

## 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 [<sup>11</sup>C]phosgene in a one-pot two-step process, via the intermediate preparation of an [<sup>11</sup>C]isocyanate derivative. Optimized conditions for the preparation of (*S*)-[<sup>11</sup>C]-**1** were the following: (1) Trapping of [<sup>11</sup>C]phosgene (radiosynthesized from cyclotron-produced [<sup>11</sup>C]methane via [<sup>11</sup>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 [<sup>11</sup>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*)-[<sup>11</sup>C]-**1** and (3) high-performance liquid chromatography (HPLC) purification on a semi-preparative Waters Symmetry<sup>®</sup> C18. Starting from a typical 1.2 Ci (44.4 GBq) batch of [<sup>11</sup>C]methane, 25–35 mCi (0.92–1.29 GBq, 6.8–9.6% decay-corrected yield based on starting [<sup>11</sup>C] methane, *n* = 5) of (*S*)-[<sup>11</sup>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<sup>®</sup> 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

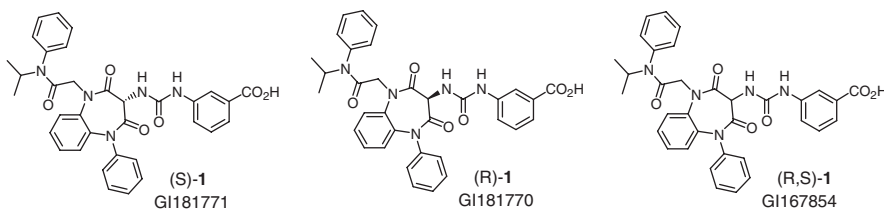
## Introduction

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.<sup>1,2</sup> CCK receptors have been already investigated in relation to a variety of disorders.<sup>3</sup> 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.<sup>4,5</sup> Among a series of 1,5-benzodiazepine selective CCK-A receptor agonists, GI181771, namely (*S*)-3-(3-{1-[(isopropylphenylcarbonyl)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.<sup>6</sup>

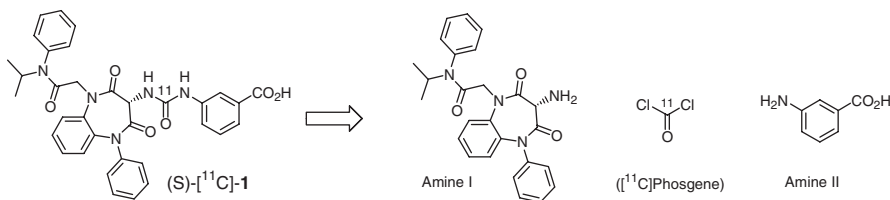
Positron emission tomography (PET) is a high-resolution, sensitive, molecular and functional imaging technique, which permits repeated, non-invasive assessment and quantification of specific biological and pharmacological processes. It is also the most advanced technology currently available for studying *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*)-[<sup>11</sup>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-[(isopropylphenylcarbonyl)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*)-[<sup>11</sup>C]-1)**

radiosynthesis of iso-[<sup>11</sup>C]propyl iodide (from [<sup>11</sup>C]carbon dioxide via [<sup>11</sup>C]acetone formation and consecutive reduction to iso-[<sup>11</sup>C]propanol<sup>7</sup>) and uses the corresponding secondary amide as non-radioactive precursor for labelling. A second approach involves the free carboxylic acid site, which directly uses [<sup>11</sup>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<sup>8</sup>) as a nonradioactive precursor for labelling. A third approach involves the urea site, which requires the preparation of [<sup>11</sup>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 [<sup>11</sup>C]phosgene technology is routinely used on-site, we chose to radiosynthesize (*S*)-[<sup>11</sup>C]-1 using the urea site approach, which we describe herein.

## Results and discussion

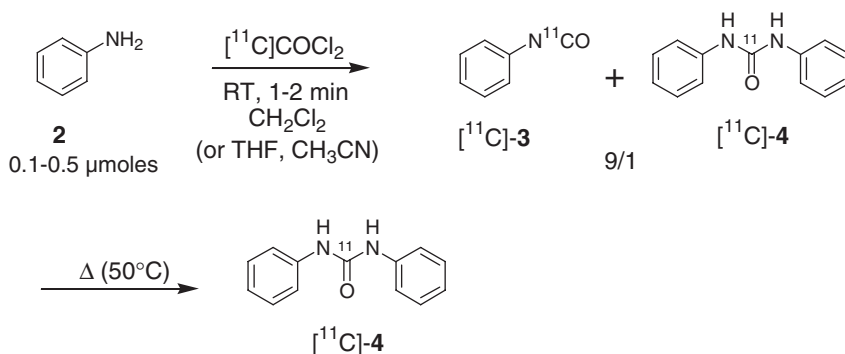
No-carrier-added [<sup>11</sup>C]phosgene ([<sup>11</sup>C]COCl<sub>2</sub>) was synthesized according to Scheme 2.

[<sup>11</sup>C]phosgene was radiosynthesized from cyclotron-produced [<sup>11</sup>C]methane ([<sup>11</sup>C]CH<sub>4</sub>) via [<sup>11</sup>C]carbon tetrachloride ([<sup>11</sup>C]CCl<sub>4</sub>) using minor modifications of published processes<sup>9–18</sup> (Scheme 2). Briefly, [<sup>11</sup>C]CH<sub>4</sub> was separated from the target contents, trapped and concentrated on Porapak-Q. [<sup>11</sup>C]CH<sub>4</sub> 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.<sup>12</sup> The on-line synthesized [<sup>11</sup>C]CCl<sub>4</sub> 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,<sup>9</sup> without intentional addition of oxygen to the system, giving [<sup>11</sup>C]COCl<sub>2</sub> in an average of 30–50% decay-corrected radiochemical yield, based on starting [<sup>11</sup>C]CH<sub>4</sub>. The whole process took 12–13 min.<sup>14–18</sup>

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



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



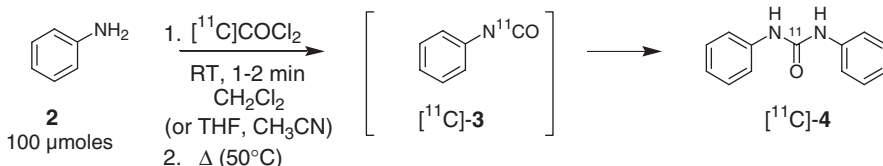
**Scheme 3. Radiosynthesis of phenyl[ $^{11}\text{C}$ ]isocyanate ([ $^{11}\text{C}$ ]-3) and [ $^{11}\text{C}$ ]carbanilide ([ $^{11}\text{C}$ ]-4) from [ $^{11}\text{C}$ ]phosgene and aniline (2)**

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

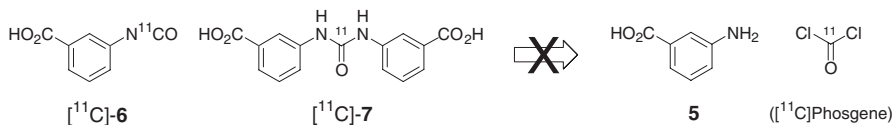
No-carrier-added [ $^{11}\text{C}$ ]phosgene was trapped at room temperature for 1–2 min in 300–500 μl of CH<sub>2</sub>Cl<sub>2</sub> (or THF, or CH<sub>3</sub>CN) containing 0.1–0.5 μmol of aniline (2). According to HPLC analysis, both phenyl[ $^{11}\text{C}$ ]isocyanate ([ $^{11}\text{C}$ ]-3) and [ $^{11}\text{C}$ ]carbanilide ([ $^{11}\text{C}$ ]-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 [ $^{11}\text{C}$ ]-4 was then predominantly formed and the intermediate isocyanate [ $^{11}\text{C}$ ]-3 could hardly be detected.

When [ $^{11}\text{C}$ ]phosgene was trapped at room temperature for 1–2 min in 300–500 μl of CH<sub>2</sub>Cl<sub>2</sub> (or THF, or CH<sub>3</sub>CN) containing a large excess of aniline (2, 100 μmol), the isocyanate ([ $^{11}\text{C}$ ]-3) was still the major product when compared to the urea ([ $^{11}\text{C}$ ]-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 [ $^{11}\text{C}$ ]carbanilide ([ $^{11}\text{C}$ ]-4) was then quantitatively formed.

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



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



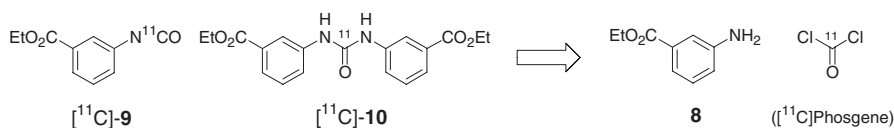
**Scheme 5.** Attempted radiosynthesis of 3- $[^{11}\text{C}]\text{isocyanatobenzoic acid}$  ( $[^{11}\text{C}]\text{-6}$ ) and 1,3-*bis*-(3-carboxyphenyl) $[^{11}\text{C}]\text{urea}$  ( $[^{11}\text{C}]\text{-7}$ ) from  $[^{11}\text{C}]\text{phosgene}$  and 3-aminobenzoic acid (**5**)

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

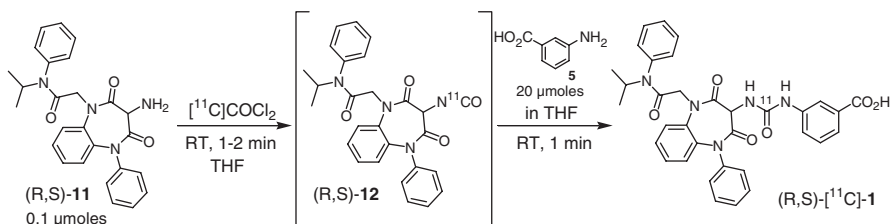
The conditions described above were therefore applied to the preparation of 3- $[^{11}\text{C}]\text{isocyanatobenzoic acid}$  ( $[^{11}\text{C}]\text{-6}$ ) and 1,3-*bis*-(3-carboxyphenyl) $[^{11}\text{C}]\text{urea}$  ( $[^{11}\text{C}]\text{-7}$ ) (Scheme 5).

$[^{11}\text{C}]\text{phosgene}$  was trapped at room temperature in 300–500  $\mu\text{l}$  of THF containing 100  $\mu\text{mol}$  of 3-aminobenzoic acid (**5**) and the reaction mixture was then concentrated to dryness. The expected 1,3-*bis*-(3-carboxyphenyl) $[^{11}\text{C}]\text{urea}$  ( $[^{11}\text{C}]\text{-7}$ ) could not be detected by HPLC analysis, neither using these conditions, nor in those where this symmetrical  $[^{11}\text{C}]\text{urea}$   $[^{11}\text{C}]\text{-7}$  was expected to be the minor product and the intermediate isocyanate ( $[^{11}\text{C}]\text{-6}$ ) the major one (using only 0.1–0.5  $\mu\text{mol}$  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- $[^{11}\text{C}]\text{isocyanatobenzoic acid}$  ( $[^{11}\text{C}]\text{-6}$ ).

On the other hand, 1,3-*bis*-(3-carbomethoxyphenyl) $[^{11}\text{C}]\text{urea}$  ( $[^{11}\text{C}]\text{-10}$ ) and 3- $[^{11}\text{C}]\text{isocyanatobenzoic acid ethyl ester}$  ( $[^{11}\text{C}]\text{-9}$ ) could be synthesized from 3-aminobenzoic acid ethyl ester (**8**) (Scheme 6). When  $[^{11}\text{C}]\text{phosgene}$  was trapped at room temperature for 1–2 min in 300–500  $\mu\text{l}$  of  $\text{CH}_2\text{Cl}_2$  (or THF, or  $\text{CH}_3\text{CN}$ ) containing 0.5  $\mu\text{mol}$  of 3-aminobenzoic acid ethyl ester (**8**), the expected ethyl 3- $[^{11}\text{C}]\text{isocyanatobenzoate}$  ( $[^{11}\text{C}]\text{-9}$ ) could be detected as the



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

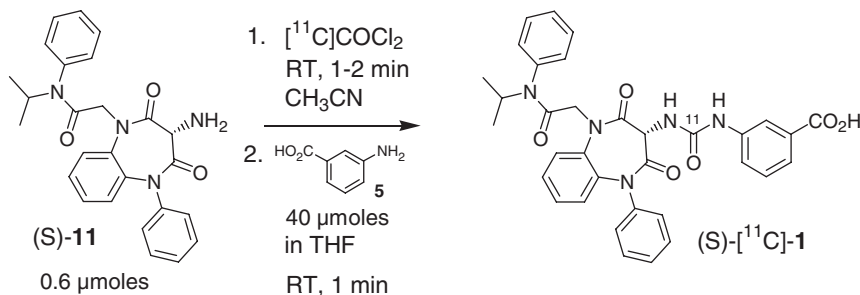


**Scheme 7.** Radiosynthesis of the racemic unsymmetrical urea (R,S)-<sup>11</sup>C]-1

major product according to HPLC analysis. When [<sup>11</sup>C]phosgene was trapped at room temperature in 300–500 μl of CH<sub>3</sub>CN 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) [<sup>11</sup>C]urea ([<sup>11</sup>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-<sup>11</sup>C]isocyanatobenzoate ([<sup>11</sup>C]-9) could be observed as the major product besides the urea ([<sup>11</sup>C]-10).

Following these experiments, we concluded that a free carboxylic acid function appeared to be incompatible with an [<sup>11</sup>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)-<sup>11</sup>C]-1 where the preparation of a structurally complex and chiral [<sup>11</sup>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, [<sup>11</sup>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)- $[^{11}\text{C}]\text{-1}$

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  $[^{11}\text{C}]\text{CH}_4$ , only 3–5 mCi of the desired unsymmetrical racemic urea (*R,S*)- $[^{11}\text{C}]\text{-1}$  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<sup>®</sup> 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  $\frac{1}{2}$  ratio of (*R*)- and (*S*)- $[^{11}\text{C}]\text{-1}$ .

Conditions for the preparation of the target unsymmetrical and chiral urea (S)- $[^{11}\text{C}]\text{-1}$  (Scheme 8) were finally optimized and included the use of enantiomerically pure amine (S)-**11** (0.6  $\mu\text{mol}$  in 300  $\mu\text{l}$  of  $\text{CH}_3\text{CN}$ ) and 40  $\mu\text{mol}$  of 3-aminobenzoic acid (**5**). Starting from a typical 1.2 Ci batch of  $[^{11}\text{C}]\text{CH}_4$ , 25–35 mCi (0.92–1.29 GBq, 6.8–9.6% decay-corrected yield based on starting  $[^{11}\text{C}]\text{CH}_4$ ,  $n = 5$ ) of (S)- $[^{11}\text{C}]\text{-1}$  could be obtained within 35 min of radiosynthesis (HPLC purification and formulation included) with specific radioactivities ranging from 500 to 1500 mCi/ $\mu\text{mol}$  (18.5–55.5 GBq/ $\mu\text{mol}$ ). 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)- $[^{11}\text{C}]\text{-1}$ ) 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:  $^1\text{H-NMR}$ , 300 MHz,  $\text{DMSO-d}_6$ :  $\delta$ : 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);  $^{13}\text{C-NMR}$ , 75 MHz,  $\text{DMSO-d}_6$ :  $\delta$ : 165.6 ( $2 \times \text{C}$ ); 152.4 (C); 139.9 ( $2 \times \text{C}$ ); 130.4 ( $2 \times \text{C}$ ); 129.0 ( $2 \times \text{CH}$ ); 122.8 ( $2 \times \text{CH}$ ); 122.6 ( $2 \times \text{CH}$ ); 118.7 ( $2 \times \text{CH}$ ); 60.6 ( $2 \times \text{CH}_2$ ); 14.1 ( $2 \times \text{CH}_3$ ); MS ( $\text{DCI}/\text{NH}_4^+$ ):  $\text{C}_{19}\text{H}_{20}\text{N}_2\text{O}_5$ : 357 [ $\text{M} + \text{H}^+$ ]. 1,3-*bis*-(3-carboxyphenyl)urea (**7**), 3-(3-{1-[(isopropylphenylcarbonyl)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.<sup>6</sup>

**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<sup>®</sup> SiO<sub>2</sub>, Merck (250 × 10 mm; porosity: 7 μm); solvents and conditions: isocratic elution with CH<sub>2</sub>Cl<sub>2</sub>/EtOH/H<sub>2</sub>O/EtNH<sub>2</sub>: 98/1.924/0.038/0.038 (v:v:v); flow rate: 8.0 ml/min; temperature: RT; absorbance detection at λ = 254 nm.

[HPLC 2]: Equipment and column: see HPLC 1; solvents and conditions: isocratic elution with CH<sub>2</sub>Cl<sub>2</sub>/MeOH/TFA: 97/3/0.2 (v:v:v); flow rate: 7.0 ml/min; temperature: RT; absorbance detection at λ = 254 nm.

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



elution with CH<sub>3</sub>CN/H<sub>2</sub>O/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<sup>®</sup> C-18, Waters (150 × 3.9 mm; porosity: 5  $\mu$ m); solvents and conditions: isocratic elution with solvent A/solvent B: 8/92 (v:v); solvent A: H<sub>2</sub>O containing Low-UV PIC<sup>®</sup> B7 reagent (20 ml for 1000 ml); solvent B: H<sub>2</sub>O/CH<sub>3</sub>CN: 50/50 (v:v) containing Low-UV PIC<sup>®</sup> B7 reagent (20 ml for 1000 ml) (Low-UV PIC<sup>®</sup> 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<sub>2</sub>O containing Low-UV PIC<sup>®</sup> B7 reagent (20 ml for 1000 ml); solvent B: H<sub>2</sub>O/CH<sub>3</sub>CN: 50/50 (v:v) containing Low-UV PIC<sup>®</sup> B7 reagent (20 ml for 1000 ml) (Low-UV PIC<sup>®</sup> 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<sup>®</sup> OD-R, Daicel (240 × 4.6 mm; porosity: 5  $\mu$ m); solvents and conditions: isocratic elution with CH<sub>3</sub>CN/H<sub>2</sub>O/CH<sub>3</sub>CO<sub>2</sub>H/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 [<sup>11</sup>C]CH<sub>4</sub> was produced on a CGR-520 MeV cyclotron by irradiation of a target consisting of an ultrapure Air Liquide 95/5 mixture of N<sub>2</sub>/H<sub>2</sub> (target holder: 668 ml operated at 8 bar) by the <sup>14</sup>N[p, $\alpha$ ]<sup>11</sup>C nuclear reaction using a 20 MeV proton beam. Typical production: 1.20 Ci (44.40 GBq) of [<sup>11</sup>C]CH<sub>4</sub> at the end of bombardment (EOB) for a 30  $\mu$ A, 30 min (54 000  $\mu$ C) irradiation.

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

### Radiochemistry

*Preparation of [<sup>11</sup>C]COCl<sub>2</sub>.* 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 [<sup>13</sup>N]ammonia (side-product of the irradiation) and subsequently through a guard of P<sub>2</sub>O<sub>5</sub> (glass tube, 70 mm length, 10 mm internal diameter) in order to remove moisture. [<sup>11</sup>C]CH<sub>4</sub> 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  $-186^{\circ}\text{C}$  (liquid argon).  $[^{11}\text{C}]\text{CH}_4$  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).  $[^{11}\text{C}]\text{CH}_4$  was then passed through a guard of  $\text{P}_2\text{O}_5$  (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  $-186^{\circ}\text{C}$  (liquid argon). On average, about 1.20 Ci (44.4 GBq, at EOB) of  $[^{11}\text{C}]\text{CH}_4$  is routinely produced for a  $30\ \mu\text{A}$ , 30 min (54 000  $\mu\text{C}$ ) irradiation and then transferred and concentrated in 4–5 min using the process described above.  $[^{11}\text{C}]\text{CH}_4$  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  $[^{11}\text{C}]\text{CH}_4$ –chlorine mixture was passed through an empty horizontal glass tube (215 mm length, 7 mm internal diameter) at a temperature of  $510^{\circ}\text{C}$ . The thus formed  $[^{11}\text{C}]\text{CCl}_4$  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  $290$ – $310^{\circ}\text{C}$ . Finally, the gaseous reaction mixture now containing  $[^{11}\text{C}]\text{COCl}_2$  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  $[^{11}\text{C}]\text{isocyanates}$  (general procedure).* The on-line synthesized  $[^{11}\text{C}]\text{COCl}_2$  was trapped (bubbling through) at room temperature or lower ( $0^{\circ}\text{C}$ ) in a reaction vessel containing 0.1–0.5  $\mu\text{mol}$  of the appropriate aniline derivative dissolved in 300–500  $\mu\text{l}$  of  $\text{CH}_2\text{Cl}_2$ , THF or  $\text{CH}_3\text{CN}$ . Trapping of  $[^{11}\text{C}]\text{COCl}_2$  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 $[^{11}\text{C}]\text{isocyanate}$  ( $[^{11}\text{C}]\text{-3}$ ):* The procedure described above was used with aniline (**2**, 0.2  $\mu\text{mol}$ ) to give predominantly phenyl $[^{11}\text{C}]\text{isocyanate}$  ( $[^{11}\text{C}]\text{-3}$ ) (HPLC 1,  $R_t$ : 13.5 min).  $[^{11}\text{C}]\text{carbanilide}$  ( $[^{11}\text{C}]\text{-4}$ ) could also be detected (HPLC 1,  $R_t$ : 5.0 min).

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

*Preparation of model symmetrical [ $^{11}\text{C}$ ]ureas (general procedure).* The on-line synthesized [ $^{11}\text{C}$ ]COCl<sub>2</sub> 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<sub>2</sub>Cl<sub>2</sub>, THF or CH<sub>3</sub>CN. Trapping of [ $^{11}\text{C}$ ]COCl<sub>2</sub> 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 [ $^{11}\text{C}$ ]carbanilide ([ $^{11}\text{C}$ ]-4):* The procedure described above was used with aniline (**2**) to give [ $^{11}\text{C}$ ]carbanilide ([ $^{11}\text{C}$ ]-4) (HPLC 1,  $R_t$ : 5.0 min).

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

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

*Optimized conditions for the preparation of [ $^{11}\text{C}$ ]GII67854 ((*R,S*)-1) and [ $^{11}\text{C}$ ]GII81771 ((*S*)-1)*

*[ $^{11}\text{C}$ ]GII67854 ((*R,S*)-1). Radiosynthesis and purification:* The on-line synthesized [ $^{11}\text{C}$ ]COCl<sub>2</sub> 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 (GII55056, (*R,S*)-**11**) dissolved in 250 μl of CH<sub>3</sub>CN. Trapping of [ $^{11}\text{C}$ ]COCl<sub>2</sub> 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<sup>®</sup> 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  $\mu\text{m}$  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.<sup>19</sup>

$[^{11}\text{C}]\text{GI181771}$  ((*S*)-**1**). The procedure described above was used with 0.6  $\mu\text{mol}$  (265  $\mu\text{g}$ ) 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  $[^{11}\text{C}]\text{GI167854}$  ((*R,S*)-**1**) and  $[^{11}\text{C}]\text{GI181771}$  ((*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*)-[(isopropylphenylcarbomoyl)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  $[^{11}\text{C}]$ phosgene, via the intermediate preparation of an  $[^{11}\text{C}]$ 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/ $\mu\text{mol}$  (18.5–55.5 GBq/ $\mu\text{mol}$ )) with high (>99%) chemical, radiochemical and enantiomeric purities.

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