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Preparation of high specific activity tritium labeled 6α,9,-difluoro-11β,21-dihydroxy-16α,17-[(1-methylethylidene)bis(oxy)]pregna-1,4-diene-3,20-one, fluocinolone acetonide

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Fluocinolone acetonide was tritiated by selective reduction of the 1,2-double bond of the *O*-protected analog under tritium, followed by re-establishment of the 1,2-double bond and deprotection. Protection of both hydroxyl functionalities was required. The product was obtained with specific activity 36.8 Ci/mmol.

Keywords: NSC92339; tritium; oxidative de-hydrogenation

Introduction

The glucocorticoid 6α,9,-difluoro-11β,21-dihydroxy-16α,17-[(1-methylethylidene)bis(oxy)]pregna-1,4-diene-3,20-one, fluocinolone acetonide (**1**) was introduced in 1965 and has been approved by FDA for dermatologic use. It is usually applied as a cream, gel, lotion, or ointment. Although not approved for such use, fluocinolone acetonide has also been used topically in the treatment of inflammatory eye, ear, and nose disorders.[1] Recent applications include its use as a component in the topical treatment of facial melasma,[2] and in an implantable devices to treat diabetic retinopathy[3] and uveitis.[4] Radiolabeled fluocinolone acetonide labeled with carbon-14 in the acetonide moiety was used for distribution studies in mice[5] and for metabolism studies in man.[6] Tritium labeled fluocinolone acetonide does not appear to have been reported.

Results and discussion

To prepare high specific activity fluocinolone acetonide [^{3}**H**]-**1** we planned to take advantage of some of the known chemistry of this compound class. Since selective reduction of the 1,2-double bond[7] and oxidative de-hydrogenation to re-introduce the 1,2-double bond[8] have been reported, the approach depicted in Scheme 1 was appealing as a straightforward synthetic approach to [^{3}**H**]-**1**. The product was expected to retain at least one tritium atom and thus have specific activity $>$20 Ci/mol, suitable for binding studies. At the same time, this product should be suitable for use in metabolism and distribution studies since vinylic protons are not subject to exchange or degradation. While all the steps in Scheme 1 were based on the literature, and thus seemed highly likely to succeed, it was tempting to determine whether reduction of **1** to [^{3}**H**]-**7**, followed by oxidative de-hydrogenation of [^{3}**H**]-**7**, would afford [^{3}**H**]-**1** (Scheme 2). Pilot scale experiments demonstrated that selective reduction of the 1,2-double bond in **1** could be accomplished with minor modification of the literature procedure[7] to give **7** in quantitative yield. However, selenium oxide oxidative de-hydrogenation of **7** resulted in a complex product mixture. This was not entirely unexpected since the literature[8] reports a low yield (∼10%) for this process, even when the 21-hydroxy group was protected as the acetate. Nevertheless, it was decided to investigate whether acetylation of the 21-hydroxy group would result in a cleaner product mixture (Scheme 3). Treatment of **7** with acetic anhydride in pyridine following the literature conditions[8] to acetylate the 21-hydroxyl functionality gave not only 21-*O*-acetyl acetonide **2**, but also about 29% of 11,21-*O*-diacetyl acetonide **8**. Acetylation of **1** with acetic anhydride using trimethylsilyltrifluoromethanesulfonate in dichloromethane[9,10] cleanly gave the 21-*O*-acetyl acetonide **2**. Selective hydrogenation of **2** to give 4-ene intermediate **9** was carried out using Wilkinson's catalyst ((triphenylphosphine)rhodium (I) choride)[7] in a 2/1 mixture of toluene and THF since the solubilities of the substrate and the catalyst were not high enough in toluene. Oxidative de-hydrogenation of **9** (selenium dioxide, *tert*-butanol, pyridine under reflux[8]) gave the 1,4-diene **2**. Optimized reaction conditions (methanol/water/sodium bicarbonate) were determined to selectively deacetylate 21-*O*-acetyl group in **2** to give **1** without hydrolysis of the acetonide. Purification of the crude product by prep-HPLC gave pure **1**.

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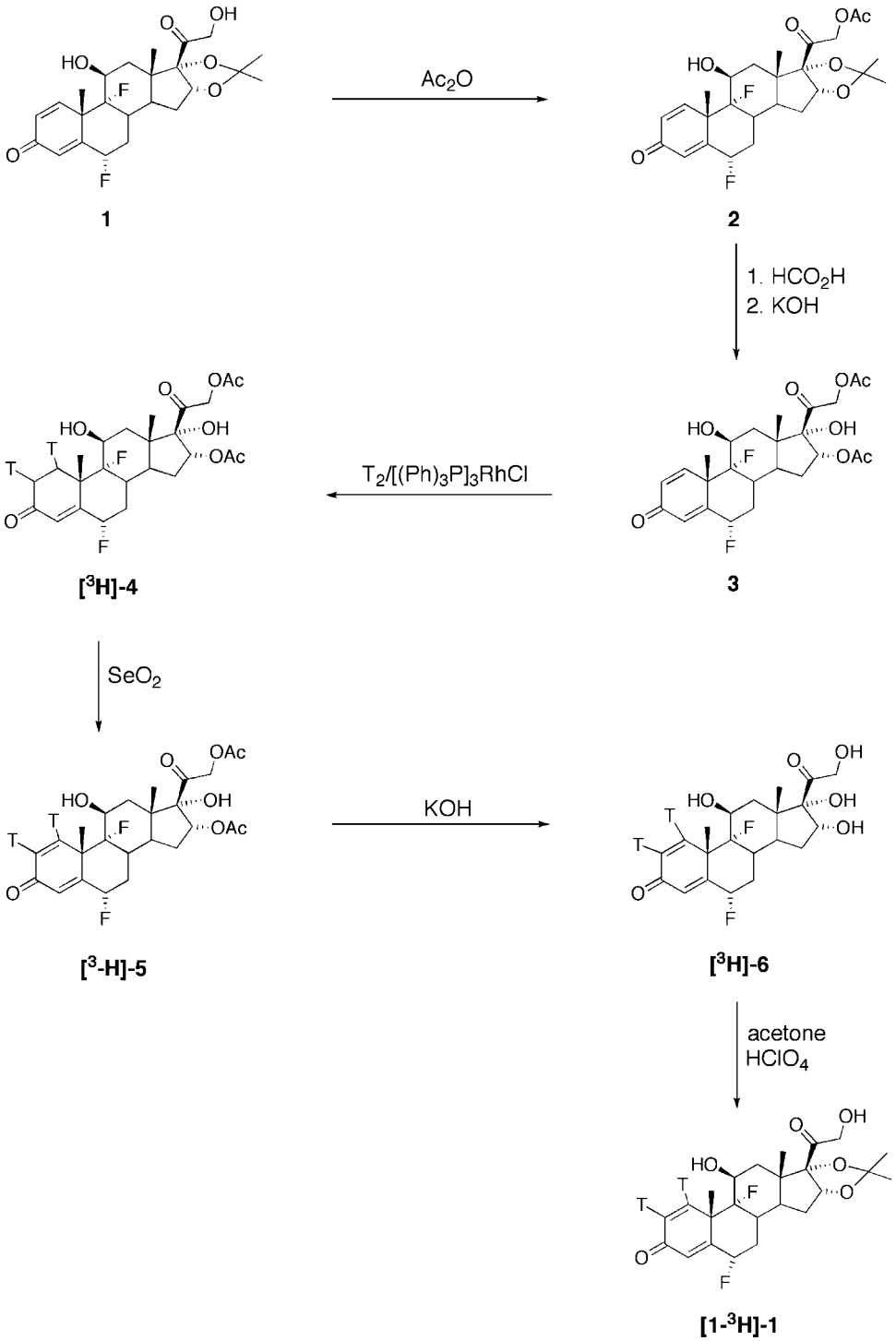
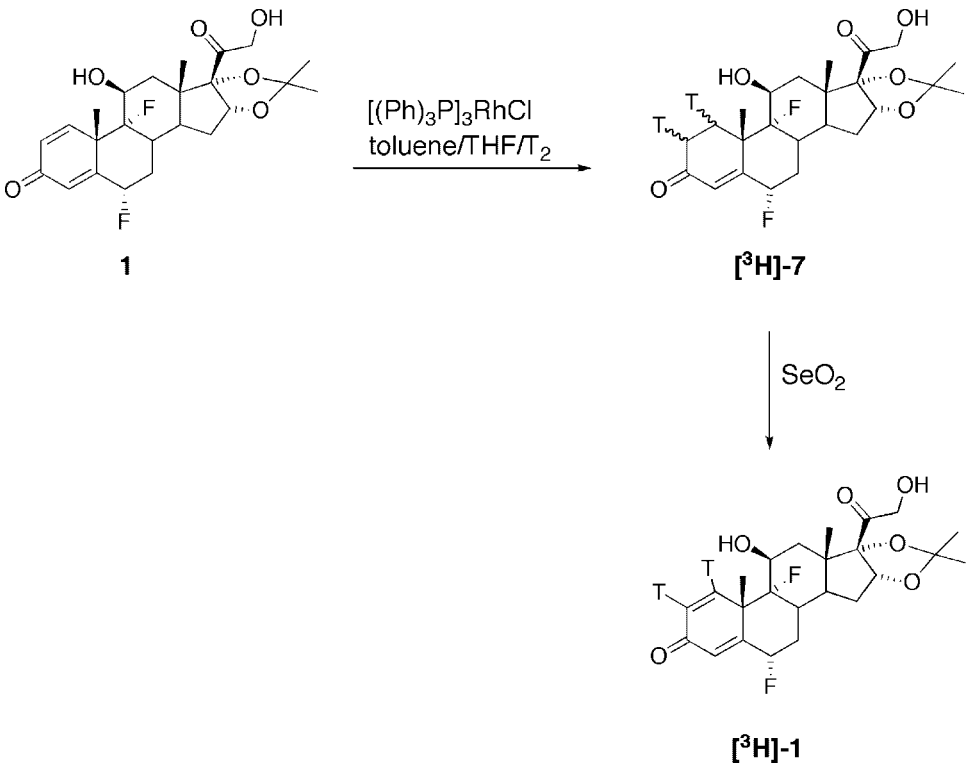
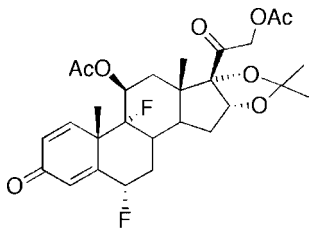


Scheme 1.

The Master Synthesis of tritium labeled fluocinolone acetonide ([^{3}H]-**1**) was undertaken using the optimized reaction conditions (Scheme 3). Selective tritiation of 21-*O*-acetyl acetonide **2** using Wilkinson's catalyst (chlorotris(triphenylphosphine)-rhodium (I)) in toluene/THF (2/1) gave a good yield of [^{3}H]-**9** (317 mCi) and this material was subjected to oxidative detritiation using selenium dioxide in refluxing *tert*-butanol and pyridine. By contrast to the de-hydrogenation of unlabeled **9** the reaction of [^{3}H]-**9** was very sluggish and none of the expected intermediate [^{3}H]-**2** was detected by HPLC (Ram) although [^{3}H]-**9** was disappearing. In addition, HPLC analysis indicated that most of the radioactivity was in the solvent front. These observations, taken together, led to the conclusion that due to the slow detritiation process, presumably associated with the isotope effect, extensive tritium exchange with solvent was taking place during the reaction. Therefore, reactions proceeding in non-exchanging media were explored. Attempted oxidative de-hydrogenation of unlabeled **9** with



Scheme 2.



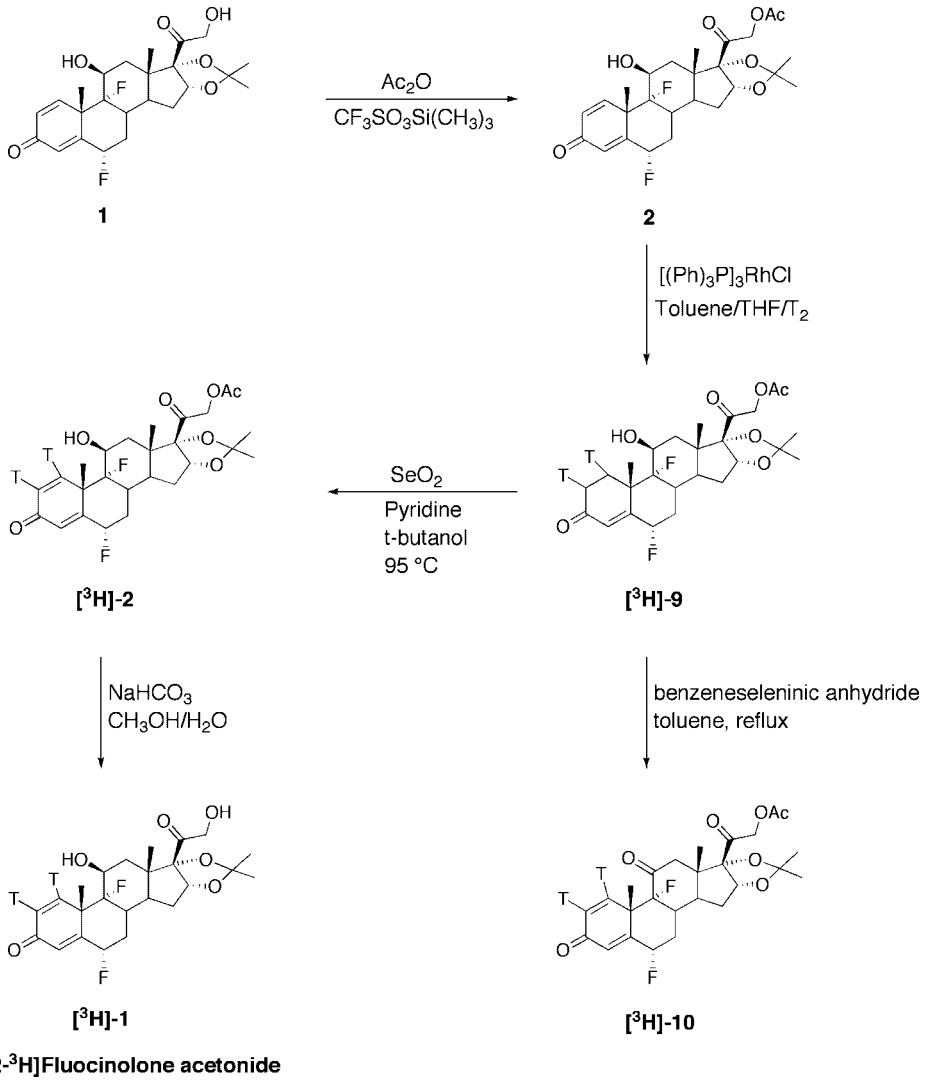
8

2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in refluxing benzene following conditions used for analogous oxidations[11] gave the 1,4-diene **2** cleanly in 14 h. Based on this promising result the tritiation of **2** was repeated to give [^{3}H]-**9** (500 mCi) and a portion (275 mCi) was treated with DDQ. Unfortunately this reaction failed; unreacted [^{3}H]-**9** was recovered.

Since the oxidative de-hydrogenation of unlabeled **9** was evidently not a useful model for oxidative detritiation, [^{3}H]-**9** was used in a series of pilot reactions to identify suitable conditions for the conversion of [^{3}H]-**9** to [^{3}H]-**2**. Of the conditions tried (selenium dioxide/dioxane (reflux),[12] hν/DIB/I_2/CH_2Cl_2,[13] hν/DIB/I_2/toluene,[13] iodoxybenzene/benzeneseleninic anhydride/toluene (reflux),[14,15] and benzeneseleninic anhydride/-toluene (reflux)[16]) only the latter gave an intermediate, which, after deacetylation, gave a product that showed two vinyl resonances in the tritium NMR spectrum, indicating that oxidative de-hydrogenation had taken place. However, the proton NMR of this material was different from that of authentic fluocinolone acetonide (**1**) as was the HPLC retention time. Reasoning that transition metal complexation (with rhodium from Wilkinson's catalyst used in the tritiation) could be responsible for the different NMR and HPLC profiles, the product was treated with several metal scavenging agents.[17] Although these treatments had some effect on the NMR chemical shifts, the isolated product was not identical to **1**. It, thus, appeared that although the intermediate obtained after oxidative de-hydrogenation had the 1,4-diene structure, it was not the intended intermediate [^{3}H]-**2**. Mass spectrometric analysis of the unlabeled intermediate obtained by the probe oxidative de-hydrogenation of **9** followed by de-acetylation showed that oxidation of the 11-hydroxy functionality had taken place to give **10**. It follows that in this instance oxidative de-hydrogenation of [^{3}H]-**9** using DDQ gave [^{3}H]-**10** although the literature[18] indicated that oxidative hydrogenation could be accomplished without compromising the unprotected 11-hydroxy group. It was therefore obvious that the 11-hydroxy group needed to be protected.

Treatment of unlabeled **9** with acetic anhydride gave the 11,21-diacetoxy intermediate **11** (Scheme 4) and this material was used to probe the oxidation and deprotection reactions. It was found that the oxidation conditions used for [^{3}H]-**9** could be adopted for the 11,21-*O*-diacetate intermediates by increasing the reaction time, but the conditions of the de-acetylation reaction required modification. Thus, attempted de-acetylation of **8** using $NaHCO_3$/CH_3OH/H_2O was found to be very slow (due to the slow de-acetylation of 11-*O*-acetate) and the prolonged reaction time led to decomposition of the product **1**. Replacing the weaker base ($NaHCO_3$) with a stronger base (K_2CO_3) gave a cleaner reaction with an 80% yield of **1**.

In continuation of the Master Synthesis, recovered [^{3}H]-**9** (261 mCi) was purified using aluminum oxide N solid phase extraction (SPE) cartridge, and then treated with acetic anhydride in pyridine and 4-dimethylaminopyridine to give 11,21-*O*-diacetate [^{3}H]-**11** (238 mCi). Reaction of [^{3}H]-**11** with benzeneseleninic anhydride proceeded much more sluggishly than the reaction of



Scheme 3.

unlabeled **11** (37 h versus 6 h) but was as clean as the reaction of unlabeled **11**. Purification of the crude product [^{3}**H**]-**8** with a C-18 SPE cartridge gave two fractions: 71.3 mCi of 98% radiochemical purity and 21 mCi of 76% radiochemical purity. Separate de-acetylation (with $K_2CO_3/CH_3OH/H_2O$) of the two fractions gave crude [^{3}**H**]-**1**, 60.4 mCi of 49% radiochemical purity, and 15.9 mCi of 58% radiochemical purity, respectively, after work-up with a C-18 SPE cartridge. The fractions were combined and purified by preparative HPLC to give pure final product, [1,2-^{3}H]fluocinolone acetonide ([^{3}**H**]-**1**) in 12% radiochemical yield from [^{3}**H**]-**9**. The identity of the product was confirmed by HPLC co-injection with authentic **1**. The radiochemical purity, based on HPLC-β-Ram was $>$97% and the chemical purity, based on HPLC-UV was $>$95%. The specific activity calculated using the mass obtained from a Beer's Law calibration curve was 36.8 Ci/mmol. The material was formulated in 95% ethanol at a concentration of 0.3 mCi/mL and stored at -70°C.

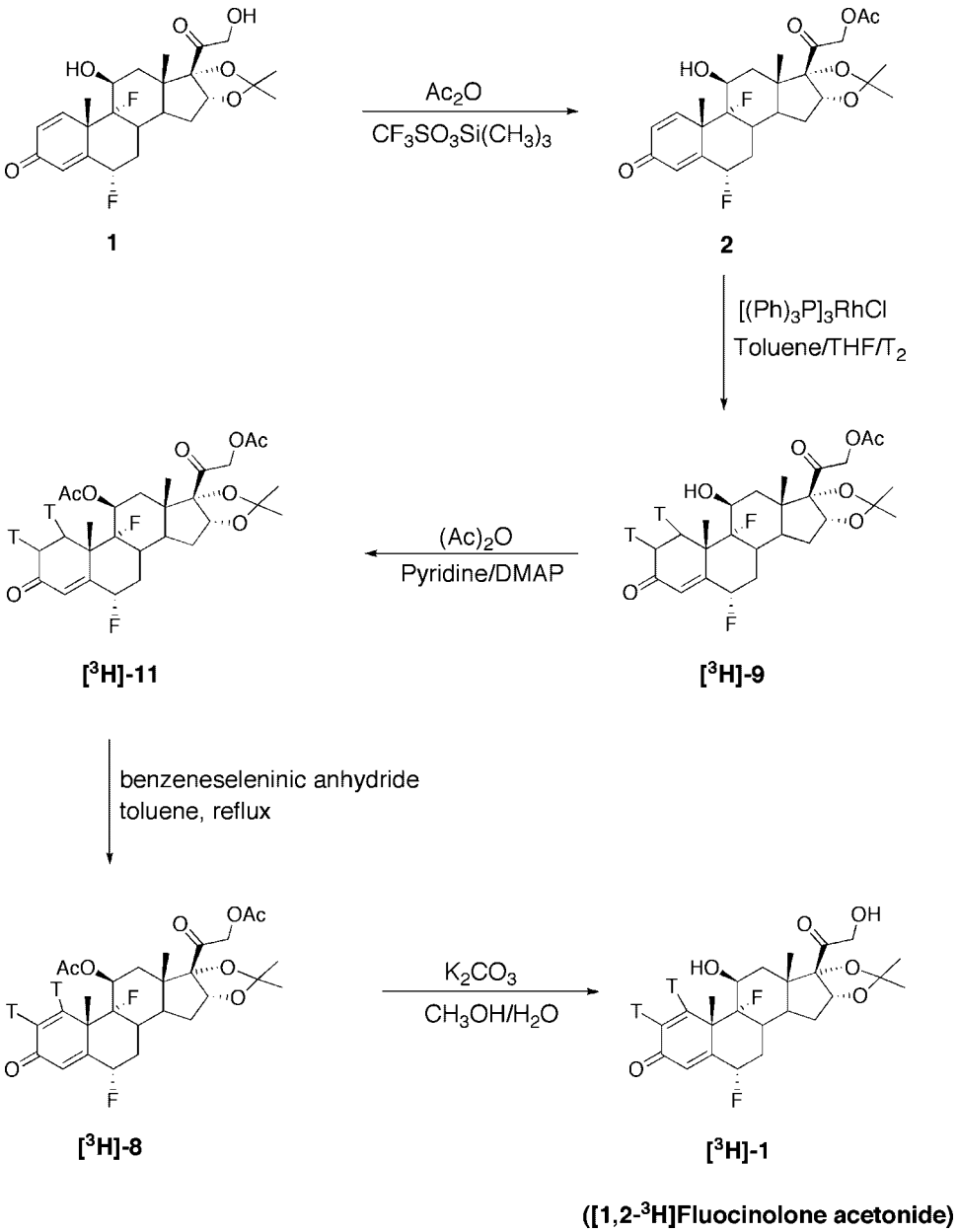
Experimental

Proton and carbon-13 nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 300 MHz NMR spectrometer. MS were determined on a Perkin-Elmer Sciex API 150EX mass spectrometer outfitted with an APCI source. TLC analyses were carried out on commercial pre-coated silica gel 60F254 glass plates (E. Merck: 5 $\times$ 20 cm). The HPLC analyses were performed on a dual pump system (Rainin HPXL solvent delivery system) with a Rheodyne injector, a Varian ProStar 325 UV-Vis detector, and an IN/US Systems β-RAM radio detector connected after the UV-Vis detector, controlled by Varian Star Workstation software. HPLC column and other analysis details are summarized below. The water used in HPLC analyses was obtained from a Millipore Milli-Q Plus Ultra-Pure Water System. Radioactive samples were counted using a Tri-Carb Liquid Scintillation Analyzer (Packard Bioscience model 2100TR) utilizing IN-FLOW 3 (IN/US Systems) as the liquid scintillation counting cocktail.

HPLC conditions: Phenomenex Gemini C18 column (3 $\times$ 150 mm, 5 μm), gradient (solvent A: H_2O, solvent B: CH_3CN, 0–20 min 20–90% B, 20–30 min 90% B), flow rate: 0.8 mL/min, UV1: 235 nm, UV2: 245 nm), β-RAM detector.

HPLC calibration of authentic fluocinolone acetonide, (1)

Authentic fluocinolone acetonide (20.0 mg) was dissolved in CH_3OH (5 mL) in a volumetric flask (5 mL). From the solution was withdrawn 5 μL (20.0 μg) and diluted with



Scheme 4.

UV (area)	Injected amount (μg)
531243	0.02
1029107	0.04
1381733	0.06
1879758	0.08
2402975	0.1

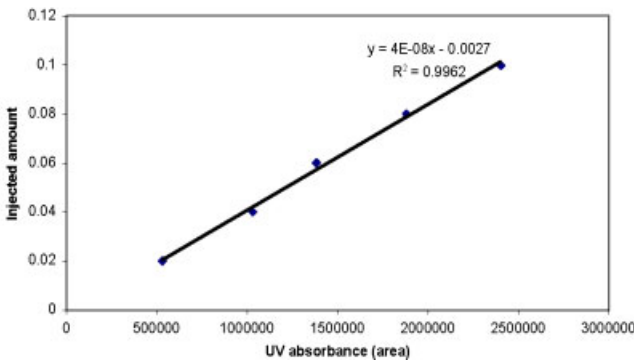


Figure 1. Calibration curve for fluocinolone acetonide (low concentration).

CH_3OH (5 mL) in another volumetric flask (5 mL). The HPLC peak areas of aliquots (5, 10, 15, 20, and 25 μL) of the solution were measured at 235 nm and a calibration curve was generated (Figure 1) for use in the determination of the specific activity of **[^{3}H]-1**.

21-Acetoxy-6α,9,-difluoro-11β-hydroxy-16α,17-[(1-methylethylidene)bis(oxy)]pregna-1,4-diene-3,20-one (21-*O*-acetyl fluocinolone acetonide, (2)

To a mixture of fluocinolone acetonide (**1**) (203 mg, 0.44 mmol) in CH_2Cl_2 (5 mL, anhydrous) was added acetic anhydride (0.050 mL, 54 mg, 0.53 mmol, 1.2 eq.) followed by a solution (1 M) of trimethylsilyl trifluoromethanesulfonate (0.040 mL, 442 mg, 2.0 mmol) in CH_2Cl_2. The mixture was stirred at RT for 35 min at which time more acetic anhydride (50 μL, 54 mg, 0.53 mmol, 1.2 eq.) was added. Stirring at RT was continued for 35 min more, then the excess acetic anhydride was destroyed by addition of CH_3OH (0.5 mL). The mixture was mixed with H_2O (10 mL) and CH_2Cl_2 (20 mL). The organic phase was separated, washed with H_2O (10 mL), then dried over Na_2SO_4. The solvent was evaporated, leaving a white solid (130 mg, 0.26 mmol, 59%), m.p. $>250^\circ$C. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.94 (s, 3H, H-18), 1.22 (s, 3H, H-23), 1.44 (s, 3H, H-22), 1.53 (s, 3H, H-19), 1.58–1.87 (m, 4H, H-7α, H-12β, H-15), 2.19 (s, 3H, H-24), 2.08–2.59 (m, 4H, H-7β, H-8, H-12α, H-14), 4.42 (d, $J=8.2$ Hz, 1H, H-11), 4.9 (bs, 2H, H-21), 5.00 (d, $J=4.6$ Hz, 1H, H-16), 5.50 (qd, $J=48.7$ Hz, 1.7 Hz, 1H, H-6), 6.38 (dd, $J=10.1$ Hz, 1.7 Hz, 1H, H-2), 6.44 (m, 1H, H-4), 7.13 (dd, $J=10.1$ Hz, 1.0 Hz, 1H, H-1). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm): 16.7, 20.8, 23.4, 23.5, 26.1, 26.9, 32.1, 32.2, 32.3, 32.5, 33.9, 34.2, 34.4, 37.5, 43.1, 45.9, 48.2, 48.5, 68.0, 71.8, 72.4, 82.2, 85.5, 87.9, 97.7, 97.8, 112.1, 121.5, 121.7, 130.6, 150.7, 161.4, 161.6, 171.0, 185.8, 203.9. MS *m/z* calcd for $C_{29}H_{32}F_2O_7$: 494.21; found (APCI) 495 (M+H). Anal calcd for $C_{29}H_{32}F_2O_7$: C 63.15; H 6.52; F 7.68; found: C 62.89; H 6.53; F 7.47.

[1,2-^{3}H]-21-acetoxy-6α,9,-difluoro-11β-hydroxy-16α,17-[(1-methylethylidene)bis(oxy)]pregn-4-ene-3,20-one (21-*O*-acetyl 1,2-dihydrofluocinolone acetonide, [^{3}H]-9)

The light yellow solution of **2** (3.8 mg, 0.0077 mmol), Wilkinson's catalyst (chlorotris(triphenylphosphine)-rhodium (I)) (3.8 mg, 0.0041 mmol) in toluene (filtered through an aluminum oxide pad packed in a disposable glass pipette, 0.8 mL) and THF (filtered through an aluminum oxide pad packed in a disposable glass pipette, 0.4 mL) in a round bottomed flask (2 mL) equipped with a stirrer bar was de-gassed three times on a tritiation system by freezing/evacuating/thawing/the content in the flask. The mixture was stirred under tritium gas (658 mmHg) for 48 h. The black reaction mixture was filtered through a Celite pad packed in a disposable glass pipette. The reaction flask was washed with CH_3OH (4 × 1 mL) and the washings were filtered through the same Celite pad. The combined filtrate was concentrated to dryness using a rotary evaporator. The residue was dissolved in CH_3OH (2 mL) and the solution was mixed with H_2O (9 mL). The slightly cloudy solution was passed through a Supelco Discovery DSC-18 SPE cartridge (500 mg/3 mL, activated with 3 mL CH_3OH and equilibrated with 3 mL H_2O before use). The cartridge was washed with H_2O (4 mL) and eluted with CH_3OH (2 mL), leaving 500.2 mCi crude **[^{3}H]-9** in the CH_3OH solution. A portion of this material was purified as follows. A Supelco LC-Alumina N SPE cartridge (500 mg/3 mL) was equilibrated with hexane (6 mL). Crude **[^{3}H]-9** (200 mCi) in toluene was mixed with hexane (12 mL) and the mixture was passed through the cartridge. The cartridge was eluted with hexane (2 mL), hexane/EtOAc (1/1, 2 × 2 mL), EtOAc (2 × 2 mL). Most of the activity was found in the hexane/EtOAc (1/1, 2 × 2 mL) and EtOAc (2 × 2 mL) eluents (171 mCi/91% radiochemical purity in the combined fraction).

11β,21-Diacetoxy-6α,9,-difluoro-16α,17-[(1-methylethylidene)bis(oxy)]pregna-1,4-diene-3,20-one (1β,21-*O*-,*O*-diacetyl fluocinolone acetonide, 8)

A solution of **1** (201 mg, 0.44 mmol) in pyridine (1.3 mL, 1.277 g, 16.1 mmol, anhydrous) and acetic anhydride (0.67 mL, 0.724 g, 7.09 mmol) was heated under N_2 for 2 h. The solution was cooled to RT, stirred at RT for 2 h, then mixed with H_2O (0°C, 20 mL). The mixture was centrifuged at 3000 rpm for 2 min and the supernatant was removed. The white solids were washed with H_2O (0°C, 2 × 20 mL), freeze-dried overnight, leaving a white solid (208 mg) containing 77% of 21-acetate **2** and 23% of 11,21-diacetate **8**.

The white solid (206 mg) was mixed with 4-dimethyaminopyridine (DMAP, 35 mg, 0.29 mmol), pyridine (1.3 mL, 1.277 g, 16.1 mmol, anhydrous), and acetic anhydride (0.67 mL, 724 mg, 7.09 mmol). The light yellow solution was stirred at RT for 18 h, mixed with H_2O (0°C, 30 mL). The mixture was centrifuged at 3000 rpm for 2 min and the supernatant was removed. The off-white solids were washed with H_2O (0°C, 30 mL), citric acid (0°C, 0.1 M, 2 × 30 mL), H_2O (0°C, 2 × 30 mL), then freeze-dried overnight, leaving a white solid (**8**) (207 mg, 0.38 mmol, yield: 86%, purity: 97%), m.p. $>250^\circ$C ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.84 (s, 3H, H-18), 1.22 (s, 3H, H-23), 1.37(s, 1H, H-22), 1.44 (s, 3H, H-19), 1.57–1.88 (m, 4H, H-7α, H-12β, H-15), 2.13 (s, 3H, H-24), 2.17 (s, 3H, H-25), 2.19-2.54 (m, 4H, H-7β, H-8, H-12α, H-14), 4.73 (d, $J=17.8$ Hz, 1H, H-21), 4.96 (d, 1H, $J=17.9$ Hz , H-21), 5.01 (d, $J=4.6$ Hz, 1H, H-16), 5.31 (qd, $J=48.6$ Hz, 1.8 Hz, 1H, H-6), 5.46–5.50 (m, 1H, H-11), 6.38 (dd, $J=10.1$ Hz, 1.8 Hz, 1H, H-2), 6.45 (m, 1H, H-4), 6.78 (dd, $J=10.1$ Hz, 1.4 Hz, 1H, H-1), in good agreement with the literature,[9] ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm): 16.3, 20.7, 21.8, 23.3, 23.4, 26.0, 26.8, 32.6, 32.8, 32.9, 33.0, 33.5, 33.8, 34.0, 34.2, 42.7, 42.8, 45.4, 67.8, 71.3, 71.3, 82.0, 85.2, 87.6, 97.4, 112.2, 121.8, 122.0, 131.2, 148.9, 169.0, 170.5, 185.1, 203.8, MS (APCI) $m/z=537$ ($M+H^+$).

[1,2-^{3}H]-11β,21-diacetoxy-6α,9,-difluoro-16α,17-[(1-methylethylidene)bis(oxy)]pregn-4-ene-3,20-one (1β,21-*O*-,*O*-diacetyl dihydrofluocinolone acetonide,[^{3}H]-11)

Crude **[^{3}H]-9** recovered from a failed DDQ oxidation (255 mCi, 5.6 mg DDQ, and 0.27 mL benzene) was purified using the procedure described in the preparation of **[^{3}H]-9** affording 90.3 mCi (89% radiochemically pure). This material was combined with separately purified **[^{3}H]-9**. The total (261 mCi) was combined with DMAP (4-dimethylaminopyridine) (2.5 mg, 0.02 mmol), pyridine (0.13 mL, 128 mg, 1.62 mmol), and acetic anhydride (0.067 mL, 72 mg, 0.71 mmol) in a 2-dram vial and stirred under N_2 at RT for 15 h. The reaction mixture was mixed with hexane (4 mL)/EtOAc (1 mL), and the solution was washed with citric acid (0°C, 0.1 M, 3 × 2 mL), H_2O (2 mL), dried over Na_2SO4, leaving **[^{3}H]-11** (238 mCi/97% radiochemical purity). The solvent was evaporated and the residue was dried by dissolving the residue in CH_3CN (1 mL) and evaporating the solvent twice.

[1,2-^{3}H]-11β,21-diacetoxy-6α,9,-difluoro-16α,17-[(1-methylethylidene)bis(oxy)]pregna-1,4-diene-3,20-one (1β,21-*O*-,*O*-diacetyl fluocinolone acetonide, [^{3}H]-8)

A mixture of **[^{3}H]-11** (238 mCi) and benzeneseleninic anhydride (5.1 mg, 0.0014 mmol) in toluene (0.4 mL, anhydrous, filtered through aluminum oxide pad) was stirred at 110°C for 37 h. The solvent was evaporated with a jet of N_2 and the residue was mixed with CH_3OH (2 mL) and H_2O (4 mL). The mixture was then passed through a Supelco Discovery DSC-18 SPE cartridge (500 mg/3 mL, activated with 3 mL of CH_3OH and equilibrated with 3 mL of H_2O before use). The reaction vial was washed with H_2O (1 mL) and the washing was passed through the cartridge. The cartridge was washed with H_2O/CH_3OH (3/1, 2 mL), H_2O/CH_3OH (1/1, 2 mL), H_2O/CH_3OH (1/2, 2 mL); eluted with H_2O/CH_3OH (1/3, 2 mL), CH_3OH (1st 2 mL), CH_3OH (2nd 2 mL). Most of the activity was found in the H_2O/CH_3OH (3/1, 2 mL) fraction. HPLC indicated the material in this fraction to coelute with authentic **8** (71.3 mCi, 98% radiochemical purity). The CH_3OH fractions contained additional **[^{3}H]-8** (21 mCi, 76% radiochemical purity).

[1,2-^{3}H]-6α,9,-difluoro11β,21-diacetoxy-16α,17-[(1-methylethylidene)bis(oxy)]pregna-1,4-diene-3,20-one (fluocinolone acetonide, [^{3}H]-1)

The two fractions of **[^{3}H]-8** were deacetylated separately, as follows:

To the larger fraction of **[^{3}H]-8** (71.3 mCi) in H_2O/CH_3OH (3/1, 2 mL) was added aqueous $K2CO_3$ (10 µL, saturated). After stirring at RT for 100 min the reaction mixture was diluted with H_2O (2.5 mL) and passed through a Supelco Discovery DSC-18 SPE cartridge (500 mg/3 mL, activated with 6 mL CH_3OH and equilibrated with 6 mL H_2O before use). The reaction vial was washed with H_2O/CH_3OH (3/1, 4 mL), eluted with CH_3OH (2 mL). Most of the activity was found in the CH_3OH (2 mL) fraction. HPLC analysis showed that 49% of the activity (60.4 mCi) coeluted with **1**.

To the smaller fraction of **[^{3}H]-8**) (21 mCi) in CH_3OH (2 mL) was added H_2O (0.8 mL) and the solution was de-acetylated and purified following the above procedure, leaving crude **[^{3}H]-1** (15.9 mCi, 58% radiochemical purity).

HPLC purification of [1,2-^{3}H]fluocinolone acetonide ([^{3}H]-1)

Crude [^{3}H]-**1** containing fractions were combined (73.3 mCi), concentrated to about 0.8 mL under N_2. The solution was loaded onto a Phenomenex Gemini C18 column (3 × 150 mm, 5 µm) and eluted with a gradient of 80% H_2O–90% CH_3CN, over 20 min and then holding for 10 min with a flow rate of 0.8 mL/min. The eluent was monitored at λ 235 and 245 nm. The eluent was collected into 1-dram vials and the activity in each vial was counted with a liquid scintillation counter. Most of the activity (30.8 mCi) was found in the fractions collected between 12.6 and 13.2 min. The radiochemical purity and the specific activity of the product were determined by HPLC. HPLC conditions: Phenomenex Gemini C18 column (3 × 150 mm, 5 µm), gradient (solvent A: H_2O, solvent B: CH_3CN, 0–20 min 20–90% B, 20–30 min 90% B), flow rate: 0.8 mL/min, λ 235 and 245 nm, retention time: 11.5 min (UV), 12.2 min (β-RAM)). The product identity was confirmed by co-injection with the unlabeled authentic sample **1**. It was found that the radiochemical purity of [^{3}H]-**1** was 97% by radio-HPLC, and the specific activity was 36.8 Ci/mmol by HPLC. The solvent in the fraction was evaporated under N_2 and the residue was formulated in 100 mL 95% EtOH and stored at −70°C.

Conclusions

Relatively large amounts (>30 mCi) of high specific activity (36.8 Ci/mmol) tritium labeled fluocinolone acetonide have been prepared from authentic fluocinolone acetonide by selective reduction of the 1,2-double bond under tritium and reintroduction of the double bond using benzeneseleninic anhydride. This reagent gave a higher product yield than previously reported conditions (selenium dioxide).

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