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Research Article

Radiosynthesis of 2-[6-chloro-2-(4-iodophenyl)imidazo [1,2-*a*]pyridin-3-yl]-*N*-ethyl-*N*-[^{11}C]methyl-acetamide, [^{11}C]CLINME, a novel radioligand for imaging the peripheral benzodiazepine receptors with PET

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Abstract: Recently, a new 2-(iodophenyl)imidazo[1,2-*a*]pyridineacetamide series has been developed as iodine-123-labelled radioligands for imaging the peripheral benzodiazepine receptors using single photon emission tomography. Within this series, 2-[6-chloro-2-(4-iodophenyl)-imidazo[1,2-*a*]pyridin-3-yl]-*N*-ethyl-*N*-methyl-acetamide (CLINME) was considered as an appropriate candidate for positron emission tomography imaging and was isotopically labelled with carbon-11 ($T_{1/2}$: 20.38 min) at the methylacetamide side chain from the corresponding *nor*-analogue using [^{11}C]methyl iodide and the following experimental conditions: (1) trapping at −10°C of [^{11}C]methyl iodide in a 1/2 (v:v) mixture of DMSO/DMF (300 µl) containing 0.7–1.0 mg of the precursor for labelling and 3–5 mg of powdered potassium hydroxide (excess); (2) heating the reaction mixture at 110°C for 3 min under a nitrogen stream; (3) diluting the residue with 0.6 ml of the HPLC mobile phase; and (4) purification using semi-preparative HPLC (Zorbax® SB18, Hewlett Packard, 250 × 9.4 mm). Typically, starting from a 1.5 Ci (55.5 GBq) [^{11}C]CO_2 production batch, 120−150 mCi (4.44–5.55 GBq) of [^{11}C]CLINME were obtained (16–23% decay-corrected radiochemical yield, $n = 12$) within a total synthesis time of 24–27 min (Sep-pak®Plus-based formulation included). Specific radioactivities ranged from 0.9 to 2.7 Ci/µmol (33.3–99.9 GBq/µmol) at the end of radiosynthesis. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: carbon-11; PBR; 2-phenylimidazo[1,2-*a*]pyridineacetamide; CLINME

Introduction

In the mammal, two pharmacologically distinct classes of benzodiazepine receptors have been identified: the central and the peripheral receptors. While central benzodiazepine receptors (CBR) are present exclusively in the central nervous system, peripheral benzodiazepine receptors (PBR or translocator protein (18 kDa, TSPO), a new name which has been recently coined[1]) are located in the peripheral organs such as kidney, heart, adrenal cortex, testis or ovary as well as in the glial cells and olfactory bulbs in the brain.[2,3] The PBR are associated with the outer mitochondrial membrane in many tissues where they are modulated by hormones and drugs.[4] Although the functional role of PBR in the CNS has not yet been fully clarified, it has been shown that they are implicated in the regulation of steroidogenesis, immunomodulation, cellular proliferation and apoptosis.[5,6] Due to the development of microglia in pathological processes such as neurodegeneration, inflammation or neurotoxic damage, the PBR appear to be useful markers of the regions affected by these neurodegenerative disorders.[7] The concomitant increase in PBR has been reported in such diverse

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neuropathological states as Huntington's disease, Alzheimer's disease, anxiety, emotional disturbances, multiple sclerosis, cognitive impairment, stroke and cerebral ischemia.[8] Moreover, the abundance of PBR in cancers of colon, breast, ovary and liver suggests a strong implication of these receptors in oncogenesis.[9]

With the aim of finding a useful positron emission tomography (PET) imaging tool for the *in vivo* study of the PBR, several selective ligands have already been radiolabelled with the positron emitters carbon-11 (half-life: 20.38 min) and fluorine-18 (half-life: 109.8 min).[10,11] These radioligands can be classified according to their structure into eight distinct groups (Figure 1). The first group includes the 3-isoquinolinecarboxamide [^{11}C]PK11195[12–18] (compound A) and its derivatives [^{11}C]PK11211,[19] [^{11}C]PK14105[19] and [^{18}F]PK14105[19–22] (compounds B–D). The second group is represented by the atypical benzodiazepine

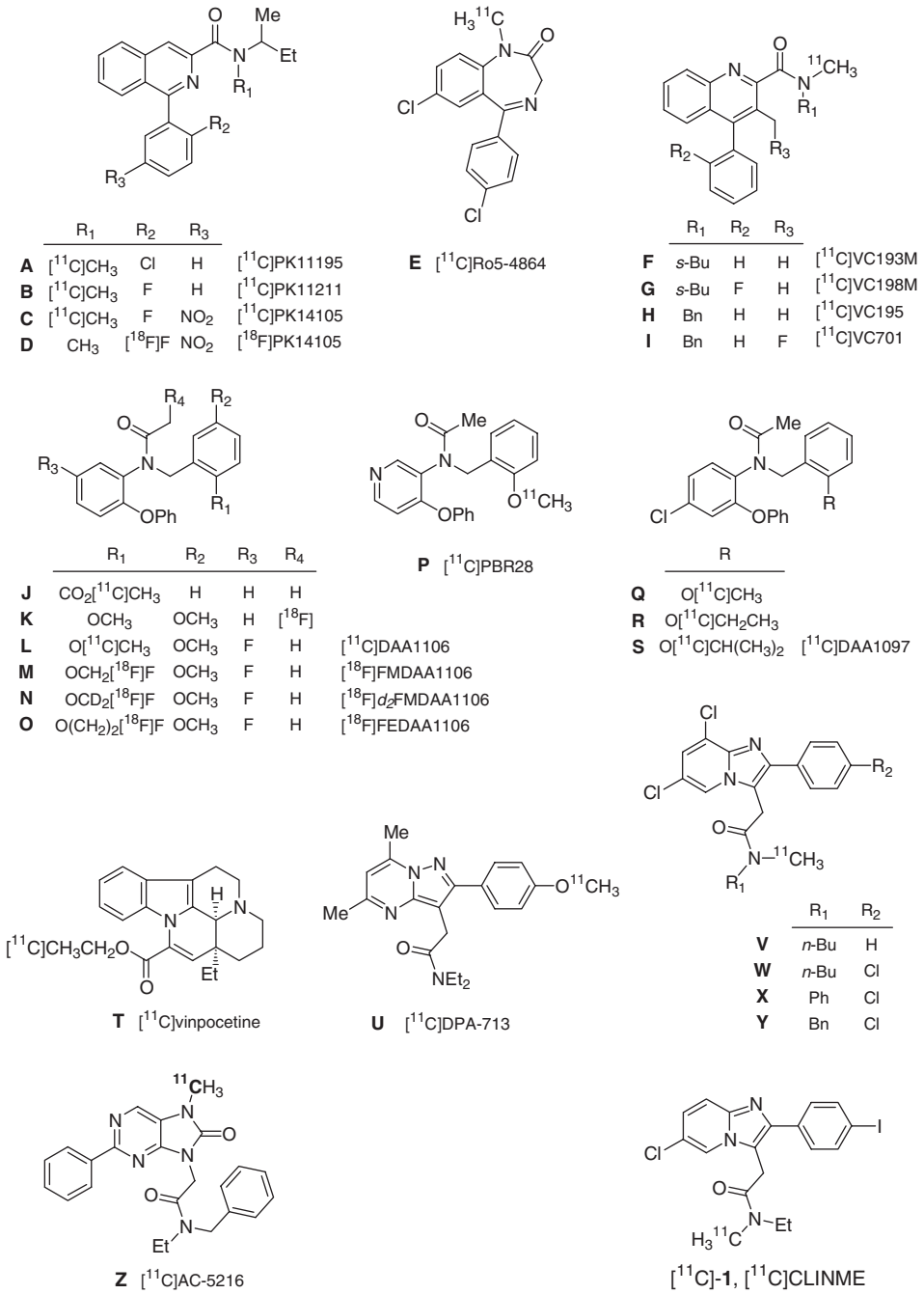

Figure 1 Chemical structures of carbon-11- and fluorine-18-labelled proposed PET ligands for PBRs, including [^{11}C]CLINME.

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4′-chlorodiazepam ([^{11}C]Ro5-4864,[23–26] compound E). The third group consists of a series of quinoline-2-carboxamides, structurally closely related to PK11195 ([^{11}C]VC193M, [^{11}C]VC198M, [^{11}C]VC195 and [^{11}C]VC701,[27–30] compounds F–I). The fourth group is made of a series of *N*-benzyl-*N*-(2-phenoxyaryl)-acetamides (compounds J–S), which includes [^{11}C]DAA1106, [^{18}F]FMDAA1106, [^{18}F]d_2FMDAA1106 and [^{18}F]FEDAA1106[31–38] as well as the recently reported pyridinylacetamide [^{11}C]PBR28[39,40] (compound P). The fifth group is represented by [^{11}C]vinpocetine[41,42] (compound T), a compound structurally related to the *Vinca minor* alkaloid vincamine. The sixth group consists of a series of 2-phenylpyrazolo[1,5-*a*]pyrimidineacetamides[43,44] and includes [^{11}C]DPA-713[45,46] (compound U). The seventh group consists of a series of 2-phenylimidazo[1,2-*a*]pyridineacetamides[47] and includes the recently reported carbon-11-labelled compounds V–Y.[48] The eighth and last group is made of a series of 2-aryl-8-oxodihydropurines, which includes the recently reported [^{11}C]AC-5216[49] (compound Z).

Among these radioligands, [^{11}C]PK11195 (Figure 1, compound A) is not only the oldest, but also the most widely used tracer for imaging the PBR. However, this tracer suffers from low brain uptake and extensive binding to plasma proteins, which complicate a quantitative analysis of PBR density.[14,15] Within the *N*-benzyl-*N*-(2-phenoxyphenyl)-acetamide series, [^{18}F]FEDAA1106 (compound O) seems to be a promising alternative to the use of [^{11}C]PK11195, based on preliminary data obtained in humans.[36] Exceptional *in vivo* binding properties have also been reported recently[45] for the 2-phenylpyrazolo[1,5-*a*]pyrimidineacetamide [^{11}C]DPA-713 (compound U), the latter being currently further evaluated in non-human primates.[50,51]

Within the 2-phenylimidazo[1,2-*a*]pyridineacetamide series, a subclass of iodophenyl derivatives has been developed as iodine-123-labelled radioligands for single photon emission tomography (SPET).[52,53] Derivative **1**, namely 2-[6-chloro-2-(4-iodophenyl)-imidazo[1,2-*a*]pyridin-3-yl]-*N*-ethyl-*N*-methyl-acetamide (CLINME, Figure 1) represents the first radioligand of this subclass to be labelled with carbon-11, which work we present herein.

Discussion

2-[6-Chloro-2-(4-iodophenyl)-imidazo[1,2-*a*]pyridin-3-yl]-*N*-ethyl-*N*-methyl-acetamide (CLINME, **1**) and its nor-methyl derivative **2** (2-[6-chloro-2-(4-iodo-phenyl)-imidazo[1,2-*a*]pyridin-3-yl]-*N*-ethyl-acetamide) as a precursor for carbon-11 labelling via *N*-amide methylation using [^{11}C]methyl iodide were synthesized from 2-(6-chloro-2-(4-iodophenyl)*H*-imidazo[1,2-*a*]pyridin-3-yl) acetic acid by procedures which will be reported elsewhere.[54]

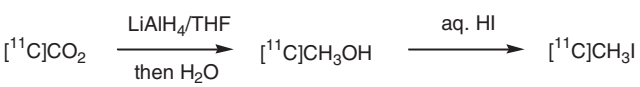
[^{11}C]Methyl iodide was prepared from cyclotron-produced [^{11}C]carbon dioxide using the well-known two step protocol, consisting of the trapping of [^{11}C]carbon dioxide in lithium aluminium hydride forming [^{11}C]methanol, followed by iodination using aqueous hydriodic acid giving [^{11}C]methyl iodide (Scheme 1).[55] On average, about 650 mCi (24.1 GBq) of [^{11}C]methyl iodide was routinely obtained in 6–7 min after end of bombardment (EOB) in 70% decay-corrected yield, based on starting [^{11}C]carbon dioxide.

In a first attempt we employed the standard conditions we routinely use in our laboratory for the radiosynthesis of [^{11}C]-(*R*)-PK11195. The conditions used were the following: (1) trapping at -10°C of [^{11}C]methyl iodide in a 1/2 (v:v) mixture of dimethylsulphoxide and *N*,*N*-dimethylformamide (300 μl) containing 1.5–2.0 mg of precursor **2** (3.4–4.6 μmol) and 15–20 mg of finely powdered potassium hydroxide (large excess); (2) heating the reaction mixture for 3 min at 110°C whilst passing through a nitrogen stream; (3) diluting the crude reaction mixture with 0.6 ml of the HPLC mobile phase; and (4) purification using semi-preparative HPLC on a SymmetryPrep® C18 column (HPLC A).

Using these conditions, the desired and expected compound [^{11}C]-**1** was found present in the reaction mixture, but only as a minor product. Using the HPLC system mentioned above (HPLC A), [^{11}C]-**1** could be well separated (Peak A, R_t: 7.0 min) from unlabelled precursor **2** (R_t: 4.5 min), and also from a radiochemical by-product at $R_t = 9.5$ min (Peak B), which was the major product of the reaction.

The radiochromatogram suggested that the ratio of the desired product [^{11}C]-**1** (Peak A) to the by-product (Peak B) was only 15:85. Further analysis of Peak A on an analytical HPLC system (HPLC C), confirmed the identity of [^{11}C]-**1** by co-chromatography with an authentic standard of **1** (R_t of 1.95 min), but also showed the presence of an additional radiochemical impurity ($R_t = 2.31$ min) totalling 20–25% of the total radioactivity. In addition, analytical HPLC analysis of Peak B, confirmed its identity as an impurity (R_t of 2.60 min).

We postulate two possible chemical structures, [^{11}C]-**3** and [^{11}C]-**4** (Scheme 2), for these two labelled



Scheme 1 Radiosynthesis of [^{11}C]CH_3I.

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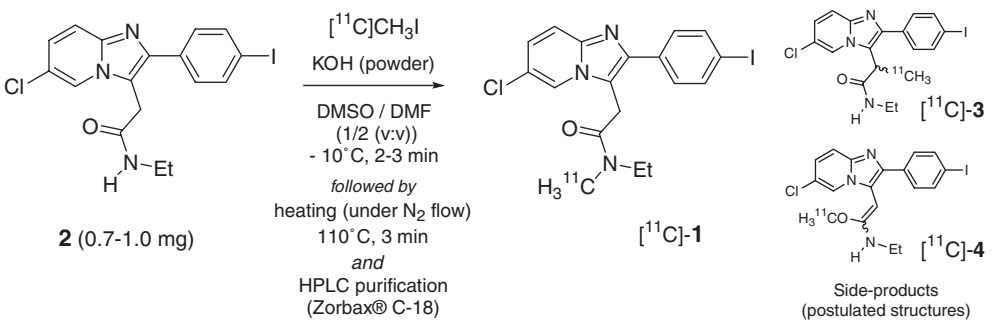
side-products, based on the following considerations. The strong basic conditions used clearly catalyse the removal of the amide-proton of the precursor **2** and therefore facilitate the subsequent [^{11}C]methylation reaction at the nitrogen-site, leading to the expected compound [^{11}C]-**1**. These basic conditions could also favour the formation of the enolate of the precursor **2**, due to the relative acidity of the methylene protons in the α-position of the amide function. Two competitive [^{11}C]methylation reactions could then occur: a *C*- or an *O*-methylation, leading to the formation of compound [^{11}C]-**3** and [^{11}C]-**4**, respectively. This ambident regioselectivity has been reported in similar systems presenting an activated methylene group.[47,56]

A different semi-preparative HPLC system was then developed in order to obtain [^{11}C]-**1** with a radiochemical purity greater than 95%. It was found that replacing the SymmetryPrep® C18 column (HPLC A) with a Zorbax SB-C18 column (HPLC B) with the same eluent as for HPLC A but with a different flow rate (9 ml/min) provided a sufficient separation of all radioactive compounds. The observed retention times were 5.4 min for the precursor **2**, 9.0 min for [^{11}C]-**1**, and 8.2 and 10.5 min for the two labelled side-products. Then, experimental conditions were modified in order to favour the formation of the desired compound **1**. Within a few additional experiments, we rapidly demonstrated that the base over precursor ratio greatly influenced the radiolabelling behaviour of the [^{11}C]methylation reaction, and therefore the final radiochemical yield observed for [^{11}C]-**1**. Moreover, we also observed that a long exposure time (30 min and more *versus* less than 5 min) of the precursor to the base, in the reaction mixture prior to the [^{11}C]methyl iodide trapping, leads preferentially towards the formation of the undesirable labelled products. Based on these observations, the optimal procedure found for the preferential formation of [^{11}C]-**1** was as follows: 0.7–1.0 mg of precursor **2** (1.6–2.3 μmol) were dissolved in a 1/2 (v:v) mixture of DMSO/DMF (300 μl) at room temperature. Immediately prior to starting the radiosynthesis (i.e. the transfer of [^{11}C]CO_2 from the target to the hot cell), the base, 3–5 mg of powdered KOH (85%, 45–76 μmol), was added into the solution of the precursor. The reaction mixture was left at room temperature for less than 5 min, then rapidly cooled to −10°C whereas [^{11}C]methyl iodide trapping started within another minute and lasted for 2 min. The reaction mixture was then heated for 3 min at 110°C whilst passing through a nitrogen stream, diluted with 0.6 ml of the HPLC mobile phase and purified by semi-preparative HPLC (HPLC B). It should be noted that lowering the amount of KOH below 2 mg led to a dramatic loss of radioactivity during the heating step. Using the conditions described above, the crude reaction mixture contained 75% of the desired product [^{11}C]-**1**, with only 10 and 15% of the by-products.

Formulation of [^{11}C]-**1** as i.v. injectable solution was performed using a home-made Sep-pak®Plus C18 device. The HPLC-collected fraction containing the radiotracer was diluted with water and the resulting solution was passed through a C18 Sep-pak® cartridge. The cartridge was then washed with water, partially dried with nitrogen and finally eluted with ethanol followed by physiological saline (less than 15% of the total radioactivity was left on the cartridge). The solution was then diluted with physiological saline to an ethanol concentration below 10%. The radiotracer preparation was a clear and colourless solution with a measured pH between 5 and 7. As demonstrated by analytical HPLC analysis, the radiotracer preparation was found to be >95% chemically and radiochemically pure (HPLC C, t_R: 1.95 min). The preparation was shown to be free of non-radioactive precursor (HPLC C, t_R: 1.53 min) and was chemically and radiochemically stable for at least 60 min.

Conclusion

The PBR ligand 2-[6-chloro-2-(4-iodophenyl)-imidazo[1,2-*a*]pyridin-3-yl]-*N*-ethyl-*N*-methyl-acetamide (CLINME, **1**) has been isotopically labelled with



Scheme 2 Radiosynthesis of [^{11}C]CLINME showing also the postulated side-products.

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carbon-11 ($T_{1/2}$: 20.38 min) at its methylamide function from the corresponding *nor*-analogue using [^{11}C]methyl iodide. Typically, starting from 1.5 Ci (55.5 GBq) of a [^{11}C]CO_2 production batch, 120–150 mCi (4.44–5.55 GBq) of [^{11}C]-**1** was obtained within 24–27 min of radiosynthesis, including HPLC purification and formulation. The total decay-corrected radiochemical yield of [^{11}C]-**1**, based on starting [^{11}C]CO_2, was 16–23% ($n = 12$). The specific radioactivity measured at the end of the radiosynthesis ranged from 0.93 to 2.74 Ci/μmol (34.4–101.4 GBq/μmol). No attempts were made to further optimize these reactions, as sufficient material was obtained to allow for radiopharmacological characterization.[50]

Experimental

General

Chemicals. Chemicals were purchased from ABX GmbH (Germany), Aldrich-, Fluka- or Sigma (France) and were used without further purification.

Spectroscopies. NMR spectra were recorded on a Bruker DPX Avance (400 MHz) apparatus using the hydrogenated residue of the deuterated solvent DMSO-d6 ($\delta = 2.50$ ppm) and/or TMS ($\delta = 0.00$ ppm) as internal standards for ^{1}H-NMR as well as the deuterated solvent DMSO-d6 ($\delta = 39.5$ ppm) and/or TMS as internal standard for ^{13}C-NMR. The chemical shifts are reported in ppm, downfield from TMS (s, d, t, m for singlet, doublet, triplet and multiplet, respectively). The mass spectra (MS, ES+) were measured on a Micromass ZMD Quadrupole spectrometer.

HPLC analysis. [HPLC A]: Equipment: Waters system equipped with a 510 pump, a Shimadzu SPD-10A UV-multiwavelength detector and a miniature ionization chamber probe; column: semipreparative Symmetry-Prep® C-18, Waters (300 × 7.8 mm); porosity: 7 μm; conditions: eluent: CH_3CN/H_2O/TFA: 30/70/0.1 (v/v/v); flow rate: 6 ml/min; temperature: RT; UV detection at λ: 253 nm. [HPLC B]: Equipment: Waters system equipped with a 510 pump, a Shimadzu SPD-10A UV-multiwavelength detector and a miniature ionization chamber probe; column: semipreparative Zorbax® SB18, Hewlett Packard (250 × 9.4 mm); porosity: 5 μm; conditions: eluent: CH_3CN/H_2O/TFA: 30/70/0.1 (v/v/v); flow rate: 9 ml/min; temperature: RT; UV detection at λ: 253 nm. [HPLC C]: 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 (50 × 4.6 mm); porosity: 5 μm; conditions: eluent: solvA/solvB: 38/62 (v/v) [solvA: H_2O containing Low-UV PIC® B7 reagent (% by weight: methanol (18–22%), heptane sulphonic acid–sodium salts (4–6%), phosphate buffer solution (3–7%), water (65–75%), pH 3, Waters), 20 ml for 1000 ml; solvB:H_2O/CH_3CN: 30/70 (v/v) containing Low-UV PIC® B7 reagent (Waters), 20 ml for 1000 ml]; flow rate: 2.0 ml/min; temperature: 30°C; UV detection at λ: 253 nm.

Radioisotope production. [^{11}C]CO_2 was produced via the ^{14}N[p,α]^{11}C nuclear reaction by irradiation of a N_2/O_2 target mixture (99.5/0.5, ultrapure, Air Liquide) with a 18 MeV proton beam (at 25 μA) on an IBA Cyclone-18/9 cyclotron (8.5 μA h in about 20 min). At the end of the bombardment, the target contents were transferred to a 5-cm-lead shielded hot cell and passed through a glass P_2O_5-guard (70 mm length, 3 mm internal diameter) in order to remove moisture. [^{11}C]CO_2 was then separated from the target gas by trapping in an empty stainless-steel coil (150 mm length, 0.51 mm internal diameter), cooled at −186°C using liquid argon. On average, at EOB about 1.20 Ci (44.4 GBq) of [^{11}C]CO_2 is routinely obtained in our laboratory for the irradiation described above.

Miscellaneous. Radiosyntheses using carbon-11, including the HPLC purifications, were performed remote-controlled in a 5.0-cm-lead shielded cell.

Chemistry

2-[6-Chloro-2-(4-iodo-phenyl)-imidazo[1,2-a]pyridin-3-yl]-N-ethyl-N-methyl-acetamide (1). To a stirred suspension of 2-(6-chloro-2-(4-iodophenyl)*H*-imidazo[1,2-*a*]pyridin-3-yl)acetic acid (2.83 g, 5.81 mmol, synthesized from α-bromo-4-iodo-acetophenone and 5-chloro-2-aminopyridine according to Reference[54]) in dry tetrahydrofuran (100 ml) at room temperature, was added, under nitrogen, 1,1′-carbonyl diimidazole (1.13 g, 7.0 mmol). The resulting mixture was stirred for 1 h at room temperature, and then heated at 55°C for another hour. After cooling to room temperature, a solution of tetrahydrofuran (20 ml) containing methylethylamine (0.550 ml, 6.4 mmol) was added. The resulting mixture was stirred for 1 h and then concentrated. The residue was taken up in dichloromethane (100 ml) and washed once with 10% sodium bicarbonate (80 ml). The aqueous layer was then extracted 3 times with dichloromethane (60 ml). The organic layers were combined, dried over Na_2SO_4, filtered and evaporated to dryness. Recrystallization from dichloromethane gave pure **1** as a white solid (1.45 g, 55%). ^{1}H-NMR ($CDCl_3$): δ: (Note: rotamers (1:1)) 1.06 and 1.13 (t, $J = 7.2$ Hz, 3H, CH_3), 2.95 (s, 3H, CH_3), 3.29 and

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3.35 (q, $J = 7.2$ Hz, 2H, CH_2), 4.03 and 4.06 (s, 2H, CH_2), 7.17 and 7.18 (dd, $J = 9.5$ and 1.9 Hz, 1H), 7.38 and 7.39 (d, $J = 8.4$ Hz, 2H), 7.56 (dd, $J = 9.5$ and 0.8 Hz, 1H), 7.80 (d, $J = 8.4$ Hz, 2H), 8.21 and 8.23 ($J = 1.8$ and 0.8 Hz). ^{13}C-NMR ($CDCl_3$): δ: 12.31, 13.52, 29.66, 30.11, 33.15, 35.05, 43.06, 44.74, 93.94, 93.97, 115.35*, 117.75*, 120.59, 120.64, 122.48, 122.54, 126.03, 126.08, 130.25*, 133.69, 133.76, 137.82*, 143.6, 143.61, 143.92, 144.01, 167.03, 167.1. (Note: Doubling up of carbons due to restricted rotation (Rotamers). The numbers with * are the only carbons not appearing as doublets.) MS: *m/z*: 456 (32% M+H)$^+$, 454 (100%, M+H)$^+$. Anal. ($C_{18}H_{17}N_3OClI$) C, H, N.

2-[6-Chloro-2-(4-iodo-phenyl)-imidazo[1,2-a]pyridin-3-yl]-N-ethyl-acetamide (2). To a stirred solution of 2-(6-chloro-2-(4-iodophenyl)*H*-imidazo[1,2-*a*]pyridin-3-yl)acetic acid (0.6 g, 1.46 mmol, synthesized from α-bromo-4-iodo-acetophenone and 5-chloro-2-aminopyridine according to Reference[54]) in triethylamine (0.29 g, 2.91 mmol) and DMF (30 ml) at room temperature, was added an excess of ethylamine gas over 1 min. The reaction was then cooled to 0°C and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP, 0.77 g, 1.74 mmol) added. After 5 min, the reaction was allowed to warm up to room temperature and stirred for another 3 h. Brine (30 ml) was then added and the mixture extracted with ethyl acetate (twice 75 ml). The organic layers were combined, dried over Na_2SO_4, filtered and evaporated to dryness. The solid residue was triturated with a 1:1 (v:v) mixture of ethyl acetate and petroleum spirit, filtered and dried in *vacuo* to give **2** (0.42 g, 65%) as a white solid. ^{1}H-NMR (DMSO-d_6): δ: 1.06 (t, $J = 7.2$ Hz, 3H), 3.13 (m, 2H), 3.99 (s, 2H), 7.32 (dd, $J = 9.5$ and 2.0 Hz, 1H), 7.59 (d, $J = 8.5$ Hz, 2H), 7.64 (dd, $J = 9.5$ and 0.8 Hz, 1H), 7.83 (d, $J = 8.5$ Hz, 2H), 8.33 (t, $J = 5.0$ Hz, 1H), 8.65 (dd, $J = 2.0$ and 0.8 Hz, 1H). ^{13}C-NMR (DMSO-d_6): δ: 16.3, 32.3, 35.4, 95.8, 118.8, 119.2, 120.7, 124.7, 127.1, 131.6, 135.3, 139.0, 144.0, 144.2, 169.2. MS: *m/z*: 442 (M+H)$^+$, 440 (M+H)$^+$. Anal. ($C_{17}H_{15}N_3OClI$) C, H, N.

Radiochemistry

Preparation of (^{11}C)CH_3I. [^{11}C]CO_2 was released from the trap by raising the stainless-steel coil temperature to ambient, swept away by a flow of nitrogen gas (40 ml/min) and trapped at −10°C (EtOH–ice bath) in 50 µl of THF containing 5 µl of a 1.0 M THF solution of lithium aluminium hydride. Concentration to dryness (evaporation of solvent at 155°C using a stream of nitrogen) followed by hydrolysis (100 µl of deionized water) of the formed aluminium complex afforded [^{11}C]CH_3OH, which was distilled using a flow of nitrogen gas into 0.8 ml of an aqueous 57% HI solution (heating block at 155°C). The [^{11}C]CH_3I thus synthesized was continuously swept away by the same flow of nitrogen gas and passed through a combined 1/1 (v/v) soda lime/P_2O_5-guard (35 mm length each, 3 mm internal diameter).

Preparation of 2-[6-Chloro-2-(4-iodo-phenyl)-imidazo[1,2-a]pyridin-3-yl]-N-ethyl-N-[^{11}C]methyl-acetamide ([^{11}C]CLINME, [^{11}C]-1). [^{11}C]CH_3I, carried by a flow of nitrogen gas, was trapped (bubbling through) at −10°C (EtOH–ice bath) in a reaction vessel containing 0.7–1 mg of 2-[6-chloro-2-(4-iodo-phenyl)-imidazo[1,2-*a*]pyridin-3-yl]-*N*-ethyl-acetamide (**2** as free base, 1.6–2.3 µmol) dissolved in a mixture of DMSO/DMF (100 µl/200 µl) and 3–5 mg finely powdered potassium hydroxide (added shortly before radioactivity arrival). Trapping of [^{11}C]CH_3I was monitored using an ionization-chamber probe. When the reading had reached its maximum (about 3 min), the reaction mixture was heated at 110°C (heating block) whilst bubbling through a nitrogen stream (40 ml/min) for 3 min. The reaction vessel was then cooled (EtOH–ice bath) and the reaction mixture was diluted with 0.6 ml of the HPLC mobile phase and was injected onto the HPLC column. The product peak [^{11}C]-**1** was collected.

Formulation of [^{11}C]CLINME ([^{11}C]-1). Formulation of the labelled product for i.v. injection was effected as follows: the HPLC-collected fraction containing the radiotracer was diluted with water (50 ml). The resulting solution was passed through a Sep-pak®Plus C18 cartridge (Waters, washed with 2 ml of EtOH and then rinsed with 10 ml of water prior to use). The cartridge was washed with water (12 ml) and partially dried by applying a nitrogen stream for 10 s. The radiotracer was eluted subsequently with 2 ml of EtOH and 8 ml of physiological saline. Finally, physiological saline was added to adjust the EtOH concentration below 10%. This whole process was performed using a remote-controlled dedicated home-made device based on a literature procedure.[57]

Quality Control of [^{11}C]CLINME ([^{11}C]-1). Quality control of [^{11}C]-1 was performed as follows: the radiotracer preparation was visually inspected for clarity, absence of colour and particulates. An aliquot of the preparation was removed for determination of pH using standard pH-paper. Chemical and radiochemical purities were also assessed on this aliquot by HPLC (HPLC C) using a sample of authentic **1** as reference. Particular attention was paid to the absence of

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non-radioactive precursor (**2**). Chemical and radiochemical stability of the entire preparation was tested by HPLC (HPLC C) at regular 10-min intervals during 60 min. Finally, specific radioactivity of the radiotracer was calculated from three consecutive HPLC (HPLC C) analyses (average) 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.

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