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Research Article

Syntheses of carbon-14 labelled AJ-9677, a specific β_3-adrenoceptor agonist

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Summary

Two ^{14}C-labelled versions of AJ-9677, a novel anti-diabetic drug candidate, have been synthesized. The first, [acetic acid-2-^{14}C]AJ-9677, was synthesized via a four-step process starting from 2-chloro-*N,N*-diethyl[2-^{14}C]acetamide (**3**), and the second, [hydroxymethine-^{14}C]AJ-9677, by a two step process from (*R*)-3-chloro[7-^{14}C]styrene oxide (**8**). When stored in the solid state both compounds undergo radiolytic decomposition in a similar manner, despite the labelled position being different, to give the dechlorinated derivative (**12a**, **12b**). Purification of the required product was achieved after derivatization to the methyl esters. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: carbon-14; AJ-9677; carbon-14 labelled AJ-9677; β_3-adrenoceptor agonist; radiolysis

Introduction

[3-[(2*R*)-[[(2*R*)-(3-Chlorophenyl)-2-hydroxyethyl]amino]propyl]-1*H*-indol-7-yloxy]-acetic acid (code name AJ-9677) has a highly selective, potent agonistic activity for β_3-adrenergic receptors[1–3] and is currently being developed as an agent to treat diabetes and obesity.

During the development program, two carbon-14 labelled forms of this compound were required for drug metabolism and pharmacokinetic studies. Both compounds degraded on storage and needed purification before use. This paper describes their syntheses, radiolytic decomposition, and purification.

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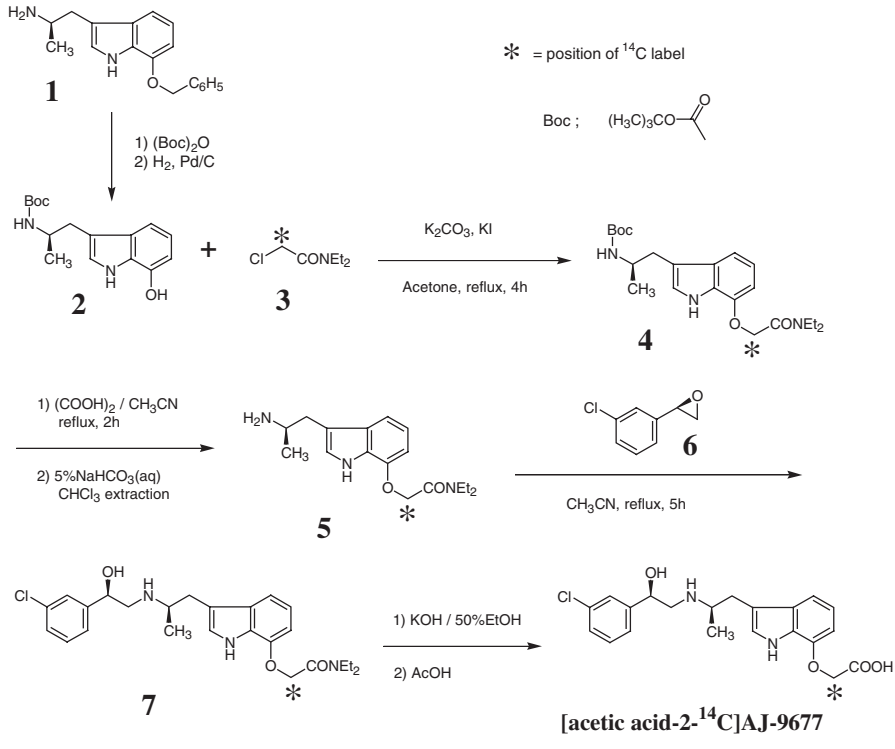
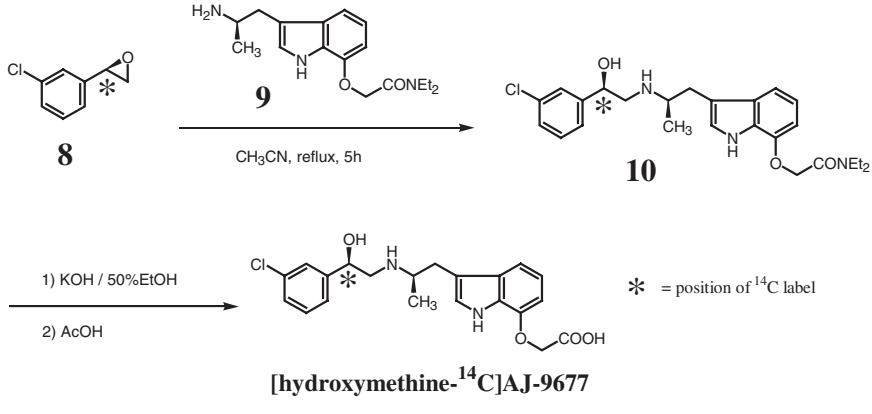
Results and discussion

The synthetic routes used to prepare the two carbon-14 labelled forms are shown in Schemes 1 and 2.



Scheme 1. Synthesis of [acetic acid-2-^{14}C]AJ-9677



Scheme 2. Synthesis of [hydroxymethine-2-^{14}C]AJ-9677

First, [acetic acid-2-^{14}C]AJ-9677 was synthesized in a four-step process (Scheme 1). (*R*)-3-(2-Aminopropyl)-7-benzyloxyindole (**1**)[3] was treated with $(Boc)_2O$ to protect the amino group[4,5] and following hydrogenation yielded

the starting material (**2**). The labelled compound, 2-chloro-*N,N*-diethyl[2-^{14}C] acetamide (**3**), was condensed with 2 equivalents of the phenol derivative **2** in acetone using K_2CO_3 and KI to give the product **4** in 81.7% yield. The latter was then treated with $(COOH)_2$ to free the amino group giving compound **5** in 98.6% yield. The amine **5** and (*R*)-3-chlorostyrene oxide (**6**) were condensed by refluxing in CH_3CN and the amide **7** was obtained after purification on column chromatography in 56.5% yield. It was then hydrolyzed with KOH in 50% C_2H_5OH and the final product crystallized in 60.0% yield. The overall radiochemical yield of the final [acetic acid-2-^{14}C]AJ-9677 was 27%. Its specific activity of 2.08 GBq (56.2 mCi)/mmol, radiochemical purity of >99%, and optical purity of >99%ee was determined by gravimetry, TLC and HPLC, and chiral HPLC, respectively. The consequent ADME studies in rats showed that the radiolabel at this position was metabolically labile. Consequently labelling at an alternative position was thus required for thorough detection of rat metabolites.

Similar to the above, [hydroxymethine-^{14}C]AJ-9677 was synthesized in 30% overall radiochemical yield from (*R*)-3-chloro[7-^{14}C]styrene oxide (**8**) as shown in Scheme 2. Synthesis of **8** and its condensation reaction using 3 equivalents of the amine **9** was performed at Amersham Biosciences Ltd., because the labelled compound **8** was thought to be unstable due to radiolysis and its purification was also difficult. The [^{14}C]AJ-9677 specific activity was 2.08 GBq (56.2 mCi)/mmol, the radiochemical purity >99% and the optical purity >99%ee. ADME studies revealed that this preparation was satisfactory for characterization of AJ-9677 metabolism in rats.

While the non-labelled version of AJ-9677 is stable for >1 year at room temperature, the labelled forms undergo radiolysis: Degradation of both [^{14}C]AJ-9677 preparations occurred at about 1% per month in the solid state at −20°C. Their degradation rates were about 1% per year in a solution of C_2H_5OH (or CH_3OH)−28%NH_3 aq. at a concentration of <1 mg/ml at −20°C. TLC and HPLC (Figure 1) analyses revealed that degradation of the two preparations occurred in a similar pattern to each other suggesting that radiolysis proceeded by a similar mechanism despite the different labelled positions. Mass spectral analysis revealed that the major degradation product (**12a** and **12b** in the figures) was the dechlorinated derivative of the [^{14}C]AJ-9677 preparations. The ^{14}C radiation probably generated free radicals[6] which attacked the C–Cl bonds leading to cleavage.

Simple recrystallization of the degraded [^{14}C]AJ-9677 preparations was not an effective purification procedure. Therefore, purification was carried out after derivatization to the methyl ester as shown in Scheme 3: Degraded preparations were esterified using p-TsOH in CH_3OH. The crude material so obtained was subjected to column chromatography to give the methyl ester **11** with a radiochemical purity of >98%; this was then hydrolyzed in aqueous

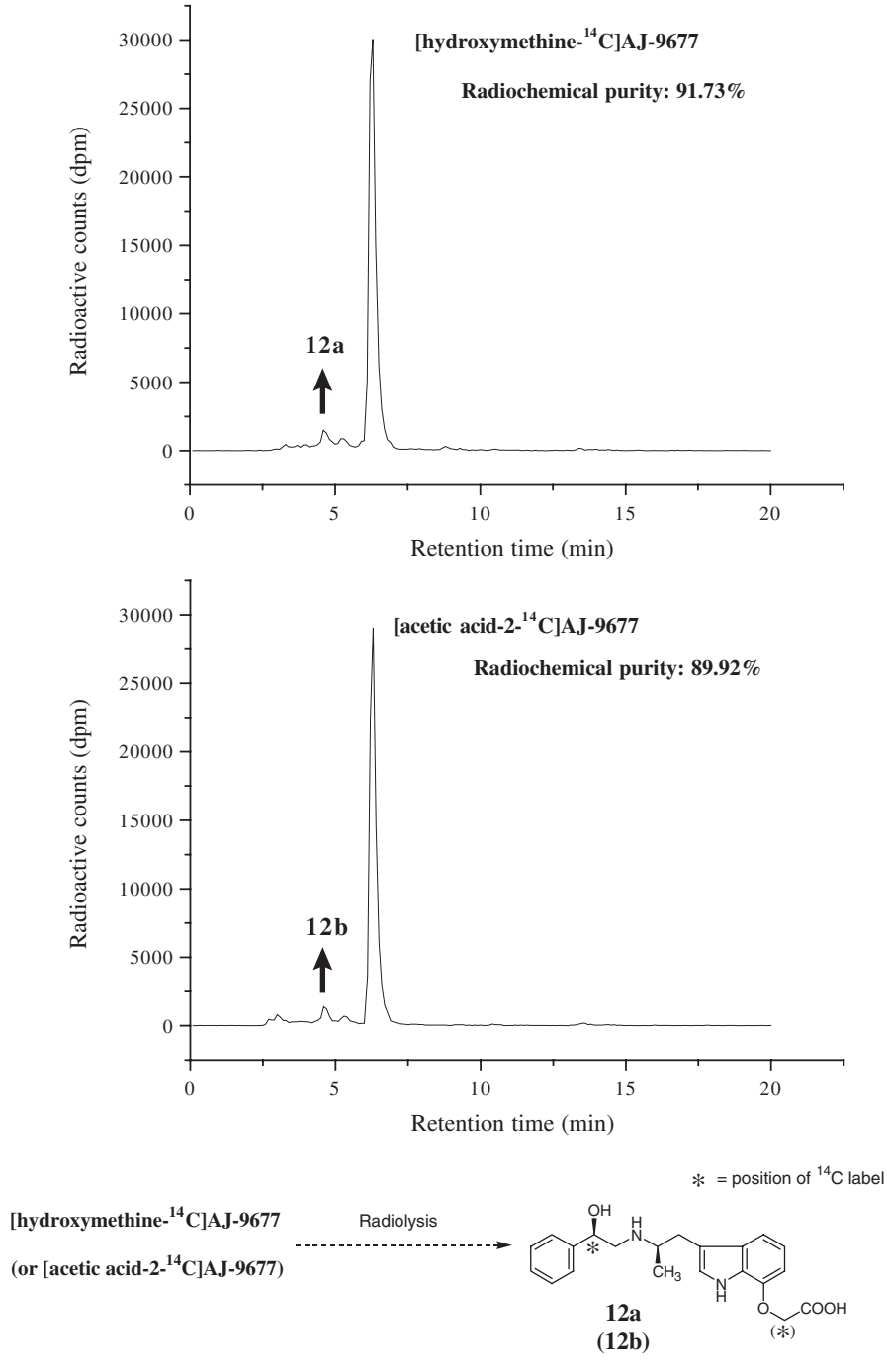
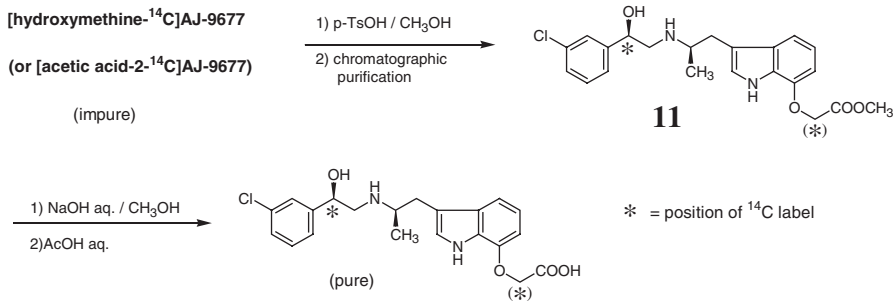


Figure 1. HPLC chromatograms of ^{14}C-labelled AJ-9677 on radiolytic decomposition



Scheme 3. Purification of degraded ^{14}C-labelled AJ-9677

NaOH. The purified compound, [acetic acid-2-^{14}C]AJ-9677 or [hydroxymethine-^{14}C]AJ-9677, was obtained in a 33–46% radiochemical recovery with a radiochemical purity of >98% and an optical purity of >99%ee.

Conclusion

[Acetic acid-2-^{14}C]AJ-9677 and [hydroxymethine-^{14}C]AJ-9677 were synthesized from commercially available labelled compounds through 4- and 2-step processes, respectively. The purification of the two labelled compounds, both of which undergo radiolytic decomposition, was achieved after derivatization to the methyl ester. ADME studies in rats revealed that the former ^{14}C-labelled version was metabolically labile and the latter was successfully used.

Experimental

Materials and methods

Silica gel column chromatography was performed using Merck silica gel 60 (70–230 mesh). 2-Chloro-*N,N*-diethyl[2-^{14}C]acetamide (**3**) and (*R*)-3-chloro[7-^{14}C]styrene oxide (**8**) were purchased from Amersham Biosciences Ltd. Unlabelled samples, compound **1** and **9**, were supplied by our Chemical Research Laboratories. Solvents and reagents used were commercially purchased.

Radioactivity was determined in a Packard Tri-carb 3100TR Liquid Scintillation Spectrometer.

Analytical TLC was performed on Merck silica gel 60 F-254 plate. The solvent systems for the final products were $CHCl_3/CH_3OH/28\%$ NH_3 aq. (50:20:1) and AcOEt/acetone/AcOH/water (5:3:1:1). After development, TLC plates were scanned for analysis of radiochemical purity by a Berthold LB-2821 Automatic TLC Linear Analyzer.

HPLC analysis of final products was carried out on a Waters 600E System Controller equipped with an FLO-ONE/Beta Radioactive Flow Detector (Packard) and a Waters 484 Tunable Absorbance Detector. The conditions for

non-chiral analysis were as follows: Column, CAPCELLPAK ODS C18 SG120 (4.6 × 250 mm, SHISEIDO); Mobile phase, 0.05% CF_3COOH aq/CH_3CN (70:30); Flow rate, 1 ml/min; Detector, UV 268 nm and radioactivity; Column temperature, 35°C. The conditions for chiral analysis were: Column, CHIRAL-AGP (4.0 × 100 mm, Chrom Tech AB); Mobile phase, [20 mM Na_2HPO_4 and 2 mM TBAS (tetrabutylammonium hydrogen sulfate), pH 7.0 with H_3PO_4 aq]/iso-PrOH (98:2); Flow rate, 0.7 ml/min; Detector, UV 220 nm and radioactivity; Column temperature, 30°C.

Mass spectrometric analysis was performed on an MS-LX2000 Mass Spectrometer (JEOL).

All labelled compounds synthesized were identified by comparative TLC with the corresponding unlabelled authentic materials and the final [^{14}C]AJ-9677 preparation, by TLC, HPLC, and MS.

Synthesis of [acetic acid-2-^{14}C]AJ-9677

*(R)-3-(N-t-Butyloxycalboxy-2-aminopropyl)-7-ol-1H-indole (**2**).* The Boc derivative[4,5] of compound **1**[3] (356 mg, 0.936 mmol) was hydrogenated with 10% palladium on carbon (50 mg) in CH_3OH (10 ml) for 2 h at room temperature. The mixture was filtered through celite and washed with CH_3OH. The filtrate was evaporated under reduced pressure to yield an oily material. This apparently unstable phenol derivative **2** was stored in a refrigerator (4°C) and used for the next reaction within 24 h.

*(R)-[3-(N-t-Butyloxycalboxy-2-aminopropyl)-1H-indole-7-yloxy]-N,N-diethyl [2-^{14}C]-acetamide (**4**).* Phenol **2** (0.936 mmol), K_2CO_3 (162 mg, 1.17 mmol) and KI (20 mg, 0.117 mmol) were mixed and to this was added 2-chloro-*N,N*-diethyl[2-^{14}C]acetamide (**3**) (978 MBq/26.4 mCi, 0.468 mmol) in acetone (2 ml) and the whole refluxed for 4 h. After cooling to room temperature, the solid was removed by filtration and washed with acetone. The filtrate and washing were combined and concentrated under reduced pressure. The concentrate was subjected to column chromatography on silica gel using $CHCl_3$/CH_3OH (99:1) to isolate the compound **4** (799 MBq/21.6 mCi) as a solid.

Radiochemical yield: 81.7%, radiochemical purity (TLC): 98.9% ($CHCl_3$/CH_3OH, 19:1).

*(R)-[3-(2-aminopropyl)-1H-indole-7-yloxy]-N,N-diethyl[2-^{14}C]acetamide (**5**).* Compound **4** (799 MBq/21.6 mCi, 156 mg) and oxalic acid (122 mg, 3.5 eq) were mixed in CH_3CN (2 ml) and the mixture was refluxed for 3 h; after cooling to room temperature, 5%$NaHCO_3$ (30 ml) was added. The whole mixture was shaken with $CHCl_3$ (2 × 30 ml). The $CHCl_3$ layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to obtain compound **5** (788 MBq/21.3 mCi).

Radiochemical yield: 98.6%, radiochemical purity (TLC): 97.4% ($CHCl_3$/CH_3OH, 9:1). This crude product was used without purification for the next reaction.

[3-[(2R)-[[(2R)-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]-1H-indole-7-yloxy]-N,N-diethyl[2-^{14}C]acetamide (7). Compound **5** (788 MBq/21.3 mCi, 115 mg) and (*R*)-3-chlorostyrene oxide (**6**) (64 mg, 1.1 eq) were mixed in CH_3CN (1 ml). The mixture was refluxed for 5 h and after cooling to room temperature, was evaporated under reduced pressure. The residue was subjected to column chromatography on silica gel using $CHCl_3$/CH_3OH (95:5) as an eluent to obtain the amide **7** (445 MBq/12.0 mCi).

Radiochemical yield: 56.5%, radiochemical purity (TLC): 98.9% ($CHCl_3$/CH_3OH, 9:1).

[3-[(2R)-[[(2R)-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]-1H-indole-7-yloxy]-[2-^{14}C]acetic acid, ([acetic acid-2-^{14}C]AJ-9677). The mixture of amide **7** (445 MBq/12.0 mCi, 98 mg) dissolved in C_2H_5OH (2 ml) and KOH (96 mg, 8 eq) as an aqueous solution (2 ml) was refluxed (bath temperature 100°C) for 3 h. After cooling to room temperature, 1 ml of aqueous AcOH (0.11 ml, 8.8 eq) was added with stirring which was continued for 1 h. The crystalline precipitate so formed was collected on filtration and washed with C_2H_5OH. Recrystallization of the precipitate was achieved by dissolving in 10 ml of C_2H_5OH/28%NH_3 aq (9:1), subsequent evaporation of the solvent until turbid (to about 3 ml) and leaving overnight in a refrigerator. The crystals were collected by filtration and washed with C_2H_5OH to obtain [acetic acid-2-^{14}C]AJ-9677 (267 MBq/7.21 mCi).

Radiochemical yield: 60.0%, specific activity: 2.07 GBq (56 mCi)/mmol, radiochemical purity (TLC and HPLC): > 99%, optical purity (chiral HPLC): > 99% enantiomer excess, MS (electrospray positive ion): $[M + H]^+$ m/z 405, 407 (fragment pattern consistent with the presence of a single chlorine substituent).

Synthesis of [hydroxymethine-^{14}C]AJ-9677

*[3-[(2R)-[[(2R)-(3-chlorophenyl)-2-hydroxy-[2-^{14}C]ethyl]amino]propyl]-1H-indole-7-yloxy]-N,N-diethylacetamide (**10**)*. Synthesis of (*R*)-3-chloro[7-^{14}C]-styrene oxide (**8**) and its condensation using 3 equivalents of amine **9** was performed at Amersham Biosciences Ltd. to give the amide **10**.

[3-[(2R)-[[(2R)-(3-chlorophenyl)-2-hydroxy-[2-^{14}C]ethyl]amino]propyl]-1H-indole-7-yloxy]acetic acid, ([hydroxymethine-^{14}C]AJ-9677). The hydrolysis of amide **10** (1.16 GBq/31.4 mCi, 248 mg) was performed under similar conditions to those in the above [acetic acid-^{14}C]AJ-9677 preparation, to obtain [hydroxymethine-^{14}C]AJ-9677 (790 MBq/21.4 mCi).

Radiochemical yield: 68.1%, specific activity: 2.08 GBq (56.2 mCi)/mmol, radiochemical purity (TLC and HPLC): >99%, optical purity (chiral HPLC): >99% enantiomer excess, MS (electrospray positive ion): $[M+H]^+$ m/z 405, 407 (fragment pattern consistent with the presence of a single chlorine substituent).

Purification of degraded [^{14}C]AJ-9677

*Methyl ester of [^{14}C]AJ-9677 (**11**).* Stored [acetic acid-2-^{14}C]AJ-9677 preparations (427 MBq/11.5 mCi) with radiochemical purity of 92% and p-TsOH (512 mg, 5 eq) were dissolved in CH_3OH (20 ml). The mixture was stirred for 2 h at room temperature, evaporated and diluted with AcOEt (100 ml) and the resultant solution was washed with 5%$NaHCO_3$ (50 ml × 2), water (50 ml), and then 10% NaCl (50 ml). The organic layer was dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to give an oily material. The oil was subjected to column chromatography on silica gel using $CHCl_3$/CH_3OH (98:2) as an eluent to obtain the ester **11** (226 MBq/6.11 mCi).

Radiochemical yield: 52.9%, Radiochemical purity (TLC): 99.1% ($CHCl_3$/CH_3OH, 9:1).

Re-purified [^{14}C]AJ-9677. The mixture of the ester **11** (226 MBq/6.11 mCi) dissolved in CH_3OH (2 ml) and 1 N NaOH (0.2 ml, 1.5 eq) was stirred for 1 h at room temperature. For crystallization, AcOH (0.02 ml, 1.7 eq) was added as an aqueous solution (1 ml) with stirring which was continued for 1 h. Recrystallization was achieved in a similar way to that in the above preparation of [^{14}C]AJ-9677. Purified [acetic acid-2-^{14}C]AJ-9677 was obtained at a specific activity of 2.08 GBq (56.2 mCi)/mmol.

Radiochemical yield: 62.4% (total 33.0%), radiochemical purity (TLC and HPLC): >98%, optical purity (chiral HPLC): >99% enantiomer excess, MS (electrospray positive ion): $[M+H]^+$ m/z 405, 407 (fragment pattern consistent with the presence of a single chlorine substituent).

*The major decomposition compound: dechlorinated forms of [^{14}C]AJ-9677, **12a** and **12b**.* The decomposition compound **12a** of degraded [hydroxymethine-^{14}C] AJ-9677 was monitored by LC/MS. Retention time: 6.3 min (HPLC). MS (electrospray positive ion): $[M+H]^+$ m/z 371. Compound **12b** of degraded [acetic acid-2-^{14}C]AJ-9677 was also identified in a similar manner.

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