Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **43**, 437-447 (2000)

Radiosynthesis of [^{14}C]- and [^{3}H]-Robalzotan a Selective 5-HT$_{1A}$ Antagonist

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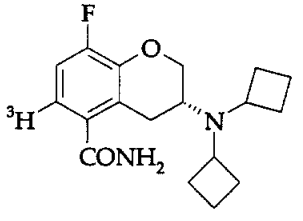
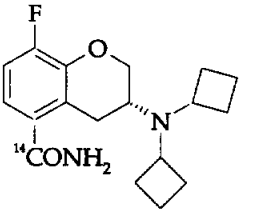
SUMMARY

The synthesis of tritium and carbon-14 labeled robalzotan is described. Tritiated robalzotan **8** was obtained after catalytic hydrogenation of (*R*)-methyl 6-bromo-3-(*N,N*-dicyclobutylamino)-8-fluoro-3,4-dihydro-2*H*-1-benzopyran-5-carboxylate (7) with tritium gas using palladium as a catalyst. The corresponding carbon-14 labeled compound **11** was obtained by cyanylation of (*R*)-3-*N,N*-dicyclobutylamino-8-fluoro-5-trifluoromethane-sulfonyloxy-3,4-*2H*-1-benzopyran. The nitrile was subsequently hydrolyzed and converted to carbon-14 labeled robalzotan.



8



11

KEY WORDS: 5HT$_{1A}$ antagonist, robalzotan, palladium catalyzed carbonylation, palladium catalyzed cyanylation.

Received 11 October 1999
Revised 25 October 1999
Accepted 6 November 1999

INTRODUCTION

Clinical data on selective serotonin reuptake inhibitors (SSRI's) have shown that the antidepressant effect of these drugs can be observed only after two to four weeks of treatment[1]. The clinical effect appears at a time when the somatodendritic $5HT_{1A}$ receptor has been functionally down regulated[2]. The $5HT_{1A}$ autoreceptor regulates the firing of serotonin, and activation of the receptor reduces the level of the transmitter in the synaptic cleft. Blocking of the somatodendritic $5HT_{1A}$ receptor would increase synaptic levels of serotonin more directly, and the $5HT_{1A}$ receptor holds promise to be the target for new and faster antidepressant treatment.

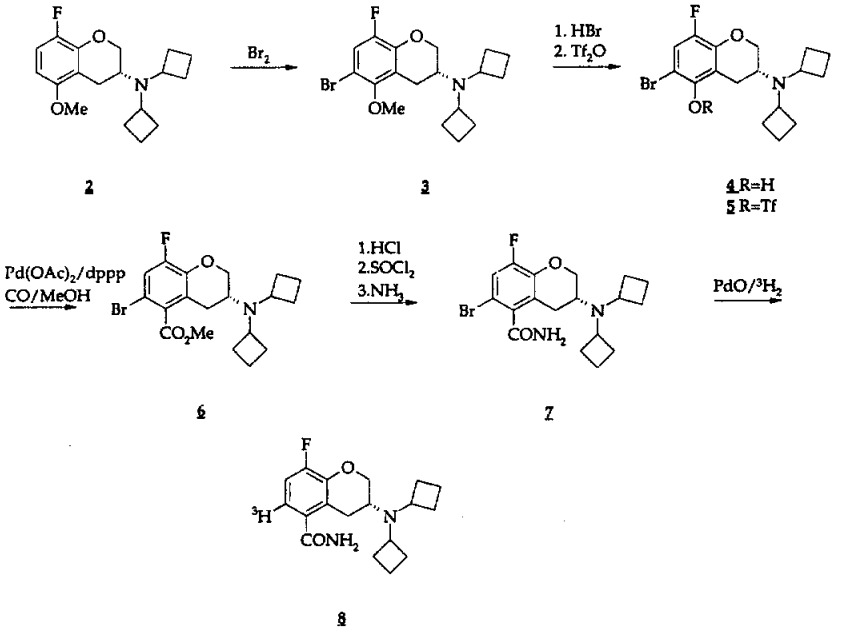
Robalzotan is a selective and highly potent $5HT_{1A}$ receptor antagonist that is currently being evaluated in clinical trials as a treatment for anxiety and depression. In order to study the tissue distribution of the drug, radiolabeled robalzotan was needed. In this paper, we describe the synthesis and characterization of [^{14}C]- and [^{3}H]robalzotan.

RESULTS AND DISCUSSION

(*R*)-8-Fluoro-3-(*N,N*-dicyclobutylamino)-5-methoxy-3,4-dihydro- *2H*-1-benzopyran hydrochloride **2**[3], was regioselectively brominated in ethanol to give **3**. Demethylation was accomplished by refluxing **3**, in hydrobromic acid. Esterification with trifluoromethanesulfonic anhydride in dichloromethane afforded the triflate **5** which subsequently was converted to the methyl ester through a palladium catalyzed carbonylation[4] in DMF-methanol.

The amide **7** was obtained after acid hydrolysis, reaction of the acid with thionyl chloride and pouring the acid chloride into ammonium hydroxide solution. 6-[^{3}H]Robalzotan, **8**, was isolated after catalytic tritiation with palladium oxide as the catalyst.

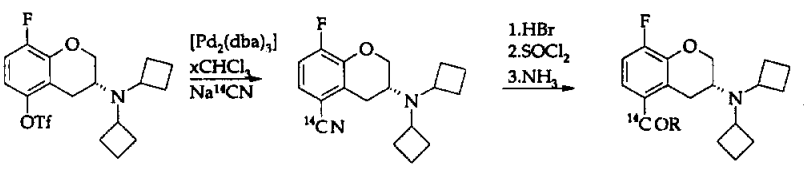
Scheme 1



2 3 4 R=H 5 R=Tf 6 7 8

To obtain [^{14}C]robalzotan, (*R*)-3-(*N,N*-dicyclobutylamino)-8-fluoro-5-trifluoromethansulfonyloxy-3,4-dihydro-*2H*-1-benzofuran[3] was treated with potassium [^{14}C]cyanide in a palladium catalyzed reaction[5] to afford the nitrile 9. The hydrochloride salt of 9 was heated in hydrobromic acid to give the corresponding acid. After reaction with thionyl chloride and pouring the obtained product into ammonium hydroxide solution, (*R*)-3-(*N,N*-dicyclobutylamino)-8-fluoro-3,4-dihydro-*2H*-1-benzofuran-5-[^{14}C]carboxamide was isolated as the L-tartrate.

Scheme 2



9 10 R=OH 11 R=NH_2

EXPERIMENTAL

Materials and Methods

Tritium gas was purchased from RC TRITEC AG, Switzerland and potassium [^{14}C]cyanide was purchased from Amersham, England. NMR spectra were recorded on a Varian Gemini 7 Tesla instrument, operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Chemical shifts are given in ppm downfield from tetramethylsilane (TMS). $CDCl_3$ containing 1% of TMS was used throughout as solvent in the NMR experiments. Resonance multiplicities are denoted s, d, t, m, q and app for singlet, doublet, triplet, multiplet, quintet and apparent, respectively. Equatorial and axial are referred to as eq and ax. Samples containing radionuclei were all run in Teflon®-tubes (Wilmad Glass Co., Inc. USA). Mass spectra were recorded on a Finnigan Mat SSQ 710, or JEOL Automass System II GC-MS instrument operating at an electron energy (EI) of 70 eV. Specific rotations were determined on a polarimeter type AA-10 from Optical Activity Ltd. Melting points were determined in capillary tubes on a Mettler FP62 automatic melting point apparatus and are uncorrected. Column chromatography was performed on silica gel 60 (230-400 mesh ASTM, Merck). Thin layer chromatography (TLC) was performed on TLC precoated plates, silica gel 60 F_{254} (Merck). Radiochemical purity was determined by TLC using a Bioscan System 200 Imaging Scanner or with a Packard 500 TR Flow Scintillation Analyzer connected to an HPLC instrument. Radioactivity was measured in a Packard 1000 liquid scintillation spectrometer using Packard Ultima Gold as a counting medium. Gas chromatographic analysis was performed on a Hewlett Packard 5890 apparatus equipped with a 10 m x 0.15 mm fused silica capillary column coated with 0.12 μm CP-Sil-5. Elemental analyses were carried out by MIKRO KEMI AB; Seminariegatan 29, S-752 28 Uppsala, Sweden.

(*R*)-6-Bromo-3-(*N,N*-dicyclobutylamino)-8-fluoro-5-methoxy-3,4-dihydro-*2H*-1-benzopyran, 3.

(*R*)-3-(*N,N*-Dicyclobutylamino)-8-fluoro-5-methoxy-3,4-dihydro- *2H*-1-benzopyran[4] (1.0 g, 3.3 mmol) was dissolved in ethanol (20 mL) and to this HCl (2M, 40 mL) was added. To the mixture was added a solution of bromine (0.2 mL, 4.0 mmol) in ethanol (20 mL) until the redish color persisted (17 mL). The excess ethanol was removed *in vacuo*, the remainder was poured into a solution of ice/NH_4OH (conc.) and then extracted, twice, with ethyl acetate. The combined ethyl acetate portions were dried ($MgSO_4$), filtered, and the solvent removed *in vacuo* to give the title compound (1.3 g; 98%) as a clear green oil. $[\alpha]^{21}D$ -82.7° (*c* 1, $CHCl_3$). ^{1}H NMR (300 MHz) δ 7.10 (d, J = 10 Hz, 1 H), 4.34-4.24 (m, 1 H), 3.83 (t, J = 11 Hz, 1 H), 3.79 (s, 3 H), 3.41 (app q, J = 8 Hz, 2 H), 3.35-3.22 (m, 1 H), 2.96-2.71 (m, 2 H), 2.13-1.92 (m, 8 H), 1.70-1.50 (m, 4 H). ^{13}C NMR (75 MHz) δ 151.1 (d, J_F = 3 Hz), 148.0 (d, J_F = 246 Hz), 142.6 (d, J_F = 13 Hz), 119.9, 117.3 (d, J_F = 18 Hz), 105.4 (d, J_F = 9 Hz), 68.8, 60.3, 53.9, 49.3, 30.8, 30.4, 24.3, 16.0. MS (70 eV) *m/z* (M+1) 385. Anal. Calcd. for $C_{18}H_{23}BrFNO_2$: C, 56.3; H, 6.0; N, 3.6. Found: C, 56.6; H, 6.0; N, 3.6.

(*R*)-6-Bromo-3-(*N,N*-dicyclobutylamino)-8-fluoro-5-hydroxy-3,4-dihydro-*2H*-1-benzopyran, 4.

(*R*)-6-Bromo-3-(*N,N*-dicyclobutylamino)-8-fluoro-5-methoxy-3,4-dihydro-*2H*-l-benzopyran hydrochloride (1.3 g, 3.1 mmol) was dissolved in HBr (48%, 40 mL) and the mixture was heated to reflux for 2 h. The reaction mixture was poured out onto ice/NH_4OH (conc.) and the ensuing mixture was extracted, twice, with ethyl acetate. The combined ethyl acetate portions were dried ($MgSO_4$), filtered, and the solvent removed *in vacuo* to give a crude residue. Chromatography (SiO_2; ethyl acetate/hexane 1:4) gave the title compound (1.2 g) as a slightly yellow

oil that solidified, mp 70.5-72.5 °C. $[\alpha]^{21}D$ -81.1° (*c* 1, $CHCl_3$). ^1H NMR (300 MHz) δ 7.05 (d, *J* = 10 Hz, 1 H), 5.4 (br s, 1 H), 4.34-4.24 (m, 1 H), 3.84 (t, *J* = 11 Hz, 1 H), 3.40 (app q, *J* = 8 Hz, 2 H), 3.45-3.23 (m, 1 H), 2.90-2.68 (m, 2 H), 2.11-1.93 (m, 8 H), 1.70-1.50 (m, 4 H). ^{13}C NMR (75 MHz) δ 146.5 (d, J_F = 2 Hz), 145.7 (d, J_F = 242 Hz), 143.1 (d, J_F = 11 Hz), 115.6 (d, J_F = 22 Hz), 112.7, 97.8 (d, J_F = 9 Hz), 69.2, 54.1, 49.4, 31.0, 30.4, 23.8, 15.4. MS (70 eV) *m/z* (M+1) 371. Anal. Calcd. for $C_{17}H_{21}BrFNO_2$: C, 55.1; H, 5.7; N, 3.8. Found: C, 55.0; H, 5.5; N, 3.7.

(*R*)-6-Bromo-3-(*N,N*-dicyclobutylamino)-8-fluoro-5- trifluoro-methanesulfonyloxy -3,4-dihydro-2*H*-1-benzopyran, 5.

(*R*)-6-Bromo-3-(*N,N*-dicyclobutylamino)-8-fluoro-5-hydroxy-3,4-dihydro-*2H*-1-benzopyran (1.2 g, 3.13 mmol) and collidine (0.58 mL, 4.38 mmol) were dissolved in anhydrous CH_2Cl_2 (50 mL) and cooled to -40 °C. Trifluoromethanesulfonic anhydride (0.63 mL, 3.76 mmol) was added dropwise and the mixture was allowed to warm to ambient temperature. The reaction was diluted with CH_2Cl_2 and washed with $NaHCO_3$ (aq., sat.), dried ($MgSO_4$), filtered, and evaporated *in vacuo* to give a crude residue. Chromatography (SiO_2; CH_2Cl_2) gave the title compound (0.9 g, 58%) as a clear oil. $[\alpha]^{21}D$ -74.7° (*c* 1, $CHCl_3$). ^1H NMR (300 MHz) δ 7.26 (d, *J* = 9 Hz, 1 H), 4.38-4.19 (m, 1 H), 3.83 (t, *J* = 11 Hz, 1 H), 3.36 (app q, *J* = 8 Hz, 2 H), 3.43-3.23 (m, 1 H), 3.00-2.88 (m, 2 H), 2.11-1.93 (m, 8 H), 1.72-1.50 (m, 4 H). ^{13}C NMR (75 MHz) δ 150.4 (d, J_F = 253 Hz), 143.2 (d, J_F = 12 Hz), 140.7 (d, J_F = 3 Hz), 121.2, 118.6 (d, J_F = 23), 118.5 (q, J_F = 321 Hz), 105.1 (d, J_F = 9 Hz), 69.2, 54.0, 48.8, 30.9, 30.3, 25.0, 15.3. MS (TSP) *m/z* (M+1) 502 (100), Anal. Calcd. for $C_{18}H_{20}BrF_4NO_4S$: C, 43.0; H, 4.0; N, 2.8. Found: C, 43.3; H, 4.1; N, 2.8.

(*R*)-Methyl 6-bromo-3-(*N,N*-dicyclobutylamino)-8-fluoro-3,4-dihydro-2*H*-1-benzopyran-5-carboxylate, 6.

(*R*)-6-Bromo-3-(*N,N*-dicyclobutylamino)-8-fluoro-5-trifluoromethanesulfonyloxy-3,4-dihydro-*2H*-1-benzopyran (0.8 g, 1.59 mmol) was dissolved in a solution of DMF/methanol (6:2, 20 mL), the solution was degassed, and then flushed three times with carbon monoxide. Under a stream of carbon monoxide palladium(II)acetate (14 mg), 1,3-bis(diphenylphosphino)propane (25 mg) and triethylamine (0.50 mL, 3.5 mmol) were added and the reaction mixture was degassed and flushed with carbon monoxide once again. The mixture was vigorously stirred at 70 °C for 6 h, under a carbon monoxide atmosphere and then cooled. The solvent was removed *in vacuo*, the remainder was taken into a NH_4OH solution (2M) and then extracted, twice, with diethyl ether. The combined ether portions were dried ($MgSO_4$), filtered, and the solvent removed *in vacuo* to give a crude residue. Chromatography (SiO_2; ethyl acetate/hexane 1:6) gave the title compound (0.3 g, 44% yield) as a clear oil that crystallized to give a waxy white solid, (mp 104.5-105.5 °C). $[\alpha]^{21}D$ -99.4° (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.16 (d, J = 10 Hz, 1 H), 4.38-4.28 (m, 1 H), 3.95 (s, 3 H), 3.84 (t, J = 10 Hz, 1 H), 3.38 (app q, J = 8 Hz, 2 H), 3.4-3.25 (m, 1 H), 3.00-2.86 (m, 1 H), 2.70-2.58 (m, 1 H), 2.11-1.93 (m, 8 H), 1.72-1.50 (m, 4 H). ^{13}C NMR (75 MHz) δ 167.2, 151.7 (d, J_F = 253 Hz), 142.3 (d, J_F = 11 Hz), 131.8 (d, J_F = 4 Hz), 124.0, 118.2 (d, J_F = 22), 108.5 (d, J_F = 9 Hz), 68.8, 53.8, 52.8, 49.2, 30.8, 30.4, 27.1, 15.3. EIMS (70 eV) *m/z* (M+1) 413. Calcd. for $C_{19}H_{23}BrFNO_3$: C, 55.4; H, 5.6; N, 3.4. Found: C, 55.3; H, 5.5; N, 3.3.

(*R*)-6-Bromo-3-(*N,N*-dicyclobutylamino)-8-fluoro-3,4-dihydro-2*H*-1-benzopyran-5-carboxamide, 7.

(*R*)-Methyl 6-bromo-3-(*N,N*-dicyclobutylamino)-8-fluoro-3,4-dihydro-2*H*-1-benzopyran-5-carboxylate (0.3 g, 0.65 mmol) was refluxed in a

HCl solution (6 M, 12 mL) for 24 h. The solution was cooled, concentrated to dryness *in vacuo*, made basic with NH_4OH (2M), washed with diethyl ether (52% starting material was recovered) and the solution was concentrated *in vacuo*. The solution was then made acidic (conc. HCl), evaporated to dryness, before adding previously dried toluene. Finally the solvent was removed *in vacuo*.

To the white solid thionyl chloride (10 mL) was added and the solution allowed to stir at room temperature overnight. The excess thionyl chloride was removed *in vacuo*, dried toluene was added and finally the solvent evaporated.

The acid chloride was dissolved in CH_2Cl_2 (10 mL) and added dropwise to an NH_4OH solution (conc., 10 mL) at 0 °C. The reaction was allowed to stir at room temperature for 2 h. The CH_2Cl_2 phase was separated and the aqueous portion was extracted three times with CH_2Cl_2. The combined CH_2Cl_2 extracts were dried ($MgSO_4$), filtered, and evaporated *in vacuo* to give a crude residue. Chromatography (SiO_2; ethyl acetate/hexane 1:1 → ethyl-acetate/hexane 3:1) gave the title compound (75 mg) as a white solid mp 167.5-169.5 °C. $[\alpha]^{21}D$ -109.9° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.13 (d, J = 10 Hz, 1 H), 6.56 (br s, 1 H), 5.92 (br s, 1 H), 4.36-4.24 (m, 1 H), 3.85 (t, J = 11 Hz, 1 H), 3.38 (app q, J = 8 Hz, 2 H), 3.4-3.24 (m, 1 H), 3.06-2.78 (m, 2 H), 2.12-1.90 (m, 8 H), 1.70-1.50 (m, 4 H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 168.8, 151.3 (d, J_F = 252 Hz), 142.4 (d, J_F = 11 Hz), 134.0 (d, J_F = 3 Hz), 123.8, 117.9 (d, J_F = 21 Hz), 107.7 (d, J_F = 9 Hz), 69.0, 53.9, 49.3, 30.8, 30.3, 26.5, 15.3. EIMS (70 eV) *m/z* (M+1) 398. Calcd. for $C_{18}H_{22}BrFN_2O_2$: C, 54.4; H, 5.6; N, 7.1. Found: C, 54.3; H, 5.5; N, 6.9.

(*R*)-3-*N*,*N*-Dicyclobutylamino-8-fluoro-[6-^{3}H]-3,4-dihydro-*2H*-1-benzopyran-5-carboxamide, 8.

(*R*)-6-Bromo-3-*N*,*N*-dicyclobutylamino-8-fluoro-3,4-dihydro- *2H*-1-benzopyran-5-carboxamide, (3.2 mg, 8.1 μmol) in DMF (0.50 mL) was

stirred over palladium oxide (3.3 mg) in an atmosphere of carrier-free $^{3}H_2$ using a tritium manifold system (RC TRITEC AG, Switzerland). After 18 h the reaction mixture was frozen and degassed, filtered and the solvent distilled *in vacuo*. Labile tritium was removed by repeated lyophilization from ethanol. The remainder was purified by chromatography (SiO_2; ethyl acetate) leaving a residue containing 152 mCi of the title compound. The radiochemical purity of the product was 98.2% and the chemical purity 97.2% (HPLC, μ-Bondapac, sodium phosphate buffer/CH_3CN 7:2, UV dectection 225 nm). The specific activity was 22 Ci/mmol as determined by quantitative HPLC analysis. ^{3}H NMR (^{1}H coupled) δ 6.98 (dd, J_F = 5.11, J_H = 9.37 Hz).

(*R*)-3-*N*,*N*-Dicyclobutylamino-8-fluoro-3,4-dihydro- *2H*-1-benzopyran-5-[^{14}C]nitrile, 9.

A mixture of (*R*)-3-*N*,*N*-dicyclobutylamino-8-fluoro-5-trifluoromethane sulfonyloxy-3,4-dihydro- *2H*-1-benzopyran[3], (376 mg, 0.89 mmol), potassium [^{14}C]cyanide (55 mCi, 0.91 mmol), tris(dibenzylideneacetone)-dipalladium(0)-chloroform adduct (45 mg, 0.043 mmol), and 1,1'-bis(diphenylphosphino)- ferrocene (89 mg, 0.016 mmol) in *N*-methyl-pyrrolidine (0.80 mL) was flushed with nitrogen and stirred at 80 °C for 2 h and then cooled. Diethyl ether (4 mL) and HCl (2N, 4 mL) were added and the mixture was stirred overnight at room temperature. The ether phase was separated, the aqueous layer was washed with diethyl ether (2x3 mL), made alkaline (pH 12) with NaOH (aq, 15%) and extracted with diethyl ether (5x2 mL). The ether extracts were combined and washed with water (2 mL), brine (2 mL) and dried (Na_2SO_4). Evaporation of the solvent gave an oil (143 mg) which was used without further purification in the next step. The radiochemical purity was 91% (TLC, SiO_2; petroleum ether (40-60)/THF, 15:3). EIMS, m/z (M+1) 302.

(*R*)-3-*N,N*-Dicyclobutylamino-8-fluoro-3,4-dihydro- *2H*-1-benzopyran-5-[^{14}C]carboxylic Acid, 10.

The above oil (143 mg) was dissolved in diethyl ether (ca. 3 mL) and an excess of ethereal HCl (ca 3 M, 1.5 mL) was added. The mixture was taken to dryness *in vacuo*, the residue was treated with HBr (48%, 4 mL) and heated to 130 °C. After 4 h the reaction mixture was evaporated to dryness *in vacuo* leaving a residue which was co-distilled with toluene (2x2 mL). The material obtained was used in the next step without further purification.

(*R*)-3-*N,N*-Dicyclobutylamino-8-fluoro-3,4-dihydro- *2H*-1-benzopyran-5-[^{14}C]carboxamide, 11.

To the above acid in toluene (1.5 mL) thionyl chloride (1.5 mL) and pyridine (2 drops) were added. The mixture was stirred overnight at room temperature. After evaporation to dryness and co-distillation with toluene (1.5 mL), the residue was dissolved in CH_2Cl_2 and added dropwise to a NH_4OH solution (3 mL) at 0 °C.

The reaction mixture was stirred at ambient temperature for 2 h. The aqueous phase was separated, extracted with CH_2Cl_2 (2 x 1.5 mL) and the CH_2Cl_2 extracts were combined and dried. Evaporation of the solvent afforded an oil (128 mg) which after purification by chromatography (SiO_2; ethyl acetate) afforded the title compound (83 mg, 29% from **8**). The specific activity of 11 was 54 mCi/mmol (liquid scintillation) and the radiochemical purity was 99% (TLC, SiO_2; ethyl acetate). ^{1}H NMR δ 6.98 (dd, *J*=8 Hz, *J*=5 Hz, 1 H), 6.90 (app t, *J*=9 Hz, 1 H), 5.92 (br s, 1 H), 5.82 (br s, 1 H), 4.32 (br s, 1 H), 3.88 (t, *J*= 10 Hz, 1 H), 3.40 (app q, *J*=8 Hz, 2 H), 3.29 (br s, 1 H), 3.13-2.94 (m, 2 H), 2.2-1.8 (br s, 8 H), 1.7-1.45 (m, 4 H). ^{13}C NMR (100 Mz) δ 170.3, 152.9 (d, J_F=250 Hz), 143.4 (d, J_F=10 Hz), 131.2, 124.4, 119.1, 113.4 (d, J_F=18 Hz), 69.1, 54.2, 49.9, 30.8, 30.2, 26.5, 15.4. EIMS, m/z (M+1) 320.

(*R*)-3-*N*,*N*-Dicyclobutylamino-8-fluoro-3,4-dihydro-2*H*-1-benzopyran-5-[^{14}C]carboxamide Hydrogen (*2R,3R*)-Tartrate Monohydrate, 12.

A solution of the base (**11**) (59 mg, 0.184 mmol) in acetone (1.2 mL) was mixed with L(+)-tartaric acid (31 mg, 0.201 mmol) in acetone (0.5 mL) and water (4 drops). The solution was kept at room temperature for 2.5 h and at 4 °C overnight.

The crystals formed were collected by centrifugation, washed with ice-cold acetone (1 mL) and dried, affording 58 mg (65%) of **12** as colourless crystals. The radiochemical purity of the product was 99% and the chemical purity 99.4% (HPLC, Inertsil ODS-2 column, 0.1 M sodium phosphate buffer/CH_3CN (6:1, pH=2.7, UV=225 nm). The enantiomeric purity was determined by HPLC (UV 220 nm) giving <0.5% of the corresponding S-enantiomer (Chiral AGP column, phosphate buffer/CH_3CN (6:1, μ=0.02, pH=7.0).

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