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Synthesis of a [2-*Pyridinyl*-^{18}F]-labelled Fluoro Derivative of (−)-Cytisine as a Candidate Radioligand for Brain Nicotinic α4β2 Receptor Imaging with PET

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Abstract—In recent years, there has been considerable effort to design and synthesize radiotracers suitable for use in Positron Emission Tomography (PET) imaging of the α4β2 neuronal nicotinic acetylcholine receptor (nAChR) subtype. A new fluoropyridinyl derivative of (−)-cytisine (**1**), namely (−)-9-(2-fluoropyridinyl)cytisine (**3**, K_i values of 24 and 3462 nM for the α4β2 and α7 nAChRs subtypes, respectively) has been synthesized in four chemical steps from (−)-cytisine and labelled with fluorine-18 ($T_{1/2}$: 119.8 min) using an efficient two-step radiochemical process [(a) nucleophilic heteroaromatic *ortho*-radiofluorination using the corresponding *N*-Boc-protected nitro-derivative, (b) TFA removal of the Boc protective group]. Typically, 20–45 mCi (0.74–1.67 GBq) of (−)-9-(2-[^{18}F]fluoropyridinyl)cytisine ([^{18}F]-**3**, 2–3 Ci/μmol or 74–111 GBq/μmol) were easily obtained in 70–75 min starting from a 100 mCi (3.7 GBq) aliquot of a cyclotron-produced [^{18}F]fluoride production batch (20–45% non decay-corrected yield based on the starting [^{18}F]fluoride). The in vivo pharmacological profile of (−)-9-(2-[^{18}F]fluoropyridinyl)cytisine ([^{18}F]-**3**) was evaluated in rats with biodistribution studies and brain radioactivity monitoring using intracerebral radiosensitive β-microprobes. The observed in vivo distribution of the radiotracer in brain was rather uniform, and did not match with the known regional densities of nAChRs. It was also significantly different from that of the parent compound (−)-[^{3}H]cytisine. Moreover, competition studies with (−)-nicotine (5 mg/kg, 5 min before the radiotracer injection) did not reduce brain uptake of the radiotracer. These experiments clearly indicate that (−)-9-(2-[^{18}F]fluoropyridinyl)cytisine ([^{18}F]-**3**) does not have the required properties for imaging nAChRs using PET.

Introduction

The nicotinic cholinergic system consists of several CNS receptor subtypes, which mediate memory and learning, drug addiction and control of pain.[1] Central nicotinic acetylcholine receptors (nAChRs) are also strongly implicated in several neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease. Imaging nAChRs using Positron Emission Tomography (PET) or Single Photon Emission Computed Tomography (SPECT) could provide useful information on the integrity of the nAChR system in vivo and could play a

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key role both in elucidating the involvement of these receptors in neurodegenerative diseases and in monitoring the effectiveness of established therapeutics or drugs under development.

In recent years, there has been considerable effort to design and synthesize radiotracers suitable for use in PET and SPECT imaging of the α4β2 nAChR subtype. Among them, radiohalogen derivatives of A-85380 (3-[(*S*)-2-azetidinylmethoxy]pyridine), the lead compound of a new series of 3-pyridyl ethers, potent and selective ligands for the human α4β2 nAChR subtype,[2–4] have been developed as high affinity radiotracers.[5–23] In particular, the [2-pyridinyl-^{18}F]-labelled fluoro derivative ($T_{1/2}$: 119.8 min), 2-[^{18}F]fluoro-A-85380,[5–8] shows high affinity and selectivity for the α4β2 nAChR subtype[24–27] and displays a safe profile — low toxicity,[28] lack of mutagenicity[28] and acceptable effective dose equivalent in dosimetric studies to the patient[29] — for its use as a PET probe in humans.

(−)-Cytisine (**1**) is a natural chiral quinolizidine alkaloid, showing a remarkable combination of properties and sharing various physiological effects with (−)-nicotine.[30] In particular, (−)-cytisine (**1**) shares with (−)-nicotine a nanomolar affinity for the α4β2 nAChR subtype (1.2 and 1.0 nM, respectively) and a low affinity for the α7 subtype.[30–33]

Only a few structure–activity relationship (SAR) studies on (−)-cytisine derivatives have been reported so far. *N*-Alkyl derivatives have been recently synthesized and characterized for their affinity for the α4β2 nAChR subtype.[34–38] In this series, the pharmacologically active *N*-methyl derivative of (−)-cytisine[39] is the only candidate which has been labelled with another positron-emitter, carbon-11 ($T_{1/2}$: 20.38 min), but appears to be unsuitable for nAChR imaging using PET.[40,41] Derivatives halogenated at the 3-position of the pyridone ring of (−)-cytisine (9-halocytisine) with 2- to 10-fold increased affinity for both α4β2 and α7 nAChRs have also been described,[38] as well as a 3-(*para*-fluorophenyl) derivative [(−)-9-(4-fluorophenyl)cytisine (**2**), Fig. 1].[42,43] Labelled with fluorine-18[42] by a four-step radiochemical procedure (in 150 min synthesis time and only 6–10% decay-corrected radiochemical yields), this radiotracer has not been evaluated further.

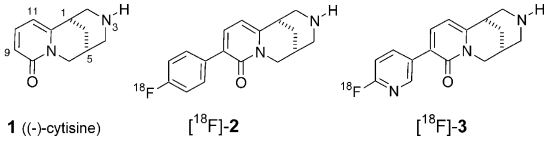


Figure 1. Chemical structures of (−)-cytisine (**1**), (−)-9-(4-[^{18}F]fluorophenyl)cytisine ([^{18}F]-**2**) and (−)-9-(2-[^{18}F]fluoropyridinyl)cytisine ([^{18}F]-**3**).

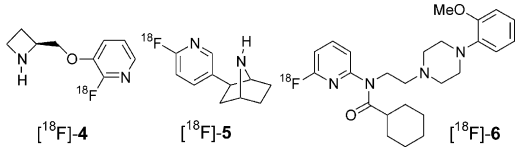


Figure 2. Chemical structures of 2-[^{18}F]fluoro-A-85380 ([^{18}F]-**4**), norchloro[^{18}F]fluoroepibatidine ([^{18}F]-**5**) and [6-pyridinyl-^{18}F]fluoro-WAY-100635 ([^{18}F]-**6**).

Compared to homoaromatic, but also aliphatic, nucleophilic radiofluorinations,[44,45] nucleophilic heteroaromatic substitution in the pyridine series, with no-carrier-added fluorine-18-labelled fluoride ion as its activated K[^{18}F]F-K_{222} complex,[46] appears today as the highest efficient method for the synthesis of radiotracers of high specific activity. *ortho*-Fluorination of the pyridine ring was first evaluated on the radiosynthesis of 2-[^{18}F]fluoropyridine[47] as a model reaction and has since been applied successfully to the preparation of various PET radiotracers such as 2-[^{18}F]fluoro-A-85380 ([^{18}F]-**4**) (05-08), norchloro[^{18}F]fluoroepibatidine ([^{18}F]-**5**).[48–54] and more recently, [6-pyridinyl-^{18}F]fluoro-WAY-100635 ([^{18}F]-**6**)[55] (Fig. 2).

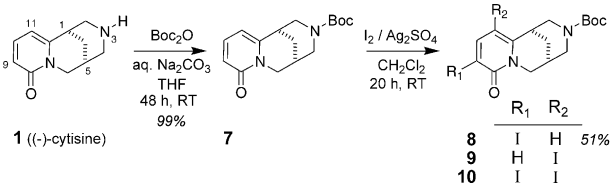
Based on these observations, we herein report the synthesis and preliminary pharmacological characterization of a new fluoropyridinyl derivative of (−)-cytisine, namely (−)-9-(2-fluoropyridinyl)cytisine (**3**), a structurally closely related analogue of the potent ligand (−)-9-(4-[^{18}F]fluorophenyl)cytisine ([^{18}F]-**2**). We also report the synthesis of the corresponding nitro-derivative (**16**) as precursor for labelling and the efficient two-step radiosynthesis of (−)-9-(2-[^{18}F]fluoropyridinyl)cytisine ([^{18}F]-**3**) by nucleophilic heteroaromatic *ortho*-radiofluorination. Finally, preliminary in vitro and in vivo evaluation of this new fluorine-18-labelled radiotracer is presented.

Results and Discussion

Chemistry

The synthesis of (−)-9-(2-fluoropyridinyl)cytisine (**3**) and the corresponding nitro derivative[16] as precursor for labelling is based on a strategy previously developed.[42] First (−)-cytisine **1**, easily extracted from commercialy available *Laburnum anagyroïdes* seeds,[56] was quantitatively protected on the amino functional group as the Boc derivative **7**. Iodination of the pyridone ring, carried out in dichloromethane using iodine and silver sulfate,[57] afforded the 9-iodocytisine derivative **8** in 51% after purification. This compound was formed along with a mixture of unreacted starting material **7**, 11-iodo regioisomer **9** and 9,11-diodocytisine derivative **10** (relative ^{1}H NMR ratio **7**/**8**/**9**/**10**: 12/64/8/16) (Scheme 1).

To prepare the target molecules **3** and **16**, a Stille type cross-coupling reaction[58] was envisaged either from 9-iodocytisine derivative **8** or from 9-trimethylstannylcytisine



Scheme 1. Preparation of (−)-*N*-*tert*-butoxycarbonyl-9-iodocytisine (**8**).

derivative **11** with a pyridine ring appropriately functionalized. The halogen-tin exchange reaction of the 9-iodocytisine derivative **8**, achieved with hexamethylditin in refluxing dioxane in the presence of $Pd(PPh_3)_4$ afforded the 9-trimethylstannylcytisine derivative **11** in 52% yield after purification (Scheme 2). Under the same conditions, the 5-trimethylstannyl-2-nitro- or -2-fluoropyridines **13** and **15** were prepared in 61 and 77% yields from 2-nitro-5-bromopyridine **12** and 2-fluoro-5-iodopyridine **14**, respectively[53] (Scheme 3).

The cross-coupling reaction of the 9-iodocytisine derivative **8** with 5-trimethylstannyl-2-nitropyridines **13** using $Pd(PPh_3)_4$ led to the 9-(2-nitropyridyl)cytisine **16** in 45% isolated yield (Scheme 4). The 9-(2-fluoropyridyl)cytisine **17** was prepared by the cross-coupling reaction of the 9-iodocytisine derivative **8** with 5-trimethylstannyl-2-fluoropyridine **15** using $Pd(PPh_3)_4$ as catalyst (51% yield) or from the 9-trimethylstannylcytisine derivative **11** with 5-iodo-2-fluoropyridine **14** and $PdCl_2(PPh_3)_2$ as the catalyst (31% yield).

Subsequent removal of the Boc protective group from **17** provided the target amine compound **3**, (−)-9-(2-fluoropyridinyl)cytisine in 86% yield (Scheme 5).

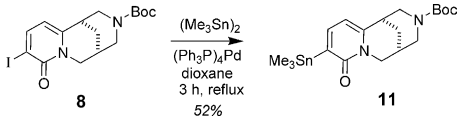
Radiochemistry

(−)-9-(2-[^{18}F]Fluoropyridinyl)cytisine ([^{18}F]-**3**) has been labelled in two radiochemical steps by (a) nucleophilic heteroaromatic nitro-to-fluoro substitution, followed by (b) TFA removal of the Boc protective group (Scheme 6).

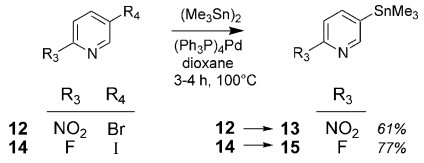
Introduction of the fluorine-18 as no-carrier-added [^{18}F]fluoride was attempted in dimethyl sulfoxide (DMSO) with the activated K[^{18}F]F-Kryptofix®222 complex[44] as the no-carrier-added radiofluorinating reactant [Kryptofix®222 (K_{222}): 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane] by conventional heating at 145 °C. In the first set of experiments, the influence of reaction time was studied. A DMSO solution (600 μL) containing 3.0 mg of the precursor for labelling **16** was transferred to 30–60 mCi of the dried K[^{18}F]F–K_{222} complex in a reaction vial (Vacutainer® tube). The tube (not sealed) was then placed in a heating block at 145 °C for 1–10 min without stirring the contents. As expected, the nitro-derivative **16** was reactive under the conditions described above. The incorporation yield increased with the reaction time up to 7 min (up to 85% yield), then remained stable until the end of the run (10 min). Moderate to high incorporation yields were observed at 4, 5 and 6 min (about 65, 75 and 80%, respectively). In each run, the remaining radioactivity at the end of the experiment was measured and over 95% of the initial radioactivity placed in the vessel was still present.

After Sep-Pak separation, the *N*-Boc-protective group of the [^{18}F]fluoropyridinylcytisine derivative [^{18}F]-**17** was rapidly (< 1 min) and quantitatively removed using TFA in dichloromethane (50/1, v/v). Finally, the reaction mixture was purified by HPLC (semi-preparative C-18 Zorbax® SB Hewlett Packard, 250×9.4 mm, 5 μm) to give pure [^{18}F]-**3**.

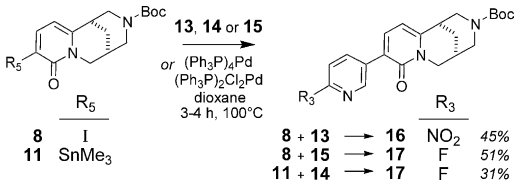
Typically, 20–45 mCi (0.74–1.67 GBq) of (−)-9-(2-[^{18}F]fluoropyridinyl)cytisine ([^{18}F]-**3**, 2–3 Ci/μmol or



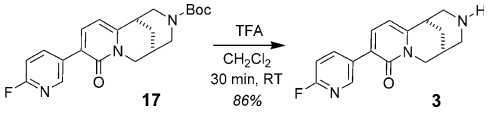
Scheme 2. Preparation of (−)-*N*-*tert*-butoxycarbonyl-9-trimethylstannylcytisine (**11**).



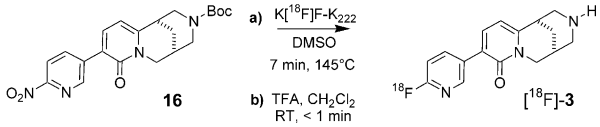
Scheme 3. Preparation of 2-nitro- and 2-fluoro-5-trimethylstannylpyridines (**13**) and (**15**).



Scheme 4. Preparation of (−)-*N*-*tert*-butoxycarbonyl-9-(2-nitropyridinyl)cytisine (**16**) and (−)-*N*-*tert*-butoxycarbonyl-9-(2-fluoropyridinyl)cytisine (**17**).



Scheme 5. Preparation of (−)-9-(2-fluoropyridinyl)cytisine (**3**).



Scheme 6. Preparation of (−)-9-(2-[^{18}F]fluoropyridinyl)cytisine ([^{18}F]-**3**).

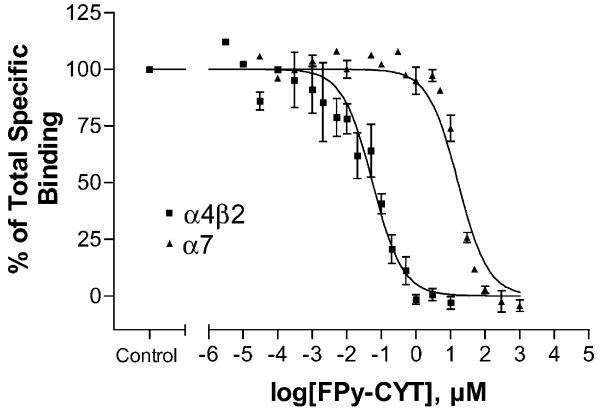


Figure 3. Competitive inhibition of (−)-[^{3}H]nicotine (α4β2) or [^{3}H]MLA (α7) binding by (−)-9-(2-[^{18}F]fluoropyridinyl)cytisine ([^{18}F]-**3**) in rat cortical or hippocampal membranes, respectively. Data are the mean (±SEM) of three independent experiments.

74–111 GBq/μmol) were easily obtained in 70–75 min starting from a 100 mCi (3.7 GBq) aliquot of a cyclotron-produced [^{18}F]fluoride production batch (20–45% non decay-corrected yield based on the starting [^{18}F]fluoride).

Pharmacology

Binding assays. (−)-9-(2-Fluoropyridinyl)cytisine (**3**) was tested for its in vitro affinity for nAChRs by radioligand binding assays. Membrane fractions isolated from Sprague–Dawley rat brain (hippocampus and cortex) were used for K_i determinations. (−)-[^{3}H]nicotine and [^{3}H]methyllycaconitine ([^{3}H]MLA) were used to determine the affinity of the compound for the α4β2 and α7 nAChR subtypes, respectively. Competition assays yielded K_i values of 23.9±2.8 nM (average value of three independent assays) and 3462±277 nM (average value of three independent assays) for the α4β2 and α7 nAChRs subtypes, respectively (Fig. 3).

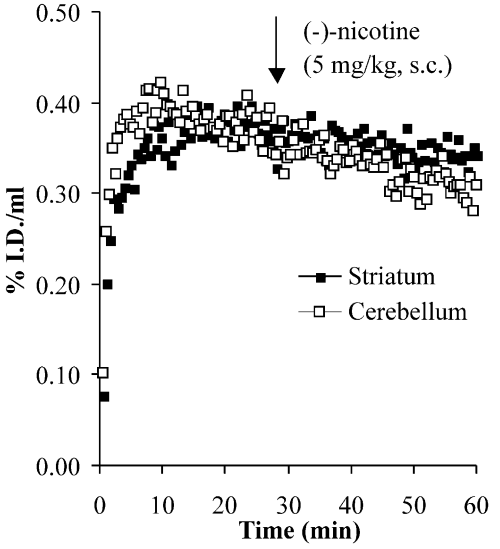


Figure 4. Radiosensitive β-microprobe acquisition: kinetics of (−)-9-(2-[^{18}F]fluoropyridinyl)cytisine ([^{18}F]-**3**) uptake into striatum and cerebellum (rat).

Radiosensitive β-microprobe acquisition. Kinetics of (−)-9-(2-[^{18}F]fluoropyridinyl)cytisine ([^{18}F]-**3**) uptake into the brain were first investigated in rats using a radiosensitive-β-microprobes device[59] (Fig. 4). Two probes were implanted, the first one in the striatum (considered as a nAChR-rich region) and the second one in the cerebellum (normally taken as a reference region poor in nAChR). Following iv injection of [^{18}F]-**3** (0.8–1.0 mCi with specific radioactivities greater than 2 Ci/μmol), no significant differences were noticed in the kinetics of uptake of the radiotracer in both structures with a maximal radioactive concentration 10 min post-injection and a slow washout phase. In order to confirm that the striatum fixation only represents non-specific binding, a displacement study was performed using (−)-nicotine (5 mg/kg, sc, 30 min after iv injection of [^{18}F]-**3**, 0.8–1.0 mCi with specific radioactivities greater than 2 Ci/μmol). No displacement of the radiotracer in the striatum was observed up to 30 min following the administration of (−)-nicotine.

Biodistribution studies. Kinetics of (−)-9-(2-[^{18}F]fluoropyridinyl)cytisine ([^{18}F]-**3**) uptake into brain were also determined in rats using conventional biodistribution experiments (Fig. 5). Following iv injection of [^{18}F]-**3** (30–60 μCi with specific radioactivities greater than 2 Ci/μmol), brain uptake was relatively low (0.3% ID/g tissue in average) and the clearance from the blood was slow. Average ratios of thalamus/blood were only 1.20 and 1.48 at 15 and 30 min post injection, respectively. Brain uptake of the radiotracer was rather uniform. For example, the uptake in the striatum (again considered as

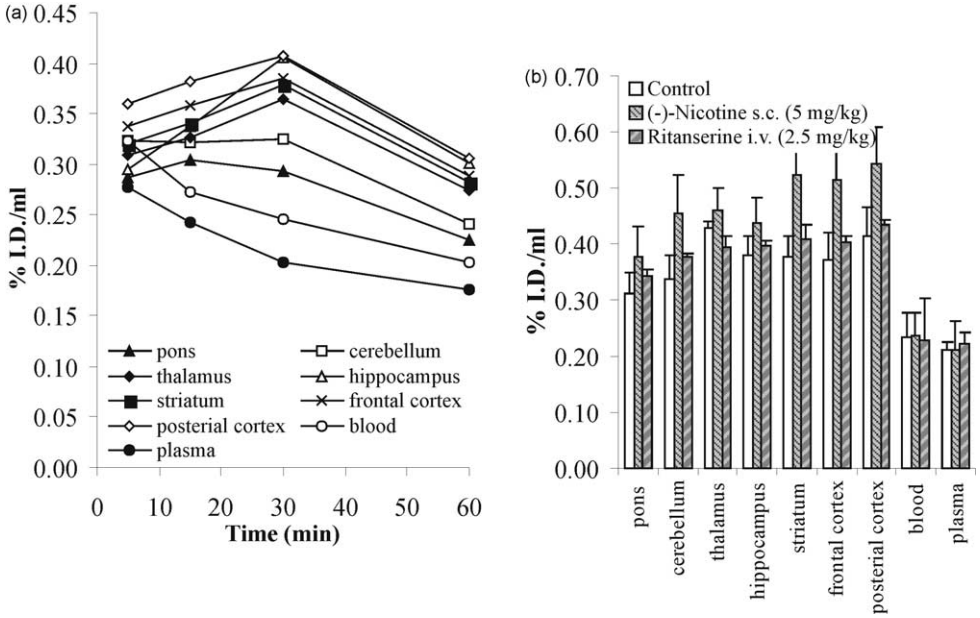


Figure 5. Biodistribution studies in rats: (a) kinetics of (−)-9-(2-[^{18}F]fluoropyridinyl)cytisine ([^{18}F]-**3**) uptake into various brain regions; (b) competition studies with (−)-nicotine and ritanserine.

a nAChR-rich region) was only slightly higher than in the cerebellum (a region poor in nAChR). The observed distribution did not match with the known regional densities of nAChRs found with other appropriate radioligands such as 2-[^{18}F]fluoro-A-85380[26,27] or norchloro[^{125}I]iodoepibatidine ((±)-exo-2-(6-[^{125}I]iodo-3-pyridyl)-7-azabicyclo[2.2.1]heptane).[60] The regional brain distribution was also significantly different from that observed for the parent compound (−)-cytisine.[61] In order to demonstrate any specific binding to the nAChRs, competition studies were performed using (−)-nicotine (5 mg/kg, sc, 5 min before the radiotracer injection). No reduction of the brain uptake of the radiotracer was observed. Finally, in view of the high concentration of the radiotracer in the frontal cortex and therefore its possible interaction with $5HT_2$ receptors, competition studies with ritanserine was performed (2.5 mg/kg, iv, 15 min before the radiotracer injection). No modification of the uptake was observed.

Conclusion

A new fluoropyridinyl derivative of (−)-cytisine, namely (−)-9-(2-fluoropyridinyl)cytisine (**3**, K_i values of 24 and 3462 nM for the α4β2 and α7 nAChRs subtypes, respectively) has been synthesized in four chemical steps from (−)-cytisine in good overall yields and labelled with fluorine-18 ($T_{1/2}$: 109.8 min) using an efficient two-step radiochemical process. The evaluation of its in vivo pharmacological profile (biodistribution studies and brain radioactivity monitoring using intracerebral radiosensitive β-microprobes, both in rats), clearly indicate that (−)-9-(2-[^{18}F]fluoropyridinyl)cytisine ([^{18}F]-**3**) does not have the required properties for imaging nAChRs using PET.

Experimental

General

Chemicals, TLCs and HPLCs. *L. anagyroïdes* seeds were purchased from Vilmorin Clause and Cie (France), and extracted according to known procedures.[56] Chemicals were purchased from Aldrich, Fluka or Sigma (France) and were used without further purification unless otherwise stated. Tetrahydrofuran (THF) was refluxed over sodium/benzophenone and diethyl ether over $LiAlH_4$ before distillation under a nitrogen atmosphere. Toluene, dichloromethane and all amines used were distilled from CaH_2. Thin layer chromatography (TLC) was performed on silica gel $60F_{254}$ plates (0.1 mm, Merck). The compounds were localized[1] when possible at 254 nm using a UV-lamp and/or[2] by iodine staining. Radioactive spots were detected using a Berthold TraceMaster 20 automatic TLC linear analyser. Flash chromatography was conducted on silica gel 40–63 μm (Merck) at 0.3 bars (Air). HPLCs: HPLC A: Equipment: Waters or Shimadzu systems. For example, Waters systems equipped with a 510 pump, 440 UV detector or 481/486 UV-multiwavelength detectors; column: semipreparative C-18 Zorbax® SB, Hewlett Packard (250×9.4 mm); porosity: 5 μm; conditions: isocratic elution with: acetonitrile/water/TFA: 13/87/0.1 (v:v:v); flow rate: 7.0 mL/min; temperature: RT; UV detection at λ: 254 nm; HPLC B: Equipment: Waters Alliance 2690 equipped with a UV spectrophotometer (Photodiode Array Detector, Waters 996) and a Berthold LB509 radioactivity detector; column: analytical Symmetry-M® C-18, Waters (4.6×50 mm, microcolumn); porosity: 5 μm; conditions: isocratic elution with solvA/solvB: 65/35 (v:v) [solvA: H_2O containing Low-UV PIC® B7 reagent (Waters), 20 mL for 1000 mL; solvB: H_2O/CH_3CN: 50/50 (v:v) containing Low-UV PIC® B7 reagent (% by weight: methanol (18–22%), heptane sulfonic acid–sodium salts (4–6%), phosphate buffer solution (3–7%), water (65–75%), pH 3, Waters), 20 mL for 1000 mL]; flow rate: 2.0 mL/min; temperature: 30 °C; UV detection at λ: 249 nm.

Spectroscopies. ^{1}H NMR, ^{19}F NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DPX 250 instrument at 250 MHz (^{1}H), 62 MHz (^{13}C) or 235 MHz (^{19}F) using $CDCl_3$ as solvent. $CDCl_3$ and TMS were used as internal references for ^{1}H NMR and ^{13}C NMR. $CFCl_3$ was used as external reference for ^{19}F NMR. The chemical shifts are reported in ppm, downfield from TMS or $CFCl_3$ (s, d, t, dd, br for singlet, doublet, triplet, doublet of doublet and broad, respectively). Mass spectra were obtained on a GC mat Jeol (EI, 70 eV) and high resolution mass spectra (HRMS) on a Jeol JMSD 300 or a Jeol AX 500 (Centre Régional Universitaire de Rouen, France). IR spectra were recorded with a FT-IR Perkin-Elmer 16 PC instrument.

Radioisotope production. No-carrier-added aqueous [^{18}F]fluoride ion was produced on a CGR-MeV 520 cyclotron by irradiation of a 2-mL water target using a 17 MeV proton beam on 95% enriched [^{18}O]water by the [^{18}O(p,n)^{18}F] nuclear reaction and was transferred to the appropriate hot cell. Typical production: 550–650 mCi (20.3–24.0 GBq) of [^{18}F]F^- at the end of bombardment for a 20 μA, 30 min (36,000 μC) irradiation. A complete description of the target hardware and operation can be found in refs 7 and 53.

Miscellaneous

Elemental analyses were obtained from a ThermoQuest CHNS-O instrument. Melting points were measured on a Gallenkamp capillary melting point apparatus and are reported uncorrected. Radiosyntheses using fluorine-18, including the HPLC purifications, were performed in a 7.5-cm lead-shielded cell using a computer assisted Zymate robot system (Zymark Corporation, USA). Microwave activation was performed with a MicroWell 10 oven (2.45 GHz), Labwell AB, Sweden. Specific radioactivity was calculated from three consecutive HPLC analyses and determined as follows: The area of the UV absorbance peak corresponding to the radiolabelled product was measured (integrated) on the HPLC chromatogram and compared to a standard curve relating mass to UV absorbance. The first injection in PET experiments was done within 15 min after the end of synthesis.

Chemistry

(−)-(1*R*,5*S*)-*N*-*tert*-Butoxycarbonyl-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-*a*][1,5]-diazocin-8-one (7). Cytisine **1** (1.0 g, 5.26 mmol) and $(Boc)_2O$ (1.377 g, 6.31 mmol) were dissolved in THF (20 mL) and water (10 mL), then a solution of Na_2CO_3 (0.669 g, 6.31 mmol) in 5 mL of water was added. The mixture was stirred for 48 h at rt, then diluted with CH_2Cl_2 (30 mL) and brine (10 mL). After separation of the layers, the aqueous phase was extracted twice with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and the solvents were evaporated under reduced pressure to give a white solid (1.520 g, 5.24 mmol, 99%): mp 156–157 °C; $[\alpha]_D^{22} = -219$ (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.25–7.40 (m, 1H), 6.41 (d, $J = 8.9$ Hz, 1H), 6.08 (d, $J = 6.6$ Hz, 1H), 4.19–4.3 (m, 3H), 3.81 (dd, $J = 6.4$ Hz, $J = 15.6$ Hz, 1H), 3.0 (br s, 3H), 2.43 (br s, 1H), 1.97 (t, $J = 13.0$ Hz, 2H), 1.27 (br s, 9H); ^{13}C NMR ($CDCl_3$) δ 163.0, 154.0, 148.7, 138.5, 116.7, 105.3 and 104.8 (conformers), 79.8, 51.3, 50.2, 49.0 and 48.5 (conformers), 34.5, 28.0, 27.7 and 27.3 (conformers), 25.8; IR (NaCl) 3056, 1844, 1640, 1412, 1156 cm^{-1}; HRMS (EI) calcd for $C_{16}H_{22}N_2O_3$ 290.16302, found 290.15970. Anal. calcd for $C_{16}H_{22}N_2O_3$ C, 66.18; H, 7.64; N, 9.65, found C, 66.48; H, 7.84; N, 9.76.

(−)-(1*R*,5*S*)-*N*-*tert*-Butoxycarbonyl-9-iodo-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-*a*][1,5]diazocin-8-one (8). Iodine (1.330 g, 5.24 mmol) was added to a mixture of **7** (1.520 g, 5.24 mmol) and silver sulfate (1.634 g, 5.24 mmol) in ice-cold CH_2Cl_2 (50 mL). After stirring 20 h at rt in the dark, 30 mL of aqueous saturated solution of sodium thiosulfate was added and the mixture was vigorously stirred for 30 min, then filtered through Celite. After separation, the aqueous layer was extracted twice with CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$ followed by evaporation of the solvent under reduced pressure. As showed by the ^1H NMR spectrum of the crude mixture, the expected product **8** was formed along with the 11-iodo regioisomer **9**, the 9,11-diodocytisine derivative **10** and some remaining starting material **7** (relative ^1H NMR ratio **7**/**8**/**9**/**10**: 12/64/8/16). The crude mixture was further purified by column chromatography on silica gel (EtOAc/CH_2Cl_2, 9:1) to afford the expected product **8** (1.110 g, 2.64 mmol, 51%, pale yellow solid) as well as side-products **9** (0.110 g, 0.262 mmol, 5%, pale yellow solid) and **10** (0.285 g, 0.524 mmol, 10%, pale yellow solid).

Analytical data for **8**: mp 159–160 °C; $[\alpha]_D^{22} = -135$ (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.85 (d, $J = 7.3$ Hz, 1H), 5.91 (d, $J = 7.3$ Hz, 1H), 4.1–4.26 (m, 3H), 3.85 (dd, $J = 6.2$ Hz, $J = 15.6$ Hz, 1H), 3.03 (s, 3H), 2.43 (s, 1H), 1.98 (s, 2H), 1.28 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 159.7, 154.0, 149.6, 147.2, 106.6 (br), 87.3, 79.9 (br), 51.1 (br), 50.1, 48.9 (br), 34.3, 27.9, 27.64, 27.3, 25.51; IR (KBr) 2972, 1678, 1642, 1421 cm^{-1}; MS (EI) *m*/*z* 416 (65), 360 (31), 343 (17), 316 (55), 286 (9), 273 (100), 260 (12), 235 (31); HRMS (EI) calcd for $C_{16}H_{21}N_2O_3I$ 416.0597, found 416.05774. Anal. calcd for $C_{16}H_{21}N_2O_3I$ C, 46.10; H, 5.09; N, 6.73, found C, 46.11; H, 5.13; N, 6.53.

Analytical data for **9**: mp 168–169 °C; ^1H NMR ($CDCl_3$) δ 7.59 (d, $J = 9.4$ Hz, 1H), 6.27 (d, $J = 9.4$ Hz, 1H), 4.37 (br d, $J = 12.4$ Hz, 1H), 4.14 (d, $J = 15.6$ Hz, 1H), 3.84 (dd, $J = 6.4$ Hz, $J = 15.6$ Hz, 1H), 3.29 (br s, 1H), 2.98 (br s, 2H), 2.40 (s, 1H), 1.99 (br s, 2H), 1.27 (br s, 9H); ^{13}C NMR ($CDCl_3$) δ 162.5, 154.0, 148.0, 147.4, 118.8, 79.8 (br), 70.2 (br), 50.1, 48.8 (br), 47.7 (br), 38.4, 28.0, 27.6, 26.7; MS (EI) *m*/*z* 416 (95), 360 (62), 343 (13), 316 (49), 273 (100), 260 (13), 235 (21).

Analytical data for **10**: mp 206–207 °C; ^1H NMR ($CDCl_3$) δ 8.23 (s, 1H), 4.35 (br s, 1H), 4.20 (d, $J = 15.7$ Hz, 1H), 3.87 (dd, $J = 6.3$ Hz, $J = 15.7$ Hz, 1H), 3.28 (br s, 1H), 2.97 (br s, 2H), 2.38 (s, 1H), 1.99 (br s, 2H), 1.27 (br s, 9H); ^{13}C NMR ($CDCl_3$) δ 159.8, 155.0, 154.2, 148.8, 89.3, 80.2 (br), 69.7 (br), 51.9, 50.1 and 48.9 (br, conformers), 47.7 (br), 38.4, 28.0, 27.7, 26.6; MS (EI) *m*/*z* 542 (100), 486 (41), 469 (12), 442 (49), 399 (81), 386 (10), 361 (27).

(−)-(1*R*,5*S*)-*N*-*tert*-Butoxycarbonyl-9-trimethylstannyl-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-*a*][1,5]diazocin-8-one (11). *N*-Boc-9-iodocytisine (**8**, 1.000 g, 2.40 mmol), hexamethyl-ditin (1.214 g, 3.72 mmol) and $Pd(PPh_3)_4$ (0.240 g, 0.25 mmol) were dissolved in 1,4-dioxane (20 mL) under a nitrogen gas flow. The reaction mixture was refluxed for 3 h, diluted with EtOAc and filtered through paper. The residue was washed with EtOAc, dried over $MgSO_4$ and concentrated under vacuum. The crude product was further purified by column chromatography on silica gel (EtOAc–CH_2Cl_2, 9:1) to afford the product as a white solid (0.570 g, 1.26 mmol, 52%): mp 130–131 °C; $[\alpha]_D^{22} = -123$ (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.34 (d, $J = 6.5$ Hz, 1H), 6.03 (d, $J = 5.3$ Hz, 1H), 4.0–4.2 (m, 2H), 3.73 (dd, $J = 6.2$ Hz, $J = 15.5$ Hz, 1H), 2.91–2.99 (m, 3H), 2.3 (d, $J = 7.0$ Hz, 1H), 1.84–1.98 (m, 2H), 1.19 (s, 9H), 0.17 (s, $J_{Sn-H} = 54$ Hz, 9H); ^{13}C NMR ($CDCl_3$) δ 166.3 and 166.03 (conformers), 165.7, 154.2, 148.7, 146.5 and 146.0 (conformers), 131.0, 106.4 and 105.9 (conformers), 80.0 and 79.5 (conformers), 51.6, 50.5, 49.1 and 48.7 (conformers), 34.7, 27.9 and 27.8 (conformers), 26.2, −9.78 ($J_{Sn-C} = 366$ Hz); IR (NaCl) 2926, 1692, 1628, 1536, 1422 cm^{-1}; MS (EI) *m*/*z* 454 (2), 443 (16), 441 (15), 440 (19), 439 (96), 438 (39), 437 (73), 436 (30), 435 (41), 383 (100), 381 (79), 380 (30), 379 (45), 337 (34), 335 (20), 309 (10), 307 (12). Anal. calcd for $C_{19}H_{30}N_2O_3$ Sn C, 50.36; H, 6.67; N, 6.18, found C, 50.43; H, 6.71; N, 6.19.

5-Trimethylstannyl-2-nitropyridine (13). 2-Nitro-5-bromopyridine (**12**, 0.40 g, 1.97 mmol), hexamethylditin (0.846 g, 2.60 mmol), $Pd(PPh_3)_4$ (0.058 g, 0.05 mmol) and dioxane (20 mL) were added in 50 mL round-bottom flask under nitrogen gas flow and heated to 100 °C for 3 h. The reaction mixture was diluted with water and extracted with diethyl ether. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under vacuum. The crude product was further purified by column chromatography on silica gel (hexane–EtOAc, 3:1) to afford the product as white solid (0.345 g, 61%): mp 109–110 °C; ^1H NMR ($CDCl_3$) δ 8.65 (t, $J = 9.5$ Hz, 1H), 8.14–8.19 (m, 2H), 0.43 (s, $J_{Sn-H} = 55.7$ Hz, 9H); ^{13}C NMR ($CDCl_3$) δ 157.2, 154.4, 147.1, 146.8, 117.0,

−9.3 (J_{Sn-C} = 367.4 Hz); IR (NaCl) 3038, 1530, 1340, 1014 cm^{-1}; MS (EI) *m/z* 288 (5), 277 (17), 275 (14), 273 (100), 272 (63), 271 (21), 270 (25). Anal. calcd for $C_8H_{12}N_2O_2Sn$ C, 33.49; H, 4.22; N, 9.76, found C, 33.57; H, 4.32; N, 9.50.

2-Fluoro-5-trimethylstannylpyridine (15). 2-Fluoro-5-iodo pyridine (**14**, 1.0 g, 4.50 mmol), hexamethylditin (1.9 g, 5.85 mmol) and $Pd(PPh_3)_4$ (0.155 g, 0.135 mmol) were dissolved in dioxane (50 mL) under nitrogen gas flow. The reaction mixture was stirred for 4 h at 100 °C. After dilution with water and two extractions with CH_2Cl_2, the combined organic layers were dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by column chromatography on silica gel (hexane–EtOAc, 3:1) to afford a yellow solid (0.90 g, 77%): mp 146–147 °C; ^1H NMR ($CDCl_3$) δ 8.15 (t, *J* = 11.0 Hz, 1H), 7.7 (dt, *J* = 1.8 Hz, *J* = 8.0 Hz, 1H), 6.84 (dd, *J* = 2.2 Hz, *J* = 7.9 Hz, 1H), 0.28 (s, J_{Sn-H} = 55.2 Hz, 9H); ^{13}C NMR ($CDCl_3$) δ 166.2 (d, J_{F-C} = 231 Hz), 153.3 (d, J_{F-C} = 12 Hz), 148.0 (d, J_{F-C} = 7 Hz), 133.6 (d, J_{F-C} = 5 Hz), 109.7 (d, J_{F-C} = 34 Hz), −9.48 (J_{Sn-C} = 367 Hz); ^{19}F NMR ($CDCl_3$) δ −68.9; IR (NaCl) 2926, 1556, 1472, 1250 cm^{-1}; MS (EI) *m/z* 261 (3), 246 (100), 242 (35), 244 (72), 216 (17), 135 (9).

(−)-(1*R*,5*S*)-*N*-*tert*-Butoxycarbonyl-9-(2-nitro-5-pyridyl)-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-*a*][1,5]diazocin-8-one (16). *N*-Boc-9-iodocytisine (**8**, 0.300 g, 0.72 mmol), 5-(trimethylstannyl)-2-nitropyridine (**13**, 0.269 g, 0.93 mmol) and $Pd(PPh_3)_4$ (0.081 g, 0.07 mmol) were solubilized in dioxane (4.5 mL) under a nitrogen gas flow and heated to 100 °C for 3 h. The reaction mixture was diluted with water and extracted twice with CH_2Cl_2. The combined organic layers were dried $MgSO_4$ and concentrated under vacuum. The crude product was purified by column chromatography on silica gel (CH_2Cl_2–MeOH, 95:5) to afford the product as a bright yellow solid (0.133 g, 45%): mp 193–194 °C; $[\alpha]_D^{22} = -160$ (*c* 0.9; $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.85 (s, 1H), 8.53 (br s, 1H), 8.26 (d, *J* = 8.5 Hz, 1H), 7.65 (d, *J* = 7.2 Hz,1H), 6.29 (d, *J* = 7.2 Hz,1H), 4.30 (d, *J* = 15.6 Hz, 3H), 3.90 (dd, *J* = 15.6 Hz, *J* = 6.3 Hz, 1H), 3.11 (br s, 3H), 2.50 (s, 1H), 1.9–2.1 (m, 2H), 1.2-1.4 (m, 9H); ^{13}C NMR ($CDCl_3$) δ 163.4, 157.3, 149.9, 141.2, 123.9, 119.6, 51.8, 37.4, 30.2, 29.8, 28.2; MS (EI) *m/z* 412 (36), 356 (15), 339 (15), 312 (100), 295 (72), 269 (723), 238 (31); HRMS (EI) calcd for $C_{21}H_{24}N_4O_5$ 412.17463, found 412.17659.

(−)-(1*R*,5*S*)-*N*-*tert*-Butoxycarbonyl-9-(2-fluoro-5-pyridyl)-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-*a*][1,5]diazocin-8-one (17). Procedure A: *N*-Boc-9-iodocytisine (**8**, 0.10 g, 0.240 mmol), 2-fluoro-5-(trimethylstannyl)-pyridine (**15**, 0.082 g, 0.312 mmol) and $Pd(PPh_3)_4$ (0.025 g, 0.021 mmol) were dissolved in dioxane (3 mL) under a nitrogen gas flow and heated to reflux temperature for 4 h. The reaction mixture was diluted with water and extracted twice with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by column chromatography on silica gel (EtOAc–CH_2Cl_2, 9:1) to afford a white solid (0.048 g, 51%).

Procedure B: *N*-Boc-9-trimethylstannylcytisine (**11**, 0.20 g, 0.441 mmol), 2-fluoro-5-iodo pyridine (**14**, 0.127 g, 0.572 mmol) and $PdCl_2(PPh_3)_2$ (0.055 g, 0.047 mmol) were added in dioxane (3 mL) under a nitrogen gas flow and heated to reflux temperature for 3 h. The warm reaction mixture was filtered and the filtrate was concentrated under vacuum. The crude product was purified by column chromatography on silica gel (EtOAc–CH_2Cl_2, 9:1) to afford a white solid (0.053 g, 31%): mp 152–153 °C; $[\alpha]_D^{22} = -159$ (*c* 1; $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.37 (s, 1H), 8.21 (br s, 1H), 7.52 (d, *J* = 3.5 Hz, 1H), 6.94 (dd, *J* = 2.9 Hz, *J* = 8.5 Hz, 1H), 6.22 (d, *J* = 6.9 Hz, 1H), 4.16–4.40 (m, 2H), 3.86 (dd, *J* = 5.9 Hz, *J* = 15.6 Hz, 1H), 3.07 (br s, 2H), 2.47 (s,1H), 1.95–2.08 (m, 2H), 1.25 (br s, 9H); ^{13}C NMR ($CDCl_3$) δ 162.6 (d, J_{F-C} = 239 Hz), 161.6, 154.4, 149.3, 146.6 (d, J_{F-C} = 15 Hz), 141.3 (d, J_{F-C} = 8 Hz), 137.1, 131.0 (d, J_{F-C} = 4 Hz), 123.4, 108.5 (d, J_{F-C} = 37 Hz), 105.8, 80.3, 50.5, 49.4, 35.0, 27.9, 27.6, 26.1; ^{19}F NMR ($CDCl_3$) δ −70.5; IR (NaCl) 2976, 1686, 1638, 1560, 1424 cm^{-1}; MS (EI) *m/z* 385 (37), 329 (17), 285 (77), 255 (18), 242 (100), 229 (25), 204 (29); HRMS (EI) calcd for $C_{21}H_{24}FN_3O_3$ 385.1801, found 385.1814.

(−)-9-(2-Fluoropyridyl)cytisine or (−)-(1*R*,5*S*)-9-(2-Fluoro-5-pyridyl)-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-*a*][1,5]diazocin-8-one (3). To a solution of *N*-Boc-9-(2-fluoropyridyl)cytisine (**17**, 0.220 g, 0.570 mmol) in CH_2Cl_2 (7.0 mL) was added trifluoroacetic acid (1.5 mL). The solution was stirred for 30 min at rt and concentrated to dryness. The residue was taken into water, basified with ammonia (28% aq solution) and extracted three times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by column chromatography on silica gel (CH_3CN–CH_2Cl_2–NH_4OH, 5:4.5:0.5) to afford the product as a white solid (0.140 g, 86%): mp 189–190 °C; $[\alpha]_D^{22} = -27$ (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.41 (d, *J* = 2.3 Hz, 1H), 8.29 (tt, *J* = 2.5 Hz, *J* = 8.0 Hz, 1H), 7.49 (d, *J* = 7.2 Hz, 1H), 6.94 (dd, *J* = 2.9 Hz, *J* = 8.5 Hz, 1H), 6.14 (d, *J* = 7.2 Hz, 1H), 4.17 (d, *J* = 15.6 Hz, 1H), 3.96 (dd, *J* = 6.5 Hz, *J* = 15.6 Hz, 1H), 3.04–3.15 (m, 4H), 3.01 (s, 1H), 2.38 (s, 1H), 1.97 (s, 2H), 1.57 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 162.5 (d, J_{F-C} = 239 Hz), 161.7, 151.7, 146.6 (d, J_{F-C} = 15 Hz), 141.3 (d, J_{F-C} = 8 Hz), 136.9, 131.1 (d, J_{F-C} = 4 Hz), 122.6, 108.4 (d, J_{F-C} = 37 Hz), 104.9, 52.9, 52.8, 50.2, 35.6, 27.7, 26.1; ^{19}F NMR ($CDCl_3$) δ −70.8; IR (NaCl) 3318, 2936, 1636, 1556 cm^{-1}; MS (EI) *m/z* 285 (91), 255 (14), 243 (37), 242 (100), 241 (98), 229 (25), 213 (10); HRMS (EI) calcd for $C_{16}H_{16}FN_3O$ 285.1277, found 285.1278.

Radiochemistry

Preparation of the K[^{18}F]F–K_{222} complex. In order to recover and recycle the [^{18}O]water target, the 2 mL of aqueous [^{18}F]fluoride from the target holder were passed through an anion exchange resin (Sep-Pak® Light Waters Accell™ Plus QMA Cartridge in the chloride form, washed with 5 mL 1 M aq $NaHCO_3$ and then rinsed with 50 mL of water) by Helium pressure (1.5–2.0 bar). Helium was blown through the column to maximally

extract the last traces of [^{18}O]water. See refs 7 and 53 for more practical details. The [^{18}F]fluoride ion was then eluted from the resin using 1.0 mL of a 4.5 mg/mL aqueous K_2CO_3 solution into a Vacutainer® tube containing 12.0–15.0 mg of Kryptofix®222 (K_{222}: 4,7,13,16,21,24 - hexaoxa - 1,10 - diazabicyclo[8.8.8]hexacosane). The resulting solution was then gently concentrated to dryness at 145–150 °C under a nitrogen stream for 10 min to give no-carrier-added K[^{18}F]F-K_{222} complex as a white semi-solid residue.

If desired, the [^{18}F]fluoride ion production batch on the cartridge can also be divided into 2–12 aliquots in order to perform parallel syntheses. To do this, the [^{18}F]fluoride ion is eluted from the resin using 1.0 mL of a 4.5 mg/mL aq K_2CO_3 solution into an empty Vacutainer® tube. In order to distribute equally this activity over *n* tubes (Vacutainer® tube, $n = 2–12$), the quantity of K_2CO_3 was firstly adjusted to *n* times 4.5 mg with a 50.0 mg/mL aqueous K_2CO_3 solution and secondly, the total volume of the solution was adjusted to 2.0 mL with water. This new aqueous [^{18}F]fluoride solution was then equally distributed over the *n* tubes each containing 12.0–15.0 mg of Kryptofix®222 (K_{222}: 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane). Finally, the volume of each fraction was adjusted to 1.0 mL with water. The resulting solutions were then independently gently concentrated to dryness at 145–150 °C under a nitrogen stream for 10 min to give no-carrier-added K[^{18}F]F–K_{222} complex as a white semi-solid residue.

Preparation of (−)-9-(2-[^{18}F]fluoropyridyl)cytisine or (−)-(1*R*,5*S*)-9-(2-[^{18}F]fluoro-5-pyridyl)-1,2,3,4,5,6-hexahydro - 1,5 - methano - pyrido[1,2-*a*][1,5]diazocin-8-one ([^{18}F]-3)

Incorporation studies. Freshly distilled DMSO (600 μL) containing 3.0 mg of the precursor for labelling ((−)-(1*R*,5*S*)-*N*-*tert*-butoxycarbonyl-9-(2-nitro-5-pyridyl)-1,2,3,4,5,6 - hexahydro - 1,5 - methano - pyrido[1,2-*a*][1,5]diazocin-8-one, **16**, 7.3 μmol) were directly added into the Vacutainer® tube containing the dried K[^{18}F]F-K_{222} complex. The tube (not sealed) was then placed in a heating block (at 145 °C for 1–10 min) without stirring the contents. The reaction vessel was then cooled using an ice-water bath and the remaining radioactivity was measured. 95% of the initial radioactivity placed in the vessel was still present. The resulting dark-coloured reaction mixture was analyzed by radiochromatography. The reaction yield was calculated from the TLC-radiochromatogram and defined as the radioactivity area of the [^{18}F]fluoropyridine derivative over total fluorine-18 radioactivity area ratio. (SiO_2–TLC, eluent: CH_2Cl_2–MeOH: 90/10 v/v, R_f: [^{18}F]fluoropyridine derivative ([^{18}F]-**17**): 0.7 and R_f: [^{18}F]fluoride ion: 0.0). Radiosynthesized [^{18}F]-**17** co-migrated with an authentic sample of fluorine-19-synthesized **17**.

Optimized conditions. Freshly distilled DMSO (600 μL) containing 3.0 mg of the precursor for labelling ((−)-(1*R*,5*S*)-*N*-*tert*-Butoxycarbonyl-9-(2-nitro-5-pyridyl)-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-*a*][1,5]diazocin-8-one, **16**, 7.3 μmol) were directly added into the Vacutainer® tube containing the dried K[^{18}F]F–K_{222} complex. The tube (not sealed) was then placed in a heating block at 145 °C for 7 min without stirring the contents. The reaction vessel was then cooled using an ice-water bath. The resulting dark-coloured reaction mixture was diluted with 1 mL of water and transferred onto a C18 Sep-Pak cartridge (PrepSep™ R-C18, Fisher Scientific). The tube was rinsed twice with 1 mL of water, which was also transferred and added to the diluted reaction mixture on the cartridge (2–3% of the total radioactivity amount engaged in the fluorination process was lost in the initial tube). The whole was then passed through the cartridge, which was then washed by 3 mL of water and partially dried for 0.5 min by applying a nitrogen stream. The [^{18}F]fluoropyridine derivative [^{18}F]-**17** was eluted from the cartridge with CH_2Cl_2 (3 mL followed by two successive rinses of 1.0 mL) into a 5 mL reaction vial containing 0.1 mL of TFA. (3–5% of the total radioactivity amount engaged in the fluorination process was left on the cartridge). The resulting CH_2Cl_2–TFA solution (50/1, v/v) was concentrated to dryness (at 65–75 °C under a gentle nitrogen stream for 4–6 min). The residue was redissolved in 2 mL of CH_2Cl_2 and concentrated again to dryness to minimize TFA presence (at 65–75 °C under a gentle nitrogen stream for 2–3 min). Finally, the residue was redissolved in 1.0 mL of the HPLC solvent used for purification and the crude was injected onto HPLC. Isocratic elution gave pure labelled (−)-9-(2-[^{18}F]fluoropyridyl)cytisine ([^{18}F]-**3**) (HPLC A, retention time: 7.0–7.5 min).

Formulation. Formulation of labelled product for iv injection was effected as follows: the HPLC-collected fraction containing the fluorine-18-labelled tracer [^{18}F]-**3** was diluted with water (50 mL). The resulting solution was passed through a C18 Sep-pak cartridge (Waters). The cartridge was washed twice with 5 mL of water and partially dried for 10 s by applying a nitrogen stream. The fluorine-18-labelled tracer [^{18}F]-**3** was eluted with 2 mL of EtOH (less than 5% of the total radioactivity was left on the cartridge) and filtered on a 0.22 μm GS-Millipore filter (vented). Finally, physiological saline was added to lower the EtOH concentration below 10%.

Quality control. The radiopharmaceutical preparation is a clear and colourless solution and its pH is between 6 and 7. As demonstrated by HPLC analysis, the radiolabelled product was found to be >99% radiochemically pure and also co-eluted with a sample of authentic (−)-9-(2-fluoropyridyl)cytisine (**3**) (HPLC B, retention time: 2.15 min). The preparation was shown to be free of non-radioactive precursor and radiochemically stable for at least 120 min.

Binding assays

Animals. Female Sprague–Dawley rats (Charles River Laboratories, Raleigh, NC, USA) weighing 155–175 g were housed individually with food (Rodent Chow 5002, PMI Nutrition International, Inc.) and water available ad libitum. The animal facility was maintained on a 12 h light/dark cycle at a temperature of 70°F (21 °C) and 50% relative humidity.

Preparation of membranes. Rats were killed by decapitation following anesthesia with 70% CO_2. Brains were removed and placed on an ice-cold platform. The hippocampus and cerebral cortex were removed. Hippocampi and cortices from 10 rats were pooled separately and homogenized by Polytron (Kinematica GmbH, Switzerland) in 10 vol (weight/volume) of ice-cold preparative buffer (KCl, 11 mM; KH_2PO_4, 6 mM; NaCl 137 mM; Na_2HPO_4 8 mM; HEPES (free acid), 20 mM; iodoacetamide, 5 mM; EDTA, 1.5 mM; 0.1 mM PMSF pH to 7.4 with NaOH). The resulting homogenate was centrifuged at 18,000*g* for 20 min at 4 °C and the resulting pellet was resuspended in 20 vol of ice-cold water. After 60 min incubation at 4 °C, a new pellet was collected by centrifugation at 18,000*g* for 20 min at 4 °C. The final pellet was resuspended in preparative buffer and stored at −20 °C. On the day of the assay, tissue was thawed, centrifuged at 18,000*g* for 20 min, then resuspended in ice-cold PBS (Dulbecco's Phosphate Buffered Saline, Life Technologies, pH. 7.4) to a final concentration of 2–4 mg protein/mL. Protein concentrations were determined using the Pierce BCA Protein Assay kit (Pierce Biotechnology, Rockford, IL, USA), with bovine serum albumin as the standard.

(−)-[^{3}H]Nicotine binding. The binding of (−)-[^{3}H]nicotine was measured using a modification of published procedures.[62,63] Briefly, samples were conducted in triplicate over a 3 h period at 4 °C in 48-well micro-titer plates. Each well contained cortical sample of approximately 400 μg/mL of protein, 5 nM (−)-[^{3}H]nicotine (80 Ci/mmol), and the test compound **3** diluted to varying concentrations in PBS (final total volume = 300 μL). Non-specific binding was determined using 10 μM (−)-nicotine in selected wells. The binding reaction was terminated by filtration of the protein containing bound ligand onto Whatman GF/B glass fiber filters using a tissue harvester (Brandel, Gaithersburg MD, USA). Filters were soaked in PBS containing 0.33% polyethyleneimine to reduce non-specific binding. Each filter was washed three times with ice-cold PBS before harvesting tissue. The filtration apparatus was cooled to 4 °C before use and was kept cold through the filtration process. Filters from binding experiments were placed in scintillation vials filled with 4 mL of scintillation fluid and allowed to equilibrate for 12 h before counting. Radioactivity bound to tissue was determined by liquid scintillation counting (2200CA Tri-Carb LSC, Packard Instruments, 50% efficiency).

[^{3}H]Methyllycaconitine ([^{3}H]MLA) binding. [^{3}H]MLA binding was determined in hippocampal membranes as described previously.[64] Briefly, samples were conducted in triplicate over a 2 h period at rt in 48-well micro-titer plates. Each well contained hippocampal sample of approximately 300 μg/mL of protein, 5 nM [^{3}H]MLA (26 Ci/mmol), and the test compound **3** diluted to varying concentrations in PBS (final total volume = 300 μL). Non-specific binding was determined using 10 μM MLA in selected wells. The binding reaction was terminated by filtration of the protein containing bound ligand onto Whatman GF/B glass fiber filters using a tissue harvester (Brandel, Gaithersburg MD, USA). Filters were soaked in PBS containing 0.33% polyethyleneimine to reduce non-specific binding. Each filter was washed three times with PBS before harvesting tissue at rt. Filters were processed as described above.

Data analysis. Data were expressed as disintegrations per min. The replicates for each point were averaged and plotted against the log of drug concentration. The IC_{50} (concentration of the compound that produces 50% inhibition of binding) was determined by least squares non-linear regression using GraphPad Prism software. K_i was calculated using the Cheng–Prussof equation.[65]

Kinetics, regional distribution and radiopharmacological characterization in rodents

Animals. Animals use procedures were in accordance with the recommendations of the EEC (86/609/CEE) and the French National Committee (decret 87/848) for the care and use of laboratory animals.

Intracerebral radiosensitive β-microprobes acquisition. Anesthesia of animals (male Sprague–Dawley rats, weighing 280–340 g) was induced with 5% isoflurane in a gas mixture of O_2/N_2O (30%/70%) and maintained with 1.5–2.5% isoflurane during the entire surgical procedure. Body temperature was monitored rectally and maintained by means of a thermoregulated blanket. Catheters were placed in both femoral veins and arteries. Animals were then mounted in a stereotaxic frame. Craniotomies were performed in order to implant radiosensitive β-microprobes (Biospace Mesures, Paris, France). Two probes were implanted, the first one in the left striatum and the second one in the cerebellum using the coordinates of implantation according to the atlas of Paxinos and Watson.[66] Data were acquired 30 min after probes implantation. Local radioactivity count rates were acquired for 30 min prior to the radiotracer injection to evaluate environmental background (approximately 5 cps) with a temporal resolution of 1 s. The radiotracer was injected in the femoral vein ([^{18}F]-**3**, 0.8–1.0 mCi with specific radioactivities greater than 2 Ci/μmol) in a volume of 1 mL over 1 min. Data were acquired for another 40–80 min with a temporal resolution of 1 s. In a displacement study, (−)-nicotine was administrated (5 mg/kg, sc) 30 min after the injection of the radiotracer and data were acquired for 60 min with the same temporal resolution. Finally, mean background noise was subtracted from the raw data and radioactive decay correction for fluorine-18 was applied to obtain quantitative time activity curves.

Biodistribution studies. Sprague–Dawley male rats weighing 200–250 g were used in all experiments. Each animal received 30 μCi of the radiotracer (with specific radioactivities greater than 2 Ci/μmol), dissolved in 0.1 mL saline, by injection in the tail vein. At designated times (5, 15, 30, 60 min) after injection of the radiotracer, animals ($n = 3$ per time point) were killed by decapitation, the brains were quickly removed, dissected, weighed and assayed for regional radioactivity in a γ-counter (Cobra Quantum, Packard). Samples of

pons, cerebellum, diencephalon, hippocampus, striata, frontal cortex, posterior cortex, blood and plasma were obtained for each animal. In vivo receptor blocking studies were also performed on three rats by administration of (−)-nicotine (5 mg/kg, subcutaneous administration), followed 5 min later by iv injection of the radiotracer ([^{18}F]-**3**, 30 μCi in 0.1 mL saline, with specific radioactivities greater than 2 Ci/μmol). Blocking studies were also performed on another three rats by pre-treatment with ritanserine (2.5 mg/kg, iv injection), followed 15 min later by iv injection of the radiotracer (30 μCi in 0.1 mL saline, with specific radioactivities greater than 2 Ci/μmol). Animals were sacrified 15 min after injection of the radioligand and the brain dissected as mentioned above. Results were expressed as % injected dose per g tissue (% ID/g tissue) after correction for the physical decay of the radioisotope.

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