JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2003; **46**: 459–464.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.687

Research Article

Synthesis of tritium labelled L783483 - a PPARδ ligand

Gunnar Grue-Sørensen* and Nicolaj Høj
LEO Pharma A/S, Medicinal Chemistry Research, Industriparken 55, DK-2750 Ballerup, Denmark

Summary

The potent peroxisome proliferator-activated receptor (PPAR) δ ligand L783483 (3-chloro-4-(3-(7-propyl-3-trifluoromethyl-benzisoxazol-6-oxy)propylsulfanyl)phenylacetic acid) has been labelled with tritium via selective tritium/bromine exchange of 5-bromo-6-(3-bromopropyloxy)-7-propyl-3-trifluoromethyl-benzisoxazole. [^{3}H]-L783483 had a specific activity of 529 GBq/mmol (14.3 Ci/mmol) and a radiochemical purity of 98%. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: L783483; PPARδ; tritium–bromine exchange

Introduction

The peroxisome proliferator-activated receptors (PPARs) have been studied intensely for more than a decade.[1] There is a need for radiolabelled ligands with high specific activities to elucidate and characterize the role of the various subtypes (α, δ, γ) of these receptors in

*Correspondence to: G. Grue-Sørensen, LEO Pharma A/S, Medicinal Chemistry Research, Industriparken 55, DK-2750 Ballerup, Denmark. E-mail: gunnar.grue-soerensen@leo-pharma.com

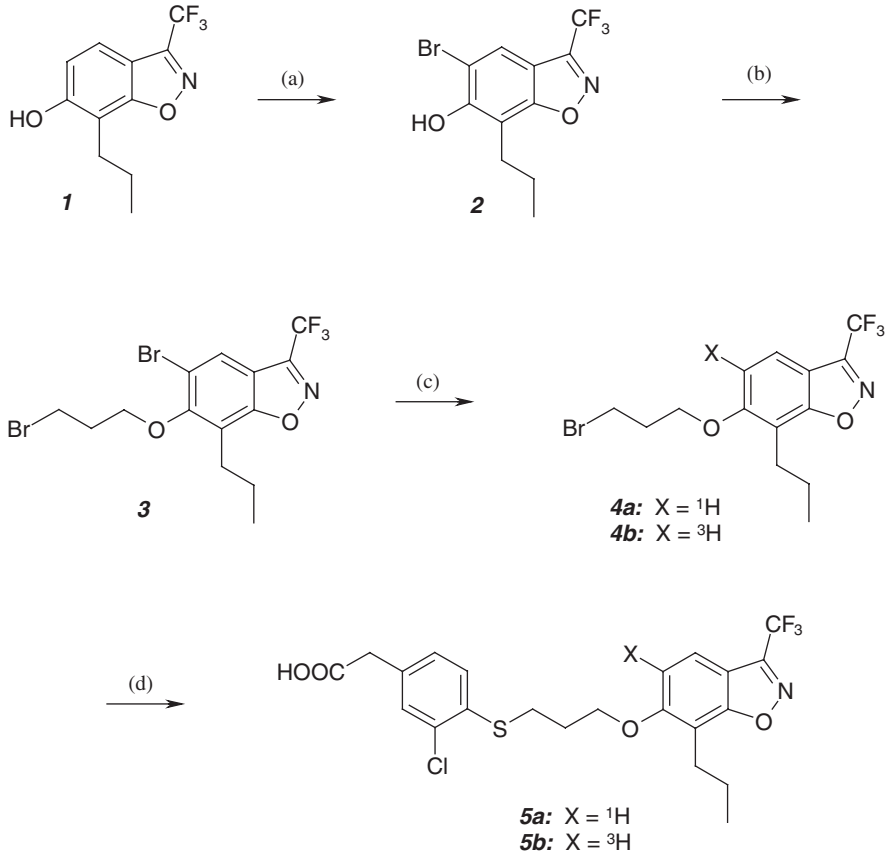
Received 17 September 2002
Revised 24 October 2002
Accepted 29 October 2002

metabolic processes and diseases. These radiolabelled ligands are also useful in screening of new ligands with optimal pharmacological properties for the treatment of PPAR related diseases. L783483 (**5a**) is such a potent PPARδ-ligand synthesised by Merck & Co.[2] [^{3}H]$_2$-L783483 with undisclosed method and sites of labelling has also been synthesised by Merck & Co (17 Ci/mmol) (*cf.* reference[3]). Here we report our synthesis of [^{3}H]-L783483 (**5b**).

Results and discussion

Our goal was to synthesize the PPAR δ ligand L783483 **(5a)** labelled with tritium and with a specific activity >10 Ci/mmol (>370 GBq/mmol). Halogen–tritium exchange is a common way of introducing tritium in aromatic compounds, provided no other functionality susceptible to catalytic reduction is present. Compound **5a** contains an aromatic chlorine substituent and selective halogen–tritium exchange of a halogen derivative of **5a** to give **5b** would most likely be difficult to control. Thus, it was decided to introduce halogen in the benzisoxazole moiety before combination with the chlorine containing part of the compound.

Bromination of 6-hydroxy-7-propyl-3-trifluoromethyl-benzisoxazole **(1)** with NBS in CCl_4 in the presence of AIBN gave the expected bromo-derivative **(2)** in 67% yield (Scheme 1). Alkylation with an excess of 1,3-dibromopropane (*cf.* reference[2]) gave the dibromo-derivative **3** in 84% yield. Selective exchange of the aromatic bromine with tritium was achieved by reaction with tritium gas in ethanol using 10% Pd/C at atmospheric pressure for 30 min. Prolonged reaction times (>3 h) resulted in a mixture where $<5\%$ of the activity belonged to the desired compound **4b**. 3-Chloro-4-(dimethylaminocarbonylsulphanyl)-phenylacetic acid methyl ester[4] was deprotected with 0.5 M MeONa/MeOH at 63°C for 70 min to give the sodium salt of 3-chloro-4-sulphanyl-phenylacetic acid methyl ester. This mixture was alkylated with **4b** in methanol at 63°C for 60 min. Finally, the reaction mixture was treated with 1 M LiOH at 63°C for 15 min to give **5b** (radiochemical purity: 98%, specific activity: 14.3 Ci/mmol) in 76% isolated radiochemical yield in step d).



Scheme 1. Synthesis of [^{3}H]-L783483. Reagents and conditions: (a) NBS, AIBN, CCl_4, 73°C, 2 days; (b) 1,3-dibromopropane, K_2CO_3, 2-butanone, reflux, 2 h; c) either [$^1H_2$], 10% Pd/C, ethanol, room temperature, 1 atm, 6 h to give 4a or [$^3H_2$], 10% Pd/C, ethanol, room temperature, 1 atm, 30 min to give 4b; (d) 3-chloro-4-(dimethylaminocarbonylsulphanyl)-phenylacetic acid methyl ester was treated with 0.5 M MeONa/MeOH, 63°C, 70 min, this mixture was added to 4a or 4b, 63°C, 60 min, then addition of 1 M LiOH, 63°C, 15 min, HPLC-purification

Experimental

General

A Packard Tri-Carb 2900TR liquid scintillation analyzer was used to determine the radioactivity in liquid samples using Pico-Fluor 40 (Packard) as scintillation cocktail. Chemical and radiochemical purity

was determined by HPLC on a Merck Hitachi apparatus (L-6200 pump) on a Spherisorb ODS1 column (150 mm × 4.6 mm) with MeCN/0.05 M phosphoric acid 73:27 as eluent (0.8 ml/min) and UV-detection (254 nm) (L-4250 UV-detector) or radioactivity detection (Packard Flow System Analyzer Model D525F1 with Ultima-Flo M (Packard) as scintillation liquid). Concentrations and specific activities were determined by HPLC by comparison of peak areas of radio-inactive reference compounds. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker ARX300 spectrometer. Chemical shifts are reported in ppm with tetramethylsilane as internal reference. ES Mass spectra were obtained on a VG Quattro II mass spectrometer in negative ion electrospray mode and EI spectra were obtained on a VG AutoSpec mass spectrometer.

5-Bromo-6-hydroxy-7-propyl-3-trifluoromethyl-benzisoxazole **(2)**. 6-Hydroxy-7-propyl-3-trifluoromethyl-benzisoxazole[2] **(1)** (4.05 g, 16.5 mmol), N-bromosuccinimide (3.30 g, 18.5 mmol) and 2,2'-azobisisobutyronitrile (62 mg) were stirred in carbon tetrachloride (60 ml) under argon at 73°C for 2 days. The reaction mixture was cooled to room temperature and filtered. Solvent was removed from the filtrate *in vacuo*. The residue was suspended in petroleum ether (bp 35–50°C) (100 ml) and the suspension was applied to a column of silica gel (43–60 μ) (8 g). The column was eluted with petroleum ether (400 ml) and the solvent was removed from the eluate to produce 5-bromo-6-hydroxy-7-propyl-3-trifluoromethyl-benzisoxazole **(2)** (3.59 g, 11.1 mmol, 67%). ^{1}H NMR (DMSO-d_6) δ 10.49 (bs, OH), 7.98 (m, 1 H), 2.94 (m, 2 H), 1.65 (m, 2 H), 0.95 (t, 3 H). ^{13}C NMR (DMSO-d_6) δ 163.9, 154.4, 148.3 (q, $J = 38.0$ Hz), 120.9, 119.9 (q, $J = 271.6$ Hz), 112.3, 112.2, 109.7, 25.9, 21.6, 13.7. MS: $[M\text{-}H]^- = 322$.

5-Bromo-6-(3-bromopropyloxy)-7-propyl-3-trifluoromethyl-benzisoxazole **(3)**. A mixture of **2** (3.32 g, 10.2 mmol), 1,3-dibromopropane (6.56 g, 32.5 mmol), potassium carbonate (4.48 g, 32.4 mmol) and 2-butanone (50 ml) was stirred at 80°C for 4 h. After cooling to room temperature ethyl acetate was added and the mixture was washed with potassium phosphate buffer (pH 4.0) (400 ml) and water (400 ml). Drying over sodium sulphate, filtration and evaporation of solvent *in vacuo* followed by removal of residual 1,3-dibromopropane at 100°C *in vacuo* gave **3** (3.82 g, 8.6 mmol, 84%). ^{1}H NMR (DMSO-d_6) δ 8.15 (s, 1 H), 4.11 (t, 2 H), 3.80 (t, 2 H), 2.97 (m, 2 H), 2.39 (m, 2 H),

1.73 (m, 2 H), 0.98 (t, 3 H). ^{13}C NMR (DMSO-d_6) δ 163.5, 155.5, 148.5 (q, J= 38.3 Hz), 121.8, 120.5, 119.6 (q, J= 271.8 Hz), 116.4, 113.7, 71.9, 32.6, 30.9, 26.4, 22.1, 13.9. MS: $M^+ = 443$.

[5-^{3}H]-6-(3-Bromopropyloxy)-7-propyl-3-trifluoromethyl-benzisoxazole **(4b)**. Compound **3** (3.8 mg, 8.5 μmol) was dissolved in ethanol (0.5 ml), 10% Pd on carbon (2.0 mg) was added and the mixture was treated with tritium gas for 30 min.Volatile tritium compounds were removed by lyophilisation with methanol (3 × 1 ml). Catalyst was removed by filtration and the filtrate was concentrated *in vacuo.* The residue was applied to a 20 × 20 × 0.05 (cm) silica gel TLC plate and eluted with *n*-hexane/ethyl acetate 4:1 (v/v). The strongest UV-band (254 nm) (R_f 0.43) containing compound **4b** was eluted with ethyl acetate, followed by removal of solvent *in vacuo*. The residue was chromatographed on a Merck Lichrosper RP-18 250-10 column with acetonitrile/0.05 M phosphoric acid 73:27 (v/v) (4 ml/min, UV-detection at 254 nm) as eluent. The fraction from 28.3–32.3 min was collected, neutralized with 2 M sodium hydroxide (0.10 ml) and concentrated to a volume of approximately 4 ml.The remaining aqueous phase was extracted with ethyl acetate (3 × 2 ml). Drying over sodium sulphate, filtration and evaporation of solvent *in vacuo* gave [5-^{3}H]-6-(3-bromopropyloxy)-7-propyl-3-trifluoromethyl-benzisoxazole **(4b)**, which was dissolved in ethanol (5.00 ml). The analysis of this solution showed a total radioactivity of 2990 MBq and a radiochemical purity of 99%. For use in the following reaction ethanol was removed *in vacuo*, and compound **4b** was transferred to a 1 ml vial with ethyl acetate followed by removal of solvent in a gentle stream of argon. Unlabelled 6-(3-bromopropyloxy)-7-propyl-3-trifluoromethyl-benzisoxazole[2] **(4a)** - used as a reference compound - had the following spectroscopic data: ^{1}H NMR (DMSO-d_6) δ 7.76 (d, 1 H), 7.39 (d, 1 H), 4.29 (t, 2 H), 3.72 (t, 2 H), 2.87 (m, 2 H), 2.34 (m, 2 H), 1.65 (m, 2 H), 0.91 (t, 3 H). ^{13}C NMR (DMSO-d_6) δ 164.0, 158.9, 149.0 (q, J= 37.8 Hz), 120.1 (q, J= 271.3 Hz), 119.0, 112.9, 112.1, 109.9, 67.0, 31.8, 31.0, 25.0, 21.7, 13.8. MS: $M^+ = 465$.

3-*Chloro-4-(3-(7-propyl-3-trifluoromethyl-[5-^{3}H]-benzisoxazol-6-oxy)-propylsulphanyl)phenylacetic acid* **(5b)** *(cf.* reference[2]*)*. 3-Chloro-4-(dimethylaminocarbonylsulphanyl)-phenylacetic acid methyl ester[4] (6.4 mg, 0.022 mmol) was stirred under argon with a solution of 0.5 M sodium methoxide in methanol (60 μl, 0.030 mmol) at 63°C for

70 min.After cooling to room temperature this solution was transferred to the vial containing [5-^{3}H]-6-(3-bromopropyloxy)-7-propyl-3-trifluoromethyl-benzisoxazole **(4b)**, (2990 MBq) in an argon atmosphere (see above). The mixture was stirred at 63°C for 1 h. An aqueous solution of 1 M lithium hydroxide (30 µl, 0.030 mmol) was added and stirring was continued at 63°C for 15 min. After cooling to room temperature acetonitrile/0.05 M phosphoric acid 73:27 (v/v) (1 ml) was added and the product was isolated by preparative HPLC on a Merck Licrospher RP-18 column (250 mm x 10 mm) with acetonitrile/0.05 M phosphoric acid 73:27 (v/v) (4 ml/min, 254 nm). The eluate containing the desired product (20.0–22.5 min, 10 ml) was concentrated to a volume of approx. 2 ml and extracted with ethyl acetate (4 × 1 ml). The combined organic phases were dried over sodium sulphate and solvent was removed *in vacuo*. The residue was dissolved in ethanol (5 ml) and this solution of **5b** was characterized by liquid scintillation counting and HPLC (by comparison with authentic unlabelled compound[2]). Radiochemical yield: 2270 MBq (61 mCi); specific activity: 529 GBq/mmol, (14.3 Ci/mmol); radiochemical purity: 98%.

Acknowledgements

We are indebted to Grethe Aagaard (NMR), Malene Mohr (MS), Dr. Karen Margrethe Engell, Dr. Lene Merete Hansen and Peter Anker Jørgensen (HPLC) (Leo Pharma A/S) and to Dr. Ewald Bannwart (catalytic tritiation) (RC TRITEC AG, Teufen, Switzerland) for invaluable assistance.

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