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Chemical Delivery System of Metaiodobenzylguanidine (MIBG) to the Central Nervous System

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The aim of the present investigation was to apply a chemical delivery system (CDS) to MIBG (**4**) with the purpose of delivering this drug to the CNS. Compound **4** has been linked to a 1,4-dihydroquinoline moiety in order to achieve its CNS penetration, and here we report the synthesis to link **4** to the chemical delivery system and the radiosynthesis with carbon-11 of the "CDS-**4** entity". After iv injection into rats of the [^{11}C]CDS-**4**, the follow-up study of the radioactivity distribution in blood samples and brain homogenates and the analysis by HPLC and LC-MS/MS have confirmed the release of **4** into the CNS.

Introduction

Drug delivery to the brain is often limited by the BBB, which regulates the exchange of substances between the peripheral circulation and the central nervous system (CNS[a]). Various attempts have been made to overcome the limited access of drugs into the brain. One of these strategies was the linking of an active drug to a brain-specific carrier, which delivers the drug specifically into the brain, where it is cleaved enzymatically from the carrier. The 1,4-dihydropyridine/pyridinium salt redox-chemical delivery systems have been used by Bodor to improve the access of therapeutic agents to the central nervous system.[1−4] The principle of the CDS is illustrated in Scheme 1. These chemical delivery systems (CDSs) based on the redox conversion of a lipophilic dihydropyridine to an ionic lipid-insoluble pyridinium salt are equivalent to the NADH/NAD$^+$ oxidation system. The important criteria for a CDS is that it should be sufficiently lipophilic to enter the central compartment and should then undergo an enzymatic conversion to promote drug retention in the CNS. Usually, the drug was conjugated through ester or amide bond to a carboxylic function of the carrier system. Nevertheless, the main drawback observed with the dihydropyridines is their instability against oxidation and mainly hydration of the 5,6-double bond.[5,6] The modest stability of this class of carriers remains an obstacle to further development of this CDS approach, and a possibility to overcome this problem was to replace the dihydropyridine/pyridinium salt system by a dihydroquinoline/quinolinium salt system.[7,8]

The delivery of radiopharmaceuticals to the CNS would constitute another interesting application of this redox CDS for brain imaging. To the best of our knowledge, only one paper has already investigated the potential of this CDS approach to improve the delivery of radiolabeled agents into the CNS.[9] However, the relative instability of the dihydropyridine carrier used in this investigation appeared also to be a major limitation, hampering severely the development of this CDS strategy. In this context, the aim of the present investigation was to apply the dihydroquinoline/quinolinium salt type CDS to the *m*-iodobenzylguanidine (MIBG) with the purpose of delivering this drug to the CNS. [^{123}I/^{131}I]MIBG imaging is the most accurate method for detection of catecholamine-secreting tumors including neuroendocrine tumors such as pheochromocytoma, paraganglioma, neuroblastoma, carcinoid, and many medullary thyroid cancers.[10] In addition to its application as a marker of adrenergic tumors, [^{123}I]MIBG has proven to be very useful for scintigraphic imaging studies of cardiac sympathetic innervation.[11] MIBG is a guanethidine derivative structurally resembling norepinephrine. Studies have demonstrated that MIBG is actively taken up into human neuroblastoma cells by the norepinephrine transporter (NET) and that MIBG uptake was attenuated into neuroblastoma cells in the presence of imipramine, an inhibitor of NET.[12,13] Cellular accumulation of MIBG occurs by two distinct mechanisms: an active uptake, which is specific, high-affinity, saturable, and ATPase-dependent and only occurs in cells that synthesize the NET, and a passive diffusion, which is nonspecific, low-level, energy-independent, and unsaturable and takes place in all cells.[14] In addition, MIBG is concentrated into storage vesicles via vesicular monoamine transporter (VMAT) and MIBG uptake can be inhibited with pretreatment with drugs such as reserpine, a specific inhibitor of VMAT.[15]

The NET is involved in several neuropsychiatric disorders and is a molecular target for the treatment of depression, attention deficit hyperactivity disorders (ADHD), and anxiety disorders.[16] MIBG does not cross the BBB, and it has been demonstrated in vitro that [^{125}I]MIBG maps the monoamine storage and release sites on rat brain slices.[17] A CDS which

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[a] Abbreviations: CDS, chemical delivery system; CNS, central nervous system; MIBG, metaiodobenzylguanidine; BBB, blood−brain barrier; NET, norepinephrine transporter; VMAT, vesicular monoamine transporter; ADHD, attention deficit hyperactivity disorders; EOS, end of synthesis; PET, positron emission tomography; SPECT, single photon emission computed tomography.

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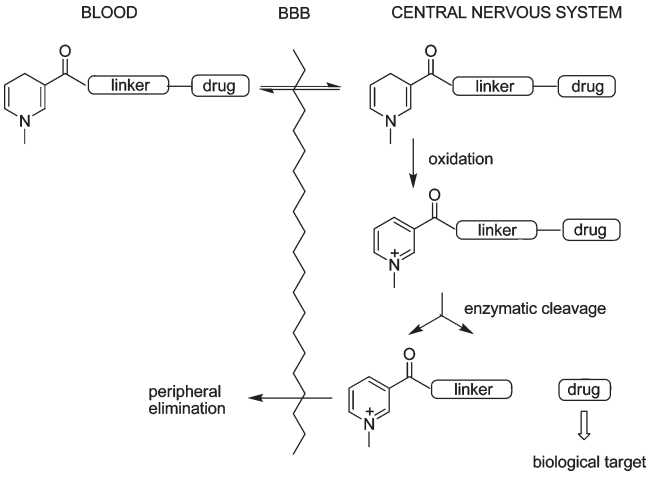
Scheme 1. Bodor's Chemical Delivery System (CDS)



would permit the crossing of MIBG through the BBB would provide a potential imaging marker of monoamine uptake, storage, and release in the brain with a wide field of application in neurodegenerative or neuropsychiatric disorders.

Previously, we have labeled with carbon-11 ($t_{1/2}$ = 20.4 min) a dihydroquinoline derivative, chosen as a model for chemical carrier in order to validate the concept of CDS described above.[18] In vivo evaluation of the *N*-[^{11}C-methyl]-dihydroquinoline was investigated in rats after iv injection where a rapid penetration into the CNS followed by a fast oxidation into the [^{11}C]quinolinium salt was identified. These encouraging results have demonstrated that this CDS approach could be used as a potential delivery system for the transport of drug which does not cross the BBB. For this reason, **4** has been linked to a 1,4-dihydroquinoline moiety in order to achieve its CNS penetration, and here we report the synthesis to link **4** to the chemical delivery system and the radiosynthesis with carbon-11 of the "CDS-**4** entity". After iv injection into rats of the [^{11}C]CDS-**4**, the distribution of the radioactivity was followed in blood samples and brain homogenates and analyzed by HPLC and LC-MS/MS.

Results and Discussion

Chemistry. The synthesis of *N*-(3-iodobenzyl)-*N*′-(1-methyl-3-carbonyl-1,4-dihydroquinoline)guanidine (**3**) was accomplished as described in Scheme 2. The key intermediate *N*-(3-iodobenzyl)-*N*′-(3-carbonyl-quinoline)-guanidine (**1**) was prepared using four different routes by reaction of several activated esters of 3-quinoline carboxylic acid with **4**. The first attempts involving the condensation of **4** with the corresponding methyl ester of 3-quinoline carboxylic acid or with a crotonamide intermediate obtained with *N*-*tert*-butyl-5-methylisoxazolium perchlorate (NBI) provided **1** in 35−37% yield.[19−25] To find a better procedure, we have also used the *N*,*N*′-carbonyldiimidazole (CDI) or *N*-hydroxysuccinimide as carboxylate activating agents.[26] The reactions of resulting activated esters with **4** showed a significant increase in reactivity and compound **1** was isolated in 59.5−61.5% yield. An alternative route to obtain **1** via the acid chloride of 3-quinoline carboxylic acid and the guanidine was unsuccessful.[27] The quaternarization reaction of **1** with methyl trifluoromethanesulfonate afforded *N*-(3-iodobenzyl)-*N*′-(1-methyl-3-carbonyl-quinolinium)-guanidine trifluoromethane sulfonate (**2**) in 50% yield. In the final step, the regioselective reduction of **2** performed with a large excess of sodium dithionite and sodium carbonate gave **3** in only a 20−52% moderate yield.[28] In contrast, the use of BNAH (1-benzyl-1,4-dihydronicotinamide) provided the corresponding final target **3** with an excellent yield of 95%.[29]

Radiochemistry. The radiosynthesis of [^{11}C]**3** was carried out as illustrated by Scheme 3. The quaternarization reaction of the quinoline **1** with [^{11}C]methyl triflate afforded the quaternary salt [^{11}C]**2** with an incorporation of [^{11}C]-CH_3OTf up to 95%. Reduction of [^{11}C]**2** was made by treatment with BNAH and provided the corresponding 1,4-dihydroquinoline [^{11}C]**3** in an excellent radiochemical yield of 95%. After HPLC purification and formulation [^{11}C]**3** was obtained with a radiochemical purity greater than 99% and specific activities ranging from 13 to 24 GBq/μmol as evaluated by analytical HPLC at the end of synthesis (EOS).

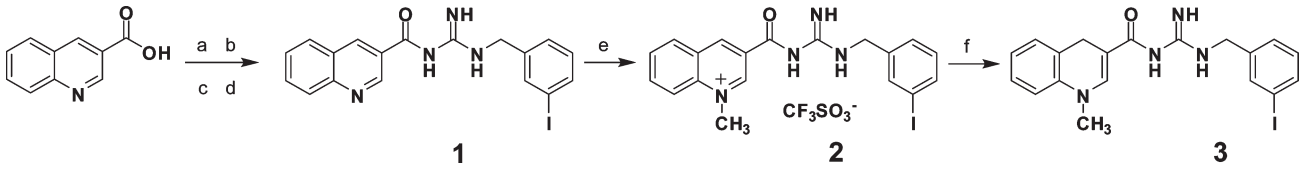
In Vitro Stability of [^{11}C]3 in Rat Blood Samples. The chemical stability of [^{11}C]**3** toward oxidation was first investigated in vitro on blood samples to establish whether it is stable enough to start developing an ex vivo evaluation. To carry out this investigation, [^{11}C]**3** was added to rat blood, and after an efficient preparation process where >90% of the radioactivity was recovered, the plasma samples were analyzed using reverse phase HPLC analysis. The chromatograms obtained at various time points (5, 15, 30, and 60 min) did not show any trace of the quaternary form-[^{11}C]**2**. Additionally, no byproduct which could have arisen from hydration reactions were detected, indicating that [^{11}C]**3** exhibit a good stability in rat blood.

In Vivo Evaluation. The aim of this work was to study the feasibility of using the 1,4-dihydroquinoline/quinolinium salt redox system as a potential chemical delivery system of **4** to the CNS. As shown in Scheme 4, we have evaluated the penetration of [^{11}C]**3** through the blood−brain barrier (BBB), its brain oxidation to the corresponding quaternary form-[^{11}C]**2** expected in vivo, and finally the release of **4** and [^{11}C]**5** after enzymatic cleavage of the quinolinium salt. The radioactivity kinetics of rat brain uptake were determined in vivo after [^{11}C]**3** injection following the sacrifice of animals at different time intervals over a period of 60 min. The radioactivity brain uptake was rapid, peaking at 0.10% ID/g at 3 min and declining to 0.06% ID/g at 60 min (Figure 1). These data indicate a moderate penetration of [^{11}C]**3** through the BBB with a progressive and slow elimination of the brain radioactivity.

According to the CDS concept described earlier, the oxidation process is very crucial to predict the ability of the 1,4-dihydroquinoline CDS to release the compound in the brain. A fast oxidation process will convert the CDS into the corresponding quaternary salt in the blood before reaching the brain. A satisfactory compromise should be obtained between the stability of the 1,4-dihydroquinoline species to ensure its survival in the blood and its subsequent oxidation in the brain. To clearly identify the radioactive species in the brain and blood, respectively, and to provide in vivo information about the [^{11}C]**3** oxidation kinetics into its corresponding quaternary form-[^{11}C]**2**, samples were treated so as to recover >90% of the radioactivity and then analyzed by radioHPLC over a period of 60 min after [^{11}C]**3** injection in rat.

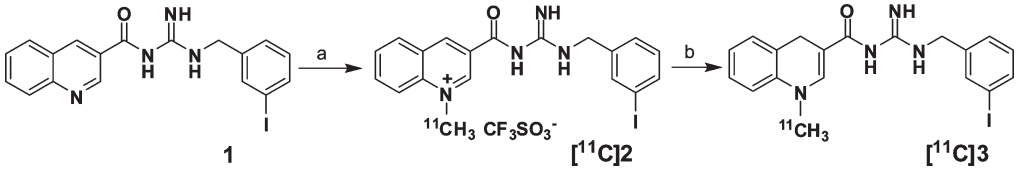
HPLC analysis of brain extracts showed two radioactive peaks, one corresponding to [^{11}C]**3** (t_R = 13 min) and the other to a polar radioactive compound (t_R = 3.5 min). The percentage of unchanged [^{11}C]**3** declined from 96% at 3 min

Scheme 2. Synthesis of **3**[a]



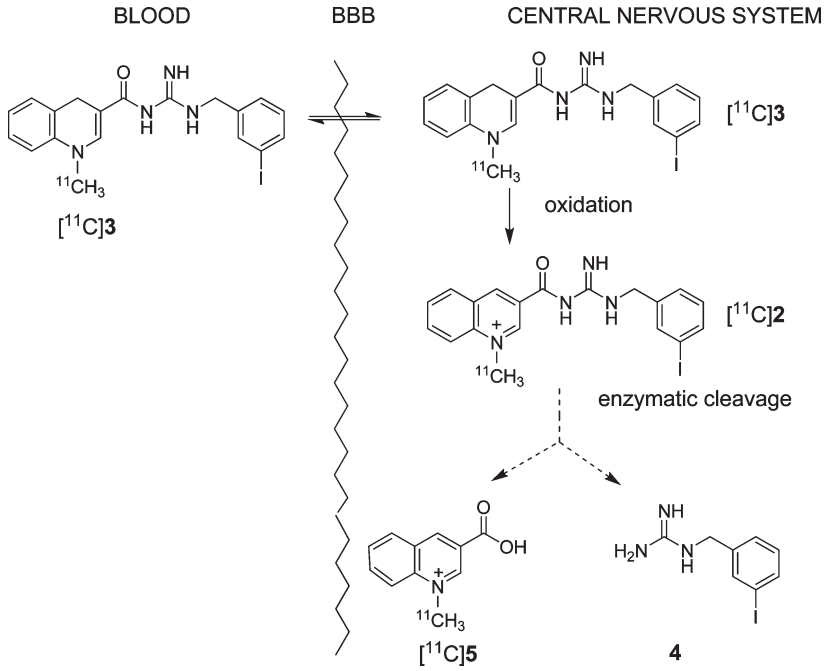
[a] Reagents and conditions: (a) H_2SO_4, MeOH, 100 °C, 12 h then **4**/DMF/100 °C, 3 h; (b) NBI, NEt_3, DMF, rt, 4 h then **4**, DMF, 140 °C, 2 h; (c) CDI, DMF, rt, 1 h then **4**, DMF, rt, 12 h; (d) HOSu, THF, DCC, rt, 12 h then **4**, DMF, 100 °C, 2 h; (e) MeOTf, CH_2Cl_2, rt, 30 min; (f) BNAH, CH_2Cl_2, rt, 12 h.

Scheme 3. Radiosynthesis of [^{11}C]**3**[a]



[a] Reagents and conditions: (a) [^{11}C]MeOTf, CH_3CN, rt, 7 min; (b) BNAH, CH_3CN, rt, 5 min.

Scheme 4. Chemical Delivery System of **4** into the CNS



to 59% at 60 min while the main polar radioactive compound appeared, which increased from 4% at 3 min to 41% at 60 min (Figure 2); in parallel, [^{11}C]**3** decreased rapidly from the blood (30% at 3 min) and was not detected beyond 20 min post injection; at this time, only the main polar radioactive compound (t_R = 3.5 min) was found (Figure 3). An extra and minor radioactive peak corresponding to the quaternary form-[^{11}C]**2** (t_R = 7.5 min) has been observed in the plasma sample only at 3 and 5 min after injection. The HPLC profiles from brain and plasma extracts let us suggest an oxidation of [^{11}C]**3** into the CNS followed by a rapid enzymatic cleavage, which could lead to the carboxylic acid [^{11}C]**5** and compound **4** (Scheme 4).

To demonstrate this hypothesis, the validity of [^{11}C]**5** was scrutinized by the synthesis of the stable analogue **5**. After **5** coinjection onto HPLC with either brain or plasma extracts, obtained from rat injected with [^{11}C]**3**, its retention time (t_R = 3.5 min) was revealed to be similar to that of the main polar radioactive compound. Because of this observation, we could assign, with confidence, the main polar radioactive compound as [^{11}C]**5**. On the basis of these findings, a complementary study has been conducted to identify by LC-MS/MS the release of **4** resulting from the amide bond cleavage of the quaternary form **2**. Previously, **4** and compounds **2** and **3** were injected as reference and the data obtained under our LC-MS/MS conditions gave similar fragmentation pattern for **2** and **3**.[30] Then, compound **3** (1 mg) was injected into rat. After sacrifice at 60 min, the brain was removed and treated, and the sample processing procedure was sufficient to provide clean filtrates before injection to the sensitive LC-MS/MS system. A typical chromatogram from brain extracts is shown in Scheme 5. The mass spectral analysis revealed molecular ion at *m*/*z* 276 assigned to **4** (t_R = 10.36 min) and which was confirmed by MS/MS data, where the fragmentation of the molecular ion generated specific product ions at *m*/*z* 233 and 217; the

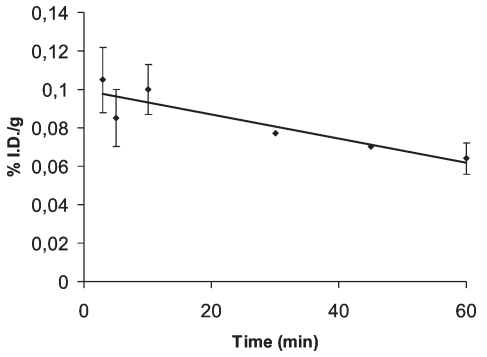


Figure 1. Ex vivo biodistribution of the radioactivity in rat brain at different time intervals following tail vein injection of [^{11}C]**3**. Results are expressed in percent of injected dose per gram (%ID/g ± SD, n = 2).

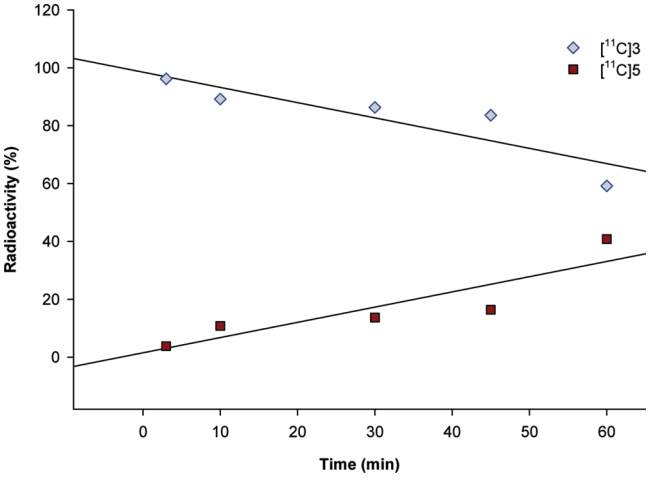


Figure 2. Percentage of unchanged [^{11}C]**3** and [^{11}C]**5** in rat brain (n = 2 for each time point).

molecular ion at m/z 445 was attributed to the parent compound (t_R = 12.76 min) with specific MS/MS fragmentation at 212 and 428. These specific ions were absent in brain extract from a control rat. These encouraging results have demonstrated, in vivo, the "proof of concept" that the 1,4-dihydroquinoline/quinolinium salt redox-chemical delivery system can be used as an effective tool to deliver drug into the brain. However, the moderate penetration of [^{11}C]**3** through the blood−brain barrier could be explained by an early oxidation in the peripheral system before reaching the brain. The improvement of this chemical delivery system CDS-**4** is based on a balance between the peripheral oxidation of the 1,4-dihydroquinoline carrier and the brain oxidation leading to the release of **4**. To tune the redox potential of the carrier, additional substituents on the phenyl ring could be easily installed and the impact of a linker between the carrier and **4** will be investigated.

In conclusion, this study provides the feasibility to use the 1,4-dihydroquinoline/quinolinium salt redox-chemical delivery system as a promising tool for the transport of drug across the blood−brain barrier. This strategy could have a real application for PET or SPECT imaging where radiopharmaceuticals are excluded from transport from blood to brain.

Experimental Section

General. All reagents were purchased from Acros Organics, Fluka, or Sigma Aldrich and were used without further purification. ^{1}H and ^{13}C NMR spectra were measured on a Bruker DPX 250 at 250 MHz (^{1}H) and 62.5 MHz (^{13}C). Samples were dissolved in an appropriate deuterated solvent ($CDCl_3$, DMSO-d_6 or CD_3OD). Chemicals shifts are reported as parts per million (δ) relative to tetramethylsilane (TMS, 0.00 ppm), which was used as an internal standard. Coupling constants are given in Hz and coupling patterns are abbreviated as: s (singulet), d (doublet), t (triplet), m (multiplet), dt (doublet of triplet), br (broad). Elemental analyses were performed on a ThermoQuest analyzer CHNS and were within ±0.44% of the calculated values. In the radiochemistry procedure, the labeled compounds were isolated on a HPLC system equipped with Merck L-6200 pump and a Merck L-4250 detector in series with Novelec β^+-flow detector. For each described labeled compound, the characteristics of the column and solvent used will be described. The identity of the labeled compounds was confirmed by its coelution with the unlabeled reference compound onto the HPLC system. Purity of the tested compounds was determined using analytical reverse phase HPLC and was found to be more than 98%.

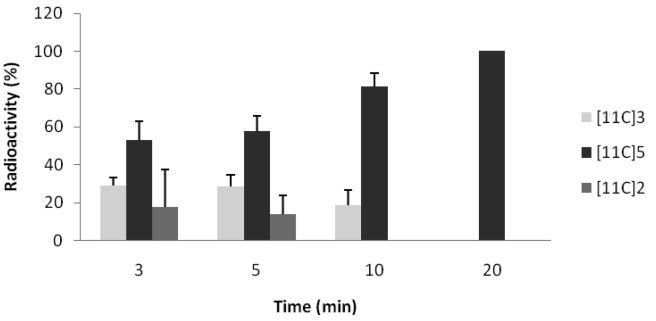
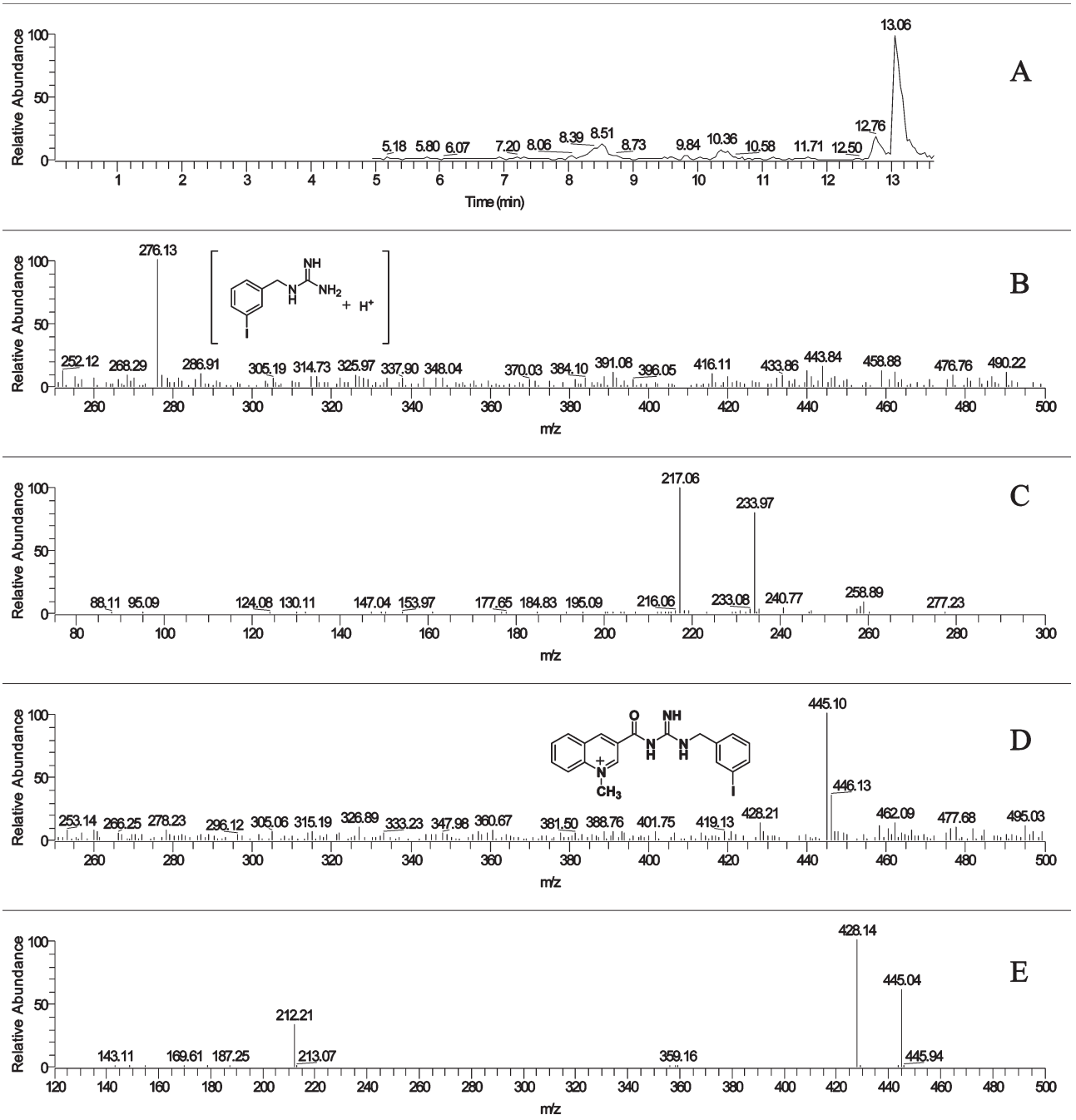


Figure 3. Percentage of unchanged [^{11}C]**3**, [^{11}C]**5**, and [^{11}C]**2** in rat plasma (n = 2 for each time point).

***N*-(3-Iodobenzyl)-*N*′-(3-carbonyl-quinoline)-guanidine (1).** (a) Via CDI. CDI (162 mg, 1 mmol) was added to a solution of 3-quinoline carboxylic acid (173 mg, 1 mmol) in dimethylformamide (3.5 mL). The resulting mixture was stirred at room temperature for 1 h and then was added to a freshly prepared guanidine base **4** obtained from guanidine hydrochloride **4**,**HCl** (311 mg, 1 mmol). The mixture was stirred at room temperature for 12 h. Afterward, acetonitrile (10 mL) was added and the precipitate was filtered. The resulting filtrate was dried under reduced pressure, and the residue was suspended in cold water (10 mL). After one week at 4 °C, the precipitate was filtered, washed with water, and dried. Compound **1** was obtained in 59.5% yield. ^{1}H NMR (CD_3OD): δ 9.51 (s, 1H), 8.95 (s, 1H), 8.00−8.10 (m, 2H), 7.75−7.90 (m, 2H), 7.60−7.70 (m, 2H), 7.41 (d, 1H, J = 7.6 Hz), 7.14 (t, 1H, J = 7.6 Hz), 4.64 (br s, 1.3H), 4.48 (br s, 0.7H). ^{13}C NMR (DMSO-d_6): δ 174.1, 161.4, 151.1, 148.8, 143.2, 137.9, 136.8, 136.2, 135.9, 131.7, 130.9, 129.9, 129.0, 127.5, 127.3, 127.1, 95.2, 43.5. Anal. ($C_{18}H_{15}IN_4O$) C, H, N. (**b**) via *N*-(3-carbonyloxy-quinoline)-pyrolidine-2,5-dione. Under nitrogen, *N*-hydroxysuccinimide (127 mg, 1.1 mmol) was added to a solution of 3-quinoline carboxylic acid (173 mg, 1 mmol) in tetrahydrofuran (5 mL) and the mixture was cooled at −5 °C. A solution of DCC (240 mg, 1.1 mmol) in tetrahydrofuran (3 mL) was added, and the resulting mixture was stirred at room temperature overnight. The suspension was filtered, and the filtrate was evaporated under reduced pressure. The residue was washed with saturated $NaHCO_3$ and extracted with chloroform (4 × 10 mL). The combined organic phases were dried and evaporated under reduced pressure. *N*-(3-Carbonyloxy-quinoline)-pyrolidine-2,5-dione was obtained in 93% yield as a white solid. ^{1}H NMR ($CDCl_3$): δ 9.47 (s, 1H), 9.00 (s, 1H), 8.21 (d, 1H, J = 7.7 Hz), 7.97 (d, 1H, J = 7.0 Hz), 7.91 (t, 1H, J = 7.0 Hz), 7.69 (t, 1H, J = 7.2 Hz), 2.96 (s, 4H). ^{13}C NMR ($CDCl_3$): δ 169.0, 160.9, 150.5, 140.4, 133.1, 129.7, 129.4,

Scheme 5. Analysis of Brain Homogenate in μLC-ESI-MS/MS[a]



[a] (A) Total ionic chromatogram. (B) MS spectrum of the peak eluted at 10.36 min. (C) MS/MS spectrum of the m/z 276. (D) MS spectrum of the peak eluted at 12.76 min. (E) MS/MS spectrum of the m/z 445.

128.1, 126.4, 118.3, 25.6. Then the compound (150 mg, 0.56 mmol) in dimethylformamide (5 mL) was added to guanidine base **4** obtained from guanidine hydrochloride **4**,**HCl** (175 mg, 0.56 mmol). The mixture was heated at 100 °C for 2 h. Then solvent was evaporated under reduced pressure, and water (10 mL) was added to the residue. The resulting mixture was kept at 4 °C for one week, and the precipitate was filtered, washed with water, and dried. Compound **1** was obtained in 66% yield.

***N*-(3-Iodobenzyl)-*N'*-(1-methyl-3-carbonyl-quinolinium)-guanidine Trifluromethanesulfonate (2).** Under nitrogen, compound **1** (100 mg, 0.23 mmol) was dissolved in dichloromethane (50 mL) at 50 °C. The solution was cooled to room temperature, and methyl trifluoromethane sulfonate (27 μL, 0.23 mmol) was added. Then the mixture was stirred at room temperature for 30 min. Diethyl ether (50 mL) was added, and after 1 h, the formed precipitate was collected, washed with diethyl ether, and dried. Compound **2** was isolated in 50% yield as a beige solid. ^{1}H NMR (CD_3OD): δ 9.84 (s, 1H), 9.68 (s, 1H), 8.61 (d, 1H, J = 8.0 Hz), 8.57 (d, 1H, J = 8.0 Hz), 8.45 (t, 1H, J = 7.6 Hz), 8.18 (t, 1H, J = 7.6 Hz), 7.84 (s, 1H), 7.72 (d, 1H, J = 8.0 Hz), 7.48 (d, 1H, J = 7.6 Hz), 7.20 (t, 1H, J = 7.6 Hz), 4.79 (s, 2H), 4.67 (s, 3H).

***N*-(3-Iodobenzyl)-*N'*-(1-methyl-3-carbonyl-1,4-dihydroquinoline)guanidine(3).** Under nitrogen, *N*-benzyl 1,4-dihydronicotinamide (BNAH) (22 mg, 0.1 mmol) was added to a mixture of quinolinium salt **2** (56 mg, 0.1 mmol) in dichloromethane (20 mL). The solution was stirred at room temperature for 12 h. The organic layer was washed three times with water and evaporated under reduced pressure. Compound **3** was obtained

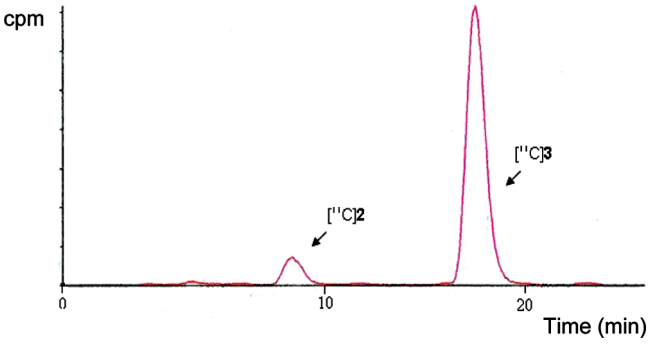


Figure 4. Radiochromatogram from HPLC analysis after [^{11}C]**3** radiosynthesis.

in 95% yield as a yellow powder. ^{1}H NMR ($CDCl_3$): δ 7.70 (s, 1H), 7.66 (d, 1H, J = 8.0 Hz), 7.56 (s, 1H), 7.32 (d, 1H, J = 7.0 Hz), 7.06−7.14 (m, 3H), 6.95 (t, 1H, J = 7.5 Hz), 6.75 (d, 1H, J = 7.8 Hz), 4.42 (s, 2H), 3.82 (s, 2H), 3.25 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 156.3, 152.8, 142.3, 136.1, 135.9, 135.2, 129.6, 129.4, 128.9, 128.4, 127.9, 126.1, 125.5, 122.3, 111.7, 93.7, 43.4, 38.0, 30.9.

1-Methylquinolinium-3-carboxylic Acid Trifluromethanesulfonate (5). Under nitrogen, 3-quinoline carboxylic acid (173 mg, 1 mmol) was dissolved in acetonitrile (20 mL) at 60 °C. Methyl trifluoromethane sulfonate (124 μL, 1.1 mmol) was added to the solution. The mixture was stirred at 60 °C for 2 h. After evaporation of the solvent, diethyl ether (3 mL) and dichloromethane (3 mL) were added, and after 12 h, the precipitate was collected and dried. Compound **5** was isolated in 45% yield as a beige solid. ^{1}H NMR (DMSO-d_6): δ 14.0 (s, 1H), 9.96 (s, 1H), 9.83 (s, 1H), 8.57−8.69 (m, 2H), 8.42 (d, 1H), 8.16 (d, 1H), 4.74 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 163.5, 150.5, 147.7, 139.3, 137.4, 131.8, 130.5, 128.4, 124.8, 119.3, 45.3.

***N*-(3-Iodo-benzyl)-*N'*-(1-[^{11}C]methyl-3-carbonyl-1,4-dihydroquinoline)-guanidine ([^{11}C]3).** [^{11}C]Methyl triflate was trapped at room temperature in a solution containing the quinoline **1** (1 mg, 3.1 μmol) in acetonitrile (150 μL).[31] When all the radioactivity was collected, a solution of BNAH (2 mg, 9.3 μmol) in CH_3CN (150 μL) was added to the mixture. The resulting mixture was kept at room temperature for 5 min. The purification was performed by reverse-phase semipreparative HPLC (Macherey Nagel nucleosil 100−5 protect1 column 10 mm × 250 mm, 4 mL/min, λ = 254 nm, $CH_3CN/H_2O/Et_3N$ 55:45:0.01) (Figure 4). The fraction containing the labeled product [^{11}C]**3** (t_R = 18.5 min) was collected into a flask containing water (25 mL). The radioactive solution was transferred onto a Macherey Nagel chromabond C18ec previously conditioned with methanol (2 mL) and water (5 mL). [^{11}C]**3** was eluted with ethanol (200 μL), and an isotonic saline solution containing 10% phosphate buffer (pH = 7.4 25 mM) was added (1 mL). The final solution was filtered through a 0.22 μm filter into a sterile vial. The radiochemical and chemical purities and specific activities were determined by reverse-phase analytical HPLC (Macherey Nagel nucleosil 100−5 protect1 column 4.6 mm × 250 mm, 1 mL/min, λ =254 nm, $CH_3CN/H_2O/Et_3N$ 45:55:0.01, t_R[^{11}C]**3** = 13 min). Radiochemical purity of [^{11}C]**3** exceeded 99% and batches of 550−740 MBq were ready for injection in about 50 min.

Biological Evaluation. 1. Animals. Animal experimental procedures were in accordance with the recommendations of the EEC (86/609/EEC) and the French National Committee (decret 87/848) for the care and use of laboratory animals and were approved by the Normandy Regional Animal Ethics Committee (saisine N/05-04-05-05). Sprague−Dawley male rats weighing 250−300 g were used in all experiments. The animals were kept at constant temperature (22 °C) and humidity (50%) with 12 h light/dark cycles and were allowed free access to food and water until experiment time. Anesthesia was induced with 5% isoflurane in a gas mixture of nitrous oxide/oxygen (70/30%) and maintained with 1.5−2.5% isoflurane during the entire surgical procedure. Body temperature was monitored rectally and kept close to 37.5 °C. A catheter was inserted into the tail vein.

2. In Vitro Stability of [^{11}C]3 in Rat Blood Samples. Compound [^{11}C]**3** (10 MBq) formulated in a solution containing NaCl 0.9%/phosphate buffer 25 mM pH = 7.4/ethanol (v/v/v 80/10/10) was added to blood. After incubation for 5, 15, 30, and 60 min at 37 °C, blood samples (1 mL) were centrifuged (2000*g*, 4 min). Plasma was mixed with an equivalent volume of acetonitrile and centrifuged (2000*g*, 10 min). The radioactivity of the precipitate was measured to quantify the efficiency of the acetonitrile extraction. Supernatants were filtered and injected onto HPLC (Macherey Nagel nucleosil 100−5 protect1 column, 4.6 mm × 250 mm, 1 mL/min, λ = 254 nm, $CH_3CN/H_2O/Et_3N$ 55:45:0.01).

3. Radioactivity Uptake in Rat Brain. Rats were injected with [^{11}C]**3** (15−120 MBq) formulated in a solution containing NaCl 0.9%/phosphate buffer 25 mM pH = 7.4/ethanol (v/v/v 80/10/10) and were killed by decapitation at 3, 5, 10, 30, 45, and 60 min post injection. Whole brain was quickly dissected and rinsed with saline solution to minimize residual blood. The brain sample was weighed, and the radioactivity was measured in a γ-counter (Cobra 2 gamma counter, Packard). Data were expressed as the percentage of injected dose (decay-corrected) per gram of tissue (% ID/g).

4. [^{11}C]3 Oxidation Rate in Blood and Brain Samples. Rats were injected with [^{11}C]**3** (15−120 MBq) formulated in a solution containing NaCl 0.9%/phosphate buffer 25 mM pH = 7.4/ethanol (v/v/v 80/10/10) and were killed by decapitation at 3, 5, 10, 20, 30, 45, and 60 min post injection. Whole brain was quickly dissected and rinsed with saline solution to minimize residual blood and intracardiac blood sample was collected. Brain was crushed (UltraTurrax T25, Janke and Kunkel) in acetonitrile (2.5 mL) and centrifuged at 2000*g* for 10 min. The radioactivity of the precipitate was measured to quantify the efficiency of the acetonitrile extraction. Supernatants were filtered and injected onto HPLC (Macherey Nagel nucleosil 100−5 protect1 column 4.6 mm × 250 mm, 1 mL/min, λ = 254 nm, $CH_3CN/H_2O/Et_3N$ 55:45:0.01). After centrifugation of blood samples (2000*g*, 4 min, 1 mL), plasma was mixed with an equivalent volume of acetonitrile and centrifuged (2000*g*, 10 min). The radioactivity of the precipitate was measured to quantify the efficiency of the acetonitrile extraction. Supernatants were filtered and injected onto HPLC as described above.

5. LC-MS/MS Analysis. Rats were injected with **3** (1 mg, 2.37 μmol) formulated in a solution containing NaCl 0.9%/phosphate buffer 25 mM pH = 7.4/ethanol (v/v/v 80/10/10) and were killed by decapitation at 30 min post injection. Whole brain was dissected, crushed (UltraTurrax T25, Janke and Kunkel) in acetonitrile (2.5 mL), and centrifuged at 2000*g* for 10 min. Supernatants were concentrated and analyzed by LC-MS/MS. Analyses were performed with a HPLC Surveyor chain connected online to an orthogonal electrospray source (Deca XP MS-n ThermoFinnigan) operated in the positive electrospray ionization mode (ESI+). The ions were focused into an ion trap capable of MS and MS/MS analyses. The mass spectra were acquired during 35 ms from 100 to 1000 m/z. The capillary exit of the electrospray ion source was set at 70 V, the octapole at 3 V, and the capillary temperature at 200 °C. A counter flow of nitrogen was used as nebulizing gas. Xcalibur data system was used to acquire the data, which were further processed with a Turbo Sequest data system. The sample was resuspended in 0.1% acetic acid in water, and 5 μL were injected onto a C18 Thermo electron corporation C18 column (0.5 mm × 50 mm, 5 μm) that had been equilibrated with 95% mobile phase A (0.1% aq acetic acid) and 5% mobile phase B (0.1% acetic acid

in acetonitrile). The peaks were eluted with a linear acetonitrile gradient of 8% per min. A split ratio of 30:1 was used to perfuse the column at a flow rate of 10 μL/min. The HPLC column was rinsed with 90% acetonitrile in 0.1% acetic acid between each injection. The MS data was acquired in scan mode considering the positive ion signal.

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Supporting Information Available: Synthesis of compounds **1** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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