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Research Article

Synthesis and characterization of tritium labeled 2,3,4,9-tetrahydro-1*H*-carbazoles as potent DP receptor antagonists

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Summary

Tritium labeled 2,3,4,9-tetrahydro-1*H*-carbazole **1a** and **2a** were prepared in good yields with a specific activity of 7.0 Ci/mmol (214 GBq/mmol) and 4.2 Ci/mmol (155 GBq/mmol), respectively. Both compounds have been synthesized in high radiochemical purity by catalytic tritium–bromine exchange of the corresponding aryl bromide precursors. The 6-bromocarbazole precursors **7** and **8** were prepared as a mixture by a three step process, involving regioselective bromination of **3c** with pyridinium tribromide, oxidation of thioether **4c** using *m*-CPBA and hydrolysis of acylsultamcarbazole **5c**. Finally, HPLC separation of the enantiomers afforded the 6-bromo precursors **7** and **8** in high diastereomeric ratio (dr 99% and dr 93% respectively). Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: tritium; labeled compounds; tritium–bromine exchange; tetrahydrocarbazole; DP receptor antagonists

Introduction

Radiolabeled compounds provide unique tools to dissect metabolic pathways, identify molecular targets and determine protein binding parameters. In this aspect, the position of the radioactive label is selected based on the molecule's metabolism profile. In this regard, a tritium labeled compound offers many advantages over its carbon-14 counterpart, mainly because of its high specific radioactivity and relative ease of preparation. During the course of our work, compound **1c** and **2c** proved to be potent prostaglandin D_2 (DP) receptor antagonists with K_i values of 2.5 nM and 1.7 nM respectively (Presented at the

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39th IUPAC Congress and the 86th Conference of the Canadian Society for Chemistry, August 10–15, 2003, Ottawa, Canada). As a result, we became interested in developing synthetic routes to prepare tritium-labeled 2,3,4,9-tetrahydro-1*H*-carbazoles, and we report herein a new and efficient radiosynthesis for such compounds.

Results and discussion

Tritium labeled compounds **1a** and **2a** were prepared in good yields with high radiochemical purity from the corresponding 6-bromocarbazole precursors (Figure 1). Attempts to directly brominate compounds **1c** or **2c** were unsuccessful despite the fact that we tried a variety of bromination conditions (Br_2, NBS, pyridinium tribromide, Br_2/Pyridine). To overcome this problem, we studied the bromination of the corresponding thioether **3**. Attempts at bromination of compounds **3a** (acid) and **3b** (methyl ester) failed to furnish any of the desired 6-bromocarbazole **4** (Scheme 1). However, the acylsultamcarbazole **3c**, which was originally synthesized to facilitate the HPLC separation of compound **3** enantiomers, reacted smoothly with pyridinium tribromide to afford the desired product **4c** in quantitative yield (For mechanistical details on indole bromination and limitations, see ref. 2). Subsequent oxidation of the thioether **4c** with *m*-CPBA afforded a 1:1 mixture of sulfoxide diastereomers **5c** in 80% yield. The acylsultamcarbazole diastereomers **5c** were then hydrolyzed with lithium hydroxide in a mixture of THF and water to obtain the corresponding acid mixture **6** in 70% yield. Separation of the diastereomers (**7**, **8**) was then carried out using flash chromatography and normal phase preparative HPLC.

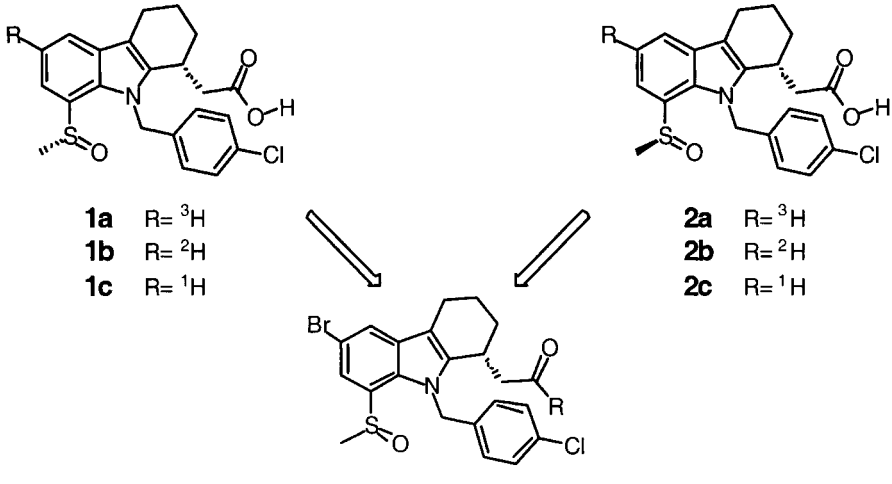
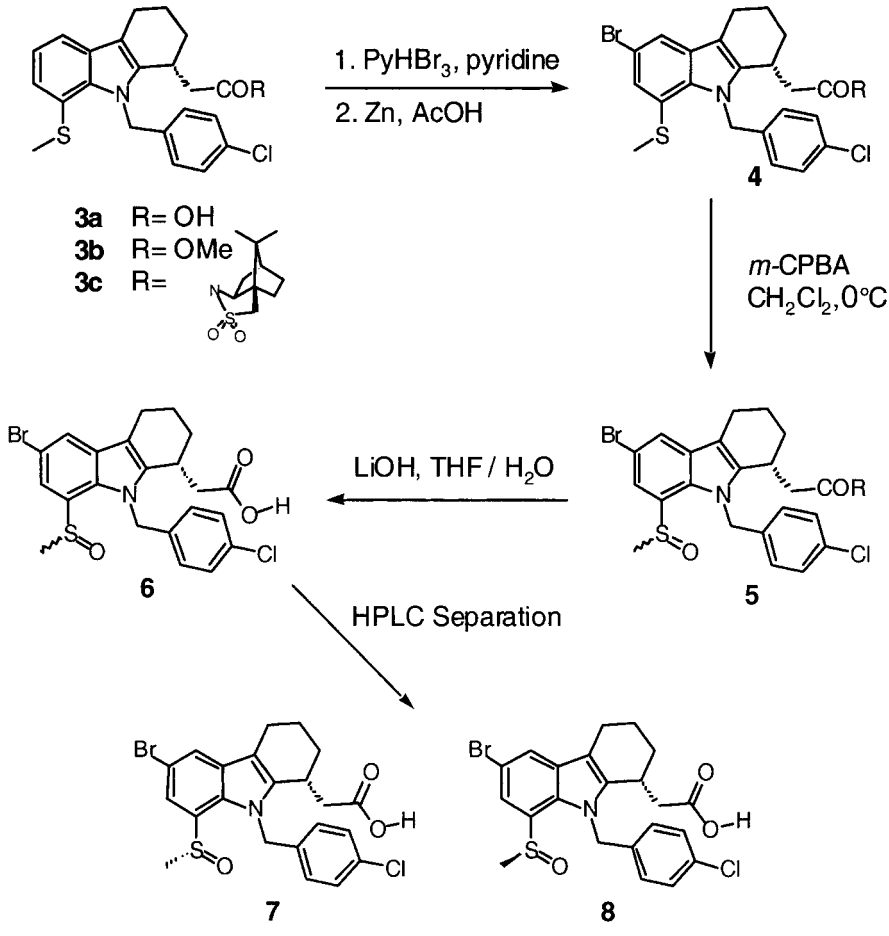
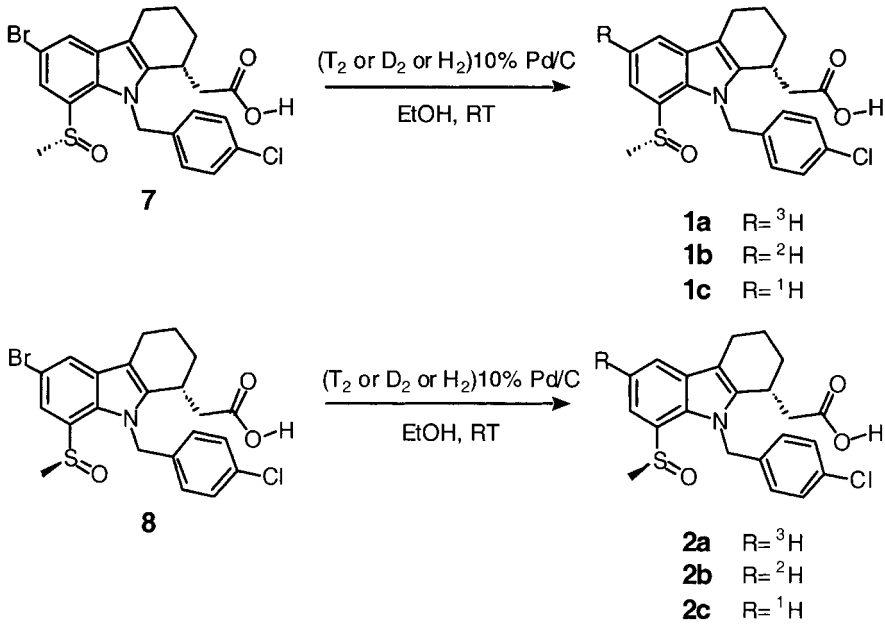


Figure 1.



Scheme 1.

Having in hand compounds **7** and **8** in high diastereomeric ratio (dr 99% and dr 93% respectively), we next investigated the catalytic deuterium–halogen exchange of the 6-bromocarbazole **7** (Scheme 2). Evaluation of deuterium incorporation by mass spectrometry indicated that 76% of compound **1b** was effectively labeled with deuterium and the remaining 24% with hydrogen. We postulated that ethanol may play an important role in donating hydrogen to the palladium in this reaction. Further modifications of the reaction conditions (solvent, catalyst, temperature, time) failed to improve the level of deuterium incorporation. Selective exchange of the aromatic bromine with tritium gas and 10% Pd/C in ethanol proceeded smoothly to give crude **1a** and **2a**. After purification, the radiochemical purity was determined to be 99 and 93% with a specific radioactivity of 7.0 and 4.2 Ci/mmol, respectively for compounds **1a** and **2a**. Tritium incorporation evaluated by mass spectrometry indicated that 24% of compound **1a** was labeled with



Scheme 2.

tritium. This result was consistent with the observed 7.0 Ci/mmol (24% of 28.8 Ci/mmol in theory) calculated by HPLC using a non-radioactive standard and a liquid scintillation counter. Despite the fact that precursors **7** and **8** contain a chlorine atom on the benzyl substituent, this halogen reacts much slower than bromine in this catalytic dehalogenation reaction and we observe only trace amounts of the tritiated benzyl analogs.

Experimental

General

All reagents and solvents are commercially available and used directly without any purification. Reactions were performed in a well ventilated fume-hood and the tritiation was done with a Tri-Sorber instrument (TS-1000) from IN/US systems. Tritium gas was released from the primary bed containing 100 Ci of tritium gas stored in the form of U^3H_3 by heating the uranium bed to 500°C. Evaporations were carried out on a Buchi evaporator *in vacuo* at bath temperatures less than 35°C. TLC was performed on Merck kGaA F_{254} precoated silica plates. Analytical HPLC was performed on a Waters instrument equipped with a Zorbax C_{18} column (4.6 × 150 mm) and peak detection was done simultaneously by UV (254 nm, Waters 996 Photodiode Array detector) and a liquid scintillation flow monitor (Packard Radiomatic 150 TR Flow Scintillation Analyzer). Tritium and deuterium incorporation were determined on a Micromass Quattro LC (electrospray mode) coupled

with a Waters 2790 HPLC and a RadioHPLC detector (β-ram model 3, IN/US) using Flo-Scint II (Packard Bioscience #6013529) as liquid scintillation cocktail. Radioactivity measurements were performed on a Beckman Coulter LS6000SC liquid scintillation counter using Ultima Gold XR (Packard Bioscience #6013119) scintillant. A Bruker 500 MHz instrument was used for proton NMR characterization and chemical shifts are reported in ppm downfield from TMS.

*(1R)-6-bromo-9-(4-chlorobenzyl)-1-{2-[(3aR)-8,8-dimethyl-2,2-dioxidotetrahydro-3a,6-methano-2,1-benzisothiazol-1(4H)-yl]-2-oxoethyl}-8-(methylthio)-2,3,4,9-tetrahydro-1H-carbazole (**4c**)*

Compound **3c** (500 mg, 0.84 mmol) was dissolved in 7 ml of pyridine at 0°C and pyridine tribromide (670 mg, 2.10 mmol) was added under nitrogen. The cooling bath was removed and the reaction was stirred for 30 min. Evaporation of the pyridine to dryness under high vacuum was done prior to addition of 7 ml of AcOH. The reaction mixture was re-cooled to 0°C and zinc dust (220 mg, 3.4 mmol) was added. The reaction mixture was stirred overnight. Quenching of the reaction was done by adding 10 ml of HCl 10%, 100 ml of EtOAc and stirred for 30 min. The reaction was extracted twice with 50 ml of EtOAc, washed with brine, dried over Na_2SO_4 and evaporated. The compound **4c** was obtained in 99% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.32 (d, 2H), 7.20 (d, 1H), 6.95 (d, 1H), 6.72 (d, 2H), 6.05 (d, 1H), 5.60 (d, 1H), 3.85 (m, 2H), 3.62 (d, 1H), 3.22 (m, 2H), 2.95-2.80 (m, 2H), 2.50 (m, 1H), 2.28 (s, 3H), 1.90-1.60 (m, 9H), 1.42 (t, 1H), 1.21 (m, 1H), 1.04 (s, 3H), 0.92 (s, 3H). Mass spectrum for $C_{32}H_{36}N_2O_3S_2BrCl$: (M-H) 675.4.

*(1R)-6-bromo-9-(4-chlorobenzyl)-1-{2-[(3aR)-8,8-dimethyl-2,2-dioxidotetrahydro-3a,6-methano-2,1-benzisothiazol-1(4H)-yl]-2-oxoethyl}-8-(methylsulfinyl)-2,3,4,9-tetrahydro-1H-carbazole (**5c**)*

Compound **4c** (360 mg, 0.53 mmol) was dissolved in 10 ml of CH_2Cl_2 under nitrogen. Addition of *m*CPBA (114 mg, 0.56 mmol) was done at 0°C and the mixture was allowed to stir for 30 min. The reaction was then quenched by addition of $NaHCO_3$ sat., extracted twice with 20 ml of CH_2Cl_2, washed with brine, dried over Na_2SO_4 and evaporated. Product **5c** was obtained in 80% yield (1:1 mixture of sulfoxide) after a quick purification through a pad of silica gel and used directly for the next step.

*[(1R)-6-bromo-9-(4-chlorobenzyl)-8-(methylsulfinyl)-2,3,4,9-tetrahydro-1H-carbazol-1-yl]acetic acid (**6**)*

Hydrolysis was performed on compound **5c** (150 mg, 0.22 mmol) by addition of lithium hydroxide (260 µl, 2N soln) at 25°C in a 5:1 mixture of THF and water. The reaction was stirred overnight and then quenched by adding HCl

2N until pH ~3. The reaction mixture was extracted twice with 50 ml of EtOAc, washed with brine, dried over Na_2SO_4 and evaporated. Purification of the crude compound **6** by flash chromatography using a gradient from 0 to 100% EtOAc in toluene and 2% AcOH afforded the desired 6-bromocarbazole precursors **7** and **8** in 70% yield. Further purification by preparative HPLC using μPorasil column (2 × 25 cm) at a flow rate of 3.8 ml/min with a mixture of solvent (20% *i*PrOH/80% Hexanes/0.2% AcOH) at 275 nm afforded compound **7** (RT = 6.2 min, dr 99%) and compound **8** (RT = 8.1 min, dr 93%). Mass spectrum for $C_{22}H_{21}NO_3SBrCl$: (M-H) 494.3.

*[(1R)-6-bromo-9-(4-chlorobenzyl)-8-((R)methylsulfinyl)-2,3,4,9-tetrahydro-1H-carbazol-1-yl]acetic acid (***7***)*

^{1}H NMR (500 MHz, acetone-d_6) δ 11.2 (bs, 1H), 7.63 (d, 1H), 7.45 (d, 1H), 7.34 (d, 2H), 6.81 (d, 2H), 5.80 (d, 1H), 5.45 (d, 1H), 3.42 (m, 2H), 2.91 (m, 1H), 2.57 (m, 2H), 2.30 (s, 3H), 1.93-1.81 (m, 4H).

*[(1R)-6-bromo-9-(4-chlorobenzyl)-8-((S)methylsulfinyl)-2,3,4,9-tetrahydro-1H-carbazol-1-yl]acetic acid (***8***)*

^{1}H NMR (500 MHz, acetone-d_6) δ 11.2 (bs, 1H), 7.61 (d, 1H), 7.45 (d, 1H), 7.27 (d, 2H), 6.74 (d, 2H), 5.63 (d, 1H), 5.37 (d, 1H), 3.35 (m, 3H), 2.64 (d, 2H), 2.60 (s, 3H), 1.93 (m, 2H), 1.81 (m, 2H).

*[(1R)-6-^{3}H-9-(4-chlorobenzyl)-8-((R)methylsulfinyl)-2,3,4,9-tetrahydro-1H-carbazol-1-yl]acetic acid (***1a***)*

Pd/C (10%, 6.0 mg) was added to a solution of compound **7** (1.8 mg, 2.6 μmol) in EtOH (0.5 ml). The reaction mixture was degassed by four freeze/thaw cycles and then stirred overnight with tritium gas (3 Ci). Excess tritium was re-adsorbed on the secondary uranium bed followed by filtration of the reaction through a short pad of celite. Co-evaporation with EtOH (3 × 3 ml) in order to remove labile tritium was done prior to purification by HPLC. Preparative HPLC using a Novapak C_{18} column (7.8 × 300 mm) with a mixture of solvent (82% MeOH/18% H_2O/0.1% AcOH) at a flow rate of 4.5 ml/min afforded the desired compound **1a** (RT = 9 min) in 99% radiochemical purity (analytical HPLC measurements) with a specific activity of 7.0 Ci/mmol. Unlabelled (**1c**), used as a reference compound, had the following spectroscopic data: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.71 (d, 1H), 7.65 (d, 1H), 7.35 (d, 2H), 7.27 (t, 1H*), 6.72 (d, 2H), 5.62 (d, 1H), 5.38 (d, 1H), 2.80 (d, 1H), 2.65-2.50 (m, 1H), 2.38-2.28 (m, 2H), 2.35 (s, 3H), 1.92-1.75 (m, 4H). *denotes the aromatic proton at the 6-position of the 2,3,4,9-tetrahydrocarbazole. Optical rotation: +121.3° (c = 0.39 in methanol).

[(1R)-6-^{3}H-9-(4-chlorobenzyl)-8-((S)methylsulfinyl)-2,3,4,9-tetrahydro-1H-carbazol-1-yl]acetic acid (**2a**)*

Compound **2a** was prepared in the same way as compound **1a**. Preparative HPLC using a Novapak C_{18} column with a mixture of solvent (90% MeOH/10% H_2O/0.1% AcOH) at a flow rate of 4.5 ml/min afforded the desired compound **2a** (RT = 13 min) in 93% radiochemical purity (analytical HPLC measurements) with a specific activity of 4.2 Ci/mmol. Unlabelled (**2c**), used as a reference compound, had the following spectroscopic data: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.66 (d, 1H), 7.63 (d, 1H), 7.34 (d, 2H), 7.28 (t, 1H*), 6.69 (d, 2H), 5.42 (d, 1H), 5.24 (d, 1H), 3.20 (d, 1H), 2.8 (d, 1H), 2.68-2.54 (m, 2H), 2.58 (s, 3H), 2.47-2.39 (m, 1H), 1.90-1.75 (m, 4H). *denotes the aromatic proton at the 6-position of the 2,3,4,9-tetrahydrocarbazole. Optical rotation: -231.9° ($c = 0.31$ in methanol).

Conclusion

In summary, we have developed an efficient synthetic pathway for the synthesis of tritium labeled 2,3,4,9-tetrahydro-1*H*-carbazoles **1a** and **2a** by use of a catalytic tritium–halogen exchange reaction on the corresponding 6-bromocarbazole precursors. A three step sequence was performed to afford the 6-bromocarbazole precursors in good overall yields. Both tritiated compounds **1a** and **2a** were obtained in high radiochemical and diastereomeric purity as well as in high radiospecific activity. Covalent binding assays and metabolism studies were performed on both compounds and these results will be reported in due course.

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