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Synthesis of [^{11}C]RPR-72840A and its Evaluation as a Radioligand for the Serotonin Reuptake Site in Positron Emission Tomography

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Abstract—RPR-72840A, an inhibitor of serotonin reuptake, was labelled with carbon-11. The synthesis of the nonradioactive precursor, which exhibited some unexpected chemistry, and its reaction with [^{11}C]phosgene affording [^{11}C]RPR-72840A are described. Biodistribution studies in rats and PET studies in baboons were conducted to evaluate [11C]RPR-72840A as a tracer for PET imaging of the serotonin reuptake system. © 1997, Elsevier Science Ltd. All rights reserved.

Introduction

The neurotransmitter serotonin, or 5-hydroxytryptamine (5-HT), is implicated in depression and other psychiatric disorders,[1] as well as in Parkinson's and Alzheimer's disease.[2] The 5-HT reuptake system plays an important role in the regulation of the 5-HT concentration in the synaptic cleft and is the target of a large class of antidepressant drugs.

The quantitative imaging of receptor distributions in the living human brain remains one of the major quests in positron emission tomography (PET).[3] Several ligands for the post-synaptic 5-HT receptor have been labelled with short-lived positron-emitting radioisotopes of high specific radioactivity and are currently used in PET,[4] for example [^{18}F]setoperone.[5] For the 5-HT reuptake site less progress has been made in this respect. A considerable number of 5-HT reuptake blockers have been labelled for PET,[6,7] but most of them showed a too high nonspecific binding in vivo. To date only one labelled ligand, [^{11}C](+)McN-5652Z, has been tried in humans[8,9] although it shows considerable nonspecific binding and requires late imaging between 1 and 2 h post-injection and consequently high doses of radioactivity to be administered.

Recently Rhône-Poulenc-Rorer published a series of new indole derivatives displaying strong 5-HT reuptake inhibition.[10] Compound RPR-72840A (**7**, Fig. 1) of this series has an IC_{50} of 1.5 nM against the standard ligand [^{3}H]paroxetine and it also shows a considerable in vivo activity. We selected this compound for labelling with carbon-11 to evaluate it as an in vivo PET tracer as it combines a high affinity and selectivity for the 5-HT reuptake site with a chemical structure compatible with carbon-11 labelling, in this case with [^{11}C]phosgene.

Herein we describe the chemical synthesis of the required nonradioactive precursor (Fig. 1, compound **5**) and its radiochemical conversion into [^{11}C]RPR-72840A (**8**) using [^{11}C]phosgene. The radiolabelled product was tested in rats and in baboons to evaluate its suitability as a PET tracer for 5-HT reuptake.

Results and Discussion

Chemistry

Carbon-11 (half-life 20.3 min) is usually produced with a cyclotron on site. It is available for radiochemistry in the form of a limited number of simple molecules. [^{11}C]phosgene[11] has proven to be a very useful radioactive precursor, not in the least in rapid ring closure reactions of the type presented in this work. The application of [^{11}C]phosgene in the synthesis of **8** required the synthesis of compound **5**, the precursor of **8** in the radiolabelling step. Figure 1 outlines the reactions employed. Our approach to **5** via reductive ring opening of the dihydroperimidine **4** was selected after several alternative routes on model compounds had failed: monoalkylation of 1,8-diaminonaphthalene with 2-piperidinoethanol using Raney nickel and $(t\text{-BuO})_3Al$[12] was unsuccessful. Neither could 2-piperidinoethanol be coupled with 1-amino-8-tosylaminonaphthalene under Mitsunobu conditions.[13] Also 1-(2-bromoethyl)piperidine·HBr failed to react with 1,8-diaminonaphthalene, probably because the former compound is unstable under the basic reaction conditions. For the synthesis of **4** from **1** we chose to make the chloro compound **2** rather than subjecting **3** to a formylmethylation. Nevertheless, we did succeed in reacting the unstable model compound piperidinoacetaldehyde, generated in situ by deprotection of its 1,3-dioxolane,[14] with 1,8-diaminonaphthalene to

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2-piperidinomethyl-1,2-dihydroperimidine (model analogue of **4**) in 66% yield. The ring opening of **4** with diisobutylaluminiumhydride (DIBAL-H) turned out to be remarkably difficult. A very long reaction time (50 h) and a large excess of DIBAL-H (20 equiv) were needed compared to the conditions usually employed in this procedure (e.g. 20 min and 6 equiv).[15] Possibly a chelate formation of an aluminium species with the piperidine nitrogen and a 1,2-dihydroperimidine nitrogen hampers ring opening. We isolated appreciable amounts of side product **6** when less, but still in excess, DIBAL-H was used. The formation and isolation of this unsaturated product is surprising as this reaction constitutes an oxidation of **4** in a reducing DIBAL-H medium. We were able to show by TLC that very soon after DIBAL-H addition **4** is completely converted into **6**, which is then very slowly turned into **5**, most likely via **4**. Perimidines too undergo reductive ring opening with DIBAL-H, via the corresponding 1,2-dihydroperimidines, just as easily as the 1,2-dihydroperimidines themselves.[15] When isolated **6** was subjected to DIBAL-H treatment, we could indeed detect **4** on TLC together with the product **5**. To our knowledge there is no precedence of this type of 'oxidation' with DIBAL-H. We can only speculate about the mechanism which we intend to make the subject of a future publication. Product **5** reacted smoothly in an immediate reaction with no-carrier-added [^{11}C]phosgene as expected, giving **8** in an approximate radiochemical yield of 70% on a 100 mCi (37 GBq) scale and with a high specific radioactivity. Evidence for the chemical identity of [^{11}C]RPR-72840A (**8**) was obtained by comparing the retention time of the radioactive product with that of authentic RPR-72840A on two different HPLC systems. In both cases the radioactive product co-eluted with authentic

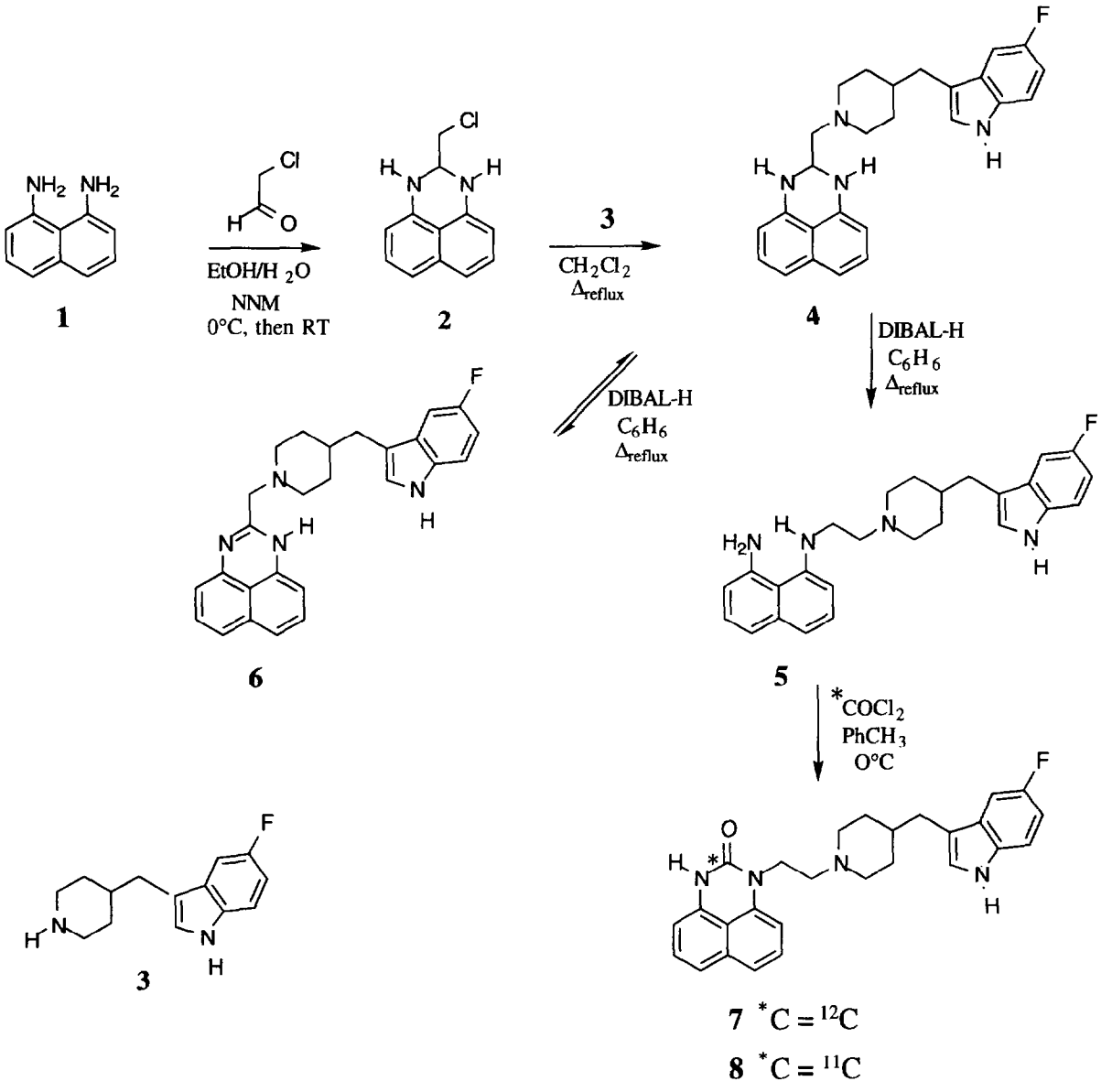

Figure 1. Synthesis of [^{11}C]RPR-72840A and its precursor.

7. Spectroscopic or elemental analysis cannot be done on no-carrier-added ^{11}C compounds; the mass quantity of our no-carrier-added [^{11}C]phosgene preparation, and thus of any product derived from it, is less than 0.5 μmol. Therefore, additional evidence for the product identity was obtained by carrying out a non-radioactive synthesis of **7** on a 50 μmol scale, using conditions similar to those of the radioactive synthesis, and analyzing the product with HPLC and NMR.

Biodistribution in rats

After formulation of [^{11}C]RPR-72840A, rats were injected with a tracer dose. Radioactivity concentrations peaked in most organs during the first minute after injection and declined or remained constant until later times. Radioactivity concentrations were highest in the lung, followed by the kidney, liver, heart and muscle, and were higher in whole blood than in plasma (Table 1). Uptake in the brain was slower, peaking at 5–10 min, and remained at constant values until 40 min after injection. Differences between brain regions were small. Radioactivity levels decreased in the order: pons, cerebellum, colliculus, posterior cortex, hippocampus, diencephalon, striatum, and anterior cortex (Table 1). Presaturation with an excess of citalopram, a selective serotonin uptake blocker, prior to [^{11}C]RPR-72840A injection, led to a decrease of radioactivity concentrations in whole blood (−60%) and lung (−40%), the latter perhaps reflecting competition for binding to the high affinity lung serotonin binding sites described in the literature.[16] Citalopram had little effect on the uptake by other organs except the liver which showed a 50% increase. In the brain, the effect of citalopram on [^{11}C]RPR-72840A uptake appeared small and highly variable among regions (Table 1). Autoradiography of sagittal rat brain sections incubated with [^{11}C]RPR-72840A (2 μCi (74 kBq)/mL, specific radioactivity 116 mCi (4.29 GBq)/μmol) during 15 or 30 min showed no clear regional difference. Competition with either 1 or 10 μM citalopram in the incubation medium had no significant effect on total or regional binding to the brain sections.

PET experiments in baboons

Three PET brain scans were performed on baboons, in one case with presaturation of the animal with citalopram. The upper row of Figure 2 shows, at different anatomical levels indicated by the MRI images (bottom row), the PET images obtained 15–40 min after injection. Little differences were found among brain regions, and the highest uptake was in adipose tissue in the retro-orbital and the peri-auditive regions. The ratio of regional uptake to uptake in the cerebellum, a region with scarce serotonin uptake sites,[17] was not significantly higher than 1 except in adipose tissue where it reached a value of 2.5 at 60 min.

Radioactivity concentrations in the brain regions, expressed in percent of the injected dose per volume of tissue (% ID/L), were corrected for ^{11}C decay and plotted against time after [^{11}C]RPR-72840A injection (Fig. 3). The peak of radioactivity uptake was reached at 5–15 min depending on the region and remained at a constant value until the end of the scan at 65 min. The major effect of citalopram preadministration was the increase of early peak uptake of [^{11}C]RPR-72840A by 50–70%. At later times, uptake continued to be higher under citalopram versus control conditions in all brain regions, with the exception of adipose tissue in which there was a 15–20% reduction of radioactivity concentrations.

Table 1. Biodistribution of [^{11}C]RPR-72840A in rats

	Radioactivity concentration (% of injected dose per gram of tissue)					Effect of citalopram preinjection (% variation at 20n min)
	1 min	5 min	10 min	20 min	40 min	
Liver	0.76	1.85	1.85	1.44	1.16	51%
Kidney	1.97	2.63	2.33	1.86	1.38	−13%
Heart	3.06	2.18	1.35	0.78	0.45	−7%
Muscle	0.55	0.42	0.54	0.58	0.26	−35%
Lung	10.84	10.64	8.40	7.58	4.77	−16%
Blood	0.50	0.29	0.33	0.31	0.22	−70%
Plasma	0.39	0.12	0.12	0.10	0.06	−9%
Whole brain	0.09	0.12	0.12	0.12	0.12	15%
Brain regions						
Pons	0.10	0.13	0.13	0.13	0.13	26%
Cerebellum	0.11	0.14	0.14	0.12	0.12	25%
Colliculi	0.09	0.12	0.12	0.12	0.12	15%
Diencephalon	0.09	0.11	0.09	0.12	0.12	−14%
Hippocampus	0.08	0.13	0.11	0.13	0.10	22%
Striatum	0.07	0.11	0.12	0.09	0.12	20%
Anterior cortex	0.08	0.10	0.10	0.10	0.12	24%
Posterior cortex	0.09	0.12	0.12	0.12	0.11	3%

In the blood, radioactivity peaked within the first minute after the injection and declined rapidly afterwards to reach low levels (1.5% ID/L) 20 min after injection. At all times, the same radioactivity concentrations were found in plasma and in whole blood, and 99% of plasmatic radioactivity was bound to proteins, even as early as 0.5 min after tracer injection. The results were similar under citalopram challenge, including the high plasmatic protein binding, except that the decrease from the initial peak was slower.

In summary, experiments in rats and baboons demonstrated that, except in rat lung tissue, [^{11}C]RPR-72840A binding was not reduced by high doses of citalopram, a specific high affinity ligand of the serotonergic uptake site. The lack of effect of citalopram on brain uptake of [^{11}C]RPR-72840A hampers the latter's use as a PET tracer of the brain serotonergic uptake sites. It is possible that the existence of other, as yet nonidentified binding sites of RPR-72840A in the brain, or a high degree of nonspecific binding in brain tissue, are responsible for this observation. Alternatively, the possibility exists that [^{11}C]RPR-72840A is rapidly metabolized in vivo, leading to labelled derivatives with little or no affinity to serotonergic uptake sites.

Experimental

Chemistry

1,8-Diaminonaphthalene, chloroacetaldehyde, diisobutylaluminiumhydride, and *N*-methylmorpholine (NMM) were purchased from Aldrich, France and phosgene (20% in toluene) was from Fluka, France. 5-Fluoro-3-[(4-piperidinyl)methyl]-1H-indole (**3**) and *N*,*N'*-naphthalene - 1,8 - diyl - *N* - [2[4((5 - fluoro - 1H - indol - 3 - yl)-methyl)piperidino]ethyl]urea (RPR-72840A, **7**) were kindly donated by Rhône-Poulenc-Rorer, Vitry-Alfortville, France. The latter two products can be synthesized according to literature procedures.[10,18]

No-carrier-added [^{11}C]phosgene was synthesized from cyclotron-produced [^{11}C]methane as described previously.[11] Synthesis of [^{11}C]RPR-72840A was carried out remote-controlled in a radiation-shielded cell.

TLC were run on precoated plates of silica gel 60F254 (Merck) unless otherwise described. The compounds were visualized using both a UV-lamp at 254 nm and iodine staining. Flash chromatography was run on silica gel (various granulometry, Merck). All solvent mixtures for TLC and flash chromatography were treated with 1 vol% of concentrated ammonia. The following HPLC

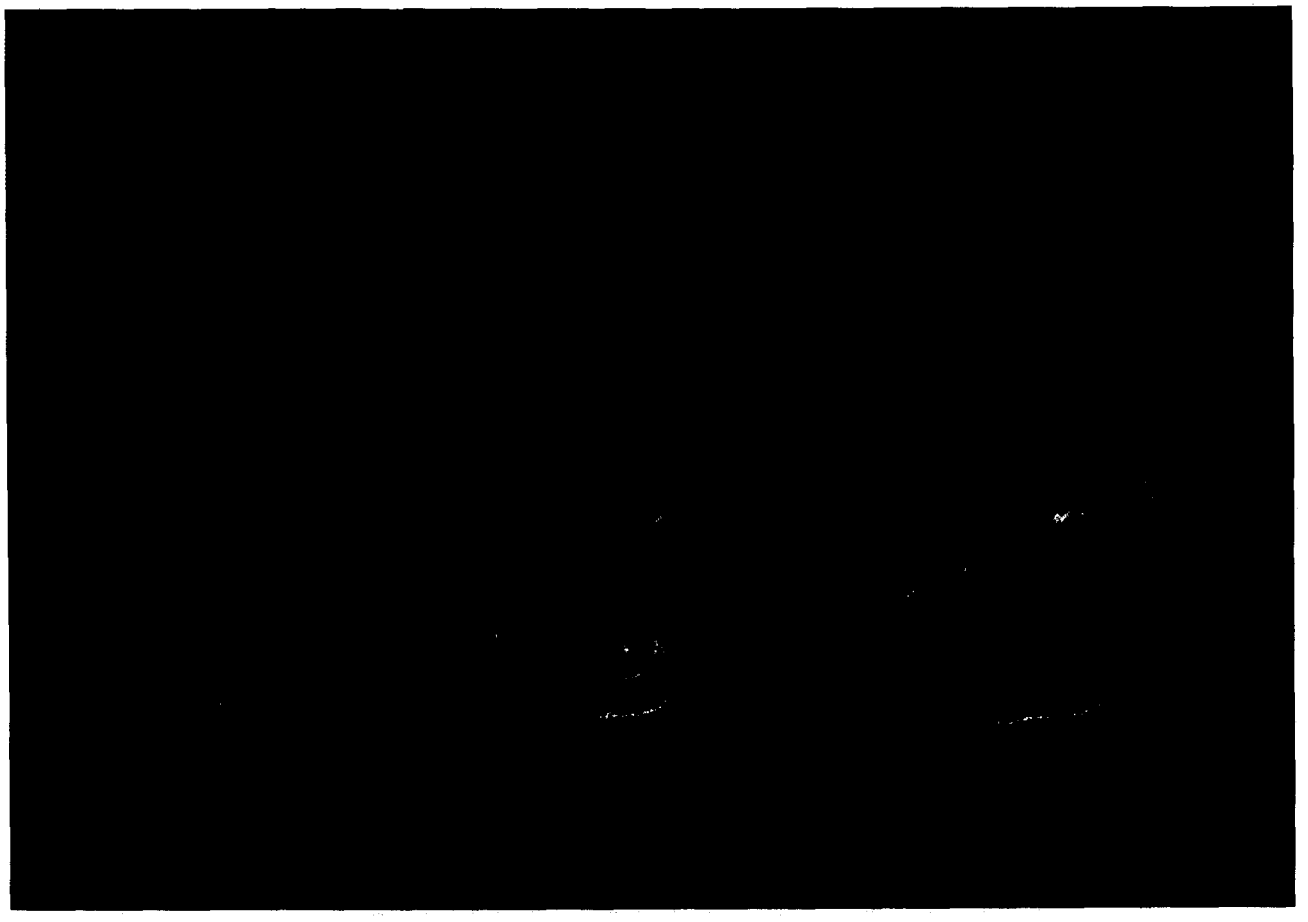

Figure 2. PET (upper row) and MRI (bottom row) images of the baboon head. Corresponding anatomical levels parallel to the orbito-meatal line are, from left to right, at the level of upper cortical areas, of the basal ganglia, and of the cerebellum and inferior temporal lobes. PET images represent the sum of counts acquired 15–40 min after injection of [^{11}C]RPR-72840A and are colour-coded from dark blue (lowest counts) to hot red (highest counts).

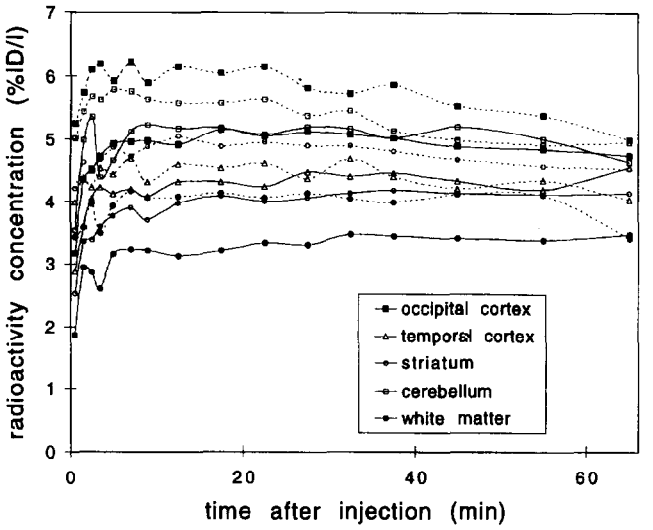

Figure 3. Kinetics of radioactivity concentrations in baboon brain after [^{11}C]RPR-72840A injection. Values expressed in percent of the injected dose per liter of tissue are shown for the occipital cortex (filled squares), temporal cortex (open triangles), striatum (open circles), cerebellum (open squares), and white matter (filled circles). Filled lines, mean of two control experiments; dotted ines, citalopram presaturation experiment.

systems were employed: System A: column: Waters, Novapak HR Silica 6 μm; 7.8 × 300 mm; mobile phase: CH_2Cl_2:EtOH:Et_3N 975/25/0.5; flowrate: 5 mL/min; UV detection at 254 nm and radioactivity detection with an ionization chamber. System B: column: Merck, Hibar RT 250–10 LiChrosorb RP18, 7 μm; mobile phase: MeCN:H_2O:Et_3N 70/30/0.1; flowrate: 5 mL/min; UV detection at 254 nm and radioactivity detection with a GM counter.

Melting points were measured on a 9200 Electrothermal instrument and are uncorrected. NMR spectra were recorded on a Bruker AMX (300 MHz) apparatus at room temperature using the hydrogenated residue of the deuterated solvents (CD_2Cl_2, $\delta = 5.32$ ppm or DMSO-d_6, $\delta = 2.49$ ppm) and/or TMS as internal standards for ^{1}H NMR as well as the deuterated solvent (CD_2Cl_2, $\delta = 53.8$ ppm) and/or TMS as internal standards for ^{13}C NMR. The chemical shifts are reported in ppm, downfield from TMS (*,†: interchangeable assignments). Air or moisture sensitive reactions were conducted in oven-dried glassware and under an argon atmosphere.

2-Chloromethyl-1,2-dihydroperimidine (2). 1,8-Diaminonaphthalene (**1**, 5 mmol, 790 mg) was dissolved in 50 mL of absolute ethanol and the soln was cooled at 0 °C. Chloroacctaldehydc (50% in H_2O, 5 mmol, 0.635 mL) was added dropwise with stirring. The mixture was stirred for another 30 min at 0 °C and subsequently for 80 min at room temperature. The solvent was then evaporated with a rotary evaporator. The residual oil was subjected to flash chromatography: silica 70–230 mesh; EtOAc:heptane 1:9 resulting in 1018 mg of **2**, an orange-brown oil that crystallized on standing; yield 95%. TLC: EtOAc:heptane 1:1: R_f 0.66; melting point 81 °C; ^{1}H NMR (CD_2Cl_2): δ 7.24–7.13 (4H, naphth. CH), 6.47 (2H, d, $J = 6.0$ Hz, naphth. CH), 4.68 (2H, br, $W_{1/2} = 30$ Hz, NH), 4.52 (1H, t, $J = 6.0$ Hz, CH), 3.48 (2H, d, $J = 6.0$ Hz, CH_2); ^{13}C NMR(CD_2Cl_2): δ 140.1 (C, aryl-1,8), 135.3 (C, aryl-10*), 127.8 (CH, aryl†-2,7), 118.3 (CH, aryl†-3,6), 113.7 (C, aryl-9*), 106.8 (CH, aryl†-4,5), 64.8 (CH), 47.3 (CH_2).

2[4((5-Fluoro-1H-indol-3-yl)methyl)piperidinomethyl]-1,2-dihydroperimidine (4). Compound **2** (1.40 mmol, 301 mg) and 30 mL of CH_2Cl_2 were placed in a reaction vessel equipped with magnetic stirring and a reflux condenser. Compound **2** was dissolved by refluxing for 10 min. To the hot soln was added successively and at once *N*-methylmorpholine (1.40 mmol, 158 μL), **3** (1.40 mmol, 325 mg) in 5 mL of CH_2Cl_2, and KI (1.40 mmol, 233 mg). The mixture was refluxed for 10 h. The orange reaction mixture, containing a white solid, was cooled to room temperature and was washed twice with H_2O. The combined aq layers were extracted once with CH_2Cl_2. The combined organic phase was dried on Na_2SO_4. After removal of the solvent the red-brown oil (556 mg) was subjected to flash chromatography: (silica 15–25 μm; ethyl acetate: heptane, 3:7) resulting in 179 mg **4**, a white solid; yield 31%; TLC: EtOAc:heptane 1:1; R_f 0.30; ^{1}H NMR (CD_2Cl_2): δ 8.15 (1H, br, $W_{1/2} = 13$ Hz, indole NH), 7.23–7.10 (6H, 4 naphth. CH + 2 indole CH), 6.97 (1H, br, $W_{1/2} = 3$ Hz, indole CH), 6.89 (1H, td, $J = 9.3$ and 2.4 Hz, indole CH), 6.49 (2H, d, $J = 7.2$ Hz, naphth. CH), 4.74 (2H, br d, $W_{1/2} = 6$ Hz, NH), 4.48 (1H, t, $J = 6.3$ Hz, CH), 2.87 (2H, br d, $J_{app} = 11.1$ Hz, piperidine CH), 2.62 (2H, d, $J = 6.6$ Hz, CH_2), 2.49 (2H, d, $J = 6.3$ Hz, CH_2), 2.02 (2H, br t, $J_{app} = 11.1$ Hz, piperidine CH), 1.7–1.5 (2 + 1 H, piperidine CH), 1.34 (2H, br q, $J_{app} = 11.7$ Hz, piperidine CH).

1-Amino-8[2[4((5-fluoro-1H-indol-3-yl)methyl)piperidino]ethyl]aminonaphthalene (5) and 2[4((5-fluoro-1H-indol-3-yl)methyl)piperidinomethyl]perimidine (6). Compound **4** (0.30 mmol, 126 mg) in 3.4 mL of dry benzene was placed in a reaction vessel with an argon atmosphere and equipped with magnetic stirring and a reflux condenser. Diisobutylaluminiumhydride (DIBAL-H, 6.08 mmol, 1.08 mL, 20 equiv) was added at once. The reaction mixture turned instantaneously bright yellow under a brief but vigorous gas development. After 50 h of reflux the still bright yellow mixture was diluted with 7 mL of benzene and cooled at 0 °C. Under vigorous stirring NaF (24.3 mmol, 1.02 g) was added followed by H_2O (18.2 mmol, 328 μL). The light-brown/yellow mixture was stirred for another 20 min while the colour gradually turned pink. The precipitate was filtered off and was washed with benzene. The combined filtrate and washings were evaporated till dryness. The residual pink oil (97 mg) contained predominantly the desired product **5** according to TLC (alumina; EtOAc:heptane 1:1; R_f 0.64). It was subjected to flash chromatography (silica, 15–25 μm; EtOAc:heptane 55:45) resulting in 42 mg of **5** (34% yield), a pink oil that may or may not crystallize (mp 49 °C). ^{1}H NMR (CD_2Cl_2): δ 8.11 (1H, br, $W_{1/2} = 11$ Hz, indole NH), 7.29–7.02 (7H, m, 4 naphth. CH + 3 indole CH), 6.90 (1H, td, $J = 9.2$ and

2.4 Hz, indole CH), 6.57 (1H, m, naphth. CH), 6.50 (1H, d, $J=7.2$ Hz, naphth. CH), 6.10 (1H, br, $W_{1/2}=17$ Hz, NH), 4.70 (2H, br, $W_{1/2}=23$ Hz, NH_2), 3.17 (2H, br t, $W_{1/2}=14$ Hz, piperidine CH), 2.89 (2H, br d, $J_{app}=11.4$ Hz, CH_2), 2.66–2.61 (4H, CH_2), 1.94 (2H, br d, $J_{app}=10.8$ Hz, piperidine CH), 1.69–1.53 (2+1H, piperidine CH), 1.38–1.20 (2H, br q, $J_{app}=12.0$ Hz, piperidine CH).

When less DIBAL-H (13 equiv) and a shorter reaction time (30 h) were employed considerably more of the yellow side product **6** was formed (yield 47%) at the expense of **5** (yield 25%). Isolation of **6** and reacting it once more with 13 equiv of DIBAL-H gave **5** in 27% yield. TLC (alumina, Merck); EtOAc:heptane 1:1; R_f 0.46. ^{1}H NMR of **6** (CD_2Cl_2) δ 8.23 (1H, br, $W_{1/2}=16$ Hz, indole NH), 7.35–7.00 (9H, 6 naphth. CH+3 indole CH), 6.91 (1H, t, $J=8.8$ Hz, indole CH), 6.27 (1H, br, $W_{1/2}=18$ Hz, NH), 3.15 (2H, s, CH_2), 2.87 (2H, br d, $J_{app}=11.4$ Hz, piperidine CH), 2.66 (2H, d, $J=6.6$ Hz, CH_2), 2.14 (2H, t, $J=10.1$ Hz, piperidine CH), 1.78–1.54 (2+1 H, piperidine CH), 1.43–1.20 (2H, piperidine CH).

***N,N'*-naphthalene-1,8-diyl-*N*-[2[4((5-fluoro-1H-indol-3-yl)methyl)piperidino]ethyl]urea or RPR-72840A (7).** Compound **5** (21 mg, 50 μmol) and triethylamine (7 μL, 1 equiv) were dissolved in 25 mL of dry toluene and cooled at 0 °C. Phosgene (1 equiv) in 5 mL of dry toluene was added dropwise while stirring. Stirring was continued for 20 min while the pink colour faded away. The mixture, which contained a white precipitate, was then washed twice with saturated bicarbonate and once with H_2O. The organic phase was dried on Na_2SO_4. Evaporation of the solvent gave 10.7 mg of the crude product **7** (48%) that was purified by flash chromatography (silica, 20–45 μm, EtOAc:heptane 60:40): a white solid which on HPLC analysis (system A) coeluted with authentic **7**. TLC (EtOAc:heptane, 60:40): R_f 0.19. ^{1}H NMR (DMSO-d_6), identical with that of authentic **7**: δ 10.88 (1H, br s, $W_{1/2}=8$ Hz, NH), 10.38 (1H, br s, $W_{1/2}=4$ Hz, NH), 7.35–7.13 (7H, m, 4 naphth. CH+3 indole CH), 6.89 (1H, t, $J=8.8$ Hz, indole CH), 6.64 (1H, d, $J=11.8$ Hz, naphth. CH), 6.56 (1H, d, $J=11.8$ Hz, naphth. CH), 3.96 (2H, br t, $W_{1/2}=20$ Hz, piperidine CH), 2.92 (2H, br d, $J_{app}=10.5$ Hz, CH_2), 2.63–2.41 (4H, CH_2), 1.94 (2H, br t, $J_{app}=10.0$ Hz, piperidine CH), 1.70–1.42 (2+1 H, piperidine CH), 1.32–1.13 (2H, piperidine CH).

***N,N'*-Naphthalene-1,8-diyl-*N*-[2[4((5-fluoro-1H-indol-3-yl)methyl)piperidino]ethyl][^{11}C]urea or [^{11}C]RPR-72840A (8).** A target holder containing $N_2+5\%$ H_2 (7 bar) was irradiated for 45 min with a cyclotron-generated 20 MeV proton beam of 25 μA. The thus produced [^{11}C]methane was then converted into [^{11}C]phosgene,[11] which contained in the vector gas $N_2+2\%$ O_2, was bubbled through a solution of 2 μmol of **5** in 300 μL of dry toluene cooled at 0 °C. Reaction of **5** with [^{11}C]phosgene giving **8** was taken to be immediate. As soon as the radioactivity in the solution levelled, the reaction vessel was heated in a Wood-metal bath of 120 °C while bubbling of the vector gas continued in order to evaporate the solvent till dryness. The heating bath was removed and the vessel was cooled down to room temperature. HPLC mobile phase (system A, 0.5 mL) was added to dissolve **8** and to inject it onto HPLC system A. The radioactive product **8**, having a retention time of 6 min, was collected at the HPLC outlet. The mobile phase was evaporated till dryness by passing a helium stream through the solution at 100 °C. The product was formulated for injection by addition, in turn and while heating gently, of 500 μL of propane-1,2-diol, 50 μL of aq NaH_2PO_4/H_3PO_4 (1 M) and 5 mL of sterile saline. At 35 min after the end of irradiation 90 mCi (3.33 GBq) of **8** could be isolated on average and with good reproducibility, with an average specific radioactivity of 320 mCi (11.94 GBq)/μmol. A sample of this preparation was diluted 3.3 times with acetonitrile for analysis on HPLC system B. Only one radioactive product eluting at 7.4 min and coeluting with authentic **7** was detected; no other nonradioactive products could be seen on UV.

Biodistribution in rats

A tracer dose of [^{11}C]RPR-72840A (100 μCi, 3.7 MBq), specific radioactivity 460 mCi (17.02 GBq)/μmol was injected in the tail vein to two groups of male adult Sprague–Dawley rats (200 g body weight; IFFA-CREDO, France). The first group received only [^{11}C]RPR-72840A, while the second group was pretreated with 1 mg/kg citalopram (Seropram®, Lundbeck A/S, Copenhagen, Denmark) iv 30 min prior to the tracer injection. Two animals from each group were killed at each of the following time points: 1, 5, 10, 20, and 40 min after [^{11}C]RPR-72840A injection. Samples of carefully dissected organs (liver, kidney, heart, lung, and skeletic muscle), whole blood, plasma, and brain regions (pons, cerebellum, colliculus, diencephalic area, hippocampus, striatum, anterior, and posterior cerebral cortex) were weighed and counted in an Intertechnique gamma well-counter calibrated for 511 keV counting efficiency with a 68 Ge source.

Autoradiography was performed on sagittal sections of adult rat brain cut at 20 μm on a cryostat, mounted onto polylysine-coated glass slides, and equilibrated in phosphate buffered saline, pH 7.4, at room temperature. After 30 min, 2 μCi (74 kBq)/mL of [^{11}C]RPR-72840A was added and incubation proceeded for either 15 or 30 min. Sections were then rinsed twice for 5 min in the same buffer, briefly in distilled water, rapidly air-dried and placed in contact with a freshly erased phosphor storage plate (Kodak) during 2 h. The phosphor plate was read in a Molecular Dynamics Storm 840 Phosphorimager, and the sections were stained with cresyl violet for anatomical identification of the structures. Competition with citalopram was conducted in the same manner, except that 1 or 10 μM citalopram was added to the incubation medium 30 min prior to [^{11}C]RPR-72840A. At least 20 sections

from 5 different animals were used for each experimental condition.

PET experiments in baboons

Two male adult baboons (*Papio papio*, 20 kg body weight) were used to evaluate the brain uptake of [^{11}C]RPR-72840A in vivo. Animals were anaesthetized with 1% isoflurane and ventilated with 1/3 O_2:2/3 N_2O under constant cardiac and respiratory monitoring. A stereotaxic head-holder was used to align the plane of the orbito-meatal lines with the plane of the PET camera. Tissue attenuation was corrected by means of a 68Ge-68Ga transmission scan, and contiguous sections were acquired in sequential time frames (4×1 min; 3×2 min; 6×5 min; 2×10 min) after injection of a tracer dose of [^{11}C]RPR-72840A 18 ± 3 mCi (666 ± 111 MBq); specific radioactivity 200 ± 95 mCi (7.4 ± 3.5 GBq)/μmol). One animal underwent two PET scans in the ECAT 953B31 camera (31 contiguous slices 2.45 mm thick, full width at half-maximum resolution 8.4×8.4 mm): a first scan under tracer dose conditions, and a second scan under presaturation conditions, that is, iv injection of 5 mg/kg citalopram 30 min before [^{11}C]RPR-72840A. The second animal underwent one PET scan in the ECAT HR+ camera (63 contiguous slices 2.425 mm thick, full width at half-maximum resolution 4.5×4.5 mm) under tracer dose conditions. Regions of interest were traced on the PET images over anatomical structures according to magnetic resonance imaging (MRI) scans of the animals placed in the same position in the head holder in a CGR MRMAX 0.5 Tesla (T1-weighted, contiguous 1-mm thick slices). Sequential blood samples were drawn from a femoral artery line into heparinized tubes and were immediately processed. Whole blood, plasma, and free- and protein-bound plasmatic radioactivity separated by trichloroacetic acid precipitation were counted in an Intertechnique gamma-counter calibrated daily with a ^{68}Ge reference source.

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