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A convenient method to produce [^{14}C]carbon monoxide and its application to the radiosynthesis of [*carboxyl*-^{14}C]celivarone, [*carboxyl*-^{14}C]SSR149744

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[*carboxyl*-^{14}C]Celivarone was synthesised from barium [^{14}C]carbonate with overall radiochemical yields in the range 49–53%. The synthetic route involves [^{14}C]carbonylation methodology, which both decreased the number of synthetic steps and increased the yields obtained from previous synthetic routes.

Keywords: [*carboxyl*-^{14}C]celivarone; antiarrhythmic; [^{14}C]carbonylation; lithium [^{14}C]formate

Introduction

The generation, transfer and subsequent reaction of radioactive gases as a means to introduce carbon-14 into target molecules remain key processes in the development of radiolabelled pharmaceuticals. We have recently investigated and developed methods to generate and capture [^{14}C]carbon monoxide from relatively non-volatile and stable precursors, providing efficient routes to the radiolabelling of small molecules. Unlabelled carbon monoxide remains, after many decades of research, possibly the most versatile C_1 building block available, giving access to a variety of functionalities.[1] [^{14}C]Carbon monoxide is seldom employed in radiochemical syntheses due to difficulties with its generation, stability, inherent toxicity and containment; however, recent advances in the generation and the use of [^{14}C]carbon monoxide have addressed many of these issues.[2–6] The use of [^{11}C]carbon monoxide is also increasingly common in connection with radiosyntheses for positron emission tomography.[7]

To this end, we have combined relevant research from the literature with our own radiochemical expertise and developed a reproducible and robust carbonylation procedure, which is compatible with standard vacuum transfer equipment. The methodology has been applied to syntheses within our portfolio. Herein, we report the application of [^{14}C]carbonylation to the radiosynthesis of the antiarrhythmic [*carboxyl*-^{14}C]Celivarone, [*carboxyl*-^{14}C]SSR149744 (**1**).

Celivarone, SSR149744C, or 2-butyl-3-{4-[3-(dibutylamino)-propyl]benzoyl}-1-benzofuran-5-carboxylate fumarate (Figure 1) (**2**),[8,9] has a strong antiarrhythmic potential.[10] It is structurally related to the marketed Amiodarone[11] and Dronedarone.[12]

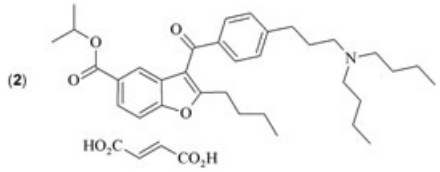

Figure 1. Structure of the potential antiarrythmic Celivarone, SSR149744C.

Results and discussion

Radiosynthesis of [*carboxyl*-^{14}C]SSR149744 (1) by [^{14}C]cyanation

The original approach towards the labelling of [*carboxyl*-^{14}C]SSR149744 (**1**) employed an initial cyanation reaction of the aryl bromide (**3**) (Scheme 1). This proceeded cleanly in 42% isolated yield. Subsequent hydrolysis, using a variety of conditions as indicated, gave only minor conversion to the corresponding carboxylic acid. The parent nitrile remained mostly intact and for this reason, the synthesis was quickly discarded.

Radiosynthesis of [*carboxyl*-^{14}C]SSR149744 (1) by [^{14}C]carboxylation

Carboxylation was then pursued as an alternative procedure to incorporate the ^{14}C-label as outlined in Scheme 2.

Introduction of the radioisotope to afford (**4**) was achieved by lithium–halogen exchange of the aryl bromide, followed by quenching with [^{14}C]carbon dioxide. The [^{14}C]carbon dioxide

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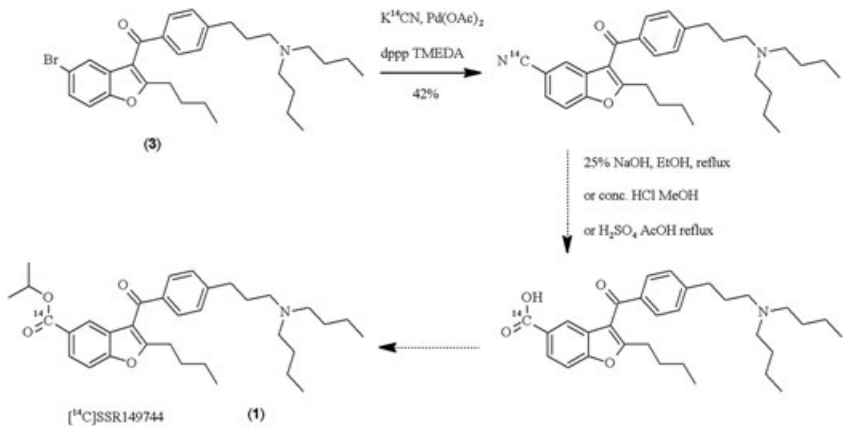


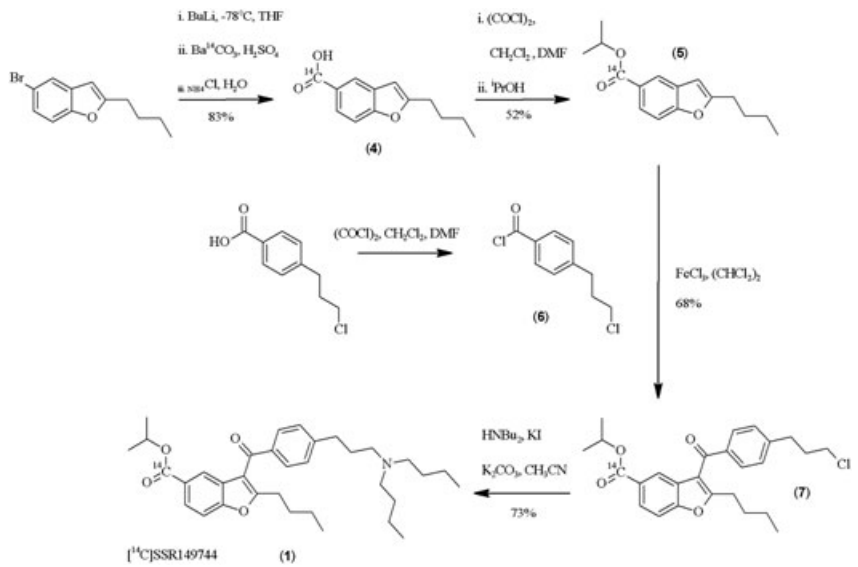
Scheme 1. Attempted radiosynthesis of [*carboxyl*-^{14}C]SSR149744 (1) by [^{14}C]cyanation.

was generated ex situ, using a standard gas manifold apparatus, by the addition of barium [^{14}C]carbonate to concentrated sulfuric acid and subsequent gas transfer under vacuum to the flask containing the pre-formed frozen lithiated species. The carboxylic acid (**4**) was isolated in good yield. Formation of the isopropyl ester (**5**) was achieved by reaction of (**4**) with oxalyl chloride and subsequent quenching with propan-2-ol. The acid chloride (**6**) was reacted using Friedel–Crafts conditions with (**5**) to afford (**7**). Reaction of (**7**) with dibutylamine, in the presence of potassium carbonate and potassium iodide, furnished the title compound [*carboxyl*-^{14}C]SR149744 (**1**) in four radiochemical steps and 21% overall radiochemical yield.

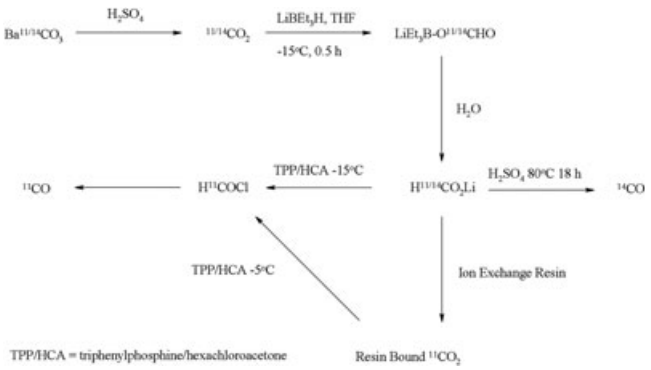
Radiosynthesis of [*carboxyl*-^{14}C]SSR149744 (1) by [^{14}C]carbonylation

Subsequently, we considered applying inhouse carbonylation methodology to the synthesis of [*carboxyl*-^{14}C]SSR149744 (**1**). This methodology is based on a combination of procedures employed to produce [^{11}C] or [^{14}C]carbon monoxide detailed in the literature.[13,14] [^{14}C]Carbon dioxide was generated on a gas manifold by addition of barium [^{14}C]carbonate to concentrated sulfuric acid. The [^{14}C]carbon dioxide produced was subsequently gas transferred *in vacuo* to a second flask and condensed onto a frozen ethereal superhydride solution. This was subsequently isolated from the manifold system and warmed to −15 °C for 0.5 h before quenching with water to give radiolabelled lithium [^{14}C]formate in reproducibly quantitative radiochemical yield. The impure lithium [^{14}C]formate was isolated as a white free-flowing amorphous salt following drying to constant mass *in vacuo* over phosphorus pentoxide. Experience within the group has shown that the residual superhydride salts appear to have no detrimental effect on the radiolabelled formate stability and neither do they affect the subsequent production of [^{14}C]carbon monoxide. For these reasons, the lithium [^{14}C]formate, generated for carbonylation use, was not purified and stored in its crude form at below −60 °C. A procedure to purify radiolabelled lithium formate by solid-phase extraction is detailed in the literature.[13] This overall process is summarised in Scheme 3.

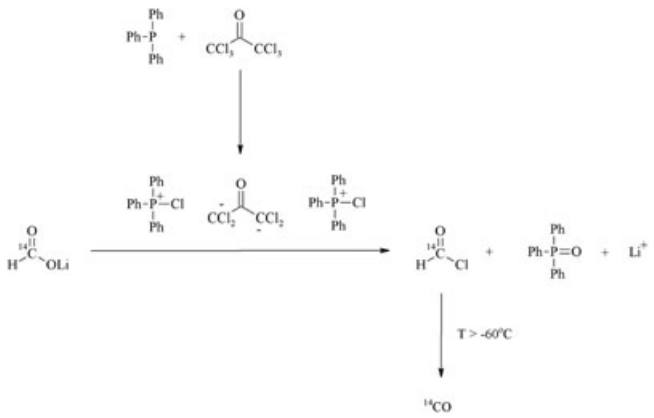
The dehydration of lithium formate in sulfuric acid generates carbon monoxide in situ.[5,15] This process requires a substantial



Scheme 2. Radiosynthesis of [*carboxyl*-^{14}C]SSR149744 (1) by [^{14}C]carboxylation.



Scheme 3. Synthetic routes to [^{14}C]carbon monoxide from [^{14}C]lithium formate.



Scheme 4. Formation of the chlorophosphonium cation complex and its reaction with [^{14}C]lithium formate.

quantity of sulfuric acid which, once contaminated with ^{14}C, requires disposal. In our hands, the conversion of lithium [^{14}C] formate to [^{14}C]carbon monoxide using this dehydration methodology was typically around 50%. Prolonged heating was required to increase these yields. We therefore searched for an alternative procedure that would liberate [^{14}C]carbon monoxide from lithium [^{14}C]formate more efficiently. A method employed in PET chemistry as described by Roeda *et al.* uses a chlorophosphonium cation to rapidly convert lithium [^{11}C]formate to [^{11}C]formyl chloride.[13] The [^{11}C]formyl chloride readily dissociates at temperatures exceeding −60 °C to give [^{11}C]carbon monoxide. By analogy, we found the addition of lithium [^{14}C]formate to the chlorophosphonium cation efficiently liberated [^{14}C] carbon monoxide in excellent yield (Table 1), quantified by trapping the radioactivity in an ethereal solution of butyl lithium (Scheme 4).[2,16]

In a process analogous to the transfer and isolation of [^{14}C]carbon dioxide, [^{14}C]carbon monoxide can be condensed using liquid nitrogen. We found the transfer of the radioactive monoxide gas under vacuum relatively efficient at this temperature. Following [^{14}C]carbon monoxide generation and transfer, the carbonylation reaction flask was isolated from the manifold apparatus and heated typically to 70 °C for a period of 18 h. Incorporation of [^{14}C]carbon monoxide into target molecules is routinely around 60%. These results are comparable with unlabelled carbonylation reactions, which typically employ a large excess of carbon monoxide in pressurised reaction vessels. Following the reaction, the set up is fully purged under controlled conditions and any unreacted radioactivity is trapped using butyl lithium scrubbers[2] and quantified. The [^{14}C]carbonylation procedure described was applied to the synthesis of a variety of functionalities as exemplified in Table 2.

Given the aforementioned background, we considered that this approach might be applied to the radiosynthesis of [*carboxyl*-^{14}C]SSR149744 (**1**). Reaction of either the iodobenzofuran or bromo-benzofuran with the acid chloride (**6**) using Friedel–Crafts conditions afforded intermediate (**8**). Subsequent reaction of (**8**) with dibutylamine, in the presence of potassium carbonate and potassium iodide, gave the arylhalide (**3**). Using a gas manifold apparatus, [^{14}C]carbon monoxide was generated and transferred *in vacuo* to a flask containing a frozen solution of (**3**), [1,1′-bis (diphenylphosphino)ferrocene]dichloropalladium(II) and diisopropylethylamine suspended in propan-2-ol. The flask was then isolated from the gas manifold and heated to 70 °C for a period of 18 h. Following purification, [*carboxyl*-^{14}C]SSR149744 (**1**) was isolated in 49% (from the brominated precursor) and 53% (from the iodinated precursor) radiochemical yield from barium [^{14}C] carbonate (Scheme 5).

Conclusion

A reproducible, robust method to produce lithium [^{14}C]formate and a procedure to subsequently liberate [^{14}C]carbon monoxide have been developed. The carbonylation reactions attempted have been successful with yields comparable to those in the literature. The methodology was applied to the synthesis of [*carboxyl*-^{14}C]Celivarone with overall purified radiochemical yields in the range 49–53% from barium [^{14}C]carbonate.

Experimental

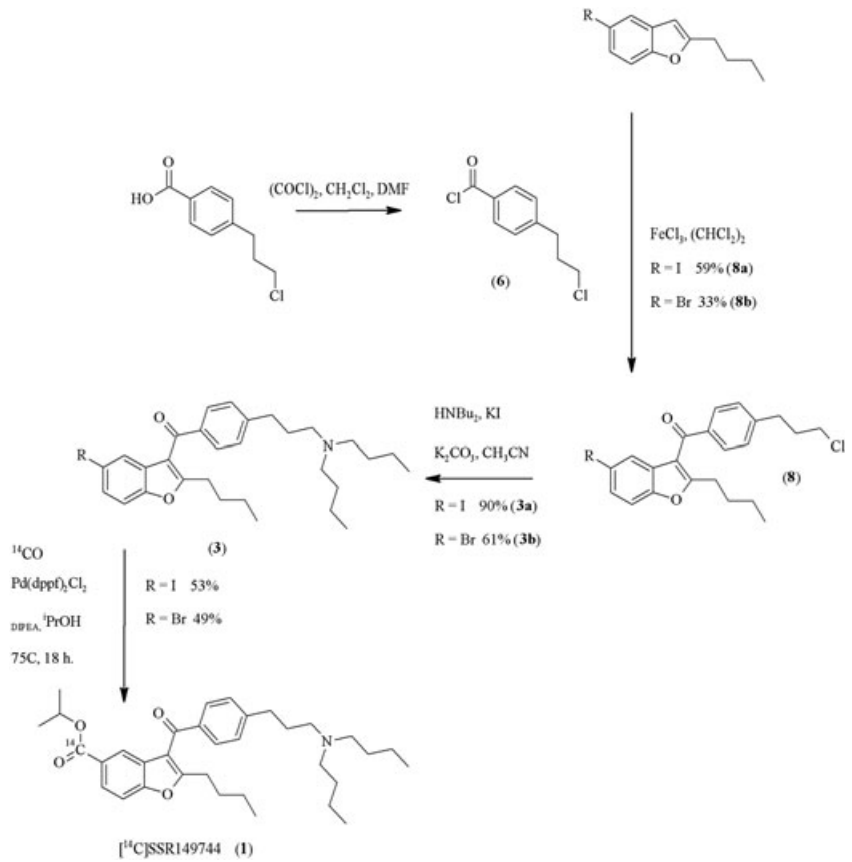
Barium [^{14}C]carbonate (2200 MBq/mmol) was purchased from American Radiolabeled Chemicals, Inc. All steps were carried out at low specific activity prior to being repeated at high-specific activity. Unless indicated otherwise, all other reagents and anhydrous solvents were obtained from Sigma-Aldrich and used without purification. High-specific activity materials were characterised by comparative TLC (to known standards), LC-MS and ^{1}H-NMR. Merck silica radio-TLC (RTLC) plates were analysed by electronic autoradiography using a Packard Instantimager. LC-MS data were obtained using an Agilent 1200 HPLC system (Agilent Technologies UK Ltd., Stockport, Cheshire, UK) connected to a Bruker micro-TOF Focus mass spectrometer operating in ESI mode with sodium formate as internal calibrant and a Phenomenex Luna C18 column. RP-HPLC analysis of (**1**) was performed using a Gilson 321 series system (Gilson, Inc.,

Table 1. Liberation of ^{14}CO from Li^{14}CO$_2$H using the chlorophosphonium cation complex

Scale mmole	Complex PPh_3Cl	Reaction conditions	Yield of ^{14}CO production (%)	Recovery of radioactivity (%)
0.8	3 equiv.	THF, −15 °C, 5 min	72	96
0.6	6 equiv.	THF, −15 °C, 5 min	98	99

Table 2. The [^{14}C]carbonylation procedure was applied to the synthesis of a range of functionalities

Aryl halide	Nucleophile	Product	Radiochemical yield (%)
Iodoanisole	ROH	Esters	40–60
Iodoanisole	RNH_2	Amides	53
Iodoanisole	H_2O	Acids	61
Iodoanisole	$ArSnR_3$	Ketones	67
Aryliodide	Triethylsilyl hydride	Aldehyde	50–60
Arylbromide	RNH_2	Isoquinolinone	44



Scheme 5. Radiosynthesis of [*carboxyl*-^{14}C]SSR149744 (1) by [^{14}C]carbonylation.

Wisconsin, USA) and using a Waters XTerra RP8 (Waters Ltd., Elstree, Hertfordshire, UK) 3.5 μm (150 × 4.6 mm) column. Samples were dissolved in methanol prior to analysis. The mobile phase is isocratic comprising 75% acetonitrile/25% sodium tetraborate aqueous buffer (2.5 mM $Na_2B_4O_7.10H_2O$ adjusted to pH 8.6 with 1 M aq. HCl) at a flow rate of 1 mL/min. The run time is 35 min and detection is by UV absorbance at 210 nm. Under these conditions, SSR149744 elutes at a retention time of ~15 min. Activities were determined by liquid scintillation analysis using a Packard Tri-Carb 1900TR (Packard Instrument Company, Meriden, Connecticut, USA, since integrated into PerkinElmer, Waltham, Massachusetts, USA). All ^{1}H-NMR data presented were recorded on a Bruker DRX 500 NMR (Bruker Biospin Corporation, Billerica, Massachusettes, USA) instrument.

Lithium [^{14}C]formate

A three-necked round-bottomed flask, attached to a manifold system and equipped with a stirrer bar, was loaded with lithium triethylborohydride (2 equiv., 3 mL, 1 M in THF) and diluted with anhydrous tetrahydrofuran (3 mL). The superhydride solution was frozen (liquid nitrogen bath) under nitrogen. Barium [^{14}C]carbonate (273 mg, 3018 MBq, 1.37 mmol, 2200 MBq/mmol) was transferred to a side-arm and connected to a second three-necked flask containing a stirrer bar and concentrated sulfuric acid (5 mL). The second flask was connected to the manifold apparatus and the acid frozen (dry-ice acetone bath). A vacuum (3.0×10^{-2} mbar) was established within the manifold and the two pendant flasks. The flask containing the sulfuric acid was defrosted and the barium [^{14}C]carbonate added portion wise to the acid. The $^{14}CO_2$ produced was gas transferred onto the frozen surface of the ethereal superhydride solution. Following completion of the gas transfer, the super hydride flask was isolated from the manifold system and the solution warmed to −15 °C and stirred for a period of 15 min. The superhydride solution was subsequently frozen and purged with nitrogen venting through two consecutive 150 mL aqueous 1 M sodium hydroxide scrubbers to trap any unreacted $^{14}CO_2$. Water (5 mL) was added and the super hydride solution defrosted. Both flasks were purged for a further 30 min with nitrogen, again venting through aqueous sodium hydroxide scrubbers. The ether was removed *in vacuo* and the crude lithium [^{14}C]

formate completely dissolved in water enabling quantification of [^{14}C] by scintillation counting (98%, 2958 MBq, 1.34 mmol). The water was subsequently removed under reduced pressure to furnish a white amorphous free-flowing solid, which was dried to constant mass (282 mg), *in vacuo* over phosphorus pentoxide. The specific activity of the impure lithium [^{14}C]formate was measured gravimetrically to be 10.5 MBq/mg. As 1.34 mmol unlabelled lithium formate has a mass of 94 mg the theoretical chemical purity of the lithium [^{14}C]formate is estimated to be 33%.

(2-Butyl-5-iodobenzofuran-3-yl)-[4-(3-chloropropyl)phenyl] methanone (8a)

A solution of 4-(3-chloropropyl)benzoic acid (2.35 g, 11.8 mmol produced in-house) in thionyl chloride (24 mL) was heated to reflux (100 °C, oil bath) for 2 h. The thionyl chloride was subsequently removed *in vacuo*. The resultant acid chloride was redissolved in anhydrous 1,2-dichloroethane (20 mL), which was subsequently removed *in vacuo*. This process was repeated to ensure that the excess thionyl chloride was removed. A second flask was loaded with a solution of 2-butyl-5-iodobenzofuan (2.30 g, 7.7 mmol) in 1,2-dichloroethane (70 mL). The solution was cooled in an ice bath and iron (III) chloride (1.1 equiv., 5 g, 9.2 mmol) added. The acid chloride (2.50 g, 11.5 mmol), dissolved in 1,2-dichloroethane (23 mL), was then added dropwise to the cooled iodobenzofuran solution over a period of 15 min. The reaction mixture was warmed to room temperature and stirred for 4 h. Water (30 mL) was added and the aqueous phase extracted with dichloromethane (3 × 30 mL). The combined organics were washed further with portions of water then dried over anhydrous magnesium sulfate. The dichloromethane was removed *in vacuo*. The residue was purified by column chromatography on silica gel (5% ethyl acetate in hexanes) to afford **8a** (2.12 g, 57%). δ_H ($CDCl_3$): 0.87 (t, 3H, J = 7.4 Hz), 1.31 (sextet, 2H, J = 7.5 Hz), 1.72 (quintet, 2H, J = 7.5 Hz), 2.15 (quintet, 2H, J = 6.4 Hz), 2.84 (t, 2H, J = 7.4 Hz), 2.90 (t, 2H, J = 7.3 Hz), 3.56 (t, 2H, J = 6.4 Hz), 7.25 (d, 1H, J = 8.6 Hz), 7.33 (d, 2H, J = 8.1 Hz), 7.56 (dd, 1H, J = 8.6, 1.8 Hz), 7.72 (d, 1H, J = 1.8 Hz), 7.74 (d, 2H, J = 8.2 Hz). LC-MS (positive ion mode) *m/z* found 481.0863 (100%, [M + H$^+$]).

(5-Bromo-2-butylbenzofuran-3-yl)-[4-(3-chloropropyl) phenyl]methanone (8b)

The procedure described for the iodo-derivative (**8a**) was applied starting from 5-bromo-2-butylbenzofuran (6.19 g, 24.5 mmol), yielding **8b** (3.49 g, 33%). δ_H ($CDCl_3$): 0.88 (t, 3H, J = 7.4 Hz), 1.33 (sextet, 2H, J = 7.5 Hz), 1.74 (quintet, 2H, J = 7.5 Hz), 2.15 (quintet, 2H, J = 6.4 Hz), 2.86 (t, 2H, J = 7.4 Hz), 2.90 (t, 2H, J = 7.3 Hz), 3.56 (t, 2H, J = 6.4 Hz), 7.33 (d, 2H, J = 8.6 Hz), 7.34 (d, 1H, J = 8.1 Hz), 7.38 (dd, 1H, J = 8.6, 1.8 Hz), 7.51 (d, 1H, J = 2.0 Hz), 7.74 (d, 2H, J = 8.2 Hz). LC-MS (positive ion mode) *m/z* found 435.0530 (100%, [M + H$^+$]).

(2-Butyl-5-iodobenzofuran-3-yl)-[4-(3-dibutylaminopropyl) phenyl]methanone (3a)

Dibutylamine (1.25 mL, 7.4 mmol), sodium iodide (0.10 g, 0.7 mmol) and (2-butyl-5-iodobenzofuran-3-yl)-[4-(3-chloropropyl)phenyl]methanone (**8a**) (0.71 g, 1.5 mmol) were combined in acetonitrile (5 mL) in a sealed 10-mL microwave vessel. The reaction mixture was heated using a CEM Discover Microwave Reactor (CEM Microwave Technology Ltd., Buckingham, UK) (300 W, 150 °C, 150 psi) for 1 h then partitioned between diethyl ether (20 mL) and water (20 mL). The ether was extracted with further portions of water (2 × 20 mL) and dried over anhydrous sodium sulfate, the solvent was removed *in vacuo* and the resultant residue purified by column chromatography on silica gel (5% ethyl acetate in hexanes) to yield **3a** (0.77 g, 90%). δ_H ($CDCl_3$): 0.86 (t, 3H, J = 7.0 Hz), 0.91 (t, 3H, J = 7.0 Hz), 1.23–1.44 and 1.68–1.85 (m, 10H), 2.40 (t, 2H, J = 7.0 Hz), 2.46 (t, 2H, J = 7.0 Hz), 2.72 (t, 2H, J = 7.5 Hz), 2.83 (t, 2H, J = 7.5 Hz), 7.24 (d, 1H, J = 8.5 Hz), 7.31 (d, 2H, J = 8.5 Hz), 7.56 (dd, 1H, J = 8.5, 2.0 Hz), 7.72 (d, 2H, J = 8.5 Hz), 7.76 (d, 1H, J = 1.5 Hz). LC-MS (positive ion mode) *m/z* found 574.2551 (100%, [M + H$^+$]).

(5-Bromo-2-butylbenzofuran-3-yl)-[4-(3-dibutylaminopropyl)phenyl]methanone (3b)

Dibutylamine (1.8 mL, 10.7 mmol) was added to a stirred suspension of (5-bromo-2-butylbenzofuran-3-yl)-[4-(3-chloropropyl)phenyl]methanone (**8b**) (1.72 g, 4 mmol) and potassium iodide (0.664 , 4 mmol) in acetonitrile (10 mL). The mixture was heated to reflux (95 °C) under nitrogen for a period of 16 h. The solvent was removed *in vacuo* and the residue suspended in dichloromethane then filtered to remove the precipitated salts. The filtrate was concentrated under reduced pressure and the crude material purified by column chromatography on silica gel (5% ethyl acetate in hexanes) to furnish the title compound (**3b**) (1.292 g, 61%). δ_H ($CDCl_3$): 0.87 (t, 3H, J = 7.0 Hz), 0.91 (t, 3H, J = 7.0 Hz), 1.27–1.44 and 1.69–1.85 (m, 10H), 2.41 (t, 2H, J = 7.0 Hz), 2.46 (t, 2H, J = 7.0 Hz), 2.73 (t, 2H, J = 7.5 Hz), 2.85 (t, 2H, J = 7.5 Hz), 7.31 (d, 2H, J = 8.5 Hz), 7.34 (d, 1H, J = 8.5 Hz) 7.37 (dd, 1H, J = 8.5, 2.0 Hz), 7.54 (d, 1H, J = 2.0 Hz), 7.73 (d, 2H, J = 8.5 Hz). LC-MS (positive ion mode) *m/z* found 528.2393 (100%, [M + H$^+$]).

[*carboxyl*-^{14}C]-2-Butyl-3-{4-[3-(dibutylamino)propyl] benzoyl}-1-benzofuran-5-carboxylate (1)

A three-necked round-bottomed flask was loaded with triphenylphosphine (3 equiv., 660 mg, 2.52 mmol), which was dissolved in anhydrous tetrahydrofuran (10 mL). The solution was degassed. A second three-necked round-bottomed flask was loaded with (5-iodo-2-butyl-2,3-dihydrobenzofuran-3-yl)-[4-(3-dibutylaminopropyl)-phenyl]methanone (**3a**) (2 equiv. 963 mg, 1.68 mmol), propan-2-ol (5 mL), [1,1′-bis(diphenylphosphino)ferrocene] dichloropalladium(II) (100 mg, 0.12 mmol) and diisopropylethylamine (1 mL) and subsequently frozen using a liquid nitrogen bath. Perchloroacetone (1.5 equiv., 191 μL, 1.26 mmol) was added neat to the triphenylphosphine solution at −15 °C and stirred under nitrogen for 5 min. The resultant chlorophosphonium complex was frozen (liquid nitrogen bath) and the manifold system evacuated to 1 mbar. The chlorophosphonium complex was warmed to −15 °C and, with stirring, 1842 MBq lithium [^{14}C]formate (10.5 MBq/mg, 175 mg, 0.84 mmol for which the estimated purity due to contamination with superhydride salts is 33%) was added portion wise. The resultant [^{14}C]formyl chloride dissociated readily producing ^{14}CO. The ^{14}CO was gas transferred to the frozen arylhalide mixture, which was then isolated from the manifold, defrosted slowly and heated with stirring to 70 °C for 18 h. Prior to purging the system, both solutions were frozen using liquid nitrogen baths. Nitrogen was carefully allowed into the flasks and vented through two in-line butyl lithium scrubber traps for 60 min whilst the contents of both flasks were defrosted. Each trap contained 5 mL of 2.5 M butyl lithium in 95 mL anhydrous THF, cooled to −78 °C using a dry-ice acetone bath. The activity recorded in the first trap was 167 MBq, no activity was recorded in the second trap. The contents of both flasks were diluted with methanol for quantification by scintillation counting. The crude product was counted at 1068 MBq (58%). The total activity recovered was 1795 MBq (97%). The solvent was removed *in vacuo* and the crude residue purified by column chromatography on silica gel (10% ethyl acetate in hexanes with 1% triethylamine) to give (**1**) (976 MBq, 53%). δ_H (DMSO-d_6): 0.79 (t, 3H, J = 7.2 Hz), 0.87 (t, 6H, J = 7.2 Hz), 1.28 (d, 6H, J = 7.4 Hz), 1.23 (m, 2H), 1.37 (m, 4H), 1.66 (m, 4H), 1.76 (m, 2H), 2.44 (m, 6H), 2.70 (t, 2H, J = 7.2 Hz), 2.80 (t, 2H, J = 7.2 Hz), 5.10 (septet, 1H, J = 7.5 Hz), 7.40 (d, 2H, J = 8.5 Hz), 7.70 (d, 2H, J = 8.5Hz), 7.74 (dd, 2H, J = 8.5, 2.0 Hz), 7.94 (dd, 1H, J = 8.5, 2 Hz), 8.10 (d, 1H, J = 2Hz). HPLC R_T = 15 min. The product was subsequently isolated as the fumaric acid salt and the specific activity determined gravimetrically to be 3.424 MBq/mg.

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Conflict of Interest

The authors did not report any conflict of interest.

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