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Synthesis of a Radiolabelled Retinoid X Receptor (RXR) Specific Ligand

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SUMMARY

Synthesis of 4-[2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)-[1,3]dioxolan-2-yl]-benzoic acid or SR11237, a specific ligand of Retinoid X Receptors, is described in a ^3H-labelled version. The labelled retinoid was prepared in 5 steps and tritium was introduced through a final reduction of an aromatic dihalide moiety with $^3H_2$.

Key words: retinoid, SR11237, tritium, synthesis.

INTRODUCTION

Retinoids, natural and synthetic analogues of vitamin A, regulate a broad range of biological processes through two subfamilies of nuclear retinoid receptors, the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs)[1-7]. In order to investigate upon the ligand-induced dissociation of RXR tetramers, controlling the formation of active RXR homo- and heterodimers, a labelled RXR specific ligand with high specific activity is required. 4-[2-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)-[1,3]dioxolan-2-yl]-benzoic acid **1**, known as SR11237, is a drug candidate that was previously described for its specific binding to RXR[8]. In this paper we wish to report the synthesis of ^3H-labelled SR11237.

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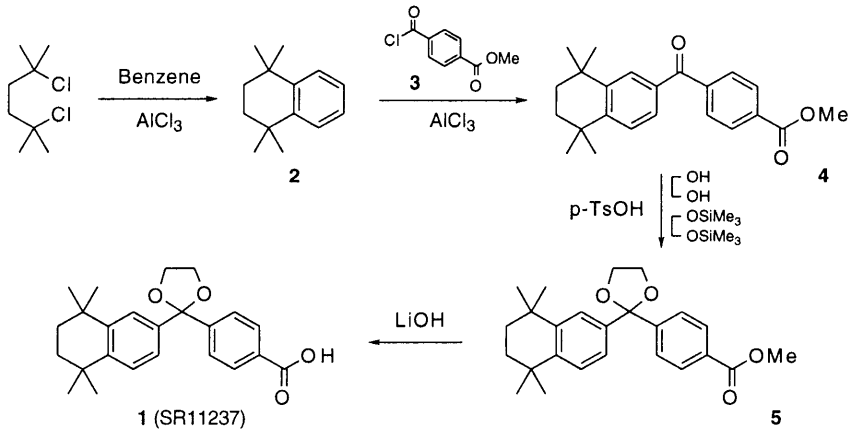
Results and Discussion

The synthesis of SR11237 has been published recently[9]. It involves a double Friedel-Crafts alkylation of benzene with 2,4-dimethyl-2,4-dichlorohexane to yield **2** (Scheme 1). A Friedel-Crafts acylation of **2** with 4-chlorocarbonyl-benzoic acid methyl ester **3** affords 4-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-naphthalene-2-carbonyl)-benzoic acid methyl ester **4**. Subsequent ketalization with ethylene glycol leads to 4-[2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)-[1,3]dioxolan-2-yl]-benzoic acid methyl ester **5** that is further hydrolyzed into SR11237, **1**.

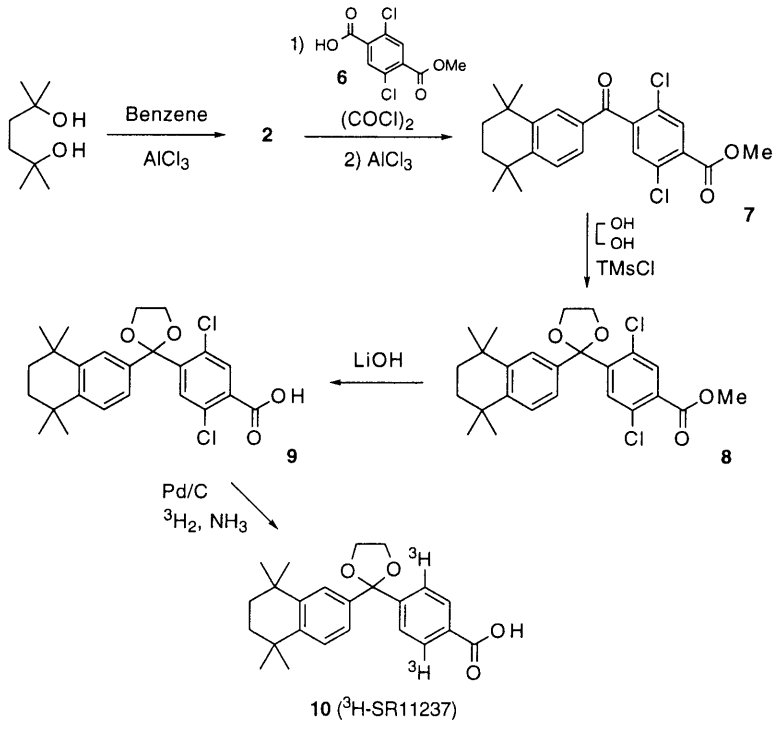


Scheme 1

Tritium labelling can be conveniently introduced onto the SR11237 molecule by catalytic reduction with $^3H_2$ of an aryl halide precursor. Consequently, the previous synthesis was modified in the second step. Condensation of 2,5-dichloro-4-chlorocarbonyl-benzoic acid methyl ester, obtained from the corresponding carboxylic acid **6**, with tetramethyl-tetrahydronaphthalene **2**†, provided bis-halide **7** (Scheme 2). The experimental conditions for the acylation reaction were slightly different from those described for the synthesis of compound **4** (reagents were brought into contact in a different sequence) and allowed to achieve a higher yield. Compound **7** was ketalized into **8** using a modification the Noyori's procedure[11]. The choice of the solvent proved to be crucial for the progress of this reaction, and the use of THF did not afford the expected compound. Furthermore to achieve a reasonable yield a larger quantity of trimethylsilyl chloride had to be used. The resulting methyl ester **8** was hydrolyzed with lithium hydroxide in aqueous DME to yield the dichloro

† The preparation of compound **2** from 2,4-dimethyl-2,4-hexanediol proved to be more efficient than a previously published procedure using 2,4-dimethyl-2,4-dichlorohexane[10]

carboxylic acid **9**, which was then reduced over Pd/C with tritium gas to afford ^{3}H-SR11237 **10**. When the reduction was performed in the absence of aqueous ammonia, removal of the ketal ring occured and ^{3}H-labelled diaryl ketone was obtained in a nearly quantitative yield.



Scheme 2

Conclusion

A ^{3}H-labelled version of retinoid SR11237 has been synthesized. Investigations on the ligand-induced dissociation of RXR tetramers are currently under progress and results will be published in due course.

Experimental

General. THF and Et_2O were distilled over Na/benzophenone and CH_2Cl_2 over CaH_2, just before use. Reactions were monitored by TLC (*Merck* precoated plates 0.25 mm, silica gel 60 F_{254}, 0.040-0.060 mm, 230-400 mesh ASTM). Liquid chromatography was performed on silica gel 60 (*Merck*, 0.040-0.060 mm, 230-400 mesh ASTM). ^{1}H- and ^{13}C-NMR spectra

were recorded on *Bruker-WP-200-Sy* and *Bruker-Avance-DPX-300* spectrometers, chemical shifts δ in ppm are relative to an internal reference (^{1}H: $CHCl_3$ at 7.27 ppm or CD_2HOD at 3.31 ppm; ^{13}C: $CDCl_3$ at 77.0 ppm or CD_3OD at 49.0 ppm). Coupling constants *J* are in Hz. ^{3}H-NMR spectra were recorded on a *Bruker* AC300 spectrometer at 320 MHz with broadband proton decoupling. The radioactivity of the tritiated samples was counted on a *Wallac 1409* apparatus. IR spectra were recorded on a *Perkin-Elmer-1600-FT* spectrometer and absorption values ν are in cm^{-1}. Mass spectra were obtained on a *Finnigan-4600* quadrupole instrument.

1,1,4,4-Tetramethyl-1,2,3,4-tetrahydro-naphthalene (**2**): $AlCl_3$ (95 mg, 0.70 mmol) is added to 2,4-dimethyl-2,4-hexanediol (100 mg, 0.68 mmol) in anhydrous benzene (3 mL) at 0-5 °C. The reaction mixture is stirred at room temperature for 40 min before a second portion of $AlCl_3$ (100 mg, 0.74 mmol) is added at 0-5 °C. The resulting suspension is vigorously stirred for 2 h at room temperature, and 6 h more at 55 °C. The brown solution obtained is pourred into iced HCl 0.4 N (15 mL) and the resulting yellowish solution is extracted with ether. The organic layer is successively washed with water, saturated aqueous $NaHCO_3$ solution, and brine, and dried over magnesium sulfate. Solvent is removed *in vacuo* to yield analytically pure tetramethyltetrahydronaphthalene (126 mg, 99 %). TLC (hexane): R_f 0.4. ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.32 (*dd*, *J* = 3.5, 43.8, 2H), 7.31 (*td*, *J* = 3.5, 28.5, 2H), 1.79 (*s*, 4H), 1.39 (*s*, 12H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 144.69, 126.44, 125.51, 35.10, 34.17, 31.89. IR ($CHCl_3$): 2964.9, 2928.0, 1486.9, 1456.3, 1385.7, 1362.8, 755.8. CI-MS (NH_3): 173 $[M-CH_4+H]^+$, 187 $[M-H_2+H]^+$, 204 $[M-H_2+NH_4]^+$.

2,5-Dichloro-terephthalic acid monomethyl ester (**6**): 2,5-Dichloro-terephthalic acid dimethyl ester (2.0 g, 7.45 mmol) and LiOH (36 mg, 1.49 mmol) are vigorously stirred in DME/H_2O 9:1 (25 mL) for 2 h at room temperature. Excess dimethyl ester is extracted with CH_2Cl_2 and the aqueous solution is acidified to pH 1-2, and washed with AcOEt. The organic layer is dried over $MgSO_4$, and removal of the solvent affords analytically pure compound **6** (0.29 g, 17 %†) as a white solid. TLC ($CHCl_3$/EtOH 7:3): R_f 0.4. ^{1}H-NMR ($CDCl_3$, 200 MHz): 8.09 (*s*, 1H), 7.95 (*s*, 1H), 3.98 (*s*, 3H). ^{13}C-NMR (CD_3OD, 50 MHz): 166.61, 165.55, 135.95, 135.60, 134.53, 134.24, 132.60, 53.41. IR ($CHCl_3$): 3100.4, 1732.2, 1712.8, 1688.1, 1479.0, 1301.0, 1249.2, 1081.9.

2,5-Dichloro-4-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-naphthalene-2-carbonyl)-benzoic acid methyl ester (**7**): Compound **6** (150 mg, 0.64 mmol) in anhydrous benzene (3 mL) is treated

with oxalyl chloride (0.3 mL, 3.37 mmol) and DMF (cat., 1 μL) for 1 h till gas evolution has ceased. Solvent is removed *in vacuo* and the residue is evaporated twice with benzene (3 mL). Methylene chloride (1.5 mL) and tetramethyltetrahydronaphthalene **2** (165 mg, 0.64 mmol) in CH_2Cl_2 (1.5 mL) are added, followed with $AlCl_3$ (190 mg, 1.40 mmol). The reaction mixture is stirred for 20 h at room temperature, quenched with dilute HCl at 0 °C, and extracted with CH_2Cl_2. The organic layer is dried, reduced *in vacuo* and the crude residue is purified by chromatography on silica gel (Et_2O/hexane: from 0:10 to 1:9) to yield diaryl ketone **7** (170 mg, 64 %) as white crystals. TLC (Et_2O/hexane 1/9): R_f 0.4. ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.95 (*s*, 1H), 7.85 (*d*, *J* = 1.8, 1H), 7.44 (*dd*, *J* = 1.8, 8.4, 1H), 7.44 (*s*, 1H), 7.40 (*d*, *J* = 8.4, 1H), 3.98 (*s*, 3H), 1.71 (*s*, 4H), 1.30 (*s*, 6H), 1.28 (*s*, 6H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 192.42, 164.35, 152.51, 145.84, 142.69, 132.64, 132.30, 131.72, 131.09, 129.50, 128.49, 127.45, 127.10, 52.86, 34.84, 34.55, 34.38, 31.66, 31.49. IR ($CHCl_3$): 2959.9, 2923.1, 2861.5, 1742.0, 1672.2, 1599.4, 1460.3, 1351.6, 1285.9, 1241.5, 1124.2, 1086.8, 1007.9. CI-MS (NH_3): 419 $[M+H]^+$, 436 $[M+NH_4]^+$.

2,5-Dichloro-4-[2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)-[1,3]dioxolan-2-yl]-benzoic acid methyl ester (**8**): Diaryl ketone **7** (75 mg, 0.18 mmol) and ethylene glycol (2 mL) in chloroform (0.5 mL) are treated with TMSCl (0.2 mL, 1.54 mmol) at room temperature for 24 h. Ether is added and the solution is washed with a diluted aqueous $NaHCO_3$ solution, water, and brine. The organic layer is dried, reduced under vacuum and purified by column chromatography (Et_2O/hexane 1/9) to yield the expected ketal **8** (59 mg, 71 %) and intact starting material (19 mg, 25 %). TLC (Et_2O/hexane 1:9): R_f 0.35. ^{1}H-NMR ($CDCl_3$, 300 MHz): 8.00 (*s*, 1H), 7.82 (*s*, 1H), 7.48 (*d*, *J* = 1.9, 1H), 7.24 (*d*, *J* = 8.3, 1H), 7.08 (*dd*, *J* = 1.9, 8.3, 1H), 4.21-4.13 (*m*, 2H), 4.09-4.00 (*m*, 2H), 3.94 (*s*, 3H), 1.66 (*s*, 4H), 1.25 (*s*, 12H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 164.61, 145.09, 144.25, 143.97, 135.94, 134.09, 131.87, 131.09, 130.42, 130.28, 126.29, 124.24, 123.38, 108.45, 65.16, 52.63, 34.95, 34.26, 34.09, 31.69. IR ($CHCl_3$): 2961.1, 1740.3, 1460.7, 1352.8, 1286.1, 1242.3, 1078.2, 999.6. CI-MS (NH_3): 463 $[M+H]^+$.

2,5-Dichloro-4-[2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)-[1,3]dioxolan-2-yl]-benzoic acid (**9**): Ester **8** (83 mg, 0.18 mmol) and LiOH (16 mg, 0.65 mmol) are stirred in DME (1.5 mL) with water (50 μL) at room temperature for 20 h. The solution is acidified to pH 2 and extracted with AcOEt. The organic layer is washed with water, brine, and is dried and reduced *in vacuo*. The residue is purified by preparative TLC ($CHCl_3$/EtOH 7:3) to yield

† Remaining starting material (87 %) is fully recovered during the first extraction with CH_2Cl_2.

the free acid **9** (62 mg, 80 %). TLC ($CHCl_3$/EtOH 7:3): R_f 0.5. ^{1}H-NMR (CD_3OD, 300 MHz): 7.81 (*s*, 1H), 7.41 (*s*, 1H), 7.39 (*d*, *J* = 1.9, 1H), 7.25 (*d*, *J* = 8.3, 1H), 7.08 (*dd*, *J* = 1.9, 8.3, 1H), 4.13-4.05 (*m*, 2H), 4.02-3.95 (*m*, 2H), 1.68 (*s*, 4H), 1.25 (*s*, 6H), 1.21 (*s*, 6H). ^{13}C-NMR (CD_3OD, 75 MHz): 165.67, 145.97, 145.11, 141.50, 138.37, 132.20, 132.05, 130.49, 129.64, 127.14, 125.75, 124.93, 109.81, 66.10, 36.15, 35.19, 35.04, 32.19. IR ($CHCl_3$): 3405.5, 2953.3, 2924.1, 2514.3, 1581.6, 1460.0, 1391.4, 1094.7, 1077.0, 996.8. CI-MS (NH_3): 449 $[M+H]^+$, 405 $[M-CO_2+H]^+$.

2,5-$^3H_2$-4-[2-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)-[1,3]dioxolan-2-yl]-benzoic acid (**10**): 2,5-Dichloro-4-[2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)-[1,3]dioxolan-2-yl]-benzoic acid **9** (3 mg, 6.7 10^{-3} mmol) and ammonia (30% solution in water, 10 µL, 53 10^{-3} mmol) were dissolved in methyl alcohol (1.5 mL). After addition of 10% Pd/C (7 mg) tritium gas (20 Ci) was introduced in the flask using a Toeppler pump. The suspension was stirred at room temperature for 3 h. Catalyst was filtered off (Millex 0.22 µm) and washed with methyl alcohol (3 mL), and the resulting solution was reduced *in vacuo*. The residue was coevaporated with MeOH (3 x 3 mL) to remove labile tritium yielding the crude compound **10** (total radioactivity 118 mCi, 62 %). Purification was achieved by HPLC (column Zorbax ODS, 4.6 x 250 mm, acetonitrile/water/triethylamine 30:70:0.1, 3.0 mL/min) and yielded **10** (65 mCi). Radiochemical purity was checked by HPLC (retention time: 15.54 min) and by TLC (chloroform/ethanol 9:1, R_f 0.4). Specific radioactivity (21.6 Ci/mmol) was determined by mass spectrometry (CI/NH_3). ^{1}H-NMR ($CDCl_3$, 300 MHz): The NMR spectra of **10** is in agreement with that of an authentic sample[9]. ^{3}H-NMR ($CDCl_3$, 320 MHz): 9.05 (46 % intensity); 7.65 (54 % intensity).

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