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Research Article

Practical (^{14}C)-synthesis of molecules containing an acetic acid moiety: application to (^{14}C)-labeled DP1 antagonists

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Abstract: Efficient carbon-14 labeling of four potent and selective DP1 antagonists is reported. The synthetic sequence began with α-hydroxylation, reduction of an ester, followed by oxidative diol cleavage and aldehyde reduction. The resulting alcohol **4** was converted to a mesylate then nucleophilic substitution with [^{14}C]-sodium cyanide was performed to yield a nitrile, which upon basic hydrolysis provided the carbon-14 labeled acid **1**. Compound **2** was obtained from the same alcohol intermediate **4** and two diastereomeric compounds **6** and **7** were easily prepared from compound **2**. Carbon-14 synthesis of compounds **1**, **2**, **6** and **7** were achieved in good yields, high radiochemical purity (>99%) and with high specific activity (45 mCi/mmol). Copyright © 2006 John Wiley & Sons, Ltd.

Keywords: [^{14}C]-synthesis; labeled compounds; tetrahydrocyclopenta[*b*]indol-3-yl acetic acid; DP1 antagonists

Introduction

The importance of labeled compounds has increased dramatically over recent years. It is worth mentioning that every preclinical candidate will have received special attention regarding its own radiosynthesis. In this regard, the specific site of incorporation of the isotope in a molecule is crucial in order to quantitatively trace the parent molecule as well as possible metabolites. Despite the fact that tritium labeled compounds are easier to synthesize and provide higher specific activity, carbon-14 labeled compounds are the most frequently used in drug pharmacokinetics and metabolic studies. The development of short and efficient procedures for the incorporation of carbon-14 in a metabolically stable position is therefore desirable. The introduction of the marker should be done preferably at the latest stage in the synthesis. During the course of our work, compound **1**, **2**, **6**, **7** proved to be potent prostaglandin D1 (DP1) receptor antagonists with Ki values of 1.5, 1.1, 0.9 and 1.0 nM, respectively.[1] Toward this end, we became interested in developing synthetic routes to prepare carbon-14 labeled 1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl acetic acid, and we wish to report herein a novel, short and efficient radiosynthesis for such compounds.

Results and discussion

The procedure described herein involves the removal of one carbon atom, located at the acetic acid moiety end, and its replacement with a carbon-14 atom originating from radiolabeled sodium cyanide. To this end, synthesis of the desired precursor **4** for the introduction of the carbon-14 labeled has been accomplished in few steps and in good yield (Scheme 1). First, the acid **1** is converted to a methyl ester using diazomethane in quantitative yield. An α-hydroxylation is then carried out using KHMDS as a base and Davis reagent[2] as the oxygen source followed by the reduction of the ester moiety with DIBAL in the same reaction flask to afford diol **3** in 70% yield. The next reaction involved an oxidative diol cleavage using sodium periodate to afford the corresponding terminal aldehyde which was then immediately reduced to alcohol **4** using DIBAL in 69% yield. No epimerization at the C_3 center has been observed during this transformation as confirmed by chiral HPLC analysis. This intermediate was used for the synthesis of our desired radiolabeled compounds.

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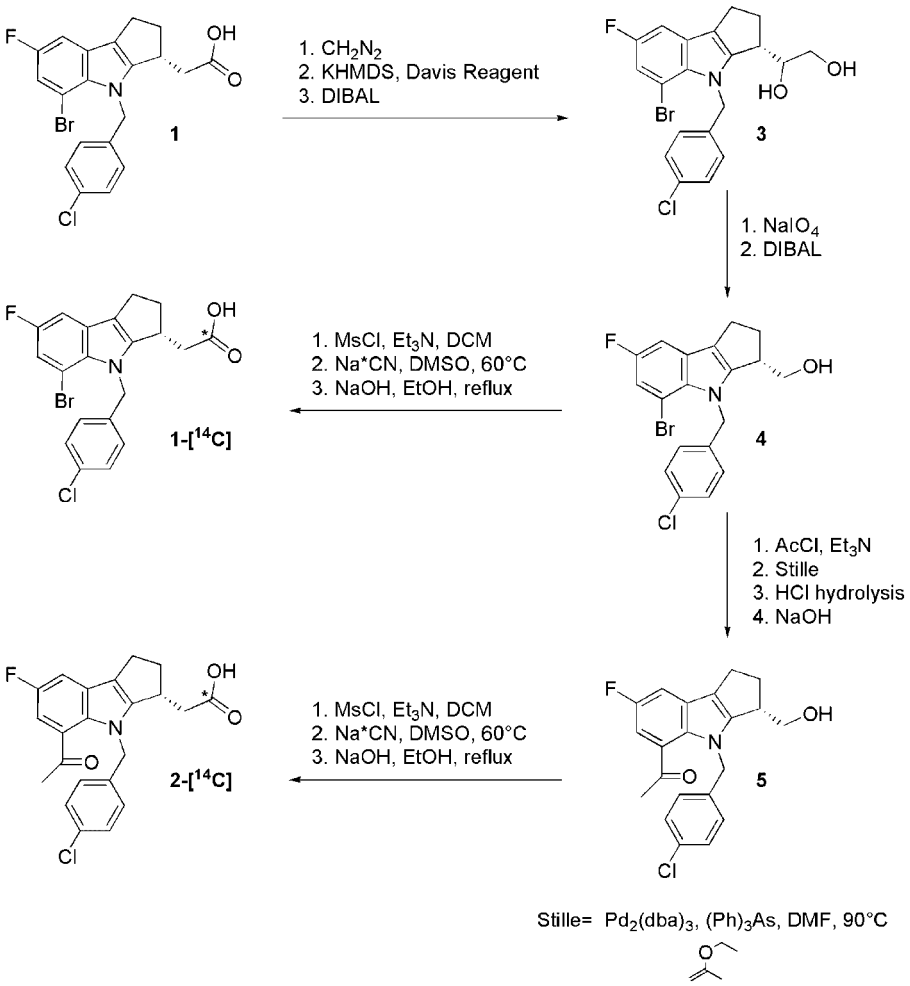

Scheme 1

At this point, the synthesis of radiolabeled compound **1** was accomplished directly in three steps by converting the alcohol **4** to a mesylate, followed by a displacement of the mesylate with [^{14}C]-sodium cyanide in DMSO at 60°C to afford the corresponding nitrile, which was directly hydrolyzed to the acid using sodium hydroxide (5 N) in refluxing ethanol to afford the carbon-14 labeled compound **1** in 70% overall yield.

The synthesis of radiolabeled compound **2** started from the same precursor **4** which was first protected as an acetate under standard conditions in quantitative yield. Secondly, a Stille (1986) coupling was used to introduce the methyl ketone at the 5-position of the core structure and finally the acetate was removed to obtain compound **5** in 67% overall yield for the three steps. Identical procedures as described above (mesylate formation, SN_2 displacement with [^{14}C]-sodium cyanide, hydrolysis) proceeded smoothly and provided carbon-14 labeled compound **2** in 75% yield.

The synthesis of compound **6** and **7** was straightforward since only a ketone reduction and an alcohol methylation were required in order to access both diastereomers (Scheme 2). Carbon-14 labeled **2** was treated first with diazomethane, and then the ketone moiety was reduced with sodium borohydride in methanol to cleanly provide a mixture of the corresponding alcohols (1.5:1), which were directly methylated using sodium hydride and methyl iodide. The ester was hydrolyzed to the acid and finally a separation was necessary to unveil the diastereomeric carbon-14 labeled **6** and **7**. All radiolabeled compounds **1**, **2**, **6**, **7** were obtained in high radiochemical purity (>99%) and with a specific activity of 45 mCi/mmol as determined by analytical HPLC and a radiometric detector. Analysis of the enantiomeric purity at the acetic acid side chain showed that no epimerization had taken place during the synthesis, neither in the SN_2 displacement with [^{14}C]-sodium cyanide nor in the hydrolysis step.

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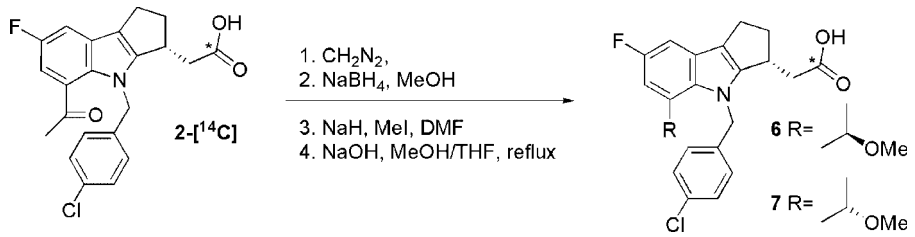

Scheme 2

Conclusion

In summary, we have developed an efficient synthetic pathway for the synthesis of carbon-14 labeled 1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl acetic acids **1**, **2**, **6** and **7**. This mild procedure can be utilized to introduce a carbon-14 label at the acid position of a molecule possessing an acetic acid moiety. The carbon-14 marker is introduced at a late stage and only two steps were required in order to successfully synthesize compounds **1** and **2** in good yields with high radiochemical purity (>99%) and high specific activity (45 mCi/mmol) from [^{14}C]-sodium cyanide. Compounds **6** and **7** were easily obtained from carbon-14 labeled compound **2** using standard chemistry.

Experimental

General

All reagents and solvents are commercially available and used directly without any purification. Davis reagent can be easily obtained according to literature procedure.[3,†] Reactions were performed in a well-ventilated fume-hood. 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. Radioactivity measurements were performed on a Beckman Coulter LS6000SC liquid scintillation counter using Ultima Gold XR. A Bruker 500 MHz instrument was used for NMR characterization and chemical shifts are reported in ppm downfield from TMS.

†The oxaziridine (Davis reagent) is readily prepared by literature procedures from benzaldehyde via an *N*-sulfonylimine.

1-((3R)-5-bromo-4-(4-chlorobenzyl)-7-fluoro-1,2,3,4-tetrahydrocyclopenta(b)indol-3-yl)ethane-1,2-diol (3)

Compound **1** (1.35 g, 3 mmol) was treated with diazomethane (1M/Et_2O) then evaporated to dryness. The resulting methyl ester was dissolved in 10 ml of THF and was slowly added to a solution of KHMDS (7.8 ml, 3.9 mmol, 0.5M/tol) in 30 ml of THF at −78°C under an inert atmosphere of nitrogen. The reaction mixture was stirred for 15 min then Davis reagent (1.57 g, 6 mmol) was added and stirred for 1h. DIBAL (15 ml, 15 mmol, 1M/Hex) was introduced and the reaction mixture was placed at −40°C for 1 h. Quenching of the reaction was done by adding 50 ml of HCl 10%, 100 ml of EtOAc and stirred for 30 min. The reaction was extracted twice with 100 ml of EtOAc, washed with 50 ml of Rochelle's salt, brine, dried over Na_2SO_4 and evaporated. The compound **3** was obtained in 70% yield and used directly for the next step.

((3R)-5-bromo-4-(4-chlorobenzyl)-7-fluoro-1,2,3,4-tetrahydrocyclopenta(b)indol-3-yl)methanol (4)

Compound **3** (910 mg, 2.1 mmol) was dissolved in 20 ml of a mixture of THF/H_2O (3:1) at room temperature and sodium periodate (890 mg, 4.2 mmol) was slowly added. After 15 min, the reaction mixture was quenched with 20 ml of H_2O, extracted twice with 50 ml Et_2O, washed with brine, dried over Na_2SO_4 and evaporated. The crude was redissolved in 10 ml of a mixture of THF/toluene (1:1) and cooled to −78°C prior to the addition of DIBAL (4.2 ml, 4.2 mmol, 1M/Hex). The mixture was stirred at −40°C for 1 h. Quenching of the reaction was done by adding 30 ml of HCl 10%, 50 ml of EtOAc and stirred for 10 min. The reaction was extracted twice with 50 ml of EtOAc, washed with 50 ml of Rochelle's salt, brine, dried over Na_2SO_4 and evaporated. Purification by flash chromatography over silica gel using 40% EtOAc/hexanes afforded the desired compound **4** in 72% yield as a colorless oil. ^{1}H NMR (acetone-d_6): δ 7.29 (d, *J*=8.3 Hz, 2H), 7.17 (d, *J*=8.9 Hz, 1H), 7.06 (d, *J*=8.9 Hz, 1H), 6.90 (d, *J*=8.2 Hz,

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2H), 5.98 (d, J_{AB}=17.8 Hz, 1H), 5.82 (d, J_{AB}=17.8 Hz, 1H), 4.02 (bs, 1H), 3.69–3.65 (m, 2H), 3.31–3.28 (m, 1H), 2.89–2.83 (m, 1H), 2.79–2.74 (m, 1H), 2.67–2.63 (m, 1H), 2.37–2.33 (m, 1H).

((3R)-5-bromo-4-(4-chlorobenzyl)-7-fluoro-1,2,3,4-tetrahydrocyclopenta(b)indol-3-yl)acetic acid (1)

Step 1: Mesylate formation. Compound **4** (45 mg, 0.11 mmol) was dissolved in 2 ml of CH_2Cl_2 at −30°C under an inert atmosphere of nitrogen. Triethylamine (25 µl, 0.18 mmol) was added followed by methanesulfonyl chloride (11 µl, 0.14 mmol) and the reaction was stirred for 30 min. The reaction was allowed to warm-up to room temperature and stirred for 30 min. Quenching of the reaction was performed using 1 ml of a saturated solution of $NaHCO_3$, extraction with 5 ml of CH_2Cl_2 twice, washing with brine, drying over Na_2SO_4 and evaporation. The crude mesylate was directly used for the next step.

Step 2: Cyanide displacement. The mesylate (40 mg, 0.08 mmol) was dissolved in 1 ml of DMSO at room temperature before addition of [^{14}C]-sodium cyanide (3.5 mg, 0.07 mmol). The reaction mixture was heated to 60°C and stirred for 18 h. The crude reaction mixture was cooled to room temperature and directly purified on silica gel using 20% EtOAc/hexanes to afford the desired nitrile as a white solid. The compound was obtained in quantitative yield and used directly for the next step.

Step 3: Hydrolysis. To a solution of the nitrile (17 mg, 0.04 mmol) in 1.2 ml of EtOH at room temperature was added sodium hydroxide 5 N (600 µl). The reaction was heated to reflux and stirred for 18 h. Quenching of the reaction was done using HCl 3 N until acidic by pH paper, dilution with 2 ml of H_2O, extracted twice with 5 ml EtOAc, washed with brine, dried over Na_2SO_4 and evaporated. Purification by prep HPLC using a Novapak C_{18} (7.8 × 300 mm) using 65% CH_3CN/H_2O + 0.1% AcOH at 3.5 ml/min at 270 nm afforded the desired [^{14}C]-acid **1** as a white solid in 70% overall yield for the three steps. Chemical and radiochemical purity were greater than 99% and the specific activity calculated to be 45 mCi/mmol. ^{1}H NMR (acetone-d_6): δ 10.69 (br s, 1H), 7.33 (d, *J*=8.3 Hz, 2H), 7.20 (dd, *J*=8.9 and 2.1 Hz, 1H), 7.09 (dd, *J*=9.0 and 2.2 Hz, 1H), 6.94 (d, *J*=8.2 Hz, 2H), 5.92 (d, J_{AB}=17.8 Hz, 1H), 5.77 (d, J_{AB}=17.8 Hz, 1H), 3.65–3.62 (m, 1H), 2.95–2.65 (m, 4H), 2.42 (dd, *J*=16.1 and 10.1 Hz, 1H), 2.32–2.27 (m, 1H); ^{13}C NMR (acetone-d_6): δ 172.2, 156.9 (d, J_{CF} = 237 Hz), 151.9, 138.7, 134.1, 132.2, 128.6, 127.5 (d, J_{CF} = 10.2 Hz), 127.2, 119.7 (d, J_{CF} = 4.8 Hz), 113.6 (d, J_{CF} = 28.7 Hz), 103.4 (d, J_{CF} = 22.7 Hz), 102.9 (d, J_{CF} = 12.1 Hz), 48.4, 38.1, 35.6, 35.2, 22.4.

1-((3S)-4-(4-chlorobenzyl)-7-fluoro-3-(hydroxymethyl)-1,2,3,4-tetrahydrocyclo-penta(b)indol-5-yl)ethanone (5)

Step 1: The common intermediate **4** (170 mg, 0.41 mmol) was dissolved in 5 ml of CH_2Cl_2 at 0°C under an inert atmosphere of nitrogen. Triethylamine (115 µl, 0.82 mmol) and DMAP (1 mg, cat.) were added followed by acetyl chloride (31 µl, 0.42 mmol). The reaction was stirred for 30 min then the cooling bath was removed for another 30 min of stirring. The reaction mixture was quenched by adding 5 ml of a saturated solution of NH_4Cl, extracted three times with 10 ml of CH_2Cl_2, washed with brine, dried over Na_2SO_4 and evaporated. The desired acetate was obtained as a colorless oil in quantitative yield and was used directly for the next step.

Step 2: To a solution of the acetate (180 mg, 0.4 mmol) in 3 ml of DMF under an inert atmosphere of nitrogen was added (1-ethoxyvinyl)tributylstannane (270 µl, 0.8 mmol). In a separate flask, triphenylarsine (50 mg, 0.16 mmol) and $Pd_2(dba)_3$ (36 mg, 0.04 mmol) were mixed in 2 ml of DMF and sonicated for 1 min. This solution was then added to the previous solution containing the acetate, purged three times with vacuum/N_2 then heated to 90°C for 18 h.

Step 3: The reaction mixture was cooled to 35°C prior to the addition of 5 ml of HCl 1 N and stirring was continued for 15 min. Filtration through a pad of celite removed palladium residues and two washes with 25 ml of EtOAc were necessary. The crude reaction mixture was extracted with three portions of 25 ml of EtOAc, washed with brine, dried over Na_2SO_4 and evaporated. Purification by flash chromatography using silica gel and 5% EtOAc/Hexanes afforded the methyl ketone in 67% yield.

Step 4: Finally, the methyl ketone (110 mg, 0.26 mmol) was dissolved in 4 ml of a mixture of THF/MeOH (1:1) at room temperature. A 2 N solution of NaOH (0.5 mL, 1 mmol) was added and the mixture was heated to reflux for 15 min. The reaction was cooled to room temperature and neutralized with 1 ml of HCl 1 N, extracted twice with 10 ml of EtOAc, washed with brine, dried over Na_2SO_4 and evaporated. Purification by flash chromatography using silica gel and a gradient of 0–30% EtOAc/Hexanes afforded the alcohol **5** in quantitative yield.

((3R)-5-acetyl-4-(4-chlorobenzyl)-7-fluoro-1,2,3,4-tetrahydrocyclopenta(b)indol-3-yl)acetic acid (2)

Compound **2** was obtained as a white powder in 70% overall yield from the previous alcohol **5** according to

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the three step procedure cited above (*mesylate formation*, *cyanide displacement* and *hydrolysis*). Chemical and radiochemical purity were greater than 99% and the specific activity calculated to be 45 mCi/mmol. ^{1}H NMR (acetone-d_6) δ 10.9 (br s, 1H), 7.38 (dd, *J*=8.9 and 2.5 Hz, 1H), 7.26 (d, *J*=8.4 Hz, 2H), 7.21 (dd, *J*=9.8 and 2.5 Hz, 1H), 6.71 (d, *J*=8.3 Hz, 2H), 5.42 (s, 3H), 3.77–3.73 (m, 1H), 3.01–2.95 (m, 1H), 2.91–2.78 (m, 3H), 2,53 (dd, *J*=16.1 and 10.0 Hz, 1H), 2.39–2.27 (m, 1H), 2.14 (s, 3H). ^{13}C NMR (acetone-d_6) δ 199.8 (d, J_{CF}= 1.5 Hz), 172.9, 156.8 (d, J_{CF}=235 Hz), 153.0, 137.6, 133.2, 133.0, 129.2, 128.9, 128.4 (d, J_{CF}=9.6 Hz), 128.2 (d, J_{CF}=7.0 Hz), 119.6 (d, J_{CF}=4.4 Hz), 109.6 (d, J_{CF}=26.5 Hz), 108.1 (d, J_{CF}=23.0 Hz), 50.1, 38.9, 36.2, 35.9, 28.7, 23.2.

{(3R)-4-(4-chlorobenzyl)-7-fluoro-5-((1S or 1R)-1-methoxyethyl)-1,2,3,4-tetrahydrocyclo-penta(b)indol-3-yl}acetic acid (6 and 7)

Step 1: Diazomethane (1 ml, 1M/Et_2O) was added to radiolabeled compound **2** (3 mg), stirred 5 min then evaporated to dryness.

Step 2: The corresponding methyl ester (~3 mg) was dissolved in 1 ml of MeOH at 0°C under an inert atmosphere of nitrogen. Sodium borohydride (10 mg) was added and stirred for 15 min. The reaction was quenched with 300 µl of AcOH and evaporated to dryness. The crude compound was quickly purified through a short pad of silica gel using 70% EtOAc/Hexanes.

Step 3: The alcohol (~3 mg) was dissolved in 1 ml of DMF and sodium hydride (5 mg) was added, stirred 15 min then methyl iodide (20 µl) was introduced. The reaction was stirred for 30 min, and then quenched by adding 200 µL of HCl 1 N, extracted twice with 2 ml of EtOAc, washed with brine, dried over Na_2SO_4 and evaporated.

Step 4: Finally, the ester (~2 mg) was hydrolyzed using 200 µl of NaOH 5 N in 1 ml of THF/MeOH (1:1) at reflux for 15 min. The reaction was cooled down to room temperature and neutralized with 1 ml of HCl 1 N, extracted twice with 2 ml of EtOAc, washed with brine, dried over Na_2SO_4 and evaporated. Purification by HPLC using a ChiralPak AD column (4.6 × 250 mm) using 7% *i*PrOH / Hexanes + 0.2% AcOH at 254 nM at a flow rate of 1 ml/min afforded cleanly carbon-14 labeled **6** and **7** with a retention time of 12 and 17 min, respectively. Radiochemical purity was greater than 99% and the specific activity calculated to be 45 mCi/mmol for both compounds.

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