^{14}C-Labeling of a Novel Atypical β-Adrenoceptor Agonist, SM-11044

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SUMMARY

(2*S*,3*R*)-2-[3-(4-Fluorophenyl)]propylamino-3-(3,4-dihydroxyphenyl)-3-hydroxypropionic acid pyrrolidine amide hydrobromide (SM-11044) was labeled with carbon-14 for use in mammalian metabolic studies. The synthesis was achieved according to the scheme shown in Fig. 5. Grignard reaction of 3,4-methylenedioxyphenylmagnesium bromide with [^{14}C]carbon dioxide liberated from barium [^{14}C]carbonate (6) gave the acid (5). Reduction of 5 with lithium aluminum hydride followed by oxidation of the resulting benzyl alcohol (11) with chromium oxide-pyridine complex afforded the aldehyde (3). Condensation of 3 with the optically active (*R*)-oxazolidinone (10) yielded the alcohol (12). Catalytic hydrogenation of 12 and subsequent hydrolysis produced the optically active β-hydroxy-α-amino acid (2), which was treated with *N*-carbomethoxyphthalimide to give the hydroxy acid (13). Reaction of 13 with pivaloyl chloride gave the corresponding mixed anhydride, which was allowed to react with pyrrolidine to provide the amide (14). Deprotection of 14 with hydrazine hydrate afforded the hydroxyamide (15). Condensation of 15 with the aldehyde (16) and subsequent reduction of the resulting imine with sodium cyanoborohydride produced the fluoride (17). Cleavage of the methylenedioxy group of 17 gave the free base of 1, which was treated with hydrobromic acid to afford 1. The overall yield was 11.1% from 6.

Key words: carbon-14, β-adrenocepter agonist, β-hydroxy-α-amino acid, chiral glycine enolate

INTRODUCTION

(2*S*,3*R*)-2-[3-(4-Fluorophenyl)]propylamino-3-(3,4-dihydroxyphenyl)-3-hydroxypropionic acid pyrrolidine amide hydrobromide (SM-11044) (Fig.1) displays β-adrenergic properties in guinea pig ileum, right atrium, trachea and in rat white

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adipocyte.[1] The physiological effects of SM-11044 were compared with those of isoproterenol and fenoterol as reference β-adrenoceptor agonists. It was revealed that SM-11044 exhibited β-adrenoceptor agonistic properties in tissues known to express mostly β_1- and β_2-adrenoceptors. In both guinea pig ileum and rat white adipocytes, however, the physiological response to SM-11044 was not significantly antagonized by propranolol in conditions where isoproterenol- and fenoterol-induced responses via β_1- and β_2-adrenoceptors were inhibited. Thus, SM-11044 showed atypical β-adrenergic properties in adipocytes and ileum tissue. For further evaluation of this compound as a pharmaceutical, it was required to synthesize radioactive SM-11044. In this report, we wish to report the synthesis of SM-11044 labeled with carbon-14 at the C3 position of the propionic acid moiety.

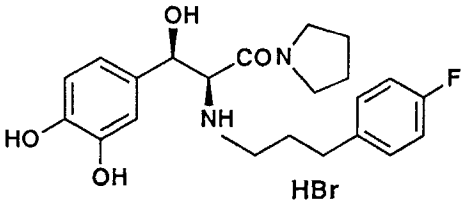


Fig. 1 Structure of SM-11044

RESULTS AND DISCUSSION

Our retrosynthetic analysis of radioactive SM-11044 is illustrated in Fig. 2. Appropriate protection of the hydroxyl groups of the catechol ring, removal of the side chain and hydrolysis of the amide led to the optically active β-hydroxy-α-amino acid (2), which was considered the most important intermediate in the present synthesis. The amino acid (2) was retrosynthetically broken by disconnection of the C2-C3 bond, leading to the aldehyde (3). The aldehyde (3) was regarded as a good candidate to afford, in the retrosynthetic direction, the amino acid (2) via condensation with glycine (4). The aldehyde (3) was traced back to the acid (5) then to barium carbonate (6), one of the readily accessible radioactive material. Included among our goals in the program were, therefore, the following: establishment of a suitable protecting group for hydroxyl groups of the catechol ring, effective conversion of the aldehyde (3) to the optically acitive β-hydroxy-α-amino acid (2) and synthesis of the aldehyde (3) from barium carbonate (6).

Since the optically active amino acid (2) was projected as a key intermediate in our synthesis, one of the main objectives was to prepare this compound from the aldehyde (3) efficiently. Several methods have appeared so far concerning the construction of the carbon skeleton of 2. These methods were usually based upon condensation of a

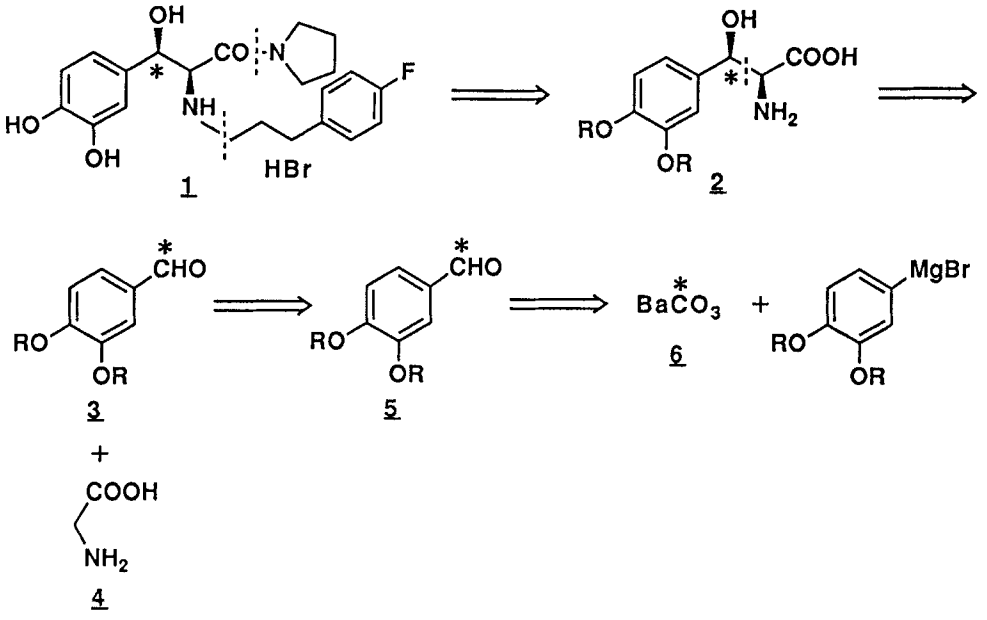


Fig. 2 Retrosynthetic analysis of SM-11044

benzaldehyde derivative with glycine.[2] Ohashi et. al. found that reaction of 3,4-methylenedioxybenzaldehyde (3) with glycine gave *threo*-β-hydroxy-α-amino acid (8) exclusively (Fig. 3).[3] Adaptation of this method directly to the present labeling work, however, seemed impractical for two reasons. Firstly, due to the formation of

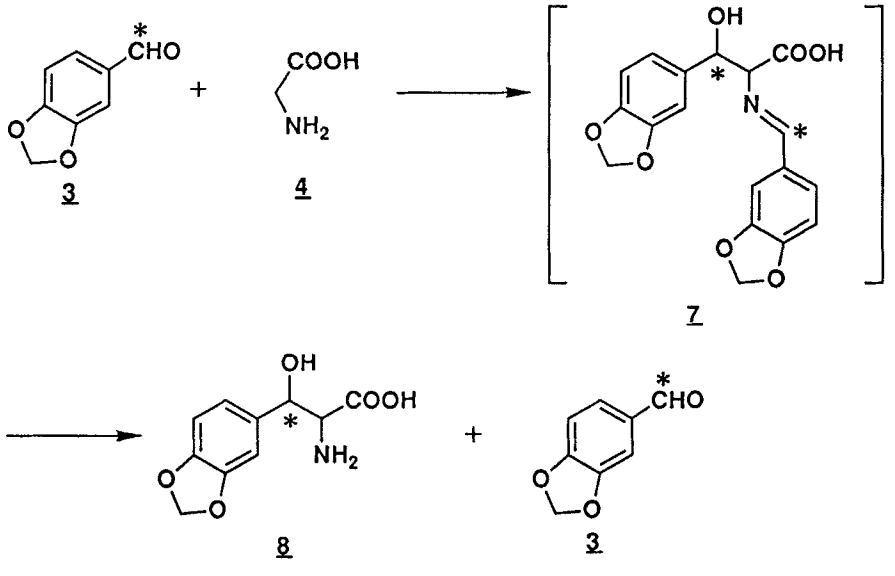
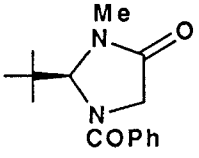


Fig. 3 Condensation of 3,4-methylenedioxybenzaldehyde with glycine

the imine (7) between the aldehyde (3) and glycine (4), the radiochemical yield of 8 based on 3 was estimated to be 50% at the highest. Secondly, optical resolution of the racemic compound through diastereomeric salt would cause a lower yield in the small scale radiosynthesis. These disadvantages prompted us to investigate an alternative method.

To overcome these problems, we turned our attention to the asymmetric synthesis of β-hydroxy-α-amino acid (2) through condensation of 3,4-methylenedioxybenzaldehyde with chiral glycine enolate equivalents. A number of methods are available for its asymmetric synthesis.[4] Among them, reaction of an aldehyde with the chiral glycine enolate precursors based on heterocyclic compounds (9 and 10) developed extensively by Seebach seemed most promising.[5] It was contemplated that use of a glycine enolate equivalent would give the amino acid in a satisfactory yield because of the absence of the imine intermediate. In addition, this method was reported to proceed with high stereoselectivity and, therefore, expected to avoid having to perform optical resolution of a radioactive material.



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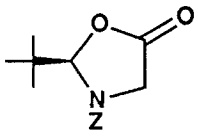

Z:$C_6H_5CH_2OCO$-

10

Fig. 4 Chiral glycine enolate equivalents

In order to explore this crucial step and establish the viability, we embarked on preliminary studies for the asymmetric synthesis of the β-hydroxy-α-amino acid (2). The chiral glycine enolate equivalents (9 and 10) were obtained by using Seebach's method. Treatment of these compounds with lithium diisopropylamide produced corresponding enolate anions, which were allowed to react with the aldehyde (3) to give the desired adducts. Acidic hydrolysis (conc. HCl, reflux) of the imidazolidinone derivative proved too destructive, giving a trace amount of the amino acid. Heat- and acid-sensitive functionalities of the resulting amino acid are presumably responsible for this unfavorable result. We then turned to the oxazolidinone derivative, which seemed to regenerate the amino acid more easily than the imidazolidinone counterpart. Catalytic hydrogenation of the adduct and subsequent hydrolysis (1N HCl, room temperature) afforded 2 in excellent yield. It was observed that condensation reaction proceeded with high stereoselectivity to give 2 of high enantiomeric purity. The optical purity was found to be 99% by chiral phase HPLC. Thus, we established an efficient method to obtain 2 in enantiomerically pure form from 3.

Our radiosynthesis began with the preparation of 3,4-methylenedioxy[carbonyl-^{14}C] benzaldehyde (3). The aldehyde (3) was prepared as depicted in Fig. 5. Grignard reaction of 3,4-methylenedioxyphenylmagnesium bromide with [^{14}C]carbon dioxide liberated from barium [^{14}C]carbonate (6) in the usual way gave the acid (5) in a quantitative yield.[6] Reduction of 5 with lithium aluminum hydride produced the corresponding benzyl alcohol (11) quantitatively. Oxidation of 11 was effected with chromium oxide-pyridine complex[7] to afford 3 in 84% yield, while oxidation under an acidic condition ($K_2Cr_2O_7$-H_2SO_4) gave unknown byproducts together with 3.

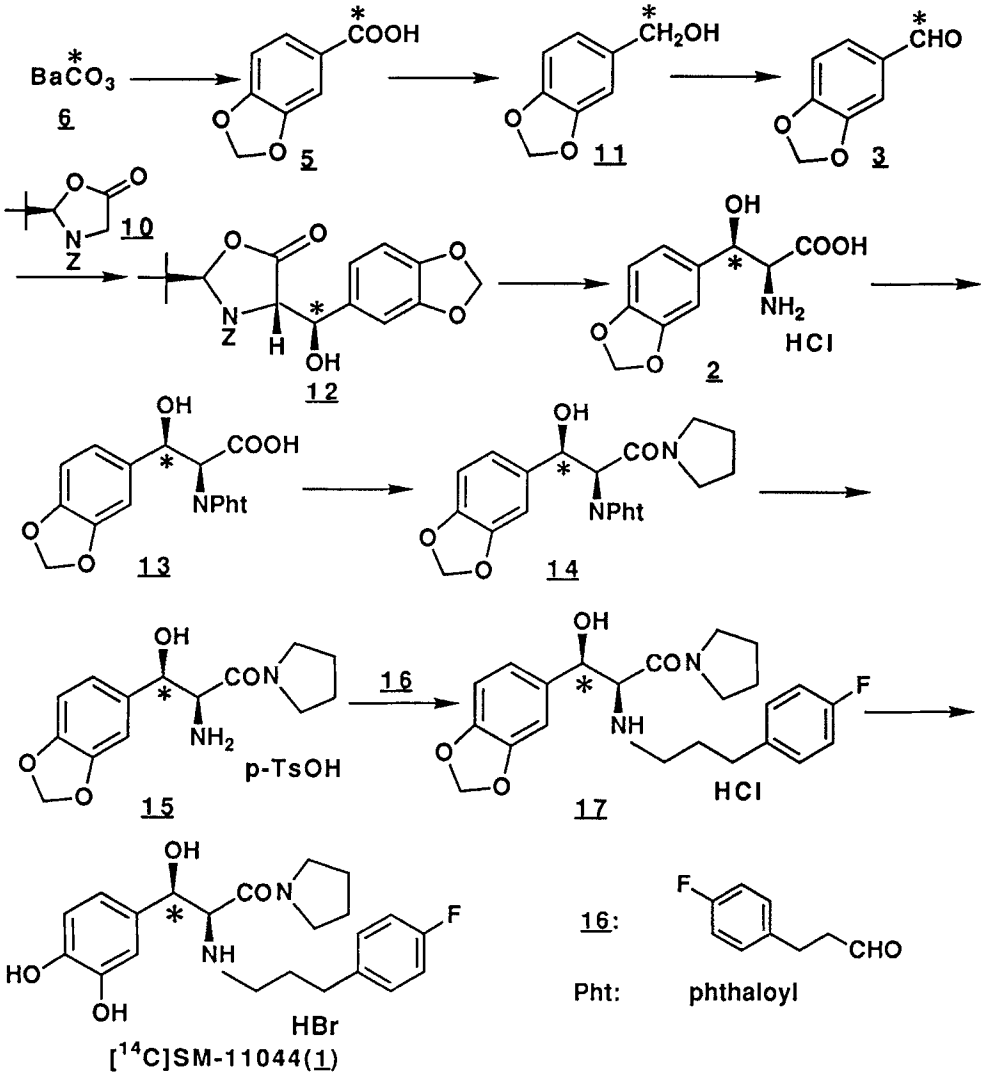

Fig. 5 Synthetic route of [propionyl-3-^{14}C]SM-11044

Now the neccessary aldehyde (3) was in hand, the stage was set for the asymmetric synthesis of β-hydroxy-α-amino acid (2). Treatment of (*R*)-oxazolidinone (10) with lithium diisopropylamide provided chiral glycine enolate anion. Without isolation, the anion was allowed to react with 3 to give the condensation product (12) in 66% yield. Catalytic hydrogenation of 12 and subsequent hydrolysis afforded the β-hydroxy-α-amino acid (2) in 93% yield. The optical purity was shown to be 100% by chiral phase HPLC.

Using the key intermediate described above, a sequence was established toward [^{14}C]SM-11044 (1) as follows. Protection of the amino group was accomplished by treating with *N*-carbomethoxyphthalimide to give the hydroxy acid (13) in 59% yield. The resulting hydroxy acid (13) was converted to its mixed anhydride with pivaloyl chloride, and further transformed to the amide (14) in 96% yield. Deprotection of the amino group was effected with hydrazine hydrate to provide the hydroxyamide (15) in 71% yield. Reaction of 15 with 3-(4-fluorophenyl)propanal (16) followed by reduction of the resulting imine with sodium cyanoborohydride afforded the fluoride (17) in 96% yield. Cleavage of the methylenedioxy group under a mild condition was achieved by using aluminum chloride/octanethiol[8] to furnish the free base of 1, which was converted to the corresponding hydrobromide to give 1 in 52% yield.

EXPERIMENTAL

Radio-thin layer chromatography (RTLC) was carried out on a Silica Gel F_{254} plate (Merck, Germany), and the radioactivity on the plate was determined by a Radio-chromanyzer (Aloka, Japan). Radio-high performance liquid chromatography (RHPLC) was conducted on a LC-3A liquid chromatograph (Shimadzu Co., Ltd., Japan) equipped with a SPD-2A UV detector (Shimadzu Co.) and a RLC-551 Radioanalyzer (Aloka). Radioactivity was measured by a TRI-CARB liquid scintillation counter (Packard Instrumet Co., USA) by using Permafluor (Packard) as the counting medium. An infrared spectrum (IR) was measured by a IR-810 grating infrared spectrophtometer (JASCO Co., Ltd., Japan). A proton nuclear magnetic resonance spectrum (NMR) was determined on a Unity 300 spectrometer (Varian, USA) or JNM FX-100 spectrometer (JEOL Ltd., Japan), and the chemical shifts (δ) for protons were quoted in ppm downfield from tetramethylsilane as the internal standard. A mass spectrum (MS) was obtained on a Hitachi M-1000 LC API (Hitachi Ltd., Japan).

3,4-Methylenedioxy[carboxyl-^{14}C]benzoic acid (5)

Under a nitrogen atmosphere, to a stirred mixture of magnesium turnings (680 mg, 28.0 mmol) and a catalytic amount of iodine in anhydrous tetrahydrofuran (16.0 ml) was added dropwise a solution of 3,4-methylenedioxybromobenzene [9](5.26 g, 26.2

mmol) in anhydrous tetrahydrofuran (25.0 ml) to keep gentle reflux during the addition. After complete addition, the mixture was refluxed for 1 h. The Grignard reagent solution was cooled, titrated (0.62 mmol/ml), and charged into two flasks (25 ml and 10 ml, respectively), which were connected to a vaccum manifold and frozen in a liquid nitrogen bath. To one flask containing 15.4 mmol of 3,4-methylenedioxyphenylmagnesium bromide was introduced [^{14}C]carbon dioxide liberated from barium [^{14}C]-carbonate (6) (9.21 GBq, 884 mg, 4.49 mmol) with concentrated sulfuric acid. The mixture was warmed to -20℃ and stirred at the same temperature for 1 h. The remaining [^{14}C]carbon dioxide gas was then confined in the other flask containing 6.2 mmol of the Grignard reagent, and this mixture was also allowed to react under the same condition described above. The reaction mixtures were decomposed with 5% hydrochloric acid, combined and extracted with ether. The ethereal solution was washed with water and extracted with 5% aqueous sodium carbonate. The alkaline solution was washed with ether, acidified with concentrated hydrochloric acid and extracted with ether. The extract was washed with water and saturated sodium chloride solution and dried over anhydrous sodium sulfate. Evaporation of the solvent gave 5 (9.95 GBq, 108%). The purity was shown to be 99% by RTLC (chloroform/methanol=9/1 v/v, Rf=0.29).

IR (ν_{max}, cm^{-1}, nujol): 3300-2600 (COOH), 1670 (CO)

3,4-Methylenedioxy[α-^{14}C]benzyl alcohol (11)

To a solution of the acid (5) (19.5 GBq, 1.58 g, 9.51 mmol) in anhydrous tetrahydrofuran (20 ml) was added lithium aluminum hydride (926 mg, 24.4 mmol) at room temperature and the mixture was stirred at the same temperature for 1 h. After addition of water and concentrated hydrochloric acid, the mixture was extracted with ether. The organic layer was successively washed with 5% aqueous sodium carbonate, water and saturated sodium chloride solution, and dried over anhydrous sodium sulfate. Evaporation of the solvent gave 11 (19.7 GBq, 101%). The purity was shown to be 97% by RTLC (chloroform/methanol=9/1 v/v, Rf=0.53).

NMR (δ, ppm, $CDCl_3$): 2.11 (1H, s, OH), 4.50 (2H, s, $C\underline{H}_2OH$), 5.85 (2H, s, OCH_2O)
6.65-6.80 (3H, m, phenyl)

3,4-Methylenedioxy[carbonyl-^{14}C]benzaldehyde (3)

To anhydrous pyridine (30.0 ml) was added chromium oxide (2.70 g, 27.0 mmol) at 0℃ and the mixture was stirred at the same temperature for 0.5 h. To this mixture was added dropwise a solution of the alcohol (11) (19.7 GBq, 1.46 g, 9.59 mmol) in anhydrous pyridine (15 ml), and the mixture was stirred at the same temperature for 0.5 h and further at room temperature for 2 h. After dilution with water, the mixture was

extracted with ether. The organic layer was successively washed with water, 5% hydrochloric acid, water, 5% aqueous sodium carbonate, water and saturated sodium chloride solution, and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue, which was chromatographed on silica gel with ethyl acetate to give 3 (16.5 GBq, 83.7%). The purity was shown to be 97% by RTLC (chloroform, Rf=0.34).

IR (ν_{max}, cm^{-1}, liquid film): 1695 (CO)

NMR (δ, ppm, $CDCl_3$): 6.01 (2H, s, OCH_2O), 6.80-6.92 (1H, m, phenyl), 7.28-7.43 (2H, m, phenyl), 9.75 (1H, s, CHO)

(*R*)-2-(*t*-Butyl)-3-benzyloxycarbonyloxazolidin-5-one (10)

The racemic oxazolidinone was prepared according to the method by Seebach.[5] The racemic compound (4.03 g, 14.5 mmol) was optically resolved by chiral phase HPLC (column CHIRALCEL OF 10 mmID × 25 cm, mobile phase hexane/2-propanol= 75/25 v/v, flow rate 4.0 ml/min, detector UV (254 nm), retention time *R* 13 min *S* 18 min) to give 10 (1.76 g, 43.7%). The optical purity was shown to be 100% by chiral phase HPLC (column CHIRALCEL OF 4.6 mmID × 25 cm, mobile phase hexane/2-propanol= 80/20 v/v, flow rate 1.0 ml/min, detector UV (254 nm), retention time *R* 16 min *S* 23 min).

NMR (δ, ppm, $CDCl_3$): 0.96 (9H, s, $C(CH_3)_3$), 3.85 (1H, d, J=17 Hz, H-C(4)), 4.31-4.37 (1H, m, H-C(4)), 5.16 5.20 (each 1H, d, J=11 Hz, $C\underline{H}_2C_6H_5$), 5.64 (1H, s, H-C(2)), 7.30-7.42 (5H, m, phenyl)

(2*R*,4*S*,4'*S*)-3-Benzyloxycarbonyl-2-(*t*-butyl)-4-[hydroxy-(3,4-methylenedioxyphenyl)-[^{14}C]methyl]oxazolidin-5-one (12)

Under a nitrogen atmosphere, to a solution of hexamethyldisilazane (3.99 ml, 18.9 mmol) in anhydrous tetrahydrofuran (10.0 ml) was added dropwise a solution of n-butyllithium in anhydrous hexane (1.6M, 11.2 ml, 18.0 mmol) at -78℃, and the solution was stirred at the same temperature for 1 h. To this solution was added dropwise a solution of the oxazolidinone (10) (4.18 g, 15.1 mmol) in anhydrous tetrahydrofuran (8.0 ml), and stirred at the same temperature for 1 h. To this mixture was added dropwise a solution of the aldehyde (3) (16.5 GBq, 1.21 g, 8.26 mmol), and stirred at the same temperature for 3.5 h. After addition of 1N acetic acid in tetrahydrofuran and saturated ammonium chloride solution, the mixture was extracted with ether. The organic layer was washed with saturated sodium chloride solution and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue which was chromatographed on silica gel with hexane/ethyl acetate (1/1 v/v) to afford (12) (10.9 GBq, 66.0%).

IR (ν_{max}, cm^{-1}, liquid film): 3500 (OH), 1715 (CO)
NMR (δ, ppm, $CDCl_3$): 0.88 (9H, s, $C(CH_3)_3$), 3.93 (1H, br s, CHOH), 4.54 (1H, d, J=4.8 Hz, H-C(4)), 5.20 (1H, s, H-C(2)), 5.29 (2H, m, $CH_2C_6H_5$), 5.93 (2H, s, OCH_2O), 6.57-6.60 (1H, m, phenyl), 7.39 (7H, br s, phenyl)
MS (positive, m/z): 427, 429 $(M+H)^+$

(2*S*,3*R*)-2-Amino-3-(3,4-methylenedioxyphenyl)-3-hydroxy[3-^{14}C]propionic acid hydrochloride (2)

To a solution of the alcohol (12) (10.9 GBq, 2.27 g, 5.30 mmol) in ethanol (50.0 ml) was added palladium on carbon (10%, 1.0 g), and the mixture was stirred under a hydrogen atmosphere at room temperature for 4 h. To this mixture was added 1N hydrochloric acid (5.50 ml, 5.50 mmol), and the mixture was stirred at room temperature for 1 h. The catalyst was filtered off and washed with ethanol. The combined filtrate was evaporated to give 2 (10.1 GBq, 92.6%). The optical purity was found to be 100% by chiral phase RHPLC (column SUMICHIRAL OA-5000, 5 μm, 4.6 mmID × 15 cm, mobile phase 0.02M aqueous copper sulfate/2-propanol=95/5 v/v, flow rate 1.0 ml/min, detectors UV (254nm) and radiodetector, retention time 9.6 min).
NMR (δ, ppm, CD_3OD): 4.06 (1H, s, CHCOOH), 5.22 (1H, s, CHOH), 5.96 (2H, s, OCH_2O), 6.83-6.98 (3H, m, phenyl)
MS (positive, m/z): 225, 227 $(M-HCl)^+$

(2*S*,3*R*)-2-Phthalimido-3-(3,4-methylenedioxyphenyl)-3-hydroxy[3-^{14}C]propionic acid (13)

The amino acid (2) (10.1 GBq, 1.29 g, 4.92 mmol) was suspended in water (5.0 ml) and acetic acid (0.1 ml), and the pH of the suspension was adjusted to 5.94 with 5% aqueous sodium hydroxide. To this mixture was added *N*-methoxycarbonylphthalimide (2.05 g, 10.0 mmol) and sodium carbonate (795 mg, 7.50 mmol), and the mixture was stirred at room temperature for 5 h. To the mixture was added again *N*-carbomethoxyphthalimide (1.00 g, 5.00 mmol) and sodium carbonate (400 mg, 3.75 mmol), stirred at the same temperature for 1 h. The pH was adjusted to 2.0 with 5% sulfuric acid, and the mixture was stirred for 1 h. The resulting precipitate was filtered off, dissolved in ethyl acetate, washed with water and dried over anhydrous sodium sulfate. Evaporation of the solvent gave 13 (5.40 GBq, 58.7%). The purity was shown to be 98% by RHPLC (column SUMIPAX ODS A-112, 5 μm, 6 mmID × 15 cm, mobile phase water/acetonitrile/acetic acid=70/30/1 v/v/v, flow rate 1.0 ml/min, detectors UV (254nm) and radiodetector, retention time 19.5 min). The optical purity was reconfirmed to be 100% by chiral phase HPLC (column SUMICHIRAL OA-3300, 5 μm, 4 mmID × 25 cm, mobile phase 0.01M ammonium acetate methanolic solution, flow rate 1.0 ml/min, detectors UV (254nm) and radio- detector, retention time 43 min).

NMR (δ , ppm, $CDCl_3$): 5.48 (1H, d, J=4.3 Hz, CHCOOH), 5.67 (1H, d, J=4.3 Hz, CHOH), 5.87 (2H, s, OCH_2O), 6.67-6.83 (3H, m, phenyl), 7.70-7.83 (4H, m, phenyl)

MS (negative, m/z): 354, 356 $(M-H)^-$

(2*S*,3*R*)-2-Phthalimide-3-(3,4-methylenedioxyphenyl)-3-hydroxy[3-^{14}C]propionic acid pyrrolidine amide (14)

Traces of water in the hydroxy acid (13) (5.40 GBq, 934 mg, 2.63 mmol) was azeotropically distilled away with benzene. To a solution of the residue in anhydrous dichloromethane (9.0 ml) and triethylamine (0.38 ml, 2.70 mmol) was added pivaloyl chloride (0.33 ml) at -20℃, and the solution was stirred at the same temperature for 1 h. To this solution was added pyridine (0.22 ml, 2.70 mmol) and pyrrolidine (0.23 ml, 2.70 mmol), and the solution was stirred at the same temperature for 1 h. To this was again added triethylamine (0.38 ml, 2.70 mmol) and pivaloyl chloride (0.33 ml, 2.7 mmol), stirred for 1 h, then added pyridine (0.22 ml, 2.7 mmol) and stirred for 1 h. After dilution with water, the mixture was extracted with dichloromethane. The organic layer was successively washed with 5% hydrochloric acid, 5% aqueous sodium hydrogen carbonate and 5% aqueous sodium chloride and dried over anhydrous sodium sulfate. Evaporation of the solvent gave 14 (5.27 GBq, 96.2%). The purity was shown to be 99% by RHPLC (column SUMIPAX ODS A-212, 5 μ m, 4.6 mmID × 25 cm, mobile phase water/aetonitrile/acetic acid=300/200/0.5 v/v/v, flow rate 1.0 ml/min, detectors UV (287 nm) and radiodetector, retention time 11 min).

NMR (δ , ppm, $CDCl_3$): 1.69-1.84 (4H, m, pyrrolidine), 3.08-3.60 (4H, m, pyrrolidine), 4.52 (1H, d, J=6.0 Hz, CHCO), 5.10 (1H, d, J=6.0 Hz, CHOH), 5.90 (2H, s, OCH_2O), 6.70-7.00 (3H, m, phenyl), 7.80-8.01 (4H, m, phenyl)

(2*S*,3*R*)-2-Amino-3-(3,4-methylenedioxyphenyl)-3-hydroxy[3-^{14}C]propionic acid pyrrolidine amide *p*-toluenesulfonic acid salt (15)

Under a nitrogen atmosphere, a mixture of the hydroxy amide (14) (5.27 GBq, 1.04 g, 2.54 mmol), hydrazine hydrate (0.80 ml), dichloromethane (3.0 ml) and water (6.0 ml) was refluxed for 5.5 h. After cooling to room temperature, dichloromethane (6.0 ml) and water (6.0 ml) were added, and the pH was adjusted to 8.9 with 5% aqueous sodium hydroxide. The mixture was extracted with dichloromethane, and the organic layer was washed with 5% aqueous sodium chloride and dried over anhydrous sodium sulfate. After filtration, to the filtrate was added *p*-toluenesulfonic acid (608 mg, 3.20 mmol) and the solution was stirred at room temperature for 1 h. After evaporation of the solvent, the residue was dissolved in ethyl acetate, cooled to 0℃ and stirred at the same temperature for 1 h. The precipitate was filtered off, washed

with ethyl acetate and dried under reduced pressure to give 15 (3.70 GBq, 71.0%). The purity was shown to be 99% by RHPLC (column SUMIPAX ODS A-212, 5 μ m, 4.6 mmID × 25 cm, mobile phase PIC B7/methanol=1/1 v/v, flow rate 1.0 ml/ min, detectors UV (287 nm) and radiodetector, retention time 8.4 min).

NMR (δ, ppm, $CDCl_3$): 1.36-1.43 (4H, m, pyrrolidine), 2.29 (3H, s, CH_3), 3.02-3.38 (4H, m, pyrrolidine), 4.10-4.22 (1H, m, CHCO), 4.73-4.80 (1H, m, CHOH), 5.89 (2H, s, OCH_2O), 6.70-7.00 (3H, m, phenyl), 7.10-4.90 (4H, m, phenyl).

(2*S*,3*R*)-2-[3-(4-Fluorophenyl)]propylamino-3-(3,4-methylenedioxyphenyl)-3-hydroxy-[3-^{14}C]propionic acid pyrrolidine amide hydrochloride (17)

To a solution of the hydroxyamide (15) (3.70 GBq, 1.85 mmol) in methanol (6.5 ml) was added acetic acid (0.7 ml), and the pH was adjusted to 5.4 with 20% aqueous sodium hydroxide. To the solution was added dropwise a solution of the aldehyde (16) (310 mg, 2.04 mmol) in methanol (1.0 ml) at 0℃, and the solution was stirred at the same temperature for 1 h then at room temperature for 1 h. After cooling to 0℃, to the solution was added sodium cyanoborohydride (128 mg, 2.04 mmol), and the mixture was stirred at room temperature overnight. After dilution with ethyl acetate and water, the mixture was extracted with ethyl acetate. The organic layer was washed with saturated sodium chloride and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue, which was dissolved in ethyl acetate (6.0 ml). To this solution was added concentrated hydrochloric acid (0.22 ml, 7.13 mmol), and the solution was stirred at 0℃ for 1 h. The resulting precipitate was filtered off, washed with ethyl acetate and dried under reduced pressure to give 17 (3.54 GBq, 95.6%). The purity was shown to be 99% by RHPLC (column SUMIPAX ODS A-212, 5 μ m, 4.6 mmID × 25 cm, mobile phase 0.005M PIC B8 (pH 2.1)/acetonitrile=65/35 v/v, flow rate 1.0 ml/ min, detectors UV (280 nm) and radiodetector, retention time 17 min).

NMR (δ, ppm, $CDCl_3$): 1.50-2.18 (8H, m, pyrrolidine), 2.64-3.16 (6H, m, $CH_2CH_2CH_2$), 4.20 (1H, d, J=9.0 Hz, CHCO), 4.89 (1H, d, J=9.0 Hz, CHOH), 5.95 (2H, s, OCH_2O), 6.82-7.40 (7H, m, phenyl)

(2*S*,3*R*)-2-[3-(4-Fluorophenyl)]propylamino-3-(3,4-dihydroxyphenyl)-3-hydroxy-propionic acid pyrrolidine amide hydrobromide (SM-11044) (1)

Under a nitrogen atmosphere, the pH of the mixture of the fluoride (17) (83.9 mCi, 1.55 mmol) in 1,2-dichloroethane (10 ml) and water (30 ml) was adjusted to 7.8 with 5% aqueous sodium hydroxide. After extraction with 1,2-dichloroethane, the organic layer was dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue, which was dissolved in 1,2-dichloroethane. Traces of water was azeotropically distilled with 1,2-dichloroethane to give the free base of 17. Under a

nitrogen atmosphere, to a mixture of anhydrous aluminum chloride (984 mg, 7.38 mmol) in 1,2-dichloroethane was added octanethiol (1.28 ml, 7.38 mmol), and the mixture was stirred at room temperature for 1 h. To this mixture was added dropwise a solution of the free base described above in 1,2-dichloroethane (5.0 ml) at -20℃, and the solution was stirred at the same temperature for 3.5 h. After dilution with ice-cold 1% hydrochloric acid, the mixture was stirred at room temperature for 1 h. The aqueous layer separated was washed with 1,2-dichloroethane, and sodium chloride was added to the solution. After extraction with *sec*-butyl alcohol, the alcohol layer was washed with 20% aqueous sodium chloride. To this solution was added 20% aqueous sodium chloride, and the pH of the solution was adjusted to 8.05 with 5% aqueous sodium hydroxide. The organic layer separated was concentrated under reduced pressure to give a residue, which was dissolved in 2-propanol. To the solution was added 31% hydrogen bromide in acetic acid (415 mg, 5.12 mmol) at 0℃, and the solution was allowed to stand at the same temperature overnight. The resulting precipitate was filtered off, washed with ice-cold 2-propanol and dried under reduced pressure to give 1 (1.61 GBq, 52.0%). The radiochemical and chemical purities were shown to be 97% and 98%, respectively by both methods of RTLC (acetonitrile/benzene/acetic acid=45/45/10 v/v/v, Rf=0.13; acetonitrile/water/acetic acid=18/1/1 v/v/v, Rf=0.20) and RHPLC (column SUMIPAX ODS A-212, 5 μm, 6 mmID × 15 cm, mobile phase 0.005 M PIC B8 (pH 2.1)/ acetonitrile=65/35 v/v, flow rate 1.0 ml/ min, detectors UV (280 nm) and radiodetector, retention time 9.6 min).

NMR (δ, ppm, CD_3OD): 1.37-2.25 (8H, m), 2.62-3.37 (6H, m), 4.01 (1H, d, J=10 Hz, C<u>H</u>CO), 4.69 (1H, d, J=10Hz, C<u>H</u>OH), 6.70-7.24 (7H, m, phenyl)

MS (LC, negative, m/z): 483, 485 $(M-H)^-$

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