly increased to 300°C in 12 min and maintained there for 2 min. LC–MS was performed using an API 150EX LC/MS system from Applied Biosystems. Chiral HPLC was performed on a Gilson Series SF3 System for packed-column supercritical fluid chromatography (Column: Diacel OD, 4.6×250 mm, 10 mm, 10% modifier: methanol with 0.5% trifluoroacetic acid and 0.5% diethylamine, flow: 2 ml/min).

All irradiations were carried out with the Scanditronix MC32 cyclotron at the PET and Cyclotron Unit at Copenhagen University Hospital, Rigshospitalet. [^{11}C]Methane-d_4 and [^{11}C]methane were produced in the $^{14}N(p,\alpha)^{11}C$ nuclear reaction by bombarding an aluminum gas target containing a mixture of nitrogen (AGA 6.0 nitrogen) and 5% deuterium (AGA 2.8 deuterium) or nitrogen (AGA 6.0 nitrogen) and 5% hydrogen (AGA 2.8 hydrogen) gas, respectively. Radiolabelled compounds were purified on a semi-preparative normal phase HPLC column and analyzed on a reverse phase analytical HPLC column at room temperature using Gilson 306 pumps and a Gilson 117 UV detector (254 nm) followed by a radioactivity detector.

Citalopram (**3**) and escitalopram (**4**) were gifts from H. Lundbeck A/S, Copenhagen, Denmark.

Chemistry

(S)-1-(3-Methylamino-propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile (6). A solution of escitalopram (**4**) (23.0 g, 71 mmol) dissolved in toluene (100 ml) was boiled under reflux when 2,2,2-trichloroethyl chloroformate (16.0 ml, 0.12 mol) was added drop wise during 30 min. After addition, the mixture was boiled under refluxed for further 2 h. After cooling to room temperature, Et_2O (100 ml) and 4 M aqueous HCl (50 ml) was added and the phases were separated. The aqueous phase was extracted with Et_2O (2×100 ml). The combined organic phases were dried ($MgSO_4$) and the volatile solvents evaporated *in vacuo* yielding 43.4 g of yellow oil. The crude mixture was dissolved in THF (200 ml) and 1.5 M aqueous KH_2PO_4 (200 ml) and heated to 50°C. To the mechanically stirred solution zinc dust (50 g, 0.76 mol) was added portion wise over 30 min. The resulting mixture was

boiled under reflux for further 2 h. After cooling to room temperature the suspension was filtered through celite. The phases were separated and the organic layer was evaporated *in vacuo* to give a mixture of **6** and **7**. Et_2O (200 ml) and water (200 ml) were added to the residue and the aqueous phase was acidified with 4 M aqueous HCl. The phases were separated and the aqueous phase was extracted with Et_2O (3×100 ml). The combined organic phases were dried ($MgSO_4$) and the solvents evaporated *in vacuo*. The residue was purified by column chromatography (EtOAc/heptane 1:3) yielding 2.4 g (11%) of **7** as a colorless oil. The remaining aqueous phase was made alkaline with 25% aqueous NH_3 and extracted with Et_2O (3×200 ml). The combined organic phases were dried ($MgSO_4$) and the solvent was evaporated *in vacuo* yielding 15.3 g (70%) of **6** as an yellow oil.

LC/MS (m/z): 311 ($M^+ + 1$), purity >97%.

^{1}H NMR ($CDCl_3$) δ 1.35 (m, 1 H), 1.48 (m, 1 H), 2.15 (m, 1 H), 2.23 (m, 1 H), 2.36 (s, 3 H), 2.85 (t, $J = 7.1$ Hz, 2 H), 5.15 (d, $J_{AB} = 13.0$ Hz, 1 H), 5.19 (d, $J_{AB} = 13.0$ Hz, 1 H), 7.00 (*t*, $J = 8.5$ Hz, 2 H), 7.40 (m, 3 H), 7.50 (s, 1 H), 7.58 (d, $J = 8.0$ Hz, 1 H).

^{13}C NMR ($CDCl_3$) δ 24.3, 36.4, 39.0, 51.8, 71.2, 91.1, 111.6, 115.1, 115.3, 118.5, 122.7, 125.1, 126.6, 126.7, 131.7, 139.5, 140.3, 149.3, 162.0 (d, $J_{C-F} = 239$ Hz).

(S)-1-(3-Chloropropyl)-1-(4-fluorophenyl)-1,3-dihyroisobenzofuran-5-carbonitrile (7). A mixture of *S*-desmethylcitalopram (**6**) (15.0 g, 48 mmol) in EtOH (100 ml), K_2CO_3 (13.8 g, 100 mmol) and ethyl bromoacetate (6.0 ml, 54 mmol) was boiled under reflux for 4 h. After cooling to room temperature the mixture was filtered and the volatile solvents were evaporated *in vacuo*. The remaining residue was dissolved in Et_2O (200 ml) and washed with brine. The aqueous phase was extracted with additional Et_2O (2×200 ml). The combined organic phases were dried ($MgSO_4$) and the solvent was evaporated *in vacuo* yielding 16.3 g of a yellow oil. The residue was dissolved in toluene (100 ml) and the resulting mixture was added dropwise to a refluxing mixture of ethyl chloroformate (42.1 ml, 0.44 mol) in toluene (100 ml) and boiled under refluxed for 2 h. After cooling the low boiling components were removed *in vacuo*. Purification by column chromatography (EtOAc/heptane 1:3) gave 8.1 g (52%) of **7** as a colorless oil. The overall yield of **7** from **4** amounts to 10.5 g (47%) taking into account the amount of **7** formed in the dealkylation of **4**.

^{1}H NMR ($CDCl_3$) δ 1.66 (m, 1 H), 1.79 (m, 1 H), 2.26 (m, 1 H), 2.34 (m, 1 H), 3.56 (m, 2 H), 5.14 (d, $J_{AB} = 13.2$ Hz, 1 H), 5.19 (d, $J_{AB} = 13.2$ Hz, 1 H), 7.02 (t, $J = 8.5$ Hz, 2 H), 7.42 (m, 3 H), 7.52 (s, 1 H), 7.61 (d, $J = 8.0$ Hz, 1 H).

^{13}C NMR ($CDCl_3$) δ 27.3, 38.5, 45.0, 71.3, 90.8, 111.9, 115.4, 115.5, 118.6, 122.7, 125.3, 126.7, 126.8, 132.0, 139.1, 140.3, 149.1, 162.1 (d, $J_{C-F} = 252$ Hz).

(S)-3-[5-Cyano-1-(4-fluorophenyl)-1,3-dihydro-isobenzofuran-1-yl]-propionic acid (9). A solution of **7** (2.4 g, 7.6 mmol) and sodium iodide (20 g, 133 mmol) in acetone (20 ml) was boiled under reflux overnight. After cooling to room temperature, brine (100 ml) was added. The aqueous phase was extracted with Et_2O (3 × 100 ml) and the combined organic phases were dried ($MgSO_4$) and the solvents were evaporated *in vacuo*. The remaining oil (3.2 g) was dissolved in DMSO (50 ml) and heated to 120°C. Sodium hydrogencarbonate (10 g) was added and the mixture was stirred for further 20 min at 120°C. The hot mixture was poured into ice-water and extracted with Et_2O (3 × 100 ml). The combined organic phases were dried ($MgSO_4$) and evaporated *in vacuo* giving 2.4 g of **8** as a crude product. A small sample was purified by column chromatography (EtOAc/heptane 1:3) yielding 45% of **8**.

^{1}H NMR ($CDCl_3$) δ 2.35 (m, 2 H), 2.45 (m, 1 H), 2.59 (m, 1 H), 5.13 (s, 2 H), 7.00 (t, J = 8.7 Hz, 2 H), 7.42 (m, 3 H), 7.52 (s, 1 H), 7.61 (d, J = 8.0 Hz, 1 H), 9.70 (s, 1 H).

^{13}C NMR ($CDCl_3$) δ 33.6, 38.9, 71.0, 90.3, 112.0, 115.4, 115.5, 118.4, 122.6, 125.3, 126.6, 126.6, 132.0, 138.7, 140.2, 148.3, 162.0 (d, J_{C-F} = 252 Hz), 201.0.

A solution of crude **8** (2.4 g) in ethanol (25 ml) was added to a mixture of 2.5 M aqueous $AgNO_3$ (8 ml) and 5 M aqueous NaOH (8 ml) at 0°C and stirred for 30 min. Water was added and the brown precipitate was filtered off. The filtrate was extracted with Et_2O (100 ml). The phases were separated and the organic phase was discarded. The aqueous phase was acidified with 4 M aqueous HCl and extracted with Et_2O (3 × 100 ml). The combined organic phases were dried ($MgSO_4$) and the solvents were evaporated *in vacuo*. Purification by column chromatography (EtOAc/heptane 1:3 + 1% CH_3COOH) gave 860 mg (36%) of **9** as a colorless oil.

^{1}H NMR ($CDCl_3$) δ 2.30 (m, 2 H), 2.45 (m, 1 H), 2.56 (m, 1 H), 5.14 (d, J_{AB} = 13.0 Hz, 1 H), 5.19 (d, J_{AB} = 13.0 Hz, 1 H), 7.02 (t, J = 8.7 Hz, 2 H), 7.42 (m, 3 H), 7.51 (s, 1 H), 7.60 (d, J = 8.0 Hz, 1 H), 10.0–11.0 (broad s, 1 H).

^{13}C NMR ($CDCl_3$) δ 29.1, 35.8, 71.3, 90.2, 112.0, 115.4, 115.5, 118.4, 122.7, 125.3, 126.6, 126.7, 131.9, 138.6, 140.2, 148.3, 162.0 (d, J_{C-F} = 252 Hz), 179.1.

(S)-1-(4-Fluorophenyl)-1-(3-hydroxy-[3-d_2]-propyl)-1,3-dihydro-isobenzofuran-5-carbonitrile (10). Boron trifluoride diethyl etherate (300 µl, 2.44 mmol) was added under N_2 to a suspension of sodium borodeuteride (77 mg, 1.83 mmol) in dry THF (5 ml) cooled to 0°C. The mixture was stirred at 0°C for 1 h. The suspension was slowly (1 h) added to a solution of **9** (475 mg, 1.53 mmol) in dry THF (5 ml) cooled to 0°C. The reaction mixture was allowed to warm to room temperature and stirred over night. Ice and saturated aqueous $NaHCO_3$ was added and the resulting mixture was extracted with Et_2O (3 × 75 ml). The combined organic phases were dried ($MgSO_4$) and the solvent evaporated *in vacuo*. Purification by column chromatography (EtOAc/heptane 1:3) gave 345 mg (76%) of **10** as a colorless oil.

^{1}H NMR ($CDCl_3$) δ 1.44 (m, 1 H), 1.55 (m, 1 H), 1.88 (broad s, 1 H), 2.21 (m, 1 H), 2.31 (m, 1 H), 5.15 (d, J_{AB} = 13.0 Hz, 1 H), 5.20 (d, J_{AB} = 13.0 Hz, 1 H), 7.01 (t, J = 8.7 Hz, 2 H), 7.41 (m, 3 H), 7.50 (s, 1 H), 7.59 (d, J = 7.1 Hz, 1 H).

^{13}C NMR ($CDCl_3$) δ 27.0, 37.6, 61.8 (q, $^1J_{C-D}$ = 21.6 Hz, CD_2), 71.2, 91.0, 111.7, 115.2, 115.4, 118.5, 122.7, 125.2, 126.6, 126.7, 131.8, 139.2, 140.1, 149.2, 162.0 (d, J_{C-F} = 238 Hz).

(S)-1-(4-Fluorophenyl)-1-(3-methylamino-[3-d$_2$]-propyl)-1,3-dihydro-isobenzofuran-5-carbonitrile (11). A solution of **10** (266 mg, 0.89 mmol) in pyridine (1.3 ml) was cooled to 0°C. Methanesulfonyl chloride (84 µl, 1.07 mmol) was added and the resulting mixture was stirred for 30 min at 0°C followed by 30 min at room temperature. Water (50 ml) was added and the resulting mixture was extracted with toluene (3 × 75 ml). The combined organic phases were dried ($MgSO_4$) and the solvents were evaporated *in vacuo* and re-evaporated *in vacuo* 3 times from toluene. The crude product was dissolved in a 2 M solution of methylamine in THF (6 ml) and heated at 80°C for 15 h in a sealed tube. After cooling to room temperature, the solvents were evaporated *in vacuo* to give 217 mg (78%) of **11**. The oxalate was crystallized from acetone yielding 130 mg (36%).

Anal. ($C_{19}H_{17}FN_2OD_2.C_2H_2O_4$) calcd. C: 62.68, H: 5.26, N: 6.96. Found C: 62.25, H: 5.45, N: 6.82. Mp. = 170–172°C. LC–MS (m/z): 313 [$M^+ + 1$].

^{1}H NMR (DMSO-d_6) δ 1.39 (m, 1 H), 1.48 (m, 1 H), 2.25 (m, 2 H), 2.46 (s, 3 H), 5.16 (d, J_{AB} = 13.4 Hz, 1 H), 5.21 (d, J_{AB} = 13.4 Hz, 1 H), 7.20 (t, J = 8.9 Hz, 2 H), 7.60 (m, 3 H), 7.74 (d, J = 7.5 Hz, 1 H), 7.80 (d, J = 8.0 Hz, 1 H).

^{13}C NMR (DMSO-d_6) δ 20.4, 32.2, 36.9, 47.4 (q, $^1J_{CD}$ = 18.8 Hz, CD_2), 71.0, 90.2, 110.6, 115.0, 115.2, 118.7, 123.0, 125.7, 126.8, 126.9, 132.0, 139.8, 140.0, 148.8, 161.3 (d, J_{C-F} = 238 Hz), 164.6.

The final product was analyzed on a chiral HPLC column applying supercritical fluid flow conditions. No indications of racemisation could be determined, ee > 98% as in the starting material.

Radiochemistry

Synthesis of [^{11}C]methyl iodide-d$_3$. Bombarding a gas target (20 µA, 10 min) containing a mixture of N_2 and D_2 (95/5) with 16 MeV protons produced [^{11}C]methane-d_4. [^{11}C]Methane-d_4 was transferred from the target to the hot cell and trapped on a Porapak N column cooled to −190°C with liquid nitrogen. After trapping the Porapak N trap was heated to room temperature and [^{11}C]methane-d_4 was liberated. In several cycles [^{11}C]methane-d_4 was passed through a quartz tube at 720°C containing I_2 vapor. At the end of each cycle [^{11}C]methyl iodide-d_3 was trapped on the Porapak N column at room temperature. Unreacted [^{11}C]methane-d_4 was re-circulated through the quartz

tube until the radioactivity at the Porapak N column reached a maximum. At this point [^{11}C]methyl iodide-d_3 was liberated by purging the Porapak N trap, now at 190°C, with helium. From a typical bombardment (~3.4 μAh) 5–7 GBq of [^{11}C]methyl iodide-d_3 was obtained. By changing the target gas to N_2/H_2 (95/5) [^{11}C]methyl iodide was obtained similarly.[42]

Radiosynthesis of S-[N-methyl-d_3-^{11}C]citalopram ([^{11}C]-12), S-[N-methyl-^{11}C]-citalopram ([^{11}C]-4) and S-[N-methyl-^{11}C]citalopram-α,α-d_2 ([^{11}C]-13). In separate runs [^{11}C]methyl iodide or [^{11}C]methyl iodide-d_3 was trapped in a reaction vessel cooled to −45°C containing EtOH (740 μl), the *S*-desmethyl-precursor (**6** or **11**) (4–8 mg) and 1,2,2,6,6 pentamethyl piperidine (6 μl). After trapping the sealed reaction vessel was heated to reflux for 5 min followed by evaporation of the solvent leaving 50–100 μl in the vessel. The residue was dissolved in chloroform (1 ml), which was automatically injected to the semi-preparative HPLC column. The product was collected after 7.5 min. The solvent was evaporated with a stream of nitrogen at atmospheric pressure at 70°C and the product was formulated in 2% EtOH in water (2 ml).

Preparative HPLC: The column used was a Knauer nucleosil-100 Si (5 μm, 250 × 8 mm). The products were eluted isocratic with as mixture of $CHCl_3$:CH_3OH:28% NH_4OH (90:10:0.1) and a flow rate of 3 ml/min. The analytical column used was a Merck LiChrospher® 60 RP-select B (5 μm, 250 × 4 mm). The mobile phases were A) CH_3CN:0.05 M ammonium acetate (60:40) and B) CH_3CN. The product was eluted with a flow rate of 1 ml/min. starting with A:B (90:10) for 5 min and then linearly increased to A:B (50:50) over a period of 13 min.

The radiochemical purity was >98% determined with analytical HPLC. The identity of the product was verified by co-elution of an authentic sample of citalopram, which showed an identical retention time. **[^{11}C]-4**, **[^{11}C]-12** and **[^{11}C]-13** eluted after approximately 16 min and the precursors after approximately 13 min. The specific radioactivity was calculated by comparing the UV-response of the product with a standard curve of UV-responses.

Ex vivo rat studies

Male Sprague-Dawley rats (254–311 g) were used for *ex vivo* rat studies. Sixty minutes after tracer injection (300 μl, 5–25 MBq) the rats ($n = 3$ in each group) were decapitated and seven brain regions (olfactory tuberli, hypothalamus, cerebellum, hippocampus, corpus striatum, thalamus, frontal cortex, cortex) were rapidly dissected out, weighed and the radioactive content was measured. For blocking studies paroxetine (3 mg/kg) dissolved in saline was injected 15 min prior to tracer injection. ROI to cerebellum ratios were determined as [ROI(cpm)/ROI(mg)]/[cerebellum(cpm)/cerebellum(mg)].

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