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Synthesis and Biological Evaluation of Novel Carbon-11-Labelled Analogues of Citalopram as Potential Radioligands for the Serotonin Transporter

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Abstract—Three serotonin reuptake inhibitors where the 5-cyano group in citalopram [1-(3-dimethylamino-propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile (**1**)] was replaced with a methyl, acetyl and piperidinyl carbonyl group, respectively, were synthesized. In a Stille reaction applying [^{11}C]methyl iodide the labelled compound [5-methyl-^{11}C]{3-[1-(4-fluorophenyl)-5-methyl-1,3-dihydroisobenzofuran-1-yl]-propyl}-dimethylamine (**[^{11}C]-2**) was synthesized in 60–90% radiochemical yield. [5-carbonyl-^{11}C]{1-[1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-yl]-1-piperidin-1-yl-methanone} (**[^{11}C]-3**) was synthesized in 62% radiochemical yield in a palladium mediated cross-coupling reaction utilizing [^{11}C]carbon monoxide. The specific activity of **[^{11}C]-2** was highly dependent on whether the corresponding trimethyltin or tributyltin precursor was applied. In ex vivo rodent studies compound **[^{11}C]-2** exhibited a good blood–brain barrier (BBB) penetration whereas **[^{11}C]-3** did not. The brain distribution of **[^{11}C]-2** was investigated in a non-human primate using PET. There was a rapid uptake of radioactivity into the brain. Accumulation of the radiotracer was in agreement with the known distribution of serotonin transporters. The maximal thalamus to cerebellum ratio of 1.3 was reached after 85 min and the specific binding was partly blocked after pre-treatment with citalopram. Thus, **[^{11}C]-2** does not exhibit appropriate properties as radioligand for visualization of the serotonin transporter in vivo.

Introduction

Development of radiotracers for in vivo visualization of the serotonin transporter (SERT) using positron emission tomography (PET) and single photon emission computed tomography (SPECT) has been a challenge for many years.[1,2] Even though candidate tracers possess high affinity and selectivity for the SERT most of them have not proven suitable for in vivo imaging.[3,4] At present, only a few radiolabelled ligands have been successfully used for in vivo studies of the SERT. [^{123}I]β-CIT[5,6] displays high affinity for the SERT and the dopamine transporter (DAT) as well as moderate affinity for the noradrenaline transporter (NAT) and has frequently been applied for SPECT studies of the DAT in humans.[7] Despite the non-selective nature of [^{123}I]β-CIT, a reduced SERT availability in drug-free depressed patients has been reported.[8] Furthermore, blockade of the SERT in depressed patients treated with the selective serotonin reuptake inhibitors (SSRI's) citalopram[9] or fluoxetine[10] has been demonstrated. Recently, several ^{123}I-labelled ligands based on the diaryl sulfide structure

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(e.g., [^{123}I]ADAM) with high affinity and selectivity for SERT compared to DAT and NAT, have been reported to be promising ligands for the in vivo imaging of the SERT in the non-human primate and human brain using SPECT.[11–15]

Until recently [^{11}C](+)McN5652[16] has been the only available ligand for PET studies of the SERT with a sufficient target-to-background ratio in humans.[17,18] [^{11}C]McN5652 suffers, however, from a high degree of non-specific binding and slow equilibration rate which complicates quantification. Two new diaryl sulfide derivatives, [^{11}C]DASB[19,20] and [^{11}C]MADAM,[21] have recently been reported to be promising PET ligands for the human SERT.[22–24] A comparison of [^{11}C]DASB and [^{11}C](+)McN 5652 have shown that [^{11}C]DASB is superior in terms of specific binding signal and clearance rate from the brain.[25] Furthermore, [^{11}C]DASB has been successfully applied in a study of SERT occupancy in depressed patients treated with the SSRI's citalopram and paroxetine, respectively.[26]

Escitalopram, the biologically more active enantiomer of citalopram,[27] is the most selective SSRI on the market.[28] Escitalopram displays high affinity and selectivity for the SERT compared to the other monoamine transporters as well as a reasonably low lipophilicity,[27] and does thus fulfils many of the criteria for being a suitable PET ligand.[29] However, *S*-[*N*-methyl-^{11}C]citalopram exhibits a relatively low, but acceptable, target-to-background ratio in ex vivo investigations of the rat brain,[30] whereas only a very low specific signal in human volunteers studied with PET is observed.[31] It was speculated that a high degree of non-specific binding and/or formation of radiolabelled metabolites might be responsible for this finding.

The structure–activity relationship for analogues of citalopram (**1**) has been extensively studied.[32] It appears that relatively large variations in the 5-position are allowed without a subsequent loss of affinity for the SERT or selectivity with respect to the DAT and the NAT. These observations prompted us to explore the possibility of introducing a ^{11}C-labelled substituent in the 5-position using the 5-bromo substituted citalopram analogue **5** as starting material. The radiosynthesis of the corresponding ^{11}C-labelled derivatives was performed in palladium mediated reactions via a Stille cross-coupling reaction with [^{11}C]methyl iodide or in a carbonylation reaction with [^{11}C]carbon monoxide. The structural changes might be associated with improved pharmacokinetics, which might result in improved properties as a PET ligand. We report herein the synthesis and pharmacological evaluation of new citalopram analogues where the 5-cyano group has been replaced with a methyl, an acetyl or a 1-piperidinyl carbonyl group. The corresponding radiolabelled compounds were subsequently evaluated in vitro and in vivo to validate their potential as PET ligands.

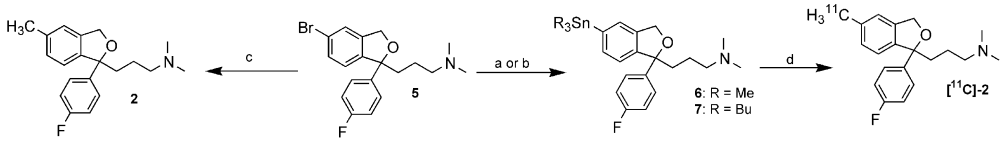
Results and Discussion

Chemistry and radiochemistry

The analogue of citalopram **2**, where the cyano group is replaced with a methyl group, was obtained in a Stille reaction.[33–35] The corresponding 5-bromo derivative **5**[32] was treated with tetramethyltin and a catalytic amount of tetrakis(triphenylphosphine)palladium(0) in DMF, as outlined in Scheme 1.[36–40] The reaction proceeded smoothly in a sealed tube at 100 °C, and **2** was obtained in 78% yield.

The radiosynthesis of **[^{11}C]-2** was performed in analogy with a recently described radiochemical variant of the Stille reaction using [^{11}C]methyl iodide as electrophile.[41] The reaction conditions have been optimized for radiolabelling purposes, where fast and efficient reactions under mild conditions, are required.[42–44] Two precursors, trimethylstannane **6** and tributylstannane **7** were prepared for the radiosynthesis of **[^{11}C]-2**. The trimethylstannane **6** was obtained in 53% yield from the corresponding 5-bromo derivative **5** by lithiation followed by addition of trimethyltin chloride in dry THF whereas the tributylstannane **7** was obtained in 55% yield in a palladium catalyzed cross-coupling reaction using hexabutylditin and a catalytic amount of tetrakis(triphenylphosphine)palladium(0) in toluene.[45]

The ^{11}C-labelled product **[^{11}C]-2** was synthesized from either of the two stannanes **6** or **7**. [^{11}C]Methyl iodide, obtained from the reduction of [^{11}C]carbon dioxide, was trapped in a vessel containing tris(dibenzylideneacetone)dipalladium(0) and tri-*o*-tolylphosphine dissolved in DMF. After trapping, the radioactive solution was transferred to a second vial. The second vial contained a freshly prepared solution of potassium carbonate, copper(I)chloride and the trialkyltin precursor (**6** or **7**) in DMF. The vial was heated to 60 °C for 5 min before purification on a semi-preparative HPLC column. [^{11}C]Methyl iodide and **[^{11}C]-2** eluted after 5.5 and 8 min, respectively, and were separated. **[^{11}C]-2** was synthesized within 45 min in 65–90% decay-corrected isolated radiochemical yield calculated from the amount of trapped [^{11}C]methyl iodide. The radiochemical purity was >98% determined by analytical HPLC. After for-



Scheme 1. Reaction conditions (a) **6**: n-BuLi, Me_3SnCl, THF, −50 to −5 °C, 1 h, 53%, (b) **7**: Bu_6Sn_2, 5 mol% $Pd(PPh_3)_4$, toluene, reflux, 18 h, 55%, (c) $(CH_3)_4Sn$, $Pd(PPh_3)_4$, DMF, 100 °C, 18 h, 78%, (d) [^{11}C]CH_3I, Pd_2dba_3:P(*o*-Tol)$_3$ (1:4), K_2CO_3, CuCl, DMF, 60 °C, 5 min. Preparation of **2** and organotin derivatives **6** and **7** applied as precursors in the radiosynthesis of **[^{11}C]-2**.

mulation in phosphate buffer and sterile filtration the purified radioligand **[^{11}C]-2** was used for biological studies.

We found that the specific activity of **[^{11}C]-2** was strongly dependent on the choice of precursor. Thus, under similar irradiations conditions (~5 μAh), the trimethyltin derivative **6** resulted in very low specific activities of **[^{11}C]-2**, whereas compound **[^{11}C]-2** obtained from the tributyltin derivative **7** resulted in significantly higher specific activities (Table 1).

To the best of our knowledge, this is the first example of a direct comparison between a trimethyltin derivative and a tributyltin derivative applied as precursors in the radiochemical variant of the Stille reaction with [^{11}C]methyl iodide showing that the former result in lower specific activity.

The specific activities of previously reported radiolabelled compounds synthesized in a Stille reaction with [^{11}C]methyl iodide and the corresponding trimethyltin derivatives varies. A relatively high specific activity (50 GBq/μmol) of a ^{11}C-labelled derivative of A-85380[46] was reported, whereas a very low specific activity (<0.8 GBq/μmol) was observed for [^{11}C]FMAU.[47] The Stille reaction between methyl iodide and a large excess (40 equivalent) of trimethylphenylstannane has been shown to result in >100% yield of toluene.[43] The mechanism for the formation of low specific activity has not been fully clarified. However, transfer of a methyl group in the trimethyltin moiety was suggested.[43,47]

In the synthesis of **[^{11}C]-2**, transfer of one of the methyl groups in the trimethyltin derivative **6** will contribute to the amount of unlabelled product and consequently result in low specific activity.

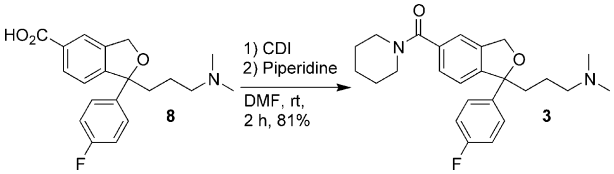
To ensure high specific activity, only the tributyltin derivative **7** was used as precursor for radioligands applied in biological experiments.

The 1-piperidinyl carbonyl analogue **3** was synthesized from the carboxylic acid derivative **8**. Treatment of **8** with 1,1′-carbonyldiimidazole (CDI) and piperidine in DMF gave amide **3** in 81% yield (Scheme 2).

Table 1. The choice of trialkyltin precursor strongly affects the specific activity

Tin precursor	Specific activity of **[^{11}C]-2**[a]
6 (R = Me)	0.1–2.5 GBq/μmol (n = 6)
7 (R = Bu)	10–35 GBq/μmol (n = 5)

[a]Specific activities are corrected to 1 h after EOB.



Scheme 2. Synthesis of amide derivative **3**.

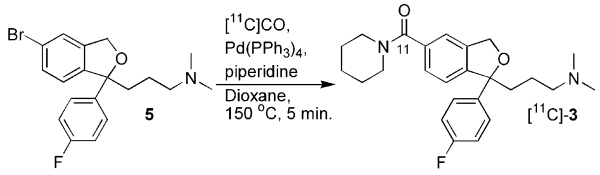
The synthesis of **[^{11}C]-3** was investigated using [^{11}C]carbon monoxide since this approach recently has been demonstrated to be very efficient in the synthesis of ^{11}C-labelled amides,[48] imides,[49] carboxylic acids[50] and hydrazides.[51] The ^{11}C-labelled amide **[^{11}C]-3** was synthesized from the corresponding bromide **5** in a palladium mediated reaction with [^{11}C]carbon monoxide and piperidine. Variations in precursor as well as amine concentrations were investigated to optimize yield and reliability. A high amine concentration was necessary for the reaction to be robust and to proceed in good to high yield (Scheme 3).

The crude reaction mixture of **[^{11}C]-3** was obtained in a pre-evacuated 2-mL vial. Based on the amount of radioactivity in the crude mixture before and after flushing the vial with air the trapping efficiency of [^{11}C]carbon monoxide was determined to be 95–98% in all cases. The ^{11}C-labelled amide derivative **[^{11}C]-3** was synthesized and purified within 40 min in 16–62% decay-corrected radiochemical yield calculated from the amount of trapped [^{11}C]carbon monoxide. In a typical experiment, approximately 500 MBq of **[^{11}C]-3** was collected after HPLC purification. The identity of the final product was confirmed by LC–MS. The purified product was formulated in phosphate buffer and used for initial biological studies in vitro.

The acetyl derivative **4** was synthesized by addition of methylmagnesium bromide to citalopram (**1**) followed by acidic hydrolysis (Scheme 4).

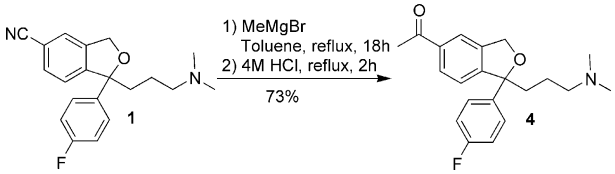
The synthesis of **[^{11}C]-4** via palladium mediated cross-coupling reactions were investigated.

Palladium-mediated synthesis of simple ^{11}C-labelled ketones with [^{11}C]carbon monoxide from the corre-



5 (μmol)	$Pd(PPh_3)_4$ (μmol)	Piperidine (μmol)	Yield (%)
8	1.7	44	< 5
12	1.7	250	16
26	1.7	250	62

Scheme 3. Radiosynthesis of the ^{11}C-labelled amide **[^{11}C]-3**.



Scheme 4. Synthesis of acetyl derivative **4** from citalopram (**1**).

sponding halides have been reported.[52–55] The reaction mixtures obtained from the reaction of trialkyltin derivative **7**, methyl iodide, [^{11}C]carbon monoxide and a palladium(0) catalyst in DMF were complicated and not purified. Similar negative results were obtained when the reaction between bromide **5**, tetramethyltin, [^{11}C]carbon monoxide and a palladium(0) catalyst was tested. Despite several attempts **[^{11}C]-4** was not successfully purified.

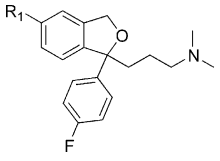
Pharmacology

The IC_{50} values of the new citalopram derivatives **2**, **3** and **4** for the monoamine transporters are shown in Table 2. The derivatives **2**, **3** and **4** exhibit high to medium affinity for the SERT and an acceptable selectivity compared to DAT and NAT. The LogP values are in the upper end of what is usually considered suitable for a PET ligand, which is in the range 2-3.[1]

The radioligand **[^{11}C]-3** showed a very low penetration of the blood–brain barrier (BBB) in tissue distribution studies in the rat (data not shown). The low BBB penetration of **[^{11}C]-3** was somewhat unexpected due to the relative high lipophilicity of **[^{11}C]-3** (Table 2). It is possible that **[^{11}C]-3** is a substrate for specific efflux systems such as P-glycoprotein expressed in the BBB, as has been the case for other lipophilic drugs.[56,57] In contrast, **[^{11}C]-2** readily penetrated the BBB. The tissue-to-cerebellum SUV ratios in the cortex and in the thalamus were 1.3 and 1.5, respectively, 35 min after injection. **[^{11}C]-2** was further studied with autoradiography and in vivo as a potential candidate for visualization of the SERT.

The binding of **[^{11}C]-2** to SERT examined by frozen section autoradiography showed an increased uptake of the radioligand in the thalamus and in the cortex in both rat and monkey brain slices. Non-specific binding, as defined by incubation of the brain sections with 1 μM citalopram before addition of the radioligand, was high, as can be seen in the saturation plot (Fig. 1). Approximately 40% of the binding at 1 nM constituted of specific binding in both species. An incubation time of 20–40 min seems appropriate for frozen section autoradiography with **[^{11}C]-2** (data not shown). The K_d and B_{max} values obtained in the study are presented in Table 3.

Table 2. Lipophilicity and 5-HT, DA and NA uptake inhibition of citalopram (**1**) and derivative **2**, **3** and **4**



Compd	R_1	Inhibition of [^{3}H]amine uptake, IC_{50} (nM)			
		5-HT	DA	NA	LogP[a]
Citalopram **1**	CN	1.8	40,000	6100	3.6
2	CH_3	3.5	720	790	4.6
3	$CONC_5H_{10}$	41	Not tested	1600	4.5
4	$COCH_3$	6.4	1000	320	3.8

5-HT, serotonin; DA, dopamine; NA, noradrenaline.
[a]Calculated with QikProp.[66]

The affinity obtained by autoradiography is lower than the affinities obtained for other SERT tracers in rat brain homogenate, for example [^{3}H]McN5652, $K_d = 0.11$ nM.[29]

The high affinity of **2** to the SERT in rat brain synaptosomes (Table 2) was confirmed with frozen section autoradiography using the labelled compound, which was displaced with unlabelled citalopram.

B_{max} values determined by autoradiography showed somewhat higher values than the reported values determined in brain homogenate (B_{max} in the monkey thalamus has been estimated to 6 pmol/g tissue).[58] Such discrepancies between B_{max} values and to some extend k_d values determined by autoradiography and brain homogenate, respectively, has previously been found.[59] A higher B_{max} value in monkey brain slices compared to rat brain slices is difficult to explain and is not in agreement with the literature.

PET imaging

After intravenous injection in a Rhesus monkey the radioligand **[^{11}C]-2** passed the BBB. PET images showed preferential distribution of radioactivity in the striatum, thalamus, and frontal cortex as early as 20 min after injection. After injection of **[^{11}C]-2**, the radioactive concentrations in regions known to contain high density of serotonergic nerve terminals were compared with cerebellum, a region poor in SERT binding sites.[60–62] The time–activity curves showed a rapid

Table 3. Scatchard plot analysis of [^{11}C]-**2** thalamus binding in frozen section autoradiography[a]

Brain tissue	$K_d \pm$SD (nM)	$B_{max} \pm$SD (fmol/mm^2)	$B_{max} \pm$SD (pmol/g wet tissue)
Rat	9.0±5.5	2.6±1.5	104±60
Monkey	22±15.3	4.8±2.7	192±108

[a]K_d and B_{max} values are means (±SD) of **4** independent experiments. B_{max} values were recalculated to picomole per gram wet tissue, taking the section thickness (25 μm) into account.

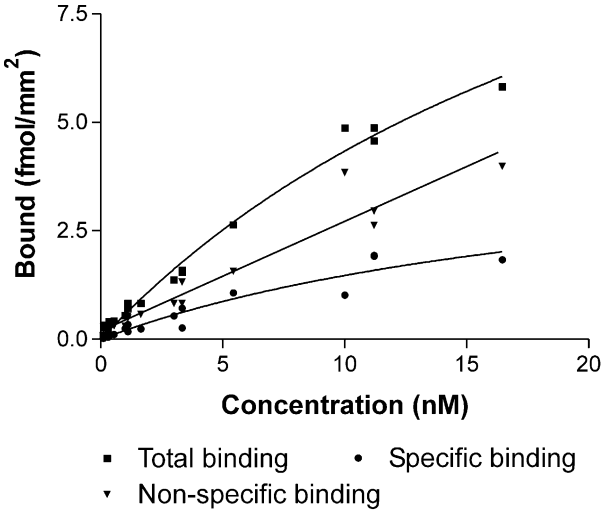


Figure 1. Saturation curve of **[^{11}C]-2** binding in frozen sections of Rhesus monkey brain (thalamus).

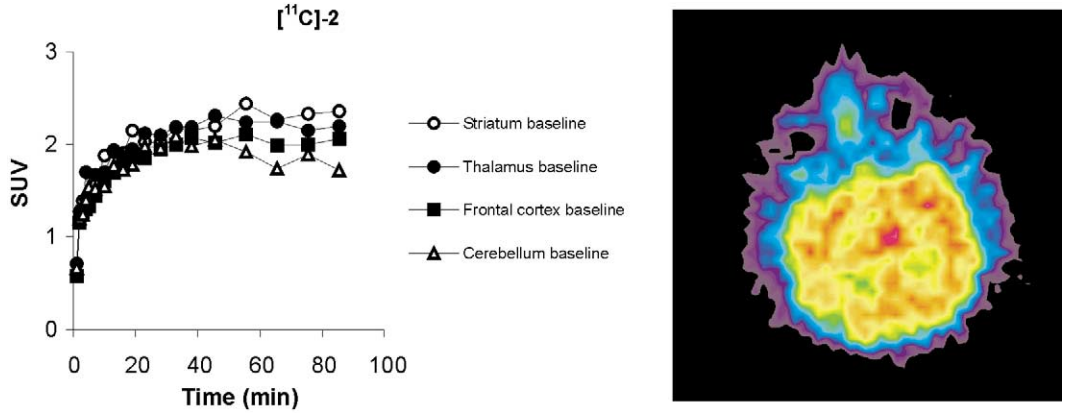


Figure 2. Time–activity curves for **[^{11}C]-2** from the baseline scan and a transverse summed image (45–85 min) for **[^{11}C]-2**. The image is at the level of striatum.

accumulation in the striatum, thalamus, and frontal cortex. In the cerebellum, however, the radioactivity was only slightly lower (Fig. 2). As an index of specificity, 85 min post-injection, the highest uptake was in striatum (SUV 2.4) and the lowest in cerebellum (SUV 1.7).

Radioactivity concentration ratios for the different brain regions relative to that in the cerebellum were calculated as indexes of SERT binding. Thalamus-, striatum-, frontal cortex-to-cerebellum ratios of SUV at 85 min post-injection were 1.3, 1.4, and 1.2, respectively.

Saturation of SERT binding sites was investigated in two conditions. Citalopram was infused intravenously over 15 min in doses of 1 and 4 mg/kg, respectively. The tracer was injected 30 min after the citalopram infusion was stopped and the distribution of **[^{11}C]-2** was studied. After intravenous injection of citalopram, the uptake was reduced in all SERT-rich tissues but also in cerebellum (Fig. 3).

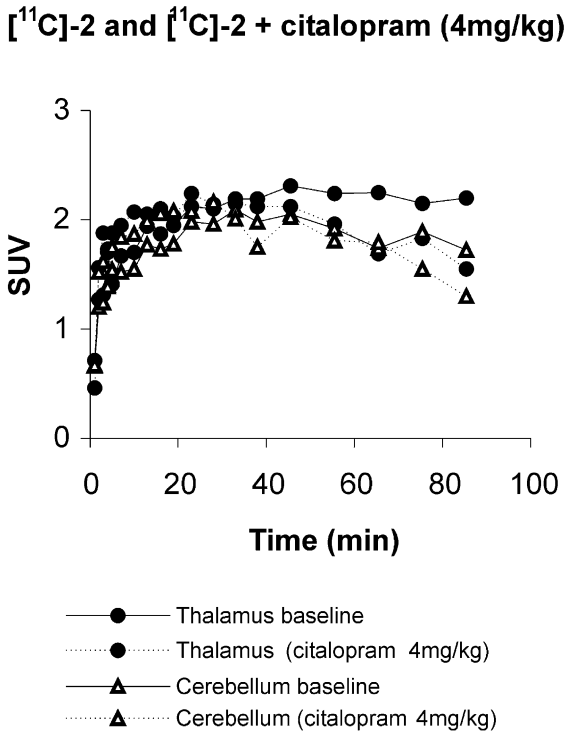


Figure 3. Time–activity curves for **[^{11}C]-2** from the baseline scan (solid lines) and after pre-treatment with 4 mg/kg citalopram (dotted lines).

The result of the PET study shows only limited specific binding of **[^{11}C]-2** to the SERT in the living Rhesus monkey brain. The radiotracer shows preferential uptake in the SERT-rich regions and the rank order correlates well with known regional SERT densities in the primate brain.[63] The tissue-to-cerebellum ratio is usually used as an estimate of the total-to-non-specific binding as cerebellum has low binding. However, from the present study, it is difficult to determine whether the relatively low tissue-to-cerebellum ratios are due to a high uptake in the cerebellum or due to a low uptake in the target regions. The specificity of **[^{11}C]-2** binding to the SERT was suggested by a slight decrease in brain radioactivity induced by citalopram blockade in SERT-rich regions. However, a reduction in specific binding using the cerebellum as a reference tissue was not evident, since also uptake in the cerebellum was affected.

Conclusion

Three new citalopram derivatives (**2**, **3** and **4**) were synthesized. Compounds **2** and **3** were successfully labelled with ^{11}C in palladium-mediated reactions using [^{11}C]methyl iodide or [^{11}C]carbon monoxide, respectively. In contrast to **[^{11}C]-3**, **[^{11}C]-2** readily penetrated the BBB and was studied in vivo as a potential candidate for visualization of the SERT. According to autoradiography studies, approximately 40% of the total **[^{11}C]-2** binding constituted of specific binding. PET imaging demonstrated a regional distribution of **[^{11}C]-2** in the Rhesus monkey in agreement with the known distribution of serotonin transporters. However, with target-to-background ratios of only 1.3-1.4, **[^{11}C]-2** does not seem to be an appropriate alternative to other SERT specific tracers such as [^{11}C]McN5652 and [^{11}C]DASB.

Experimental

General. NMR spectra were obtained using a 200 or 500 MHz spectrometer. 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 and m = multiplet. 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 a Micromass VG Quattro with electrospray ionization or a API 150EX LC/MS system from Applied Biosystems. Melting points were obtained on a Büchi B-540 apparatus and are uncorrected. HPLC was performed with a Beckman 126 gradient pump and a Beckman 166 variable wavelength UV-detector in series with a β^+-flow detector.

All reactions were carried out in oven-dried reaction flasks and freshly distilled solvents were used. All non-radioactive products were purified using flash chromatography (silica gel, 230–400 mesh). Reagents were purchased from Aldrich Chemical Co., except from tetrakis(triphenylphosphine)palladium(0), which was purchased from Lancaster Synthesis Limited, and used without further purification.

With a Scanditronix MC-17 at Uppsala University PET Center [^{11}C]carbon dioxide was produced in the ^{14}N(p,α)^{11}C reaction in a gas target containing nitrogen (AGA nitrogen 6.0) with 0.05% oxygen (AGA oxygen 4.8) with 16 MeV protons. [^{11}C]Methyl iodide was synthesized from [^{11}C]carbon dioxide using $LiAlH_4$ and HI.[64] [^{11}C]Carbon monoxide was produced by reduction of [^{11}C]carbon dioxide in a zinc oven and applied for synthesis in a semi-automatic system as previously reported.[48,53]

1-(3-Dimethylamino - propyl) - 1 - (4 - fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile (**1**), {3-[5-bromo-1-(4-fluorophenyl) - 1,3 - dihydroisobenzofuran - 1 - yl]-propyl}-dimethylamine (**5**) and 1-(3-dimethylamino-propyl)-1-(4-fluorophenyl) - 1,3 - dihydroisobenzofuran-5-carboxylic acid (**8**) were donated by H. Lundbeck A/S.

Chemistry and Radiochemistry

{3-[1-(4-Fluorophenyl) - 5 - methyl - 1,3 - dihydroisobenzofuran-1-yl]-propyl}-dimethylamine (2). A solution of {3-[5-bromo-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl]-propyl}-dimethylamine (**5**) (1.56 g, 4.1 mmol) in DMF (20 mL) was flushed with argon for 15 min, then tetrakis(triphenylphosphine)palladium(0) (239 mg, 0.2 mmol, 5 mol%) and Me_4Sn (0.67 mL, 5.0 mmol) was added. The resulting mixture was heated at 100 °C for 17 h in a sealed tube under argon atmosphere. The reaction mixture was allowed to cool to room temperature and Et_2O (200 mL) and saturated $NaHCO_3$ (200 mL) was added. The layers were separated and the aqueous layer was extracted with Et_2O (2×200 mL). The combined organic phases were dried ($MgSO_4$) and the solvents evaporated in vacuo. Purification by flash chromatography (EtOAc/pentane 1:4 + 2.5% Et_3N) yielded 1.0 g (78%) of **2** as a colourless oil. The oxalate was crystallized from acetone. Anal. ($C_{20}H_{24}FNO\cdot C_2H_2O_4$) calcd C: 65.49, H: 6.50, N: 3.47. Found C: 64.81, H: 6.48, N: 3.40. Mp = 131–132 °C.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.42 (m, 1H), 1.52 (m, 1H), 2.16 (m, 2H), 2.30 (s, 3H), 2.63 (s, 6H), 2.97 (t, J = 8.0 Hz, 2H), 5.07 (d, J_{AB} = 12.8 Hz, 1H), 5.13 (d, J_{AB} = 12.8 Hz, 1H), 7.13 (m, 4H), 7.36 (d, J = 8.0 Hz, 1H), 7.55 (m, 2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 19.1, 20.7, 37.4, 42.0, 56.5, 71.2, 89.8, 114.7, 114.9, 121.4, 121.6, 126.7, 126.7, 128.2, 137.0, 138.6, 140.8, 141.4, 161.0 (d, J_{C-F} = 239 Hz), 164.6. LC–MS: m/z 314 [$M^+ + 1$].

1-[1-(3-Dimethylaminopropyl) - 1 - (4 - fluorophenyl)-1,3-dihydroisobenzofuran-5-yl]-1-piperidin-1-yl-methanone (3). A solution of 1-(3-dimethylamino-propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxylic acid (**8**) (475 mg, 1.38 mmol) in DMF (25 mL) was flushed with argon for 15 min. 1,1′-carbonyldiimidazole (1.12 g, 6.92 mmol) was added and the resulting mixture was stirred for 40 min before piperidine (275 μL, 2.76 mmol) was added. The mixture was stirred at room temperature for further 1 h before saturated $NaHCO_3$ (50 mL) was added and the mixture was extracted with Et_2O (3×75 mL). The combined organic phases were dried ($MgSO_4$) and the solvents were evaporated in vacuo yielding 460 mg (81%) of **3** as yellow oil with purity > 91% (HPLC-UV). The oxalate was crystallized from THF. Anal. ($C_{25}H_{31}FN_2O_2\cdot 1\frac{1}{2}\ C_2H_2O_4$) calcd C: 61.53, H: 6.45, N: 5.13. Found C: 61.64, H: 6.93, N: 5.22. Mp = 87–90 °C.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.35–1.65 (m, 8H), 2.20 (m, 2H), 2.63 (s, 6H), 2.98 (t, J = 8.0 Hz, 2H), 3.22 (broad s, 2H), 3.56 (broad s, 2H), 5.14 (d, J_{AB} = 13.0 Hz, 1H), 5.20 (d, J_{AB} = 13.0 Hz, 1H), 7.16 (t, J = 8.7 Hz, 2H), 7.29 (m, 2H), 7.59 (m, 3H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 19.1, 23.9, 25.1, 25.8, 37.3, 42.0, 42.2, 47.9, 56.5, 71.3, 89.9, 114.9, 115.1, 119.8, 121.7, 126.1, 126.8, 136.1, 136.9, 138.6, 140.8, 144.6, 161.1 (d, J_{C-F} = 251 Hz), 164.5, 168.5. LC–MS: m/z 411 [$M^+ + 1$].

1-[1-(3-Dimethylamino-propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-yl]-ethanone (4). Methylmagnesium bromide (0.15 mL, 0.48 mmol) was added slowly to a stirred solution of citalopram (**1**) (140 mg, 0.43 mmol) in dry toluene (5 mL). The resulting mixture was heated under reflux overnight. After cooling to room temperature, the mixture was poured into ice-cold 1 M aqueous Na_2CO_3 (20 mL) and extracted with EtOAc (2×25 mL). The combined organic layers were washed with 4 M aqueous HCl (2×15 mL). The combined aqu-

eous phases were heated under reflux for 2 h. After cooling to room temperature the aqueous phase was made alkaline with 2 M aqueous NaOH and extracted with EtOAc (2×50 mL). The combined organic phases were dried (Na_2SO_4) and the solvent was removed in vacuo. Purification was performed by flash chromatography (EtOAc/pentane 1:1 + 2.5% Et_3N) yielding 108 mg (73%) of **4** as a colourless oil. The oxalate was crystallized from acetone.

Anal. ($C_{21}H_{24}FNO_2.C_2H_2O_4$) calcd C: 64.02, H: 6.07, N: 3.25. Found C: 63.84, H: 6.18, N: 3.24. Mp = 143–146 °C.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.43 (m, 1H), 1.52 (m, 1H), 2.23 (t, J = 7.8 Hz, 2H), 2.57 (s, 3H), 2.62 (s, 6H), 2.98 (t, 8.0 Hz, 2H), 5.17 (d, J_{AB} = 13.0 Hz, 1H), 5.24 (d, J_{AB} = 13.0 Hz, 1H), 7.17 (t, J = 9.0 Hz, 2H), 7.59 (m, 2H), 7.65 (d, J = 8.0 Hz, 1H), 7.91 (m, 2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 19.1, 26.8, 37.0, 42.0, 56.4, 71.2, 90.0, 114.9, 115.1, 121.4, 122.0, 126.8, 126.9, 128.1, 136.6, 139.1, 140.4, 148.4, 161.2 (d, J_{C-F} = 239 Hz), 164.5, 197.3. LC–MS: m/z 342 [M^+ + 1].

{3-[(4-Fluorophenyl)-5-trimethylstannanyl-1,3-dihydroisobenzofuran-1-yl]-propyl}-dimethylamine (6). A solution of {3-[5-bromo - 1 - (4 - fluorophenyl)-1,3-dihydroisobenzofuran-1-yl]-propyl}-dimethylamine (**5**) (170 mg, 0.45 mmol) in dry THF (4 mL) was cooled to −40 °C under argon, and n-BuLi (0.43 mL, 0.54 mmol) was added slowly. During 1 h, the temperature was increased to −10 °C. Trimethyltin chloride (180 mg, 0.9 mmol) dissolved in dry THF (0.5 mL) was added to the yellow solution. After addition, the yellow color disappeared. The reaction mixture was allowed to heat to room temperature and stirred for further 45 min. The reaction was quenched with 1 M aqueous Na_2CO_3 (20 mL) and extracted with Et_2O (2×25 mL). The combined organic layers were concentrated in vacuo and the remaining oil re-dissolved in ether (25 mL). The organic phase was washed with 20% aqueous KF (10 mL) and the layers separated. The organic phase was dried (Na_2SO_4) and the solvent was evaporated in vacuo. Flash chromatography (EtOAc/pentane 1:4 + 2.5% Et_3N) yielded 110 mg (53%) of **6** as an yellow oil.

^{1}H NMR (200 MHz, $CDCl_3$) δ 0.3 (s, 9H), 1.3–1.6 (m, 3H), 2.15 (s, 6H), 2.25 (m, 3H), 5.2 (s, 2H), 7.0 (t, J = 7.0 Hz, 2H), 7.25–7.55 (m, 5H).

^{13}C NMR (50 MHz, $CDCl_3$) δ−9.4, 22.3, 39.4, 45.4, 59.7, 71.9, 90.9, 114.7, 115.1, 121.4, 126.6, 126.8, 128.3, 134.6, 138.6, 141.2, 141.4, 144.2, 161.7 (d, J_{C-F} = 245 Hz). GC-MS: m/z 463[M^+].

{3-[(4-Fluorophenyl)-5-tributylstannanyl-1,3-dihydroisobenzofuran-1-yl]-propyl}-dimethylamine (7). A solution of {3-[5-bromo - 1 - (4 - fluorophenyl) - 1,3 - dihydroisobenzofuran-1-yl]-propyl}-dimethylamine (**5**) (200 mg, 0.34 mmol), hexabutylditin (0.51 mL, 1 mmol), tetrakis(triphenylphosphine)palladium(0) (30 mg, 26 μmol, 7.6 mol%) and toluene (10 mL) was heated under reflux overnight. After cooling to room temperature the solvent was removed in vacuo. The remaining residue was re-dissolved in a mixture of ether (20 mL) and 20% aqueous KF (20 mL). After vigorous stirring for 1 h, the precipitate was filtered off and the filtrate was extracted with ether (3×20 mL). The combined organic phases were dried (Na_2SO_4) and the solvents evaporated in vacuo. Flash chromatography (EtOAc/pentane 1:6 + 2.5% Et_3N) yielded 170 mg (55%) of **7** as colourless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.9 (t, J = 6.2 Hz, 9H), 1.1 (m, 6H), 1.3 (m, 7H), 1.5 (m, 7H), 2.2 (m, 10H), 5.2 (s, 2H), 7.0 (t, J = 7.5 Hz, 2H), 7.3–7.5 (m, 5H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 9.6, 13.6, 22.3, 27.3, 29.0, 39.5, 45.4, 59.7, 71.9, 90.9, 114.6, 115.0, 121.2, 126.7, 128.7, 135.1, 138.4, 141.1, 143.8, 161.7 (d, J_{C-F} = 251 Hz). HRMS: $C_{31}H_{48}NOF^{116}Sn$, calc. 586.2816, found 586.2796 [M^+ + 1].

[5-methyl-^{11}C]{3-[1-(4-Fluorophenyl)-5-methyl-1,3-dihydroisobenzofuran-1-yl]-propyl}-dimethylamine ([^{11}C]-2). Tris(dibenzylideneacetone)dipalladium(0) - chloroform adduct (1 mg, 1 μmol) and tri-o-tolylphosphine (0.9 mg, 4 μmol) was transferred to a pre-dried 1-mL vial. The vial was capped and flushed with nitrogen before DMF (300 μL) was added. [^{11}C]Methyl iodide was trapped at room temperature in this vial. After trapping, the radioactive mixture was transferred to a second vial containing 3–5 mg precursor (**6** or **7**), CuCl (1.2 mg, 12 μmol), K_2CO_3 (1.4 mg, 10 μmol) and DMF (100 μL). The resulting mixture was heated at 60 °C for 5 min and added to a solid phase extraction cartridge (SPE, C-18, EC, 100 mg). The product was eluted with CH_3CN (1 mL). The eluate was diluted with 1 mL 50 nM ammonium formate before injection on a semi-preparative HPLC column. The product containing fraction was collected, evaporated in vacuo and the residue was formulated in phosphate buffer and sterile filtrated. **[^{11}C]-2** was isolated in 65–90% decay corrected radiochemical yield calculated from trapped [^{11}C]methyl iodide. The total synthesis time was 45 min. The radiochemical purity (>98%) was determined by analytical HPLC. The identity of the final product was determined by co-elution with the reference substance **2** and by LC–MS. The specific activity of **[^{11}C]-2** was determined by comparing the amount of unlabelled substance with a standard curve.

HPLC conditions: The semi-preparative column was a Jones Chromatography Genesis C_{18}, 7 μm, 250×10 mm (id). Solvents were A: 50 mM ammonium formate pH 3.5 and B: CH_3CN/H_2O (50/7). A 50:50 mixture (A:B) eluted isocratic with a flow rate of 5 mL/min. The analytical column was a Jones Chromatography Genesis C_8, 4 μ, 150×4.6 mm (id). Solvents were A: 50 mM ammonium formate pH 3.5 and B: CH_3CN/H_2O (50/7). A 60:40 mixture (A/B) eluted isocratic with a flow rate of 1.5 mL/min.

[5-Carbonyl-^{11}C]{1-[1-(3-dimethylaminopropyl) - 1 - (4 - fluorophenyl)-1,3-dihydroisobenzofuran-5-yl]-1-piperidin-1-yl-methanone} ([^{11}C]-3). {3-[5-Bromo - 1 - (4 - fluorophenyl)-1,3-dihydroisobenzofuran-1-yl]-propyl}-dime-

thylamine (**5**) (4 mg, 10 μmol) was dissolved in 1,4-dioxan (300 μL) and transferred to a nitrogen-flushed vial containing tetrakis(triphenylphosphine)palladium(0) (2 mg, 1.7 μmol). The mixture was left for 2 min before piperidine (25 μL, 250 μmol) was added. The resulting mixture was transferred to a micro autoclave together with [^{11}C]carbon monoxide and pressurized to approximately 35 MPa. After heating, the reaction chamber at 150 °C for 5 min the reaction mixture was emptied into a pre-evacuated 2-mL vial. The crude reaction mixture was diluted with mobile phase (2 mL) prior to injection on semi-preparative HPLC. The isolated decay corrected radiochemical yield of **[^{11}C]-3** was 62% calculated from trapped [^{11}C]carbon monoxide. The total synthesis time was 40 min. The identity and radiochemical purity (>98%) was determined by analytical HPLC.

HPLC conditions: The semi-preparative column was a Jones Chromatography Genesis C_{18}, 4 μm, 250×10 mm(id). Solvents were A: 50 mM ammonium formate pH 3.5 and B: CH_3CN/H_2O (50/7). A 40:60 mixture (A:B) eluted isocratic with a 5 mL/min flow rate. The analytical column was a Jones Chromatography Genesis C_8, 150×4.6 mm (id). Solvents were A: 50 mM ammonium formate pH 3.5 and B: CH_3CN/H_2O (50/7). A 60:40 mixture (A/B) eluted isocratic with a flow rate of 1.5 mL/min flow rate.

Pharmacology. Inhibition of serotonin (5-HT), dopamine (DA) and noradrenaline (NA) uptake in vitro was measured in rat brain synaptosomes using a modification of a previously described protocol.[65] In brief, tritium-labelled amines were used to measure uptake into synaptosomes from whole rat brain (excluding cerebellum) ([^{3}H]serotonin), rat striatal synaptosomes ([^{3}H]dopamine) or into rat cortical synaptosomes ([^{3}H]noradrenalin). The dissected rat brain regions were homogenized in 0.40 M sucrose supplemented with 1 mM nialamid and centrifuged at 1000*g* for 10 min. The supernatants were further centrifuged for 30 min at 20.000*g*, 4 °C and resuspended in Krebs–Ringer buffer, pH 7.4 supplemented with 0.2 g/L ascorbic acid. Test compounds and membranes were added in 96-well plates and the incubation was started by adding either 10 nM [^{3}H]serotonin, 12.5 nM [^{3}H]dopamine or 10 nM [^{3}H]noradrenaline for 15 min at 37 °C except for [^{3}H]dopamine uptake (5 min at 20 °C). Non-specific uptake was defined as uptake in the presence of 10 μM citalopram, 100 μM benztropin or 20 μM talsupram, respectively and accounted for 5–10% of total uptake. Samples were filtered over Whatman GF/C filters and the IC_{50} values were estimated using non-linear regression analysis from at least eight points dose–response curves with triplicate determinations. LogP values were calculated with QikProp 2.0.[66]

In biodistribution studies rats (n=4–5, Sprague–Dawley, 275–458 g) received 5–10 MBq of either **[^{11}C]-2** or **[^{11}C]-3** via the tail vein. The animals were sacrificed 35 min after tracer injection and thalamus, cortex and cerebellum was rapidly removed. The tissue samples were weighed and transferred to tubes and the radioactivity was measured in a γ-counter [Well-type, NaI(Tl) scintillation counter] for about 30 s per sample. The radioactivity concentration in the tissue was corrected for physical decay and normalized to the total amount of injected radioactivity and for the body weight of the rat. Standardized uptake value (SUV), defined as radioactivity concentration in tissue divided by the ratio of total administered radioactivity and body weight, was calculated.

Brain slices from male Sprague–Dawley rats or Rhesus monkeys were used in frozen section autoradiography with **[^{11}C]-2**. Coronal sections (25 μm) were cut with a cryostat microtome (SLEE Technik GmbH), mounted on gelatin-coated glass slides, dried at room temperature, and stored at −20 °C until used within 4 weeks. A brain atlas was used to identify the thalamic level of the brain while sectioning. Whole brain sections were pre-incubated in buffer containing Tris–HCl (50 mM), NaCl (120 mM), KCl (5 mM), $CaCl_2$ (2.5 mM) and $MgCl_2$ (1 mM) at pH 7.4 for 10 min at 37 °C. For determining the total binding, the sections were incubated at different concentrations of **[^{11}C]-2** in the same buffer at 37 °C for 40 min. Binding characteristics of **[^{11}C]-2** was studied using tracer concentrations of 0.1–17 nM. Non-specific binding was determined in adjacent sections by pre-incubation with 1 μM citalopram before addition of the radiotracer. In a parallel, the time course of tracer binding was examined with incubation times of 10, 20, 30, 40 and 50 min. After the incubation, sections were washed for 2 min with the incubation buffer three times and dried. The sections exposed phosphor image plates sensitive to positrons for 40 min. The plates were scanned with a laser beam in the image-reading unit of the PhosporImager (Molecular Dynamics). Scanning and image display and analysis were performed by ImageQuant software (Molecular Dynamics). For quantification, calibration standards were prepared for each set of brain sections exposed to the same imaging plate. The standard was a 20-μL drop of **[^{11}C]-2** with known concentration placed on a absorbent paper (Bench-Guard). The average counts per pixel of the background area were subtracted from the average counts of the corresponding region of interest (ROI).

Knowing the concentration and volume of the standard sample, the amount of substance in femtomole substance was calculated. The total counts over the standard allowed the calculation of a calibration factor in counts per femtomole. The signal measured in the target region, thalamus, was given as average counts per pixel. Knowing the pixel size, the value was recalculated to counts per square millimeter and, using a calibration factor, converted into femtomoles per square millimeters. The maximum binding capacity (B_{max}) and affinity constant (K_d) were calculated utilizing linear fits in Scatchard plots.

PET studies. The PET imaging experiment was carried out using a female Rhesus monkey (*Macaca mulatta*) weighing 6.8 kg from the Primate Research Laboratory of Uppsala University. The study was approved by the Animals Ethics Committe of the Uppsala University

(permission C29/1). Anesthesia was induced with 100 mg of intramuscular ketamine (Ketalar®, Parke Davis).

After tracheal intubation, muscle relaxation was achieved with 5 mg/kg/h intravenous atracurium (Tracrium, Wellcome). Supported ventilation was afforded with 30% oxygen in air. Anesthesia was continued with infusion of propofol (10 mg/kg/h). The PET system used was a Hamamatsu SHR-7700 (Hamamatsu Photonics, Japan).

This scanner allows simultaneous acquisition of 31 slices with a slice thickness of 3.7 mm with transaxial resolution of 5.5 mm (3-D mode). Images were reconstructed and ROIs were delineated in the thalamus, striatum, frontal cortex, and cerebellum using a Rhesus monkey brain atlas. Radioligand uptake data were plotted after correction for physical decay and normalization for injected dose of radioactivity and body weight of the monkey.

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