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Research Article

Radiosynthesis of carbon-11-labelled GI181771, a new selective CCK-A agonist

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Summary

The novel CCK-A agonist, (*S*)-3-(3-{1-[(isopropylphenylcarbamoyl)methyl]-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydro-1H-benzo[*b*][1,4]diazepin-3-yl}ureido)benzoic acid, GI181771 ((*S*)-**1**) has been isotopically labelled with carbon-11 at its urea site using [^{11}C]phosgene in a one-pot two-step process, via the intermediate preparation of an [^{11}C]isocyanate derivative. Optimized conditions for the preparation of (*S*)-[^{11}C]-**1** were the following: (1) Trapping of [^{11}C]phosgene (radiosynthesized from cyclotron-produced [^{11}C]methane via [^{11}C]carbon tetrachloride using minor modifications of published processes) at room temperature for 1–2 min in 300 µl of acetonitrile containing 0.6 µmol of the appropriate (structurally complex) chiral-amine giving the corresponding [^{11}C]isocyanate followed by (2) addition of an excess of 3-aminobenzoic acid (40 µmol in 100 µl of THF) as the second amine giving the desired urea derivative (*S*)-[^{11}C]-**1** and (3) high-performance liquid chromatography (HPLC) purification on a semi-preparative Waters Symmetry® C18. Starting from a typical 1.2 Ci (44.4 GBq) batch of [^{11}C]methane, 25–35 mCi (0.92–1.29 GBq, 6.8–9.6% decay-corrected yield based on starting [^{11}C] methane, $n = 5$) of (*S*)-[^{11}C]-**1** could be obtained within 35 min of radiosynthesis (HPLC purification and formulation as an *i.v.* injectable solution using a home-made Sep-pak®Plus C18 device included) with specific radioactivities ranging from 500 to 1500 mCi/µmol (18.5–55.5 GBq/µmol). The radiotracer preparation was a clear and colourless solution and its pH was between 5 and 7. As demonstrated by HPLC analysis, the radiolabelled product was found to be >99% chemically and radiochemically pure and the preparation was shown to be free of non-radioactive precursors (starting amines) and radiochemically stable for at least 60 min. Finally, enantiomeric purity was found to be >99%

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according to chiral HPLC, demonstrating the absence of racemization during the process. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: carbon-11; positron emission tomography; PET; phosgene; isocyanate

Introduction

Cholecystokinin (CCK) is an important mammalian hormone binding to specific receptors named CCK-A and CCK-B predominately located in the gastrointestinal system and in the CNS, respectively.[1,2] CCK receptors have been already investigated in relation to a variety of disorders.[3] In particular, studies demonstrating that exogenous CCK can diminish meal duration and size, have triggered the development of selective and potent synthetic CCK-A ligands as satiety agents for the treatment of obesity.[4,5] Among a series of 1,5-benzodiazepine selective CCK-A receptor agonists, GI181771, namely (*S*)-3-(3-{1-[(isopropylphenylcarbamoyl)methyl]-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydro-1H-benzo[*b*][1,4]diazepin-3-yl}ureido)benzoic acid ((*S*)-**1**, Figure 1), has been identified as an orally active satiety agent in a rat-feeding model.[6]

Positron emission tomography (PET) is a high-resolution, sensitive, molecular and functional imaging technique, which permits repeated, non-invasive assessment and quantification of specific biological and pharmacological processes. It is also the most advanced technology currently available for studying *in vivo* molecular interactions and plays an increasing role in both drug discovery and development of pharmaceuticals, by assessing their *in vivo* distribution, pharmacokinetics and dynamics.

The present study was undertaken to investigate the radiolabelling feasibility of GI181771 with the positron emitter carbon-11 ($t_{1/2}$: 20.38 min).

Regarding the chemical structure of the target unsymmetrical chiral urea GI181771 ((*S*)-[^{11}C]-**1**, Figure 1), three sites are potentially available for isotopic carbon-11-labelling: The first approach involves the *N*-isopropylamide site, which requires the rather complicated and scarcely documented

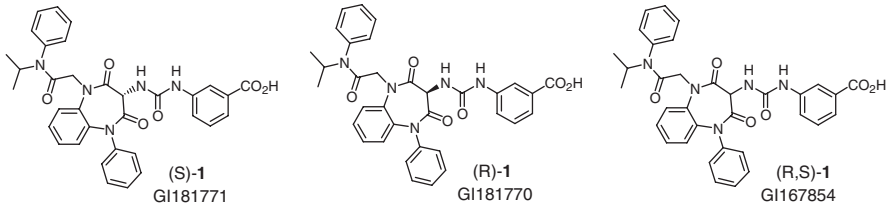
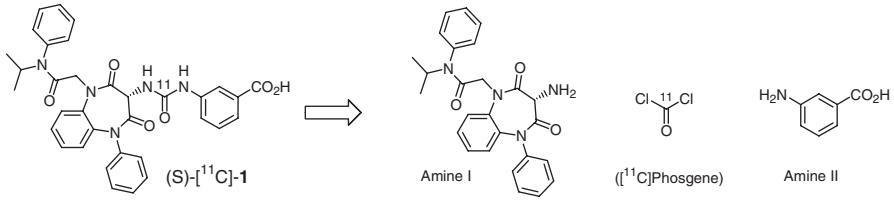


Figure 1. Chemical structures of 3-(3-{1-[(isopropylphenylcarbamoyl)methyl]-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydro-1H-benzo[*b*][1,4]diazepin-3-yl}ureido)benzoic acid: GI181771 ((*S*)-1), GI181770 ((*R*)-1) and GI167854 ((*R*,*S*)-1)



Scheme 1. Retrosynthetic approach designed for the preparation of the target unsymmetrical urea GI181771 ((*S*)-[^{11}C]-1)

radiosynthesis of iso-[^{11}C]propyl iodide (from [^{11}C]carbon dioxide via [^{11}C]acetone formation and consecutive reduction to iso-[^{11}C]propanol[7]) and uses the corresponding secondary amide as non-radioactive precursor for labelling. A second approach involves the free carboxylic acid site, which directly uses [^{11}C]carbon dioxide (no secondary carbon-11-labelled precursors) but requires, immediately before the radiosynthesis process itself, the preparation of the corresponding (relatively unstable) organometallic compound (lithio or more likely Grignard, from the corresponding halogen compound[8]) as a nonradioactive precursor for labelling. A third approach involves the urea site, which requires the preparation of [^{11}C]phosgene, and uses the two amine derivatives coded *amine* I and *amine* II as shown in the following retrosynthetic scheme (Scheme 1).

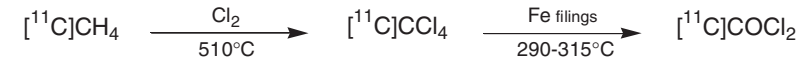
As both amines were available to us off-shelf and the [^{11}C]phosgene technology is routinely used on-site, we chose to radiosynthesize (*S*)-[^{11}C]-**1** using the urea site approach, which we describe herein.

Results and discussion

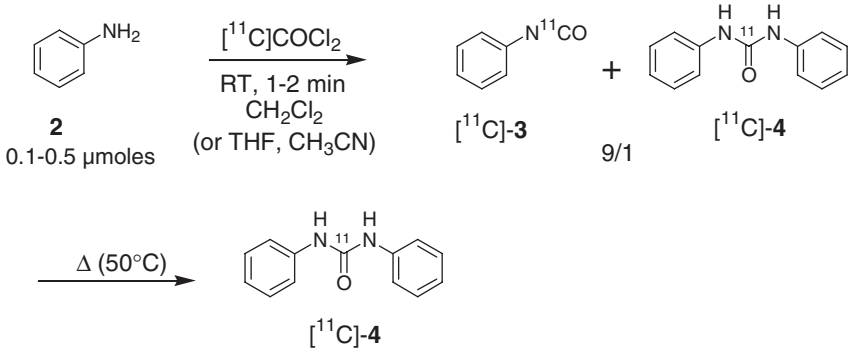
No-carrier-added [^{11}C]phosgene ([^{11}C]$COCl_2$) was synthesized according to Scheme 2.

[^{11}C]phosgene was radiosynthesized from cyclotron-produced [^{11}C]methane ([^{11}C]CH_4) via [^{11}C]carbon tetrachloride ([^{11}C]CCl_4) using minor modifications of published processes[9–18] (Scheme 2). Briefly, [^{11}C]CH_4 was separated from the target contents, trapped and concentrated on Porapak-Q. [^{11}C]CH_4 was then released by a flow of helium gas, mixed with 3 ml of chlorine and the mixture passed through an empty linear horizontal glass tube at a temperature of 510°C.[12] The on-line synthesized [^{11}C]CCl_4 was continuously swept away by the same helium vector gas and finally passed through a glass U-tube containing iron filings at a temperature of 290–310°C,[9] without intentional addition of oxygen to the system, giving [^{11}C]$COCl_2$ in an average of 30–50% decay-corrected radiochemical yield, based on starting [^{11}C]CH_4. The whole process took 12–13 min.[14–18]

Before starting with the preparation of the target GI181771 ((*S*)-[^{11}C]-**1**), in-house standard conditions for the formation of carbon-11-labelled ureas



Scheme 2. Radiosynthesis of [^{11}C]phosgene from cyclotron-produced [^{11}C]methane via [^{11}C]carbon tetrachloride



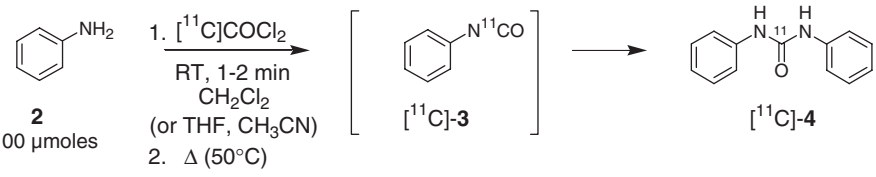
Scheme 3. Radiosynthesis of phenyl[^{11}C]isocyanate ([^{11}C]-3) and [^{11}C]carbanilide ([^{11}C]-4) from [^{11}C]phosgene and aniline (2)

and -isocyanates were set up again and verified using the radiosynthesis of 1,3-diphenyl[^{11}C]urea ([^{11}C]carbanilide, [^{11}C]-**4**) as a model reaction (Scheme 3).

No-carrier-added [^{11}C]phosgene was trapped at room temperature for 1–2 min in 300–500 µl of CH_2Cl_2 (or THF, or CH_3CN) containing 0.1–0.5 µmol of aniline (**2**). According to HPLC analysis, both phenyl[^{11}C]isocyanate ([^{11}C]-**3**) and [^{11}C]carbanilide ([^{11}C]-**4**) were present in the reaction mixture (in a $\frac{9}{1}$ ratio) at this stage. When the reaction mixture was concentrated to dryness (or gently heated at 50°C), the urea [^{11}C]-**4** was then predominantly formed and the intermediate isocyanate [^{11}C]-**3** could hardly be detected.

When [^{11}C]phosgene was trapped at room temperature for 1–2 min in 300–500 µl of CH_2Cl_2 (or THF, or CH_3CN) containing a large excess of aniline (**2**, 100 µmol), the isocyanate ([^{11}C]-**3**) was still the major product when compared to the urea ([^{11}C]-**4**) according to HPLC analysis (in a $\frac{3}{1} - \frac{2}{1}$ ratio), for up to 5 min at room temperature (Scheme 4). When the reaction mixture was concentrated to dryness (or gently heated at 50°C), the [^{11}C]carbanilide ([^{11}C]-**4**) was then quantitatively formed.

Our initially designed one-pot two-step radiosynthetic pathway for the preparation of the target unsymmetrical urea (*S*)-[^{11}C]-**1** was (a) the preparation of 3-[^{11}C]isocyanatobenzoic acid ([^{11}C]-**6**) from [^{11}C]phosgene and 3-aminobenzoic acid (**5**) as the first (structurally simple and commercially

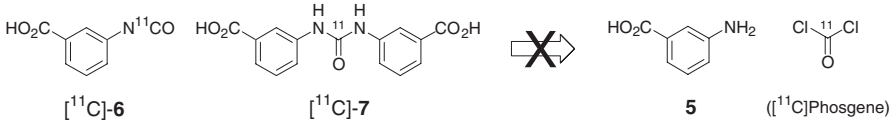


2
100 μmoles

[^{11}C]-**3**

[^{11}C]-**4**

Scheme 4. Radiosynthesis of [^{11}C]carbanilide ([^{11}C]-4) from [^{11}C]phosgene and aniline (2)



[^{11}C]-**6**

[^{11}C]-**7**

5

([^{11}C]Phosgene)

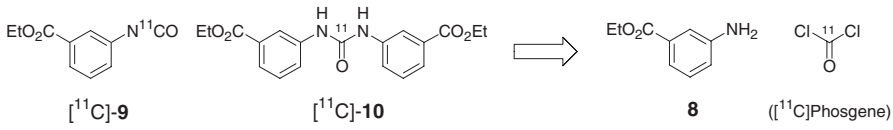
Scheme 5. Attempted radiosynthesis of 3-[^{11}C]isocyanatobenzoic acid ([^{11}C]-6) and 1,3-*bis*-(3-carboxyphenyl)[^{11}C]urea ([^{11}C]-7) from [^{11}C]phosgene and 3-aminobenzoic acid (5)

available) amine (coded *amine* II in Scheme 1) and (b) condensation of this [^{11}C]isocyanate with an excess of the second amine (chiral and more structurally complex, coded *amine* I in Scheme 1).

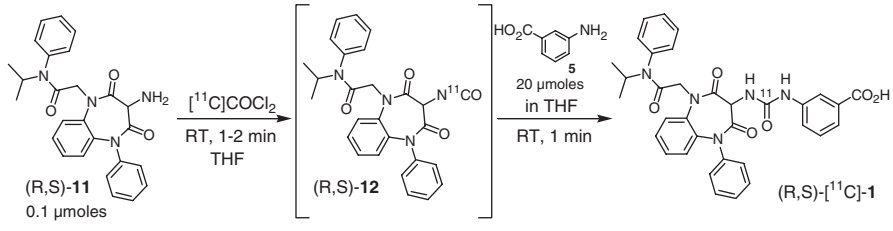
The conditions described above were therefore applied to the preparation of 3-[^{11}C]isocyanatobenzoic acid ([^{11}C]-**6**) and 1,3-*bis*-(3-carboxyphenyl)[^{11}C]urea ([^{11}C]-**7**) (Scheme 5).

[^{11}C]phosgene was trapped at room temperature in 300–500 μl of THF containing 100 μmol of 3-aminobenzoic acid (**5**) and the reaction mixture was then concentrated to dryness. The expected 1,3-*bis*-(3-carboxyphenyl)[^{11}C]urea ([^{11}C]-**7**) could not be detected by HPLC analysis, neither using these conditions, nor in those where this symmetrical [^{11}C]urea [^{11}C]-**7** was expected to be the minor product and the intermediate isocyanate ([^{11}C]-**6**) the major one (using only 0.1–0.5 μmol of **5** as described above). Interpretation of the HPLC chromatograms remained difficult as the expected isocyanate was not available to us as a reference. No further efforts were engaged to demonstrate any formation of 3-[^{11}C]isocyanatobenzoic acid ([^{11}C]-**6**).

On the other hand, 1,3-*bis*-(3-carbethoxyphenyl)[^{11}C]urea ([^{11}C]-**10**) and 3-[^{11}C]isocyanatobenzoic acid ethyl ester ([^{11}C]-**9**) could be synthesized from 3-aminobenzoic acid ethyl ester (**8**) (Scheme 6). When [^{11}C]phosgene was trapped at room temperature for 1–2 min in 300–500 μl of CH_2Cl_2 (or THF, or CH_3CN) containing 0.5 μmol of 3-aminobenzoic acid ethyl ester (**8**), the expected ethyl 3-[^{11}C]isocyanatobenzoate ([^{11}C]-**9**) could be detected as the



Scheme 6. Radiosynthesis of 3-[^{11}C]isocyanatobenzoic acid ethyl ester ([^{11}C]-9) and 1,3-*bis*-(3-carbethoxyphenyl)[^{11}C]urea ([^{11}C]-10) from [^{11}C]phosgene and 3-aminobenzoic acid ethyl ester (8)

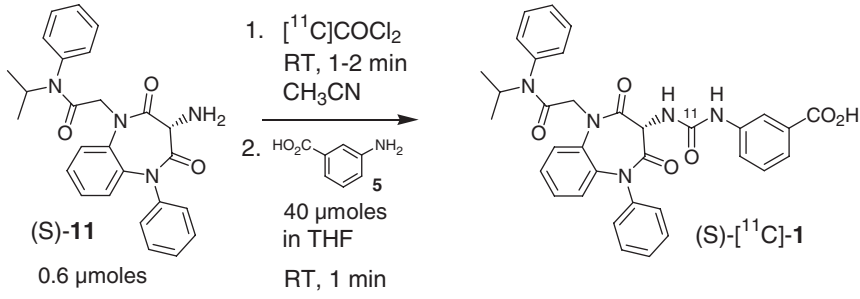


Scheme 7. Radiosynthesis of the racemic unsymmetrical urea (*R*,*S*)-[^{11}C]-1

major product according to HPLC analysis. When [^{11}C]phosgene was trapped at room temperature in 300–500 µl of CH_3CN containing 70 µmol of 3-aminobenzoic acid ethyl ester (**8**) and after having concentrated the reaction mixture to dryness, the corresponding expected 1,3-*bis*-(3-carbethoxyphenyl) [^{11}C]urea ([^{11}C]-**10**) could be obtained as the sole product as demonstrated by HPLC analysis. Finally, when the reaction mixture was not concentrated or heated, the expected ethyl 3-[^{11}C]isocyanatobenzoate ([^{11}C]-**9**) could be observed as the major product besides the urea ([^{11}C]-**10**).

Following these experiments, we concluded that a free carboxylic acid function appeared to be incompatible with an [^{11}C]isocyanate formation under our conditions and decided to design an alternative one-pot two-step radiosynthetic pathway for the preparation of the target unsymmetrical urea (*S*)-[^{11}C]-**1** where the preparation of a structurally complex and chiral [^{11}C]isocyanate derivative from the corresponding amine (coded *amine* I in Scheme 1) would be the first radiochemical step, followed by condensation with the 3-aminobenzoic acid (**5**) as the second amine (coded *amine* II in Scheme 1) as is shown in Scheme 7 for the racemate.

In order to first validate this approach, and with the intermediate isocyanate **12** not at our disposal as a reference, [^{11}C]phosgene was trapped at room temperature for 1–2 min in 300 µl of THF containing 0.1 µmol of the racemic amine (*R*,*S*)-**11**. An excess of 3-aminobenzoic acid (**5**, 20 µmol in 100 µl of



Scheme 8. Radiosynthesis of the target unsymmetrical urea (*S*)-[^{11}C]-1

THF) was then added. The reaction mixture was left at room temperature for 1 min, diluted with the HPLC solvent and finally HPLC-purified. Starting from a typical 1.2 Ci batch of [^{11}C]CH_4, only 3–5 mCi of the desired unsymmetrical racemic urea (*R*,*S*)-[^{11}C]-**1** could be isolated after HPLC purification (in 25–30 min) in the first runs. Nevertheless, it proved to be possible to formulate the labelled derivative as an *i.v.* injectable solution (in 0.9% aq. NaCl containing 10% of EtOH using our home-made Sep-pak®Plus C18 device) and to set up preliminary quality controls. HPLC-based chemical and radiochemical purities were found to be >99%. Chiral HPLC analysis gave as expected a $\frac{1}{1}$ ratio of (*R*)- and (*S*)-[^{11}C]-**1**.

Conditions for the preparation of the target unsymmetrical and chiral urea (*S*)-[^{11}C]-**1** (Scheme 8) were finally optimized and included the use of enantiomerically pure amine (*S*)-**11** (0.6 µmol in 300 µl of CH_3CN) and 40 µmol of 3-aminobenzoic acid (**5**). Starting from a typical 1.2 Ci batch of [^{11}C]CH_4, 25–35 mCi (0.92–1.29 GBq, 6.8–9.6% decay-corrected yield based on starting [^{11}C]CH_4, $n = 5$) of (*S*)-[^{11}C]-**1** could be obtained within 35 min of radiosynthesis (HPLC purification and formulation included) with specific radioactivities ranging from 500 to 1500 mCi/µmol (18.5–55.5 GBq/µmol). The preparation was a clear and colourless solution and its pH was between 5 and 7. As demonstrated by HPLC analysis, the radiolabelled product was found to be >99% chemically and radiochemically pure and the preparation was shown to be free of non-radioactive precursors (starting amines (*S*)-**11** and **5**) and radiochemically stable for at least 60 min. Finally, enantiomeric purity was found to be >99% according to chiral HPLC (only the (*S*)-enantiomer ((*S*)-[^{11}C]-**1**) could be detected), demonstrating the absence of racemization during the process. These results were in compliance with our in-house quality control/assurance specifications.

No attempts were made to further optimize these reactions, the yields being sufficient for our purposes. Nevertheless, a temperature increase, especially in the second radiochemical step where the apparently not very reactive amine **5**

is used, could have been envisaged. Also, appropriate protection of the free carboxylic function could have been another option, adding however an extra and potentially penalizing, radiochemical step.

Experimental

General

Chemicals. Chemicals, including aniline (**2**), 3-aminobenzoic acid (**5**), 3-aminobenzoic acid ethyl ester (**8**), phenylisocyanate (**3**), 3-isocyanatobenzoic acid ethyl ester (**9**) and 1,3-diphenylurea (**4**) were purchased from standard commercial sources (Aldrich, Fluka or Sigma France) and were used without further purification, unless stated otherwise. 1,3-*bis*-(3-carbethoxyphenyl)urea (**10**), needed as a standard, was synthesized in one step from 3-aminobenzoic acid ethyl ester (**8**) and 3-isocyanatobenzoic acid ethyl ester (**9**) using standard procedures (1 equivalent of each reagent in toluene at room temperature for 30 min, 65% non-optimized yield). Analytical data were in accordance with the structure: ^{1}H-NMR, 300 MHz, DMSO-d_6: δ: 8.99 (s, 2H); 8.17 (s, 2H); 7.67 (d, 7.2 Hz, 2H); 7.58 (d, 7.5 Hz, 2H); 7.43 (t, 7.8 Hz, 2H); 4.31 (q, 7.2 Hz, 4H); 1.32 (t, 7.2 Hz, 6H); ^{13}C-NMR, 75 MHz, DMSO-d_6: δ: 165.6 (2 × C); 152.4 (C); 139.9 (2 × C); 130.4 (2 × C); 129.0 (2 × CH); 122.8 (2 × CH); 122.6 (2 × CH); 118.7 (2 × CH); 60.6 (2 × CH_2); 14.1 (2 × CH_3); MS (DCI/NH_4^+): $C_{19}H_{20}N_2O_5$: 357 [$M + H^+$]. 1,3-*bis*-(3-carboxyphenyl)urea (**7**), 3-(3-{1-[(isopropylphenylcarbamoyl)methyl]-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydro-1H-benzo[*b*][1,4]diazepin-3-yl}ureido)benzoic acid (GI167854: (*R*,*S*)-**1**; GI181771: (*S*)-**1** and GI181770: (*R*)-**1**) as well as 2-(3-amino-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl)-*N*-isopropyl-*N*-phenylacetamide (GI155056: (*R*,*S*)-**11** and GW502085X: (*S*)-**11**) were available at GlaxoSmithKline and prepared according to Reference.[6]

Analytical methods. HPLCs were performed on Waters or Shimadzu systems.

[HPLC 1]: Equipment: a Waters 510 pump, a Shimadzu SPD10-AVP UV-multi-wavelength spectrometer and a Geiger–Müller detector; column: semi-preparative Lichrosorb® SiO_2, Merck (250 × 10 mm; porosity: 7 μm); solvents and conditions: isocratic elution with CH_2Cl_2/EtOH/H_2O/$EtNH_2$: 98/1.924/0.038/0.038 (v:v:v:v); flow rate: 8.0 ml/min; temperature: RT; absorbance detection at $\lambda = 254$ nm.

[HPLC 2]: Equipment and column: see HPLC 1; solvents and conditions: isocratic elution with CH_2Cl_2/MeOH/TFA: 97/3/0.2 (v:v:v); flow rate: 7.0 ml/min; temperature: RT; absorbance detection at $\lambda = 254$ nm.

[HPLC 3]: Equipment: see HPLC 1; column: semi-preparative Symmetry® C18, Waters (300 × 7.8 mm; porosity: 7 μm); solvents and conditions: isocratic

elution with $CH_3CN/H_2O/TFA$: 50/50/0.1 (v:v:v); flow rate: 8.0 ml/min; temperature: RT; absorbance detection at $\lambda = 225$ nm.

[HPLC 4]: Equipment: a Waters Alliance 2690 equipped with a Waters 996 Photodiode Array Detector and a Berthold LB509 radioactivity detector; column: analytical Symmetry-M® C-18, Waters (150 × 3.9 mm; porosity: 5 μm); solvents and conditions: isocratic elution with solvent A/solvent B: 8/92 (v:v); solvent A: H_2O containing Low-UV PIC® B7 reagent (20 ml for 1000 ml); solvent B: H_2O/CH_3CN: 50/50 (v:v) containing Low-UV PIC® B7 reagent (20 ml for 1000 ml) (Low-UV PIC® B7 reagent, Waters: % by weight: methanol (18–22%), heptanesulfonic acid–sodium salts (4–6%), phosphate buffer solution (3–7%), water (65–75%), pH 3); flow rate: 2.0 ml/min; temperature: 30°C; absorbance detection at $\lambda = 254$ nm.

[HPLC 5]: Equipment and column: see HPLC 4; solvents and conditions: isocratic elution with solvent A/solvent B: 5/95 (v:v); solvent A: H_2O containing Low-UV PIC® B7 reagent (20 ml for 1000 ml); solvent B: H_2O/CH_3CN: 50/50 (v:v) containing Low-UV PIC® B7 reagent (20 ml for 1000 ml) (Low-UV PIC® B7 reagent, Waters: see HPLC 4); flow rate: 2.0 ml/min; temperature: 30°C; absorbance detection at $\lambda = 254$ nm.

[HPLC 6]: Equipment: see HPLC 1; column: analytical Chiralcel® OD-R, Daicel (240 × 4.6 mm; porosity: 5 μm); solvents and conditions: isocratic elution with $CH_3CN/H_2O/CH_3CO_2H/TEA$: 80/20/1/0.1 (v:v:v:v); flow rate: 0.5 ml/min; temperature: RT; absorbance detection at $\lambda = 230$ nm.

Radioisotope production. No-carrier-added $[^{11}C]CH_4$ was produced on a CGR-520 MeV cyclotron by irradiation of a target consisting of an ultrapure Air Liquide 95/5 mixture of N_2/H_2 (target holder: 668 ml operated at 8 bar) by the $^{14}N[p,\alpha]^{11}C$ nuclear reaction using a 20 MeV proton beam. Typical production: 1.20 Ci (44.40 GBq) of $[^{11}C]CH_4$ at the end of bombardment (EOB) for a 30 μA, 30 min (54 000 μC) irradiation.

Miscellaneous. Radiosyntheses using carbon-11, including the HPLC purifications, were performed in a 5 cm lead-shielded cell.

Radiochemistry

Preparation of $[^{11}C]COCl_2$. At the end of the bombardment, the target contents were transferred by expansion to the 5 cm lead-shielded hot cell dedicated to the radiosynthesis of the tracer. The contents were initially passed through an empty tube (stainless-steel coil, 500 mm length, 4 mm internal diameter, cooled at −186°C using liquid argon) in order to remove [^{13}N]ammonia (side-product of the irradiation) and subsequently through a guard of P_2O_5 (glass tube, 70 mm length, 10 mm internal diameter) in order to remove moisture. $[^{11}C]CH_4$ was then separated from the target gas by trapping

in a copper U-tube (150 mm length, 4 mm internal diameter) filled with Porapak-Q (80-100 mesh, Waters) and cooled at −186°C (liquid argon). [^{11}C]CH_4 was released from the trap by warming the copper U-tube to room temperature (hot air) and swept away by a flow of helium gas (40 ml/min). [^{11}C]CH_4 was then passed through a guard of P_2O_5 (glass tube, 70 mm length, 10 mm internal diameter) and concentrated in a second smaller copper U-tube (150 mm length, 2 mm internal diameter) filled with Porapak-Q (80-100 mesh, Waters) and cooled at −186°C (liquid argon). On average, about 1.20 Ci (44.4 GBq, at EOB) of [^{11}C]CH_4 is routinely produced for a 30 μA, 30 min (54 000 μC) irradiation and then transferred and concentrated in 4–5 min using the process described above. [^{11}C]CH_4 was released from the trap by warming the latter to room temperature and swept (15 ml/min) in a volume of 1–2 ml of helium into a gas mixing chamber containing 3 ml of chlorine (99.99%, Air Liquide). Using the same helium as vector gas (15 ml/min), the [^{11}C]CH_4—chlorine mixture was passed through an empty horizontal glass tube (215 mm length, 7 mm internal diameter) at a temperature of 510°C. The thus formed [^{11}C]CCl_4 then passed on-line through a glass U-tube (200 mm length, 4 mm internal diameter) containing 1.5 g of iron filings (Telar 57, Weber) at a temperature of 290–310°C. Finally, the gaseous reaction mixture now containing [^{11}C]$COCl_2$ passed on-line through an antimony-guard (glass tube, 70 mm length, 3 mm internal diameter, containing a $\frac{2}{1}$ ratio [v/v] of antimony powder (400 mg) and glass beads (1 mm diameter) in order to remove the excess of chlorine.

Preparation of model [^{11}C]isocyanates (general procedure). The on-line synthesized [^{11}C]$COCl_2$ was trapped (bubbling through) at room temperature or lower (0°C) in a reaction vessel containing 0.1–0.5 μmol of the appropriate aniline derivative dissolved in 300–500 μl of CH_2Cl_2, THF or CH_3CN. Trapping of [^{11}C]$COCl_2$ was monitored using an ionization-chamber probe. When the reading had reached its maximum (2–3 min usually), the reaction mixture was diluted with 0.5 ml of HPLC solvent and injected onto the column.

*Radiosynthesis of phenyl[^{11}C]isocyanate ([^{11}C]-**3**)*: The procedure described above was used with aniline (**2**, 0.2 μmol) to give predominantly phenyl[^{11}C]isocyanate ([^{11}C]-**3**) (HPLC 1, R_t: 13.5 min). [^{11}C]carbanilide ([^{11}C]-**4**) could also be detected (HPLC 1, R_t: 5.0 min).

*Radiosynthesis of 3-isocyanatobenzoic acid ethyl ester ([^{11}C]-**9**)*: The procedure described above was used with 3-aminobenzoic acid ethyl ester (**8**, 0.5 μmol) to give predominantly 3-isocyanatobenzoic acid ethyl ester ([^{11}C]-**9**) (HPLC 1, R_t: 21.0 min). 1,3-*bis*-(3-carbethoxyphenyl) [^{11}C]urea ([^{11}C]-**10**) could also be detected (HPLC 1, R_t: 8.0 min).

Preparation of model symmetrical [^{11}C]ureas (general procedure). The on-line synthesized [^{11}C]$COCl_2$ was trapped (bubbling through) at room temperature or lower (0°C) in a reaction vessel containing 100 μmol of the appropriate aniline derivative dissolved in 200–500 μl of CH_2Cl_2, THF or CH_3CN. Trapping of [^{11}C]$COCl_2$ was monitored using an ionization-chamber probe. When the reading had reached its maximum (2–3 min usually), the reaction mixture was concentrated to dryness at 70–80°C under a gentle helium stream for 3–5 min, re-dissolved in 0.5 ml of HPLC solvent and injected onto the column.

*Radiosynthesis of [^{11}C]carbanilide ([^{11}C]-**4**)*: The procedure described above was used with aniline (**2**) to give [^{11}C]carbanilide ([^{11}C]-**4**) (HPLC 1, R_t: 5.0 min).

*Radiosynthesis of 1,3-bis-(3-carbethoxyphenyl) [^{11}C]urea ([^{11}C]-**10**)*: The procedure described above was used with 3-aminobenzoic acid ethyl ester (**8**) to give 1,3-*bis*-(3-carbethoxyphenyl) [^{11}C]urea ([^{11}C]-**10**) (HPLC 1, R_t: 8.0 min).

Radiosynthesis of 1,3-bis-(3-carboxyphenyl) [^{11}C]urea ([^{11}C]-7): The procedure described above was used with 3-aminobenzoic acid (**5**). The expected 1,3-dicarboxyphenyl[^{11}C]urea ([^{11}C]-**7**) could not be detected (HPLC 2, R_t: 13.0 min).

Optimized conditions for the preparation of [^{11}C]GI167854 ((R,S)-1) and [^{11}C]GI181771 ((S)-1)

[^{11}C]GI167854 ((R,S)-1). Radiosynthesis and purification: The on-line synthesized [^{11}C]$COCl_2$ was trapped (bubbling through) at room temperature in a reaction vessel containing 0.6 μmol (265 μg) of (*R*,*S*)-2-(3-amino-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl)-*N*-isopropyl-*N*-phenylacetamide (GI155056, (*R*,*S*)-**11**) dissolved in 250 μl of CH_3CN. Trapping of [^{11}C]$COCl_2$ was monitored using an ionization-chamber probe. When the reading had reached its maximum (2–3 min usually), a THF solution (100 μl) containing 40 μmol (5.5 mg) of 3-aminobenzoic acid (**5**) was rapidly added to the reaction mixture. The reaction vessel was left standing at room temperature for another 1 min. Finally, the reaction mixture was diluted with 1.0 ml of the HPLC mobile phase and was injected onto the column. HPLC purification gave (*R*,*S*)-**1** (HPLC 3, R_t: 8.1 min), well separated from starting amines (*R*,*S*)-**11** and **5** (HPLC 3, R_t: <2.0 min).

Formulation: 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 5 ml of EtOH and then rinsed with 10 ml of water prior to use). The cartridge was washed twice with 5 ml of water and partially dried by applying a nitrogen stream for 10 s. The

radiotracer was eluted with 2 ml of EtOH (less than 5% of the total radioactivity was left on the cartridge) followed by 10 ml of physiological saline and filtered on a 0.22 μm GS-Millipore filter (vented). Finally, physiological saline was added to lower the EtOH concentration below 10%. This whole process was performed using a remote-controlled dedicated home-made device based on a literature procedure.[19]

[^{11}C]GI181771 ((S)-1). The procedure described above was used with 0.6 μmol (265 μg) of (*S*)-2-(3-amino-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl)-*N*-isopropyl-*N*-phenylacetamide (GW502085X, (*S*)-**11**).

Quality control of [^{11}C]GI167854 ((R,S)-1) and [^{11}C]GI181771 ((S)-1). The radiotracer preparation was briefly visually inspected for clarity, absence of colour and particulates. An aliquot of the preparation was evaluated for pH using standard pH-paper. Chemical and radiochemical purities were also assessed on this aliquot by HPLC (HPLC 4, R_t: 4.5 min and HPLC 5, R_t: 3.0 min). Finally, specific radioactivity of the radiotracer was calculated from three consecutive HPLC 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. Enantiomeric purity was determined using chiral HPLC (HPLC 6, R_t(*S*)-**1**: 13.7 min/R_t(*R*)-**1**: 8.5 min).

Conclusion

The novel CCK-A agonist, (*S*)-[(isopropylphenylcarbamoyl)methyl]-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydro-1H-benzo[*b*][1,4]diazepin-3-yl}ureido)benzoic acid, GI181771 ((*S*)-**1**) has been successfully labelled with carbon-11 at its urea site using [^{11}C]phosgene, via the intermediate preparation of an [^{11}C]isocyanate derivative. The one-pot two-step process described herein for the preparation of (*S*)-**1** lasted 30–35 min (HPLC purification and formulation included) and afforded up to 50 mCi (1.85 GBq) of the radiotracer (with specific radioactivities ranging from 500 to 1500 mCi/μmol (18.5–55.5 GBq/μmol)) with high (> 99%) chemical, radiochemical and enantiomeric purities.

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