[^{11}C]/(^{13}C)Carbon monoxide in palladium-mediated synthesis of imides

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Received (in Cambridge, UK) 19th March 2001, Accepted 11th May 2001
First published as an Advance Article on the web 5th June 2001

[^{11}C]Carbon monoxide in low concentration with palladium(0) complexes (in the range of 5 μmol) and aryl halides (about 10 μmol) were used in the synthesis of the ^{11}C-labelled imides, thalidomide, *N*-phthaloyl-L-glutamic acid, *N*-phthaloylhexane and aniracetam. The labellings were performed with ring closure reactions except in the case of aniracetam. These imides were obtained in nearly quantitative radiochemical yields, calculated from the [^{11}C]carbon monoxide, with specific radioactivities between 70–600 GBq μmol^{-1}. The radiochemical purity of the LC-purified products exceeded 98%. *N*-(^{13}C)Phthaloyl-L-glutamic acid **2** was produced to verify the position of the label (δ 167.5 ppm) with ^{13}C NMR, and this also indicates that the discussed approach is valuable for labelling with other carbon isotopes. In a typical experiment starting with 2.8 GBq of [^{11}C]carbon monoxide, 0.6 GBq of HPLC-purified [^{11}C]-*N*-phthaloyl-L-glutamic acid **2** was obtained within 40 minutes from the start of the carbonylation reaction (84% decay-corrected radiochemical yield).

Introduction

Tracers labelled with short-lived positron emitting radionuclides (*e.g.* ^{11}C, $t_{½}$ = 20.3 minutes) are frequently used in various non-invasive *in vivo* studies in combination with positron emission tomography (PET).[1]

Although [^{11}C]carbon monoxide was one of the first ^{11}C-labelled compounds to be applied in tracer experiments in man,[2] it has until recently rarely been used as a precursor in labelling chemistry.[3] One reason for this is the difficulty in trapping [^{11}C]carbon monoxide in a reaction medium.[4] To overcome this problem a method has been developed which makes it possible to trap the [^{11}C]carbon monoxide in a micro-autoclave and to perform reactions at high pressure. The method has been used in the synthesis of a wide range of ^{11}C-labelled carbonyl compounds, *e.g.* ^{11}C-labelled amides.[5] In the present report the versatility of this approach is further explored in the ^{11}C-labelling of imides using a palladium-mediated carbonylation with [^{11}C]carbon monoxide.

The following compounds were labelled using this method: thalidomide, *N*-phthaloyl-L-glutamic acid, 2-hexylisoindoline-1,3-dione, and aniracetam.

a) Thalidomide [2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione] **1**, was developed as an alternative to barbiturates. It was withdrawn from the market in the 1960s because of its teratogenicity but has recently shown interesting antiangiogenic and anti-inflammatory properties.[6] Thalidomide has also been evaluated in the treatment of autoimmunological diseases such as human immunodeficiency virus (HIV) infection.[7]

b) *N*-Phthaloyl-L-glutamic acid **2** is a selective *N*-methyl-D-aspartate (NMDA)/glutamate receptor agonist.[8]

c) 2-Hexylisoindoline-1,3-dione **3** was chosen as a model compound.

d) Aniracetam [1-(4-anisoyl)pyrrolidin-2-one] **4** is a cognitive enhancer with pharmacological properties and a chemical structure similar to the nootropic piracetam (2-oxopyrrolidine-1-acetamide).[9]

Results and discussion

The ^{11}C-labelled imides were synthesised in a micro-autoclave (200 μl) at pressures exceeding 35 MPa using tetrakis(triphenylphosphine)palladium(0), the appropriate aryl halide and [^{11}C]carbon monoxide. The concentration ratio between [^{11}C]carbon monoxide and the substrates was in the range of 1 : 200–600. Compounds **1**, **2** and **3** (Fig. 1) were obtained through ring closure as shown in Scheme 1. Compound **4** was obtained in a carbonylation reaction using 4-iodoanisole and pyrrolidin-2-one as shown in Scheme 2. All the ring closure reactions were performed at 140 °C. For compound **4** a higher reaction temperature was required due to its lower reactivity. In

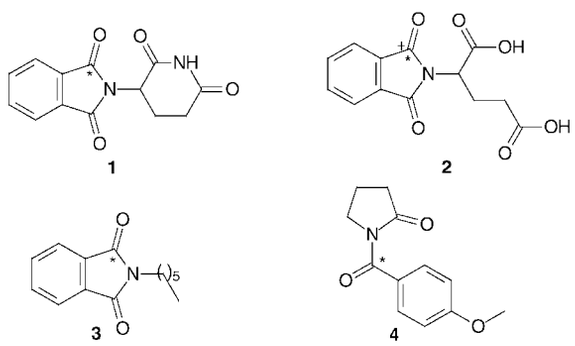
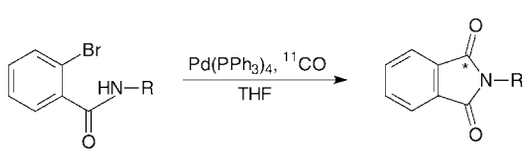
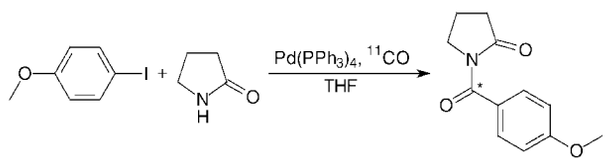


Fig. 1 Target molecules (* = ^{11}C and + = ^{13}C).



Scheme 1



Scheme 2

 DOI: 10.1039/b102524b

Table 1 Radiochemical yields and specific radioactivity for the ^{11}C-labelled amides shown in Fig 1

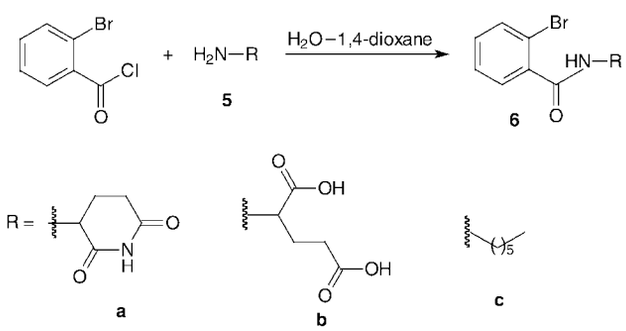
Compound	Trapping efficiency[a] (%)	Isolated rcy[b] (%)	Specific radioactivity[c] /GBq μmol^{-1}
1	88 ± 1 (2)[d]	69 (1)	72 (1)
2	85 ± 1 (1)	73 ± 4 (3)	575 ± 25 (2)
3	80 (1)	45 (1)	—
4	96 ± 2 (2)	77 ± 3 (2)	260 (1)

[a] Decay-corrected, the fraction of radioactivity left in the crude product after purging with nitrogen. [b] rcy = Radiochemical yield, decay-corrected, calculated from the amount of radioactivity in the crude product before nitrogen purge, and the radioactivity of the LC purified product. [c] Based on concentration measurements determined by LC-MS-analysis. Bombardments 2.5 μA h. [d] Number of runs.

most cases the isolated radiochemical yields were in the order of 70% as shown in Table 1.

To our knowledge, palladium-mediated carbonylation has not previously been applied in the synthesis of imides.

The identities of all compounds were confirmed by LC-MS. The identity and the labelling position of compound **1** were further confirmed by ^{13}C-NMR analysis of the corresponding carbonyl ^{13}C-substituted compound. The ^{13}C-NMR signal at 167.5 ppm (in DMSO-d_6) corresponds to the carbonyl carbon of authentic *N*-phthaloyl-L-glutamic acid. The precursor (**6a**) was obtained from (*S*)-2-benzyloxycarbonylglutamine in three steps (Scheme 3). The starting material was cyclised using



Scheme 3

thionyl chloride,[10] and hydrogenated.[11] The resulting amine hydrochloride (**5a**) was then treated with 2-bromobenzoyl chloride and sodium carbonate to obtain **6a**.

Conclusion

Carbonylation reactions using [^{11}C]carbon monoxide, as exemplified in this report, will have a significant value for synthesis of labelled compounds useful in various PET applications. Biologically active substances, which are of interest as PET-tracers, often contain carbonyl groups or functionalities that can be derived from a carbonyl group. The synthetic routes described here are tolerant of many functional groups, which means that complex building blocks can be assembled in the carbonylation step to yield the target compound. This is particularly valuable in PET-tracer synthesis where the unlabelled substrates should be combined with the labelled precursor as late as possible in the reaction sequence, in order to optimise the yield of the labelled product.[12]

The presented method is presently the only realistic alternative for ^{11}C-labelling of phthalimides such as thalidomide. The use of [^{11}C]carbon monoxide is a valuable approach in the synthesis of PET-tracers in high radiochemical yield and specific radioactivity.

Experimental

General

[^{11}C]Carbon dioxide production was performed using a Scanditronix MC-17 cyclotron at the Uppsala University PET Centre. The ^{14}N(p,α)^{11}C reaction was performed in a target gas containing nitrogen (AGA, Nitrogen 6.0) and 0.1% oxygen (AGA, Oxygen 4.8), which was, bombarded with 17 MeV protons.

[^{11}C]Carbon monoxide was produced by reduction of the [^{11}C]carbon dioxide formed in the target. The latter was trapped on a column (Porapac Q) at −196 °C and released by heating and reduced during passage through a zinc filled tube at 400 °C.[13]

Liquid chromatographic analysis (LC) was performed with a Beckman 126-gradient pump and a Beckman 166 variable wavelength UV-detector in series with a β$^+$-flow detector.[14] The following mobile phases were used: 0.05 M ammonium formate pH 3.5 (A), acetonitrile (B), 0.01 M trifluoroacetic acid (C), 0.01 M formic acid (D). For analytical LC, a Beckman Ultrasphere ODS C_{18} (5 μm, 250 × 4.6 mm id) column was used at a flow of 1.5 ml min^{-1}. For semi-preparative LC, a Beckman Ultrasphere ODS C_{18} (5 μm, 250 × 10 mm id) column was used at a flow of 4 ml min^{-1}. Synthia, an automated synthesis system,[15] was used for LC injection and fraction collection. Data collection and LC control were performed with the use of a Beckman System Gold chromatography software package.

Radioactivity was measured in an ion chamber, Veenstra Instrumenten bv, VDC-202. For crude estimations of radioactivity during synthesis, a portable dose-rate meter was used, Långenås eltekniska AB.

In the analysis of the ^{11}C-labelled compounds, reference substances were used for comparison in all the LC runs. Identities of synthesised materials were determined using both ^{1}H and ^{13}C NMR and LC-MS. NMR spectra were recorded on a Varian XL 300 (300 MHz, unless stated otherwise) using dimethyl sulfoxide-d_6 or chloroform-d as internal standards.

Mass spectra were recorded on a Finnigan GCQ mass spectrometer coupled to Finnigan Q-GC.

LC-MS was performed using a Micromass VG Quattro with electrospray ionisation. Separation and injection was performed for LC-MS analysis with a Beckman 126 pump, and a CMA 240 autosampler with a Beckman Ultrasphere ODS C_{18} (5 μm, 100 × 4.6 mm id) column. The mobile phases B, C and D were used.

THF was distilled under nitrogen from sodium–benzophenone.

The reference compounds for **1** and **2** were purchased from Research Biochemical International.[16]

N-(2,6-Dioxo-3-piperidyl)-2-bromobenzamide (6a)

A solution of (±)-3-aminopiperidine-2,6-dione hydrochloride (**5a**) (102 mg, 0.62 mmol), dioxane (0.8 ml) and $Na_2CO_{3(aq)}$ (1.7 ml, 1.1 M) was mixed with a magnetic stirrer. The mixture was cooled on an ice bath and a solution of 2-bromobenzoyl chloride (113 μl, 0.87 mmol) in dioxane (1.6 ml) was added over 1 hour. The mixture was stirred overnight, with gradual warming to room temperature. The reaction mixture was then poured into H_2O (36 ml) and extracted with methylene chloride. The pooled extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The resulting yellowish oil,

which solidified on standing, was recrystallised from EtOAc–hexane. The compound was further purified by LC as described for **1**. Concentration of the collected fraction under reduced pressure followed by vacuum gave **6a** (102 mg, 53%). δ_H(DMSO): 10.85(1H, br), 8.76(1H, d), 7.66(1H, d), 7.38(3H, m), 4.73(1H, m), 2.80–2.50(2H, m), 2.10–1.99(2H, m). ^{13}C NMR(200 MHz, DMSO): δ 173.5, 172.2, 167.6, 138.9, 133.3, 131.6, 129.4, 128.1, 119.4, 49.8, 31.3, 24.6. LC-MS (ESI+), solvent (B–C): *m*/*z* 311, 313 $[M + H]^+$.

2-(2-Bromobenzoylamino)succinic acid (6b)

This synthesis was performed as described for **6a** except that (L)-glutamic acid in 1 M $NaOH_{(aq)}$ (2 ml/1 mmol) was used instead of (±)-3-aminopiperidine-2,6-dione hydrochloride. The crude product was acidified by addition of 6 M $HCl_{(aq)}$ and washed with cold water. The resulting salt was recrystallized from water. This was further purified by LC as described for **2** to obtain the compound **6b** (yield 35%). δ_H(DMSO): 12.45(2H, br), 8,72(1H, d), 7.65(1H, d), 7.45–7.32(3H, m), 4.39(1H, m), 2.38(2H, m), 2.06(1H, m), 1.86(1H, m). ^{13}C NMR(DMSO): δ 173.8, 173.0, 167.4, 138.7, 132.7, 131.0, 129.0, 127.6, 118.9, 51.6, 30.2, 26.1. LC-MS (ESI+), solvent (B–D): *m*/*z* 330, 331 $[M + H]^+$.

N-Hexyl-2-bromobenzamide (6c)

To a solution of 2-bromobenzoyl chloride (168 mg, 0.76 mmol) in dry THF (50 ml) was added *N*-hexylamine (155 mg, 1.53 mmol). The reaction mixture was heated at 50 °C for 30 minutes. The solvent was evaporated and the residue was dissolved in ether and washed with 1 M $HCl_{(aq)}$, saturated $NaHCO_{3(aq)}$ and water. The ether phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by LC as described for **3** to give the title compound **6c** (140 mg, 64%). δ_H(DMSO): 8.38(1H, br), 7.63(1H, d), 7.29–7.42(3H, m), 3.18(2H, m), 1.49(2H, m), 1.20–1.40(6H, m), 0.86(3H, t). ^{13}C NMR(DMSO): δ 167.0, 139.5, 132.6, 130.6, 128.7, 127.5, 118.9, 31.0, 28.9, 26.1, 22.1, 14.0. LC-MS (ESI+), solvent (B–D): *m*/*z* 283, 285 $[M + H]^+$.

2-Hexylisoindoline-1,3-dione (3)

Hexylamine (50 mg, 0.49 mmol) was added to phthaloyl chloride (100 mg, 0.49 mmol) dissolved in dry THF (50 ml). The reaction mixture was stirred at room temperature for 1 hour. Imidazole (60 mg) was added and the mixture was stirred for an additional 30 minutes at 50 °C. The solvent was evaporated and the residue was heated at 150 °C for 5 minutes. After the work-up (as described for **6c**), the crude product was purified by LC to give compound **3** (87 mg, 76%). δ_H($CDCl_3$, 200 MHz): 7.66–7.85(4H, m), 3.66(2H, t), 1.64(2H, m), 1.30(6H, m), 0.86(3H, t). ^{13}C NMR($CDCl_3$, 200 MHz): δ 168.0, 133.8, 123.1, 38.0, 31.3, 28.5, 26.5, 22.5, 14.0. GC-MS: *m*/*z* = 231, 216, 202, 188, 174, 160.

1-(4-Anisoyl)pyrrolidin-2-one (4)

A mixture of pyrrolidin-2-one (2 ml, 26 mmol), triethylamine (4.3 ml, 31 mmol) and THF (100 ml) was loaded into a 250 ml round flask and cooled on an ice bath. The mixture was stirred and 4-anisoyl chloride (3.5 ml, 26 mmol) in THF (10 ml) was added over 0.5 hour. The mixture was then left overnight, with gradual warming to room temperature. The precipitated triethylamine hydrochloride was filtered and concentrated to dryness under reduced pressure. The residue was recrystallised twice from THF–pentane (4.7 g, 83%). δ_H(DMSO): 7.55(2H, d), 6.93(2H, d), 3.80(5H, m), 2.52(2H, m), 2.0(2H, m). ^{13}C NMR(DMSO): δ 174.8, 169.4, 162.2, 131.5, 126.6, 113.0, 55.4, 46.5, 32.8, 17.3. LC-MS (ESI+), solvent (B–C) *m*/*z* 220 $[M + H]^+$.

2-(2,6-Dioxo-3-piperidyl)[1-^{11}C]isoindoline-1,3-dione (1)

A capped vial (1 ml) containing a solution of tetrakis(triphenylphosphine)palladium(0) (5.7 mg, 5.2 μmol) and **6a** (3.3 mg, 10.6 μmol) in dry THF (250 μl) was flushed with nitrogen and shaken till the solution was homogeneous. The resulting reaction mixture was transferred to the micro-autoclave, which was pre-charged with [^{11}C]carbon monoxide. The micro-autoclave was heated at 140 °C for 5 minutes. The crude product was transferred to a vial with reduced pressure. The radioactivity was measured before and after the vial was flushed with nitrogen. The solvent volume was reduced to less than 0.1 ml by heating at 50 °C and purging with nitrogen. Acetonitrile (0.2 ml) followed by water (2 ml) were added and the resulting solution injected on to the semi-preparative LC. Solvent A–B (90 : 10) linear gradient to 40 : 60 during 5 minutes then to 100 : 0 during 2 minutes, flow 4 ml min^{-1}, t_R = 8.6 min. Analytical LC, same method as semi-preparative LC, flow 1.5 ml min^{-1}, wavelength 254 nm, t_R = 6.8 min. LC-MS (ESI+), solvent (B–C), *m*/*z* 259 $[M + H]^+$.

2-(1,3-Dicarboxypropan-1-yl)[1 -^{11}C]isoindoline-1,3-dione (2)

The synthesis was performed as described for **1** except that 2-(2-bromobenzoylamino)succinic acid (**6b**) was used instead of **6a**. Semi-preparative LC, solvent A–B (85 : 15) linear gradient to 40 : 60 during 9 minutes then at 10 minutes to 100 : 0 during 0.5 minutes, flow 4 ml min^{-1}, t_R = 9.4 min. Analytical LC, same method as described for **1**, flow 1.5 ml min^{-1}, wavelength 254 nm, t_R = 5.7 min. LC-MS (ESI+), solvent (B–D), *m*/*z* 260, 278 $[M + H]^+$.

N-(^{13}C)Phthaloyl-L-glutamic acid (2)

A vial (1 ml) containing tetrakis(triphenylphosphine)-palladium(0) (9.6 mg, 8.3 μmol) and **6b** (11.2 mg, 33.9 μmol) in dry THF (100 μl) and dry DMSO (100 μl) was flushed with nitrogen and shaken till the solution was homogeneous. The resulting reaction mixture and [^{13}C]carbon monoxide (1 ml) was transferred to the micro-autoclave, which was pre-charged with [^{11}C]carbon monoxide. The micro-autoclave was heated at 140 °C for 20 minutes. The crude product was transferred to a vial with reduced pressure and concentrated by heating at 50 °C and purging with nitrogen. Acetonitrile (0.2 ml) followed by water (1.8 ml) were added and the resulting solution injected on the semi-preparative LC, solvent A–B (90 : 10) linear gradient to 40 : 60 during 5 minutes then to 100 : 0 during 2 minutes, flow 4 ml min^{-1}, t_R = 8.6 min to obtain the desired product (48%). ^{13}C NMR(DMSO): δ 167.5.

2-Hexyl[1 -^{11}C]isoindoline-1,3-dione (3)

The synthesis was performed as described for **1** except that *N*-hexyl-2-bromobenzamide (**6c**) was used instead of **6a**. Semi-preparative LC, solvent A–B (60 : 40) linear gradient to 0 : 100 during 10 minutes, flow 4 ml min^{-1}, t_R = 15.1 min. Analytical LC, solvent A–B (70 : 30) linear gradient to 0 : 100 during 10 minutes, flow 1.5 ml min^{-1}, wavelength 254 nm, t_R = 9.9 min. LC-MS (ESI+), solvent A–B, *m*/*z* 232 $[M + H]^+$.

N-[1″-^{11}C](4-Anisoyl)pyrrolidin-2-one (4)

The synthesis was performed as described for **1** except that 4-iodoanisole (2 mg, 8.5 μmol) and pyrrolidin-2-one (10 μl, 130 μmol) were used instead of **6a**. The reactor was heated at 200 °C. Semi-preparative LC, solvent A–B (70 : 30) linear gradient to 30 : 70 during 8 minutes then at 0.5 minutes to 100 : 0, flow 4 ml min^{-1}, t_R = 10.3 min. Analytical LC, solvent A–B (70 : 30) linear gradient to 0 : 100 during 10 minutes, flow 1.5 ml min^{-1}, wavelength 254 nm, t_R = 5.8 min. LC-MS (ESI+), solvent (B–C) *m*/*z* 220 $[M + H]^+$.

Acknowledgements

We thank Dr Robert Moulder for checking the language. The Swedish Nature Research Council is acknowledged for its support through Grant No. K3464 (B.L.).

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