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Synthesis of Carbon-14 and Tritiated Steroidal 5α-Reductase Inhibitors

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Summary

17β-[N-(1,1-Dimethylethyl)carbamoyl]androsta-3,5-diene-4-^{14}C-3-carboxylic acid ([^{14}C]SK&F 105657) was prepared *via* a three-step sequence (t-butyl amidation, triflation and carbomethoxylation) starting from androst-4-en-3-one-4-^{14}C-17β-carboxylic acid. Its A-ring aromatic analog 17β-[N-(1,1-dimethylethyl)carbamoyl]estra-1,3,5-(10)-triene-3-carboxylic acid (SK&F 105656) was labeled with tritium by means of iridium-mediated exchange methodology.

Key Words: 5α-Reductase inhibitors, ring-labeled steroids, iridium-mediated tritium exchange, and ^{3}H NMR.

Introduction

Recent efforts in searching for improved therapeutic agents to treat benign prostatic hyperplasia (BPH) in the aging male population led to the discovery of a series of unsaturated 3-carboxysteroids.[1,2] These compounds bind to the enzyme human prostatic steroid 5α-reductase *via* a novel enzyme-NADP^{+}-inhibitor ternary complex, and inhibit the biosynthesis of 5α-dihydrotestosterone.[3,4] The latter, though it is essential for normal prostatic growth to reach puberty, also brings about the undesired effect of organ enlargement at a later age, causing, among other physiological symptoms, the indicative blockage of the urinary tract. Suppression of 5α-dihydrotestosterone synthesis would shrink prostate size and provide a potential cure for BPH.[1,2]

Being potent inhibitors of normal substrate binding to the human prostatic 5α-reductase, SK&F 105657 (**1**) and the A-ring aromatic analog SK&F 105656 (**2**), both with a 17β-t-butylcarboxamide, were targeted for further investigation. Experiments designed to profile their

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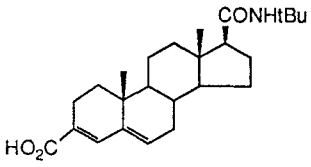
pharmacokinetics and investigate binding characteristics in various biomedia required radiolabeled analogs. Consideration of potential metabolic lability of peripheral substituents restricted the label to the tetracyclic skeleton of these steroidal drugs. Herein we describe our efforts in preparing ring-labeled [^{14}C]SK&F 105657 and [^{3}H]SK&F 105656.

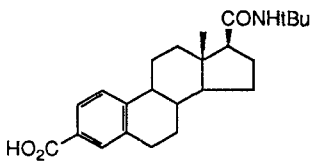
Results and Discussion

In a previously developed route, SK&F 105657 was prepared from pregnenolone *via* the methyl ester of androst-4-en-3-one-17β-carboxylic acid **3**.[5] Synthesis of the ^{14}C-labeled analog of the latter has been described, employing the Fujimoto-Belleau reaction sequence with [^{14}C]methylmagnesium iodide as the labeling source (Scheme I).[6] The carbon-14 label thereby is incorporated into the 4-position in ring A of the steroidal nucleus. This isotopic incorporation pattern fulfills our key requirement mentioned above. Our efforts thus focused on the elaboration of [^{14}C]**3** to [^{14}C]SK&F 105657.



SK&F 105657

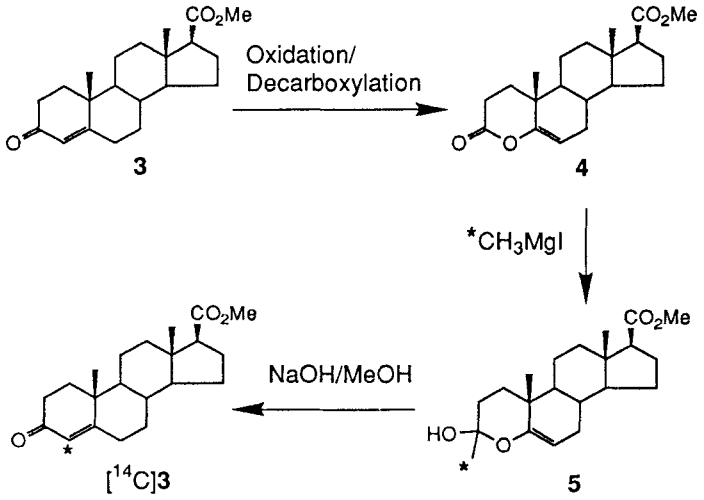
1



SK&F 105656

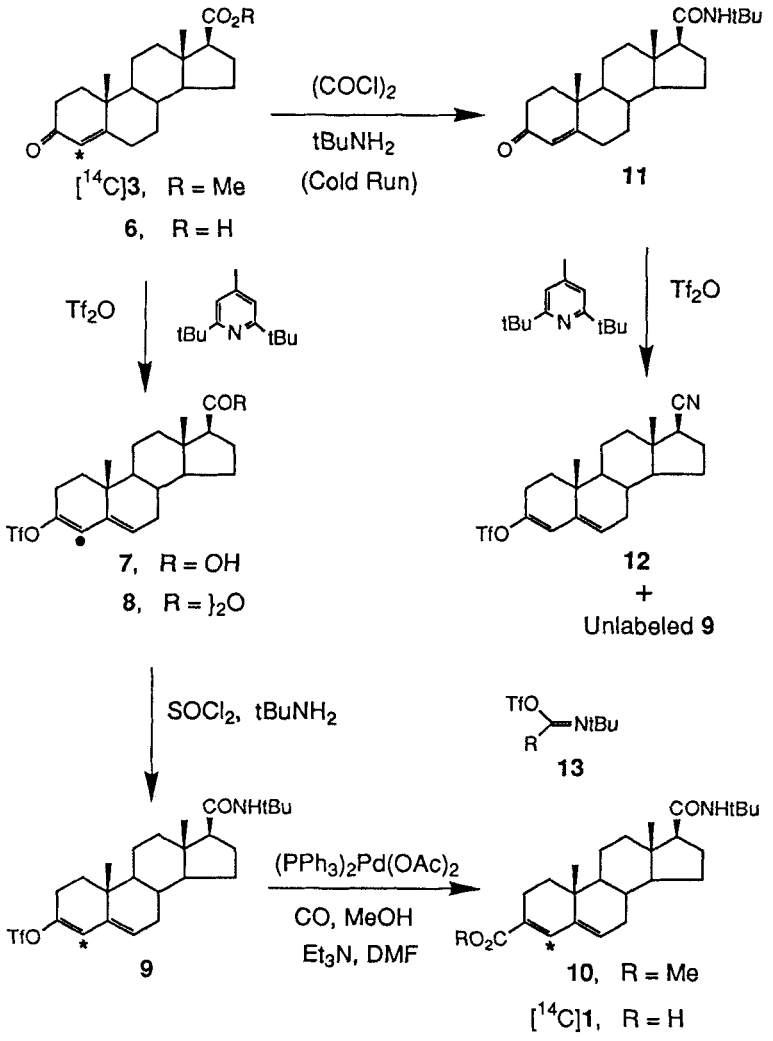
2

Scheme I



Synthesis of [^{14}C]SK&F 105657 involves essentially a three-step sequence. The methyl ester [^{14}C]**3** with a specific activity of 26 mCi/mmol was first hydrolyzed in nearly quantitative yield to 17β-acid **6** (Scheme II). Two options were available at this juncture: elaboration of the peripheral 17β-carboxylic acid to its t-butylcarboxamide derivative followed by A-ring transformation, or *vice versa*. The latter course was chosen because of complications encountered in synthetic method development. Although acid **6** could be converted cleanly to the corresponding enone 17β-t-butylcarboxamide **11**, the ensuing A-ring transformation to diene triflate **9** in the presence of triflic anhydride and

Scheme II

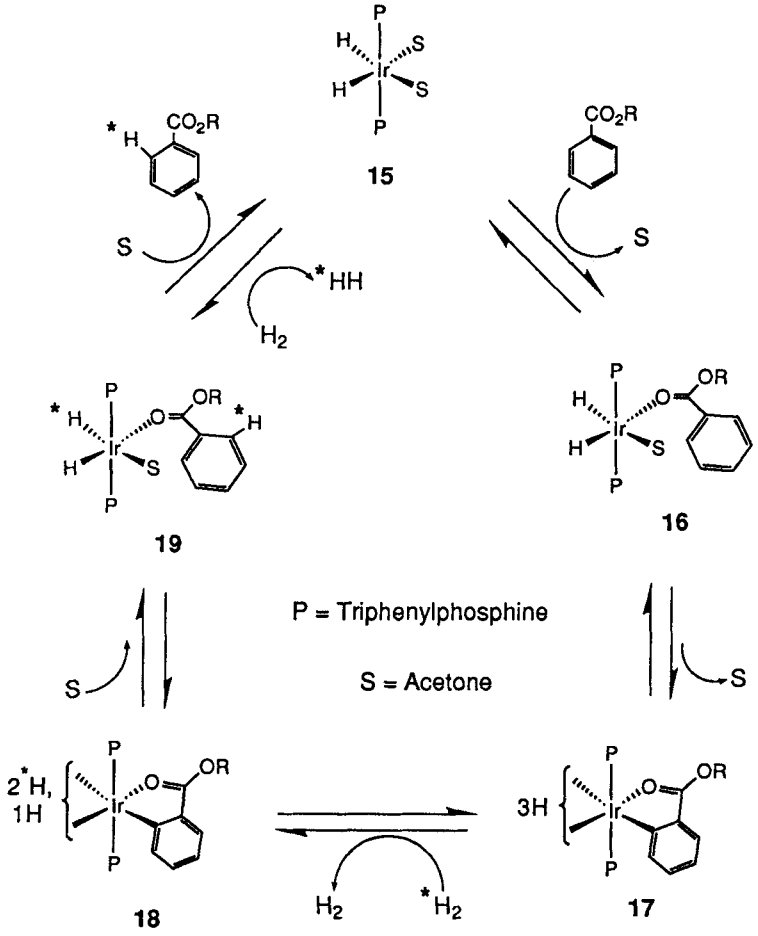


2,6-di-t-butyl-4-methylpyridine[7] was accompanied by competing conversion (25-35%) of the D-ring amide to its corresponding 17β-nitrile **12**, which not only reduced the yield of desired **9**, but also encumbered its purification. Byproduct **12** most likely arose from the reverse Ritter reaction *via* an iminotriflate intermediate **13**, followed by subsequent cleavage of the t-butyl substituent and elimination of isobutylene and triflic acid.[8] This obstacle was circumvented by reversing the reaction sequence. Under analogous triflation conditions, **6** afforded, after aqueous workup and flash column chromatography, a 1:1 ratio of 17β-acid **7** and 17β-anhydride **8**, both possessing the same diene triflate moiety. Each was independently treated with thionyl chloride and t-butylamine to furnish diene triflate 17β-carboxamide **9** in a combined 72% yield from enone acid **6**.[9] The last step comprised palladium(0)-catalyzed carbomethoxylation[10] of the diene triflate to give methyl ester **10**, which was hydrolyzed to [^{14}C]SK&F 105657 in an overall yield of 47% from [^{14}C]**3**.

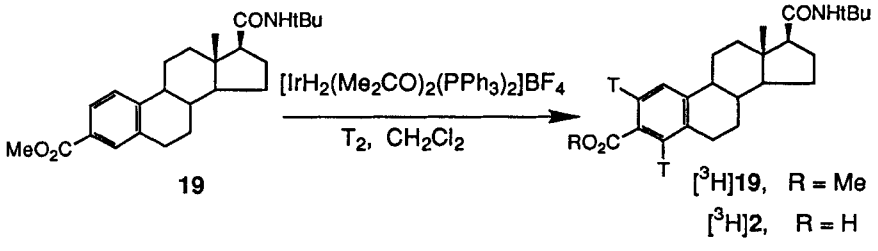
For the preparation of labeled SK&F 105656, we turned our attention to the recently developed iridium-mediated exchange methodology using $[IrH_2(Me_2CO)_2(PPh_3)_2]BF_4$.[11] According to the proposed mechanism outlined in Scheme III,[12] this catalytic cycle involves coordination of a substrate, for example, an aromatic ester, with the dihydrido-iridium-bisphosphine complex **14** followed by metal insertion into an *ortho*-C-H bond to form a 5-membered metallocyclic intermediate **16**. Simultaneous exchange of the metallohydrides with isotopically labeled hydrogen gas channels the label into the ortho-positions of the aromatic ring. SK&F 105656 represented a possible candidate for the application of this method to gain a facile entry to ring-labeled steroids. When methyl ester derivative **19** was exposed to tritium gas in the presence of $[IrH_2(Me_2CO)_2(PPh_3)_2]BF_4$, tritium labels were incorporated exclusively into the C-2 and C-4 positions of the A-ring as observed by the ^{3}H NMR spectra (Figure 1) of [^{3}H]SK&F 105656 ([^{3}H]**2**), obtained after base hydrolysis of the tritiated methyl ester [^{3}H]**19**. Figure 1b depicts the proton-decoupled tritium spectrum of [^{3}H]**2**. Both C-2 (7.66 ppm) and C-4 (7.62 ppm) tritons appear as singlets in a ratio of 9:10. When broad-brand proton decoupling is removed, the C-2 signal shows up as a doublet (J_{HT} = 9 Hz) whereas C-4 remains a singlet (Figure 1a).[13]

The specific activity level of the product, 774 mCi/mmol, was consistent with expectations based on the presence of the coordinative secondary amide function at 17β, which reduced the ability of the iridium complex to participate in the reversible interactions with the A-ring ester group which are necessary for isotopic exchange.

Scheme III



Scheme IV



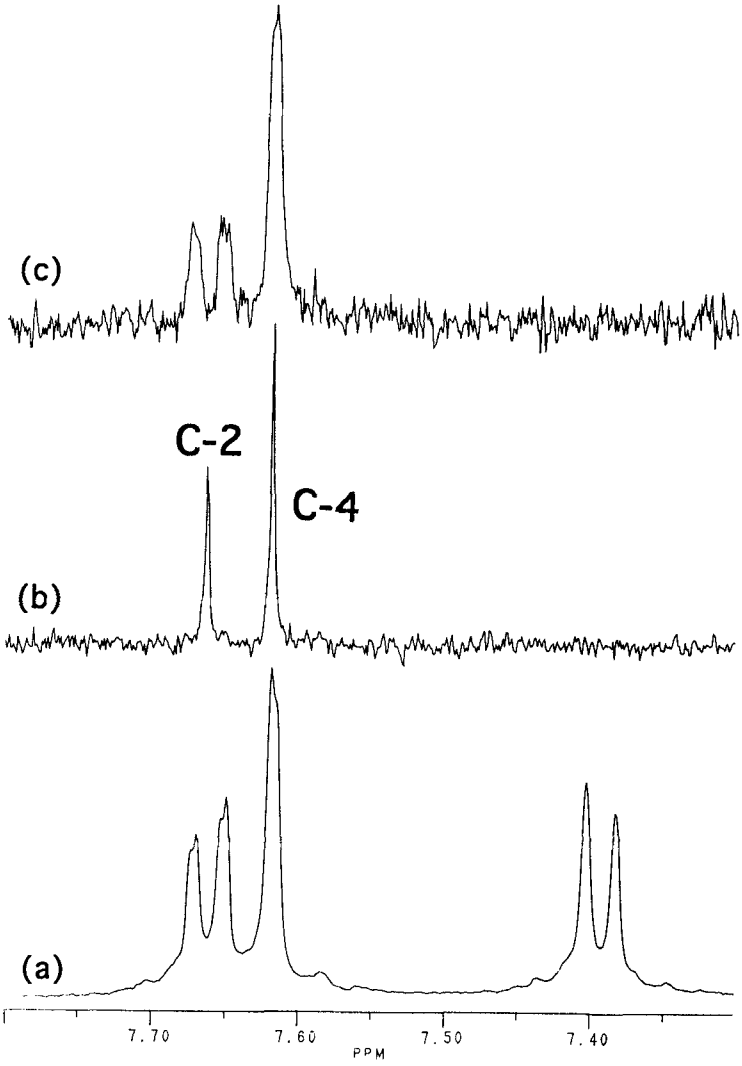


Figure 1. (a) Proton spectrum of **2**. (b) Proton-decoupled tritium spectrum of [^{3}H]**2**. (c) Proton-coupled tritium spectrum of [^{3}H]**2**.

Experimental

Chemicals were obtained from Aldrich. Reaction solvents were distilled from appropiate drying reagents prior to usage. The methyl ester of androsta-4-en-3-one-^{14}C-17β-carboxylic acid was custom-synthesized by Amersham. Radiochemical purities were measured on a Ramona-D radioactivity detector. ^{1}H NMR and ^{3}H NMR spectra were recorded on a Bruker AM-400 spectrometer in specified deuterated solvents.

Referencing of chemical shifts in ^3H NMR, measured on a 5 mCi sample in DMSO-d_6, was achieved initially by the reported ghost referencing method,[14] and later adjusted to match the appropiate signals in ^1H NMR.

Androst-4-en-3-one-4-^{14}C-17β-carboxylic acid (**6**)

Ester [^{14}C]**3** (130 mCi, specific activity: 57 mCi/mmol) was diluted with 0.90 g of cold carrier and the resulting material had a specific activity of 26 mCi/mmol. A 0.81 g portion was refluxed in CH_3OH (33 mL) and KOH (0.80 g) at 95 °C for 22 h. Workup included acidification, extraction with EtOAc and removal of organic solvent, providing 0.76 g of enone acid **6** (98% yield).

[((Trifluoromethyl)sulfonyl)oxy]androsta-3,5-diene-4-^{14}C-17β carboxylic acid (**7**) and 3-[((Trifluoromethyl)sulfonyl)oxy]androsta-3,5-diene-4-^{14}C-17β-carboxylic acid anhydride (**8**)

To a stirred solution of trifluoromethanesulfonic acid anhydride (0.8 mL, 476 mmol) in CH_2Cl_2 (25 mL) at 0 °C was added a solution of enone acid **6** (0.76 g, 2.42 mmol) and 2,6-di-t-butyl-4-methylpyridine (1.20 g, 5.85 mmol) in CH_2Cl_2 (40 mL) over a period of 2 min. The resulting mixture was stirred at 0 °C for 10 min and then room temperature for 20 min. Workup included dilution with CH_2Cl_2 (50 mL), washing with 5% aqueous HCl (25 mL), saturated $NaHCO_3$ (20 m), H_2O (15 mL), and brine (15 mL). Concentration under reduced pressure gave a crude mixture of acid and anhydride, which was chromatographed on a silica gel column with gradient elution of hexane/CH_2Cl_2 mixtures (20% increment of CH_2Cl_2). The 100% CH_2Cl_2 fraction afforded, after concentration under reduced pressure, anhydride **8** (0.56 g, 52% yield). ^1H NMR ($CDCl_3$): 0.83 (3H, s, 18-**Me**), 0.97 (3H, s, 19-**Me**), 5.58 (1H, br s, 6-**H**), 5.99 (1H, s, 4-**H**).

The column was then eluted with CH_2Cl_2/CH_3OH mixtures. The 10% CH_3OH fraction gave acid **7** (0.54 g, 50% yield). ^1H NMR ($CDCl_3$): 0.78 (3H, s, 18-**Me**), 0.97 (3H, s, 19-**Me**), 5.58 (1H, br s, 6-**H**), 5.99 (1H, s, 4-**H**).

N-(1,1-Dimethylethyl)-3-[((trifluoromethyl)sulfonyl)oxy]androsta-3,5-diene-4-^{14}C-17β-carboxamide (**9**)

A solution of triflate acid **7** (0.54 g, 1.21 mmol) and thionyl chloride (1 mL) in toluene (30 mL) was stirred at room temperature for 3 h, and then concentrated under reduced pressure. The resulting material was redissolved in pyridine (15 mL), followed by addition of t-butylamine (8 mL). The mixture was stirred at room temperature for 3 h, followed by removal of solvents. Triflate anhydride **8** (0.56 g) was treated analogously. Radio-TLCs of these two reaction mixtures showed, respectively, 76% and 91% of the total activity corresponded to the desired triflate amide **9**. The crude amides were combined and

chromatographed on a silica gel column with gradient elution of hexane/EtOAc mixtures (4% increment of EtOAc). The 24% EtOAc fraction gave the triflate amide **9** (0.89 g, 72% combined yield). ^{1}H NMR ($CDCl_3$): 0.72 (3H, s, 18-**Me**), 0.97 (3H, s, 19-**Me**), 1.36 (9H, s, C-**Me$_3$**), 5.09 (1H, s, N-**H**), 5.58 (1H, br s, 6-**H**), 5.99 (1H, s, 4-**H**); radiochemical purity: 98% (Altex Ultrasphere C_{18} column (5 μm, 4.6 mm I.D. x 25 cm), 9/1 (v/v) CH_3CN/H_2O, 1.0 mL/min, UV at 230 nm, R_t: 10.2 min).

Methyl 17β-[N-(1,1-Dimethylethyl)carbamoyl]androsta-3,5-diene-4-^{14}C-3-carboxylate (**10**)

A solution of **9** (0.89 g, 1.77 mmol), bis(triphenylphosphine)-palladium (II) acetate (0.15 g, 0.20 mmol), Et_3N (10 mL), DMF (15 mL) and CH_3OH (15 mL) were stirred under a carbon monoxide atmosphere (balloon) at 65 °C for 50 min. Concentration under reduced pressure and chromatography on a silica gel column (gradient elution with hexane/EtOAc mixtures) gave ester **10** (0.60 g, 82%). ^{1}H NMR ($CDCl_3$): 0.72 (3H, s, 18-**Me**), 0.91 (3H, s, 19-**Me**), 1.36 (9H, s, C-**Me$_3$**), 3.75 (3H, s, CO_2**Me**), 5.09 (1H, s, N-**H**), 5.81 (1H, br s, 6-**H**), 7.03 (1H, s, 4-**H**); radiochemical purity: 98% (Altex Ultrasphere C_{18} column (5 μm, 4.6 mm I.D. x 25 cm), 9/1 (v/v) CH_3CN/H_2O, 1.0 mL/min, UV at 230 nm, R_t: 8.5 min).

17β-[N-(1,1-Dimethylethyl)carbamoyl]androsta-3,5-diene-4-^{14}C-3-carboxylic acid ([^{14}C] **1**)

A solution of ester **10** (0.60 g, 1.45 mmol) and K_2CO_3 (0.65 g) in 1/9 (v/v) H_2O/CH_3OH (45 mL) was refluxed at 95 °C for 15 h. Workup included acidification with 5% aqueous HCl and extraction with EtOAc (100 mL). The organic phase was washed with H_2O (2 x 20 mL) and brine (10 mL), followed by drying over $NaSO_4$ and concentration under reduced pressure, affording 0.55 g of crude [^{14}C]SK&F 105657. Recrystallization from CH_2Cl_2 and CH_3CN gave, after drying at 110 °C for 40 h, 0.46 g (77% yield from **10**) of [^{14}C]**1**. ^{1}H NMR ($CDCl_3$): 0.72 (3H, s, 18-**Me**), 0.92 (3H, s, 19-**Me**), 1.36 (9H, s, C-**Me$_3$**), 5.10 (1H, s, N-**H**), 5.87 (1H, br s, 6-**H**), 7.15 (1H, s, 4-**H**); radichemical purity: 98% (two Altex Ultrasphere C_{18} columns (5 μm, 4.6 mm I.D. x 25 cm), 200/200/100/0.5 (v/v/v/v) $CH_3CN/CH_3OH/H_2O$/trifluoroacetic acid, 1.0 mL/min, UV at 230 nm, R_t: 16.3 min).

Methyl 17β-[N-(1,1-Dimethylethyl)carbamoyl]estra-1,3,5(10)-triene-2,4-t_2-3-carboxylate ([^{3}H]**19**)

A 6 mL round-bottomed flask containing a homogeneous solution of methyl ester **20** (12 mg, 0.030 mmol) and $[IrH_2(Me_2CO)_2(PPh_3)_2]BF_4$ (15 mg, 0.016 mmol) in 1 mL of CH_2Cl_2 was attached to a tritium stainless steel manifold. After six cycles of freeze-pump-thaw, the mixture was exposed to 15.5 Ci of tritium gas and then stirred at room temperature for 18 h.

Workup involved removal of excess tritium gas and subsequent static vacuum distillation of the reaction mixture to remove tritium labiles. Recovered in the resulting crude material was 641 mCi of activity. Flash column chromatography provided 26 mCi of [^{3}H]**19** with a radiochemical purity of 83% (Altex Ultrasphere C_{18} column (5 μm, 4.6 mm I.D. x 25 cm), 75/25/0.2 (v/v/v) CH_3CN/H_2O/trifluoroacetic acid, 1.0 mL/min, UV at 220 nm, R_t: 15.6 min).

17β-[N-(1,1-Dimethylethyl)carbamoyl]estra-1,3,5(10)-triene-2,4-t_2-3-carboxylic acid ([^{3}H]**2**)

A mixture of [^{3}H]**19** (26 mCi) and aqueous NaOH (20 mg in 0.2 mL of H_2O) in CH_3OH (2 mL) was heated at 80 °C under argon for 75 min. After cooling to ambient temperature, solvents were removed by concentration under reduced pressure. The residue was purified by HPLC (Altex Ultrasphere semi-preparative C_{18} column (5 μm, 10 mm I.D. x 25 cm), 770/280/1 (v/v/v) CH_3OH/H_2O/trifluoroacetic acid, 3.0 mL/min, UV at 220 nm, R_t: 15.2-16.8 min). Removal of HPLC solvents and dissolution in absolute EtOH (5 mL) gave 17 mCi of [^{3}H]**2** with a radiochemical purity in excess of 99% (Altex Ultrasphere C_{18} column (5 μm, 4.6 mm I.D. x 25 cm), 250/250/0.5 (v/v/v) CH_3CN/H_2O/trifluoroacetic acid, 1.0 mL/min, UV at 230 nm, R_t: 11.7 min). The specific activity was determined by a combined gravimetric assay and radioactive concentration determination by scintillation counting to be 774 mCi/mmol.

Acknowledgements

We thank D.H. Holt for helpful discussion of the synthesis, W. Kokke (C-14), S.H. Levinson (^{3}H NMR) and C.R. Newsome for performing various radioanalyses.

References and Notes

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13. Fine coupling constants between C-2 and C-4 in the proton spectrum (J_{HH} = 2 Hz) and the proton-coupled tritium spectrum (J_{HT} = 2 Hz) could be extracted by resolution enhancement through modification of the respective FIDs with appropiate gaussian window functions.

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