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Research Article

Synthesis of carbon-14 labeled (*R*)-3-fluoro-4-(2′-(5″, 6″, 7″, 8″-tetrahydro-5″, 5″, 8″, 8″-tetramethyl-2″-naphthyl)-[2′-hydroxy-^{14}C])[carbonyl-^{14}C]acetamidobenzoic acid

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Summary

Carbon-14 labeled (*R*)-3-fluoro-4-(2′-(5″, 6″, 7″, 8″-tetrahydro-5″, 5″, 8″, 8″-tetramethyl-2″-naphthyl)-[2′-hydroxy-^{14}C])[carbonyl-^{14}C]acetamidobenzoic acid, **1**, was prepared in a six step radioactive synthesis from diethyl [carboxylate-$^{14}C_{1,2}$] oxalate. The penultimate compound was purified by chiral HPLC, which following deprotection yielded **1** in an overall radiochemical yield of 6.8%. The specific activity of the final product was found to be 24.5 μCi/mg with a radiochemical purity of >99% as determined by HPLC. Derivatization of **1** via trimethylsilyl diazomethane to the corresponding methyl ester **9**, followed by chiral HPLC analysis, demonstrated that **1** had an optical purity of >99% ee. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: carbon-14; retinoid; diethyl [carboxyl-$^{14}C_{1,2}$]oxalate

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Introduction

Retinoic acid and its analogs (retinoids) act through the retinoic acid receptors (RARs), which are ligand-inducible transcription factors.[1] Among the three RAR's (α, β, and γ), it is RARγ which is responsible for retinoid activity in the skin.[2] As part of an investigation into RAR-selective retinoids for dermatological uses, it was discovered that optically active **1** was a potent and selective RARγ agonist.[3–5] Radiolabeled **1** was required for subsequent *in vivo* investigations (Figure 1).

Results and discussion

The preparation of carbon-14 labeled **1** was accomplished in a six step radioactive synthesis from diethyl[carboxyl-$^{14}C_{1,2}$] oxalate (Scheme 1). The initial reaction of the lithium salt of **2** with an excess of the diethyl[carboxyl-$^{14}C_{1,2}$]oxalate ensured a high yield of the radiolabeled monosubstituted ethyl oxalate **3**. NMR analysis of prior non-radioactive studies had shown that complete separation of the diethyl oxalate from the monosubstituted ethyl oxalate was not possible by flash chromatography. HPLC analysis of this product was made more difficult by the similar retention times of both the diethyl [carboxyl-$^{14}C_{1,2}$] oxalate and **3**. Subsequent saponification of the crude product yielded a mixture of the corresponding free acids, [carboxyl-$^{14}C_{1,2}$] oxalic acid and [2-^{14}C]oxo-2-(1′, 2′, 3′ 4′-tetrahydro-1′, 1′, 4′, 4′-tetramethyl-6′-naphthyl) [carboxyl-^{14}C] acetic acid **4**. HPLC analysis of crude **4** indicated a radiochemical purity of 87%. Subsequent conversion to the acid chloride **5** and reaction with allyl 4-amino-3-fluorobenzoate yielded **6** which was purified via flash chromatography. HPLC analysis of **6** indicated a radiochemical purity of >99%. Treatment of **6** with sodium borohydride yielded the penultimate

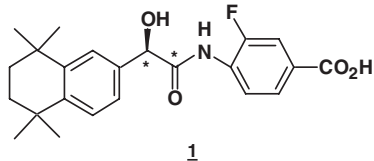

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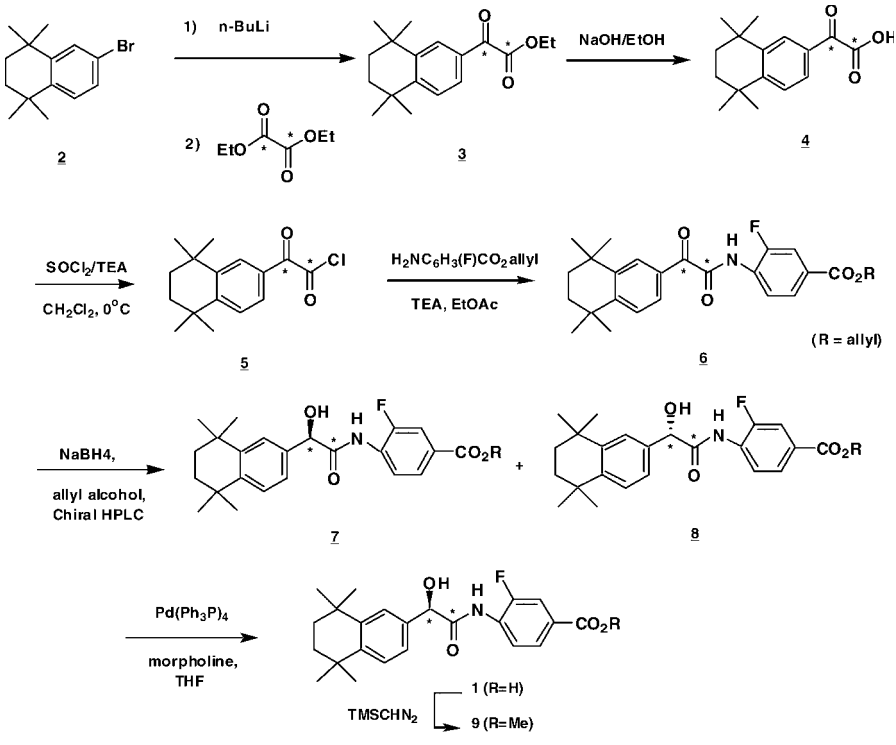
Figure 1. (R)-3-Fluoro-4-(2′-(5″,6″,7″,8″-tetrahydro-5″,5″,8″,8″-tetramethyl-2″-naphthyl)-[2′-hydroxy-^{14}C])[carbonyl-^{14}C]acetamidobenzoic acid, 1.



Scheme 1. Synthesis of ^{14}C-3-Fluoro-4-(2′-(5″, 6″, 7″, 8″-tetrahydro-5″, 5″, 8″, 8″-tetramethyl-2″-naphthyl)-[2′-^{14}C]hydroxy-[carbonyl-^{14}C]acetamido-benzoic acid 1

product as a mixture of enantiomers 7 and 8. Separation of the enantiomers by chiral HPLC was possible at the penultimate stage but not with the final product 1; therefore, chiral HPLC was used at this stage to purify the racemate and obtain the desired *R*-isomer 7. Deprotection of the allyl ester 7 yielded the desired product 1. Radiochemical purity of the final product was determined by HPLC on a cyano column with a mobile phase of acetonitrile and 0.1% trifluoroacetic acid. Determination of the optical purity of the final product 1 was achieved by derivatizing a sample of the final product with trimethylsilyl diazomethane and analyzing the resulting methyl ester 9 via chiral HPLC.[6]

Materials and methods

Previously known non-radioactive proprietary precursors and final products were prepared according to published procedures.[3–5] Diethyl

[carboxyl-$^{14}C_{1,2}$] oxalate (52 mCi/mmol) was purchased from ViTrax. $Pd(PPh_3)_4$ was obtained from Fluka. All other reagents were obtained from Aldrich and were either ACS grade or the highest quality material commercially available. The identity of intermediates and the final product was established by co-elution of the radiolabeled material with authentic unlabeled compound on HPLC.[3–5] Compound purification and analysis was performed on a Rainin Dynamax HPLC system consisting of two SD-200 pumps, a Rainin UV-I detector and an *INUS* β-RAM radioactive flow-through detector. Zorbax HPLC columns were obtained from the Hewlett-Packard Company. Chiralpak AD HPLC columns were obtained from Chiral Technologies, Exton, PA. Radiochemical purity was determined by HPLC. The specific activity of the samples were determined via HPLC. In this procedure, a radioactive sample is applied to the column, the mass of the sample was determined by comparison of the UV absorbance to a standard curve and the total radioactivity measured via liquid scintillation counting.

High performance liquid chromatography

Method A: In this method, samples are loaded onto a Zorbax cyano column ($4.6 \times 250\,mm^2$) with a mobile phase of 60% CH_3CN and 40% of a 0.1% trifluoroacetic acid (TFA) solution in water. The flow rate through the column was 1.0 ml/min and the sample was monitored by both UV (265 nm) and radioactivity.

Method B: In this method, samples are loaded onto a Zorbax cyano column ($4.6 \times 250\,mm^2$) with a mobile phase of 65% CH_3CN and 35% of a 0.1% TFA solution in water. The flow rate through the column was 1.0 ml/min and the sample was monitored by both UV (265 nm) and radioactivity.

Method C: In this method, samples are loaded onto a Chiralpak AD column ($10 \times 250\,mm^2$) with a mobile phase of 30% isopropanol and 70% hexane. The flow rate through the column was 3.0 ml/min and the sample was monitored by both UV (265 nm) and radioactivity.

Method D: In this method, samples are loaded onto a Zorbax cyano column ($4.6 \times 250\,mm^2$) with a mobile phase of 50% CH_3CN and 50% of a 0.1% TFA solution in water. The flow rate through the column was 1.0 ml/min and the sample was monitored by both UV (265 nm) and radioactivity.

Method E: In this method, samples are loaded onto a Chiralpak AD column ($4.6 \times 250\,mm^2$) with a mobile phase of 20% isopropanol and

80% hexane. The flow rate through the column was 1.0 ml/min and the sample was monitored by both UV (265 nm) and radioactivity.

Experimental

Ethyl [2-oxo-^{14}C]-2-(1′, 2′, 3′ 4′-tetrahydro-1′, 1′, 4′, 4′-tetramethyl-6′-naphthyl) [carboxyl-^{14}C]acetic acid, **3**.

Into a 25 ml RB flask containing anhydrous THF (4.5 ml) was added **2** (226 mg, 0.847 mmol) and the solution cooled to −78°C. To this was added *n*-BuLi in hexane (0.8 ml, 1.6 M, 1.28 mmol, 1.5 eq) dropwise via a syringe under nitrogen. The reaction mixture was allowed to stir at –78°C for 20 min.The aryl lithium salt was then cannulated to a second 25 ml RB flask containing a THF (4.0 ml) solution of diethyl [carboxyl-$^{14}C_{1,2}$] oxalate (75 mCi, 210 mg, 1.44 mmol, 52 mCi/mmol, 1.7 eq). The reaction mixture was stirred at –78°C for 30 min, quenched with a 1:1 solution of EtOH/CH_3CO_2H (3 ml) and then allowed to warm to room temperature. The mixture was diluted with EtOAc (25 ml) and washed with H_2O (10 ml, 2 ×); the aqueous layer was back-extracted with EtOAc (10 ml). The combined organic extracts were washed with brine and dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. Attempted purification of **3** by flash chromatography (SiO_2, 10% EtOAc/hexane) resulted in incomplete separation of **3** from diethyl [carboxyl-$^{14}C_{1,2}$] oxalate. Thus, the impure product **3** was used in the next step.

[2-oxo-^{14}C]-2-(1′, 2′, 3′ 4′-tetrahydro-1′, 1′, 4′, 4′-tetramethyl-6′-naphthyl) [carboxyl-^{14}C] acetic acid, **4**.

Into a 25 ml RB flask containing **3** was added EtOH (4 ml) and aq NaOH (4 ml, 1 M, 4 mmol). The reaction mixture was stirred at room temperature for 3 h and then concentrated *in vacuo*. The residue was dissolved in H_2O (30 ml), extracted with Et_2O (30 ml) and the aqueous layer acidified (pH ∼2–3) with conc. HCl. The acidified layer was then extracted with EtOAc (30 ml), and the organic layer was washed with brine (30 ml) and then dried ($MgSO_4$), filtered and concentrated *in vacuo* to yield **4** as a thick oil (35 mCi). HPLC analysis of **4** (method A) showed a radiochemical purity of 87% with the remaining material being [carboxyl-$^{14}C_{1,2}$] oxalic acid. In this system, **4** has a retention time of approximately 5.3 min, and [carboxyl-$^{14}C_{1,2}$] oxalic acid has a retention time of approximately 3.7 min. The crude product **4** was used as is in the next step.

Allyl 3-fluoro-4-(2′-(5″, 6″, 7″, 8″-tetrahydro-5″, 5″, 8″, 8″-tetramethyl-2″-naphthyl)-[2′-oxo-^{14}C])[carbonyl-^{14}C]acetamidobenzoate, **6**.

Into a 50 ml RB flask containing 35 mCi of **4** was added CH_2Cl_2 (10 ml) and the resulting solution cooled to 0°C. To this was added $SOCl_2$ (1.2 ml, 1.96 g, 16.45 mmol) and triethylamine (1.5 ml, 1.09 g, 10.76 mmol) and the reaction mixture stirred at room temperature for 2 h, after which time, it was concentrated *in vacuo*, and the residue was treated with hexane (20 ml) and filtered through a cotton plug (to remove triethylamine hydrochloride). The hexane was then removed *in vacuo* to yield chloride **5**.

Into a 25 ml RB flask containing **5** dissolved in EtOAc (1 ml) was added allyl 3-fluoro-4-aminobenzoate (193 mg, 0.98 mmol) dissolved in EtOAc (2 ml), and triethylamine (200 µl, 1.43 mmol). The solution was then stirred at room temperature for 1.5 h. The solution was then diluted with EtOAc (25 ml), extracted with H_2O (25 ml), and the organic layer was washed with brine (25 ml), dried ($MgSO_4$) filtered and then purified by flash chromatography (SiO_2, 3–5% EtOAc/ hexane) to yield **6** (19.5 mCi, 163 mg) as a light tan solid. HPLC analysis of **6** (method B) indicated a radiochemical purity of >99%. In this system, **6** has a retention time of approximately 8.1 min and **5** has a retention time of approximately 4.8 min.

Allyl 3-fluoro-4-(2′-(5″, 6″, 7″, 8″-tetrahydro-5″, 5″, 8″, 8″-tetramethyl-2″-naphthyl)-[2′-hydroxy-^{14}C])[carbonyl-^{14}C]acetamidobenzoate, **7**.

To a 25 ml RB flask containing **6** (19.5 mCi, 163 mg) dissolved in allyl alcohol (4 ml) was added $NaBH_4$ (13.40 mg, 0.35 mmol), and the reaction mixture was stirred for 20 min at room temperature. (A homogenous solution was not achieved.) After 20 min, the reaction was quenched with concentrated HCl (60 µl), and the mixture was extracted with EtOAc (30 ml). The organic layer was washed with sat. $NaHCO_3$ (20 ml), and the aqueous layer was back-extracted with EtOAc (20 ml). The combined EtOAc fractions were then washed with brine (20 ml), dried ($MgSO_4$), filtered and then purified by flash chromatography (SiO_2, 15–20% EtOAc/hexane). Separation of the enantiomers was then achieved by chiral HPLC (Method C) to obtain **7** (7 mCi, 60 mg) as a colorless oil. In this preparative chiral HPLC system, the desired enantiomer **7** has a retention time of approximately 14 min while the undesired enantiomer **8** has a retention time of approximately 34 min.

(R)3-Fluoro-4-(2′-(5″, 6″, 7″, 8″-tetrahydro-5″, 5″, 8″, 8″-tetramethyl-2″-naphthyl)-[2′-hydroxy-^{14}C])[carbonyl-^{14}C]acetamidobenzoic acid, **1**.

To a 25 ml RB flask containing **7** (7 mCi, 60 mg, 0.136 mmol) was added THF (1 ml), morpholine (118 mg, 1.36 mmol) and $Pd(PPh_3)_4$ (16 mg, 0.0136 mmol), and the reaction was stirred at room temperature for 30 min. Radioactive TLC analysis (SiO_2, 75% hexane/24% EtOAc/ 0.5% CH_3OH/0.5% CH_3CO_2H) indicated essentially complete conversion to **1**. The reaction mixture was then diluted with EtOAc (15 ml), washed with 1 N HCl (10 ml, 2 ×) and brine (10 ml), dried ($MgSO_4$), filtered and concentrated *in vacuo* to yield **1**. Recrystallization (EtOAc/ :hexane) afforded **1** (5.14 mCi, 126.25 μCi/mg, 40.7 mg) as a white solid. HPLC analysis of **1** (Method D) indicated a radiochemical purity of > 99% and a retention time of approximately 9.4 min.

Methyl (R)-3-Fluoro-4-(2′-(5″, 6″, 7″, 8″-tetrahydro-5″, 5″, 8″, 8″-tetramethyl-2″-naphthyl)-[2′-hydroxy-^{14}C])[carbonyl-^{14}C]acetamidobenzoate, **9**.

Analysis of the optical purity of **1** was achieved by derivatization of **1** with TMS-diazomethane to yield the corresponding methyl ester which was then analyzed by chiral HPLC (Method E). In this procedure, **1** (5 mg, 631 μCi) was dissolved in C_6H_6:CH_3OH (7:3) (0.5 ml) and treated with trimethylsilyl-diazomethane (150 μl of 2.0 M in hexane). The reaction mixture was stirred at room temperature for 10 min. The reaction was quenched with acetic acid until the yellow color dissipated and the nitrogen effervescence ceased. The solution was then concentrated *in vacuo* to yield **9**. HPLC analysis (Method E) indicated > 99% ee. The retention times of the R and S enantiomers of the methyl esters were 4.7 and 17 min, respectively.

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