SYNTHESIS OF CARBON-14 AND TRITIUM LABELED METHYLPREDNISOLONE SULEPTANATE

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SUMMARY

Methylprednisolone suleptanate was initially labeled with tritium in the A-ring of the steroid portion of the molecule, and with carbon-14 at both carboxylic carbons of the suberic acid portion of the side chain. However these labels proved to lack total metabolic stability after administration to rats. Subsequently a second pair of labeled methylprednisolone suleptanates was synthesized, with tritium at C-7 in the B-ring of the steroid and carbon-14 exclusively at the carboxamide carbon in the side chain. These labeled compounds showed excellent metabolic stability of both the tritium and carbon-14 labels, and should be well suited for conducting drug disposition studies.

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Key words: methylprednisolone suleptanate, synthesis, tritium labeled, carbon-14 labeled, metabolic stability, isomerization

INTRODUCTION

Methylprednisolone Suleptanate* (***1c***) is potentially a new water soluble pro-drug for methylprednisolone (***1a***). Solu-Medrol® sterile powder, the water soluble hemisuccinate ester (***1b***).of methylprednisolone, has been an important drug for the treatment of shock and inflammatory disorders, but its rapid *in vitro* hydrolysis gives

*Methylprednisolone is the generic name for 11β, 17α, 21-trihydroxy-6α-methylpregna-1,4-diene-3, 20-dione. Methylprednisolone suleptanate is the ester formed from the C_{21} alcohol of methylprednisolone and the monosodium salt of 8-[methyl(2-sulfoethyl)amino]-8-oxooctanoic acid.

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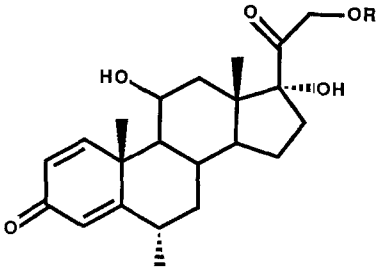
the pro-drug a short shelf life in solution. Methylprednisolone suleptanate, on the other hand, exhibits good solution stability and yet maintains the desired *in vivo* lability required of a pro-drug (1). The compound has shown particular utility in treating acute bronchospasm when administered intravenously (2,3). Radioactive forms of the compound are needed to study its absorption, distribution, biotransformation, and excretion in test animals and man. In anticipation of the need to trace the metabolic fate of both the steroid and side chain portions of *1* following its *in vivo* hydrolysis, we chose to doubly label the compound with tritium in the steroid, and with carbon-14 in the side chain.



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a) R = H METHYLPREDNISOLONE

b) R = $-\overset{O}{\overset{\|}{C}}-(CH_2)_2CO_2Na$ METHYLPREDNISOLONE HEMISUCCINATE

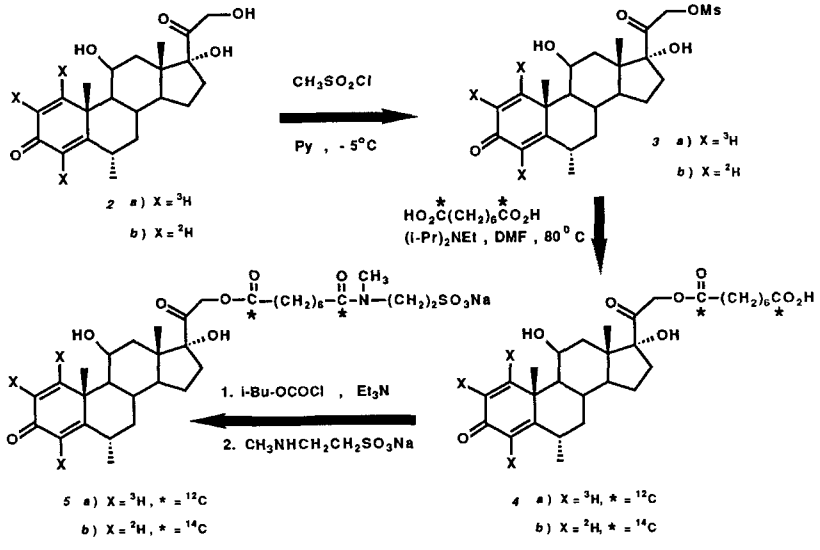
c) R = $-\overset{O}{\overset{\|}{C}}-(CH_2)_6-\overset{O}{\overset{\|}{C}}-\overset{CH_3}{\overset{|}{N}}-(CH_2)_2SO_3Na$ METHYLPREDNISOLONE SULEPTANATE

DISCUSSION AND RESULTS

Tritium labeled methylprednisolone suleptanate (***5a***) was synthesized by attaching the side chain onto radioactive methylprednisolone labeled with tritium in the steroid A-ring at C_1, C_2, and C_4, as shown in Scheme 1. The labeled starting material ***2a*** was prepared by reducing the A-ring of methylprednisolone with tritium

gas, which introduced tritium at C_1, C_2, C_4, and C_5, and reestablishing the $\Delta^{1,2}$ and $\Delta^{4,5}$ double bonds by dehydrogenation. The latter occurred predominantly from the side of the steroid molecule opposite to the face where introduction of tritium had taken place, thus leaving the tritium labels at C_1, C_2, and C_4 of the product. [1,2,4-^{3}H]methylprednisolone (***2a***) was converted to its 21-mesylate ester (***3a***). The mesylate was treated with a four-fold excess of suberic acid in the presence of diisopropylethylamine to give, in 83% yield, [1,2,4-^{3}H]methylprednisolone 21-hemisuberate (***4a***). Use of excess suberic acid was appropriate in this case in order to maximize utilization of the valuable labeled steroid. The hemisuberate ***4a*** was converted to the mixed anhydride with isobutyl chloroformate, which was used to acylate the sodium salt of N-methyl taurine (NMT-Na) to give tritium labeled ***5a***.

Scheme 1



For the synthesis of carbon-14 labeled methylprednisolone suleptanate (***5b***), we modified the procedure in order to optimize radiochemical yield, since labeled suberic acid was the more valuable material. With equimolar amounts of mesylate ***3b*** and suberic acid, although the chemical and radiochemical yield of the hemisuberate ***4b*** was only 40%, it nevertheless represented a significant improvement, in radiochemical yield, when compared with the method using suberic acid in excess. With the use of 4-fold excess of labeled suberic acid, the radiochemical yield of the hemisuberate ***4b*** would have only been 15-20%, even though a chemical yield of 80% based on the steroid would have been achieved. The conversion of the carbon-14 labeled hemisuberate ***4b*** to carbon-14 labeled ***5b***, was carried out in the same manner as the preparation of ***5a*** from ***4a***.

In a preliminary study, carbon-14 labeled ***5b*** was administered to rats. It was found that approximately 20% of the radioactivity dosed was excreted as $^{14}CO_2$ (4). Evidently the side chain, after cleavage from the drug through *in vivo* hydrolysis, underwent β-oxidation at the resulting labeled free acid end with loss of 2-carbon units, which underwent further metabolic transformations to give carbon dioxide. When tritium labeled ***5a*** was administered to rats, although there appeared to be little or no loss of the tritium labels *in vivo*, significant loss of tritium as unknown volatile material(s) was observed in collected and stored fecal samples (4). It was clear that for some of the planned metabolism studies with methylprednisolone suleptanate, labeled drug with metabolically more stable labels would be needed. Since metabolic transformations in the steroid A-ring appeared responsible for loss of the tritium label(s), we decided to next place tritium in the B-ring. For the side chain, we decided to locate the carbon-14 label exclusively at the amide carbon. The rationale for this strategy was that, following *in vivo* hydrolysis of the ester function in the pro-drug, β-oxidation of the free acid end of the side chain, with loss of two-carbon units, would hopefully proceed no further than two carbons in front of the amide nitrogen. Thus the remnant of the side chain would still bear the radiolabel and allow the tracing of side chain metabolites.

To introduce tritium into the steroid B-ring, we attempted selective catalytic reduction with tritium gas of a precursor having both A- and B-ring unsaturation.

Reduction with deuterium was used as a model for ascertaining optimum reaction conditions. 11β,17α,21-Trihydroxy-6-methylpregna-4,6-diene-3,20-dione, 21-acetate (***6***) was subjected to reduction with deuterium gas over 5% palladium on strontium carbonate in benzene (cf. 5). The reduction was slow (uptake of 1 equivalent of D_2 in 5.5 hr) and non-selective, giving a mixture of products as well as unreacted starting material. Reduction of a dieone in the presence of a base is known to increase selectivity through enolization. The rates of adsorption, reduction, and desorption of the enolates formed with base catalysis are greater than the corresponding rates of the ketonic forms (6). We therefore carried out the reduction of ***6*** in methanol containing 0.1 N potassium hydroxide in the presence of 5% palladium on carbon (Scheme 2). One molar equivalent of D_2 was taken up in 1 hr. Most of the starting material was consumed, but a mixture of products was still found, with complete deesterification at C_{21}. We wanted to avoid premature deesterification since the unprotected 21-hydroxy group might lead to undesired complications in subsequent steps. Reduction with 5% palladium on carbon in methanol without potassium hydroxide was also fast (1 molar equivalent of deuterium gas in 30 min), giving a mixture of products in which compound ***7*** predominated. The reaction rate could be attenuated by replacing methanol with varying amounts of dioxane. Use of dioxane as solvent slowed the reduction rate (uptake of 1 molar equivalent of deuterium in 15 hr)*, but the product distribution remained similar to that found using methanol (compound ***7*** major product). Use of methanol or methanol-dioxane mixtures as solvent for the reduction also produced some deesterified products.

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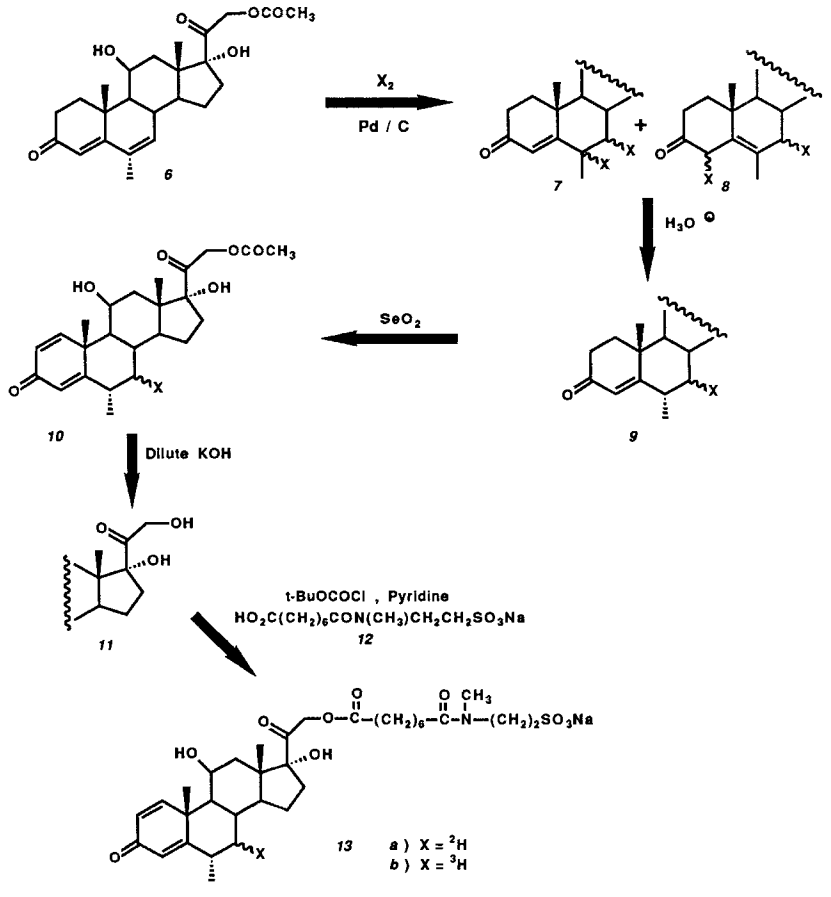
*Dioxane used in this experiment contained a trace of water. Dioxane dried over sodium required ca. 30 hr to take up 1 molar equivalent of deuterium.

Scheme 2



Reduction of ***6*** with deuterium gas using 5% palladium on carbon in methanol in the presence of triethylamine (TEA) gave a dramatic change in product distribution. The major product, a material less polar than ***7a***, was the diene 1,4-addition product ***8a***. Analogous 1,4-addition of hydrogen to dienes also occurs in the related 6-methylene-3-keto-Δ^4 steroids when they are catalytically reduced in methanol containing TEA (7). In the presence of hydrochloric acid, the 3-keto-Δ^5 steroid *8a* was readily converted to the desired Δ^4 ***9a*** with the correct stereochemistry at C_6. Simultaneously, the labile deuterium at C_4 was completely removed through exchange. Furthermore, under the same acidic condition, compound ***7a***, which was shown by NMR to be of mixed stereochemistry at C_6, also underwent isomerization, along with removal of label at C_6, to give a single clean product ***9a*** with non-exchangeable label

exclusively at C_7. Thus the mixture of all the deuterated reduction products was efficiently transformed, without separation, into the single desired labeled product ***9a*** as shown in Scheme 2. The reduction of ***6*** with tritium gas was carried out with dioxane containing TEA as the solvent, which also produced a mixture of ***7b*** and ***8b***. The choice of dioxane, although resulting in a slower reaction rate, did minimize the production of deesterified products. Acid treatment of the reduction product mixture then afforded tritium labeled ***9b***.

Labeled Δ^4-3-keto-steroid ***9a*** was converted into [^{2}H]methylprednisolone (***11a***) by dehydrogenation followed by alkaline hydrolysis. Because of the greater tendency of chloranil and selenium dioxide for causing side reactions, 2,3-dichloro-5,6-dicyano-benzoquinone (DDQ) has been considered the dehydrogenation reagent of choice (8). A solution of ***9a*** in dioxane was heated to 90°C with an excess of DDQ for 16 hr to give an 80% yield of the desired $\Delta^{1,4}$-3-keto-steroid ***10a***, which contained ~20% of an impurity as determined by HPLC analysis. Higher reaction temperatures produced larger amounts of impurity. Several attempts directed at purification of the DDQ reaction mixture by preparative HPLC failed to produce satisfactory results. Attention was therefore turned to modifying the dehydrogenation reaction.

It has been shown that DDQ reactions catalyzed by benzoic acid are more specific for Δ^1-dehydrogenation than the corresponding uncatalyzed reactions (9). In our hands, addition of benzoic acid reduced the "impurity" level to ~8% but did not eliminate it. Dehydrogenation of ***9a*** with selenium dioxide in refluxing t-butanol, however, gave ***10a*** free of the impurity associated with the DDQ oxidations. Dehydrogenation of 1.24 Ci of ***9b*** in the same manner led to a 59.2% yield (726 mCi) of ***10b*** which was 98.4% radiochemically pure. A sample of 666 mCi of ***10b*** was then carefully hydrolyzed in dilute methanolic potassium hydroxide. This ester hydrolysis was rapid, but always accompanied by base-promoted side reactions which required the product to be purified by chromatography to give 564 mCi of [^{3}H]methylprednisolone ***11b*** having a specific activity of 7.4 mCi/mg and a radiochemical purity of 98.5% by HPLC. Most of ***11b*** was stored in solution while 144 mCi was transformed into [^{3}H]methylprednisolone suleptanate (***13b***). The side chain ***12***

was activated by reaction with pivaloyl chloride to give the mixed anhydride prior to the addition of ***11b*** and unlabeled methylprednisolone to give ***13b***.

The synthesis of carboxamide carbon-14 labeled methylprednisolone suleptanate required the preparation of ^{14}C-labeled side chain, the monosodium salt of 8-[methyl(2-sulfoethyl) amino]-8-oxo-[8-^{14}C] octanoic acid (***16***). The side chain is composed of two subunits, the symmetrical diacid suberic acid, and N-methyltaurine sodium salt (NMT-Na). In order to radiolabel exclusively the amide end of the suberic acid moiety with carbon-14, a "mask" was needed to distinguish one end of the symmetrical molecule from the other. The differentiation was achieved by holding the two ends of the eight-carbon chain at dissimilar oxidation states as outlined in Scheme 3. One terminus of the eventual suberic acid moiety was held at the alcohol oxidation state until the labeled amide function was in place. The unlabeled alcohol end was then oxidized to give the properly functionalized side chain with the label exclusively located at the carboxamide carbon. 7-Bromoheptanoic acid (***14***) was reduced with borane-dimethylsulfide in tetrahydrofuran to give 7-bromoheptanol (***15***). The bromo alcohol was treated with 3,4-dihydro-2H-pyran (DHP) and the resulting bromo tetrahydropyranyl ether was reacted with sodium [^{14}C]cyanide to afford the labeled nitrile, which was hydrolyzed to the corresponding acid. The acid was then coupled with NMT-Na to complete the amide end of the side chain. Acid induced deprotection* followed by permanganate oxidation of the resulting alcohol afforded the carboxamide carbon labeled side chain ***16***, which was reacted with methylprednisolone 21-mesylate to produce the carbon-14 labeled suleptanate ***17***.

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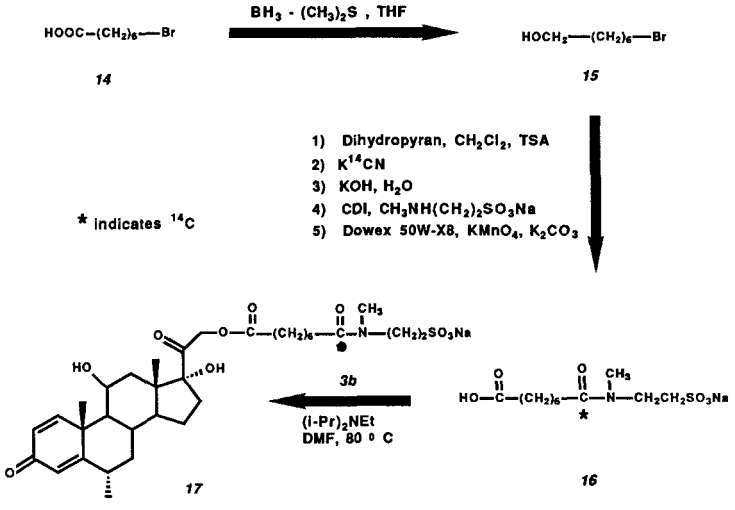
*Deprotection with ion exchange resin Dowex 50W-X8 in the acid form (10) was more convenient and rapid than using acetic acid/THF/water.

Scheme 3



The C-7 tritium labeled (***13b***) and carboxamide carbon ^{14}C-labeled methylprenisolone suleptanate (***17***) have been administered to rats. Both labels have proved to be metabolically stable (4).

EXPERIMENTAL SECTION

Radioactivity determinations were made with an LKB Instruments Model 1217 Rackbeta liquid scintillation spectrometer using the external standard method. Diotol (Burdick and Jackson laboratories; Muskegon, MI., U.S.A.) was used as the scintillation solvent. Thin layer chromatographic (TLC) analyses were done on 2.5 x 10 cm glass plates precoated with a 250 μm layer of silica gel GF (Analtech, Newark, Delaware, U.S.A.). The developed zones were visualized with the method most applicable, either with 254 nm UV light or 18N H_2SO_4 spray-char, or 0.5% bromocresol green (in ethanol)

spray. Radioactive zones were detected and quantified with a Bioscan System 200 Imaging Scanner with Auto Changer 3000. HPLC analyses were carried out with a Spectra Physics Model 8700 Solvent Delivery System using a Supelcosil LC-18 (5μ, 4.6 mm I.D. x 25 cm) or a DuPont Zorbax C-8 (6μ, 4.6 mm I.D. x 25 cm) analytical column. The mobile phases used were: A) 600:400:4 v/v methanol:water:TEA, adjusted to pH6 with phosphoric acid; B) 540:460:4 v/v methanol:water:TEA adjusted to pH6 with phosphoric acid, C) 320:680 v/v acetonitrile:H_2O; D) 540:460 v/v methanol:water; E) 180:820:8 v/v acetonitrile:water:10% tetra-n-butylammonium hydroxide adjusted to pH6 with acetic acid. Eluates were analyzed with a Spectra Physics Model 8440 Variable Wavelength UV Detector and a Berthold LB-503 HPLC Radioactivity Monitor with Flo-Scint II as the scintillator. Chromatographic data were quantified with Spectra Physics Model 4270 Computing Integrators. Nuclear Magnetic Resonance (NMR) Spectra were obtained on a Varian CFT-20, a Varian XL-200 or on a Varian XL-300 MHz instrument. Tritium reductions were done with an ambient pressure semi-micro hydrogenation apparatus as described by Thomas *et al.* (5).

[1,2,4-^{3}H]Methylprednisolone, 21-Mesylate (***3a***)

[1,2,4-^{3}H]Methylprednisolone* obtained commercially was purified by preparative HPLC using a Supelcosil LC-18 (5 μm) analytical column and mobile phase B. To the pooled fractions containing pure [1,2,4-^{3}H]methylprednisolone was added 650 mg of unlabeled methylprednisolone. The solution was acidified to pH3 with 1N HCl and concentrated at 35°C and 25 torr to remove methanol. The resulting crystals were filtered, washed with water, and dried under vacuum to give 637 mg of ***2a***, sp. act. 282 μCi/mg, >99% radiochemically pure by HPLC (Supelcosil LC-18 column, mobile phase A) and TLC (1:9 methanol:methylene chloride).

Methanesulfonyl chloride (0.25 ml) was added at -5°C with stirring under N_2 to a solution of 560 mg of ***2a*** (1.5 mmol) in 2.5 ml of pyridine. The mixture was stirred

*Supplied by Amersham Corporation, Arlington Heights, IL, USA, nominally 200 mCi, 52 Ci/mmol.

at -5°C for 1 hr and added dropwise with stirring to 25 ml of water at 5°C. The resulting solids were collected by filtration, washed with water, and dried to afford 677 mg (99% yield) of ***3a***, sp. act. 229 μCi/mg, >98% radiochemically pure by HPLC (Supelcosil LC-18 mobile phase A) and TLC (1:9 methanol:methylene chloride).

[1,2,4-^{3}H]Methylprednisolone, 21-hemisuberate (***4a***)

A solution of 676 mg of ***3a*** (1.5 mmol), 1.3 g of suberic acid (7.5 mmol), and 1.3 ml of diisopropylethylamine (7.5 mmol) in 8 ml of dimethylformamide (DMF) was stirred at 78°C under nitrogen for 16 hr. The mixture was cooled to room temperature and added dropwise with vigorous stirring to 125 ml of water. The resulting solids were collected by filtration, washed with water, and dried under high vacuum briefly. The crude solids were chromatographed on a column of 150 g of silica gel packed in and eluted with 8% v/v methanol in methylene chloride. Fractions of 13 ml each were collected at a flow rate of 4.3 ml/min. After approximately 300 ml had been collected, the eluent was changed to 10% v/v methanol in methylene chloride. Fractions 33-61 were pooled and concentrated. The residue was recrystallized from 20% v/v methanol in acetone, and heptane to give 656 mg (83%) of white crystals. The product had a specific activity of 193 μCi/mg, was >95% pure by TLC (10% v/v methanol in methylene chloride, Rf = 0.3, with a small amount of bis-methylprednisolone 21-suberate, Rf = 0.6). HPLC analysis* showed the material was >99% pure by UV (254 nm) and radioactivity detection (Supelcosil LC-18 column, mobile phase A).

[1,2,4-^{3}H]Methylprednisolone Suleptanate (***5a***)

A sample of commercially available (Pfaltz & Bauer, Waterbury, CT) NMT-Na (61.28 g, a syrupy slurry) was dissolved in 25 ml of water and filtered to remove any insoluble material. The filtrate was dried in an oven at 60°C under high vacuum for two days. The hard residue was broken up, ground in a mortar to a fine powder and further dried for 48 hours. This material weighed 41.6 g. A 2.5 g sample was dissolved in DMF

*Note: The HPLC mobile phase used does not elute bis-methylprednisolone 21-suberate from the Supelcosil LC-18 column.

(50 ml) and filtered to remove insoluble material (approximately 0.35 g). This filtrate was calculated to contain 43 mg of NMT-Na per ml of solution.

A solution of 656 mg of ***4a*** (1.24 mmol) and 0.19 ml TEA (0.19 ml, 1.36 mmol) in 2.3 ml of DMF was cooled to -5°C, and isobutylchloroformate (0.18 ml, 1.36 mmol) was added under nitrogen *via* a syringe. A precipitate formed immediately and the mixture was stirred for 20 minutes. The cooling bath was removed and NMT-Na (5.6 ml of solution in DMF described above, 1.48 mmol, and more TEA (0.19 ml, 1.36 mmol) were added, and the mixture was stirred for 40 minutes. The DMF was removed under high vacuum at 40°C and the residue was partitioned between 25 ml each of 0.6 M sodium bisulfate (pH = 1.7) and ethyl acetate. The aqueous phase was washed once more with 25 ml of ethyl acetate and then extracted with 2 x 25 ml of n-butanol. The n-butanol extracts were combined, washed with 3 x 25 ml of 0.5 M sodium sulfate solution (pH adjusted to 5.1 with dilute NaOH), and dried over anhydrous sodium sulfate. The mixture was filtered and the filtrate was concentrated under high vacuum at 40°C. The residue was dissolved in a few ml of methanol, decolorized with activated charcoal, and filtered through a bed of celite. The methanol solution was concentrated to 1 ml and added slowly to 25 ml of acetonitrile with stirring. The resulting solids were collected by filtration, dried, and recrystallized by dissolving in 4 ml of 10% v/v methanol in methylene chloride and adding the solution dropwise to 25 ml of ether with stirring. The solids were washed with ether and dried under high vacuum at room temperature to give 0.364 g of ***5a*** (43.6%), specific activity 143 μCi/mg, radiochemically pure by TLC (10% v/v methanol in methylene chloride, Rf = 0.04, single component identical to a standard. Analysis by HPLC (Supelcosil LC-18 column, mobile phases A and B) showed the materials was >99% pure by both UV and radioactivity detection.

A second crop of ***5a*** was obtained from the mother liquor by recrystallization with 120 mg of unlabeled methylprednisolone suleptanate from methanol and acetonitrile. This gave 216 g of ***5a*** with a specific activity of 53.7 μCi/mg, >99% radiochemically pure by TLC and HPLC (Supelcosil LC-18 column, mobile phases A and B; UV 254 nm and radioactivity).

Methylprednisolone, 21-[1,8-^{14}C]Hemisuberate, (***4b***)

A solution of ***3b*** (1.268 g, 2.80 mmol) diisopropylethylamine (0.975 ml, 5.6 mmol), and [1,8-^{14}C]suberic acid* (nominally 488 mg, 2.80 mmol) in DMF (7 ml) was stirred at 75°C under a nitrogen atmosphere for 16 hours. The mixture was cooled to room temperature, added dropwise with rapid stirring to 175 ml of water, and acidified to pH 1.2 with 6N HCl. The solids were collected by filtration, washed with water, and dried under high vacuum at room temperature to give 1.324 g of crude product. This was chromatographed on a column of 160 g of silica gel packed in and eluted with 7% v/v methanol in methylene chloride. Fractions of 12.5 ml each were collected at a flow rate of 4.1 ml/min. After approximately 400 ml, the eluent was changed to 10% v/v methanol in methylene chloride. Fractions 49-78 were pooled and concentrated to give 380 mg of ***4b***, while fractions 42-48 were combined and rechromatographed on a column of 40 g silica gel packed and eluted with 8% v/v methanol in methylene chloride. Fractions of 10 ml each were collected at a rate of 2.5 ml/min. Fractions 14-32 were pooled and concentrated to give 140 mg of ***4b***. The two lots of ***4b*** were combined and recrystallized from acetone-heptane to give 497 mg of ***4b*** (33.4%**), specific activity 97.3 μCi/mg, >98% pure by TLC (10% v/v methanol in methylene chloride, Rf = 0.27) >99% pure by HPLC (Supelcosil LC-18 column, mobile phase A, with both UV and radioactivity detection).

Methylprednisolone [^{14}C]Suleptanate (***5b***)

To a -5°C solution of ***4b*** (0.497 g, 0.936 mmol) and TEA (0.14 ml, 1.03 mmol) in 2 ml of DMF was added, with stirring under a nitrogen atmosphere, isobutylchloroformate (0.135 ml, 1.03 mmol) *via* a syringe. A precipitate formed immediately, and the mixture was stirred at -5°C for 2 hr. The cooling bath was removed, and NMT-Na (4.05 ml,

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*[1,8-^{14}C]suberic acid, nominally 40 mCi/mmol, was supplied by Amersham Corporation, Arlington Heights, Illinois, USA.

**This procedure normally affords the hemisuberate in 40-45% yield as a single crop of product in non-radioactive preparations.

1.07 mmol, of the above described solution in DMF) and more TEA (0.14 ml, 1.03 mmol) were added. The mixture was stirred for 30 minutes, and concentrated under high vacuum at 40°C to remove the DMF. The residue was partitioned between 25 ml each of 0.6 M sodium bisulfate (pH = 1.7) and ethyl acetate. The aqueous phase was washed with 25 ml of ethyl acetate and extracted with 2x25 ml of n-butanol. The combined butanol extracts were washed with 3x25 ml of 0.5 M sodium sulfate (pH adjusted to 5.1 with dilute NaOH) and dried over anhydrous sodium sulfate. The solution was filtered and the filtrate concentrated under high vacuum at 40°C. The residue was dissolved in a few ml of methanol, filtered through a bed of celite, and added dropwise with vigorous stirring to 30 ml of acetonitrile. The solids were collected by filtration, washed with acetonitrile and dried under high vacuum at room temperature to give 0.416 g of ***5b*** (62.6%), specific activity 76.3 μCi/mg, radiochemically pure by TLC (10% v/v methanol in methylene chloride, Rf = 0.04). HPLC (Supelcosil L-18 column, mobile phases A and B) analysis showed ***4b*** was >99% pure by UV (254 nm) and radioactivity detection.

11β,17α,21-Trihydroxy-6β-methyl[6,7-^{2}H]pregna-4-ene-3,20-dione, 21-Acetate (***7a***)

A mixture of 416 mg of 11β,17α,21-trihydroxy-6-methylpregna-4,6-diene-3,20-dione, 21 acetate (1.0 mmol, ***6***), 20 mg of 5% palladium on carbon (Aldrich), and 15 ml of methanol was vigorously stirred under deuterium gas for 50 min at room temperature. The uptake of deuterium gas stopped after 27 ml had been consumed. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was chromatographed on a column of 60 g of silica gel packed in and eluted with 20% v/v acetone in methylene chloride. Fractions containing ***7a*** (TLC:silica gel, 20% v/v acetone in methylene chloride, Rf = 0.45) were pooled and concentrated and the residue was crystallized with acetone-water to give 206 mg of ***7a*** (49.3%). In a similar preparation, from 832 mg of ***6***, 352 mg of crystallized ***7a*** (42%) was obtained. These two lots were combined and rechromatographed on a column of 80 g of silica gel packed in and eluted with 20% v/v acetone in methylene chloride. After pooling and concentrating the desired fractions, the residue was crystallized from acetone-water to give 407 mg of ***7a*** (85% recovery). HPLC analysis (Supelcosil LC-

18, 5μ analytical column, gradient elution over 40 min starting with 29:71 v/v acetonitrile:water, ending with 32:68 v/v, at 2 ml/min) showed the material was 97% pure as detected by UV at 210 nm. Deuterium label positions were determined by proton and carbon-13 NMR analysis; ^{1}H-NMR, DMSO-d_6: δ 0.8 (s, 3H, C_{18} methyl), 1.2 (s + d, 3H, C_6 methyl, singlet indicative of deuterium on methine carbon at C_6, calculated at 73%), 1.4 (s, 3H, C_{19} methyl), 2.1 (s, 3H, C_{23} methyl), 4.28 (bs, 1H, C_{11} hydroxyl), 4.32 (t, 1H, J = 3.5 Hz, C_{11} methine), 4.75, 5.10 (2d, 2H, J = 18 Hz, C_{21} methylene), 5.4 (s, 1H, C_{17} hydroxyl), 5.6 (s, 1H, C_4 vinyl, integration shows no deuterium at C_4); ^{13}C-NMR, DMSO-d_6: ppm 205.5 (C_{20}), 198.5 (C_3), 176.7 (C_{22}), 169.9 (C_5), 122.78 (C_4, no deuterium by peak height measurement), 88.8 (C_{17}), 67.6 (C_{21}), 66.2 (C_{11}), 55.3 (C_9), 51.7 (C_{14}), 47.0 (C_{13}), 39.1 (C_{12}or C_{16}), 37.9 (C_7, ~75% deuterium on C_7 by peak height measurement), 36.7 (C_6, ~75% deuterium on C_6 by peak height measurement), 36.6 (C_{12} or C_{16}), 33.7 (C_1), 33.2 (C_2), 27.0 (C_8), 23.6 (C_{15}), 23.17, 23.04 (C_{19} and C_6 methyl), 20.6 (C_{23}), 16.8 (C_{18}).

11β,17α,21-Trihydroxy-6β-methyl [7-^{2}H]pregna-4-ene-3,20-dione 21-Acetate (***9a***) *via* ***8a***

A mixture of 832 mg of ***6*** (2.0 mmol), 40 mg of 5% palladium on carbon (Aldrich), 30 ml of methanol, and 0.56 ml of TEA (4.0 mmol) was vigorously stirred under deuterium gas at room temperature for 40 min, during which 50 ml of deuterium gas was consumed. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was chromatographed on a column of 80 g of silica gel, packed in and eluted with 20% v/v acetone in methylene chloride. Fractions containing ***8a*** (TLC:silica gel, 20% v/v acetone in methylene chloride, Rf = 0.51) were pooled and concentrated and the residue was crystallized from 1.5 ml of acetone and 10 ml of water to give 207 mg of ***8a*** (24.8%). HPLC analysis (Supelcosil LC-18, 5μ analytical column, gradient elution over 40 min starting with 29:71 v/v and ending with 32:68 v/v acetonitrile:water, at 2.0 ml/min)showed the material to be 77.2% pure as determined by UV detection at 210 nm. ^{13}C-NMR analysis, due to the mixture of unknown impurities in the sample, gave an incomplete tentative assignment, in chloroform-d, ppm: 52.1 (C_4), 42.0 (C_7), both peaks showed triplets indicating deuterium enrichment. The sample, together with another 120 mg of ***8***

prepared in a similar fashion, was dissolved in 10 ml of methylene chloride and vigorously mixed with 5 ml of 6N hydrochloric acid for 3 hours at room temperature. The aqueous phase was separated from the mixture and extracted with 10 ml of methylene chloride. The combined extracts were washed with 20 ml of brine, dried over sodium sulfate, and concentrated. The residue was chromatographed on a column of 80 g of silica gel packed in and eluted with 20% v/v acetone in methylene chloride. Fractions containing ***9*** were collected, pooled, and concentrated. The residue was crystallized from 1.5 ml of acetone and 10 ml of water to give 186 mg of ***9*** (58.1%), 95% pure by HPLC analysis at UV 210 nm, with Supelcosil LC-18 column and mobile phase C; ^{1}H-NMR and ^{13}C-NMR identical to that of an authentic sample of ***9*** except for presence of deuterium at C_7. No deuterium was detected at the C_4 position.

Compound **9** *via* Mixture of ***7a*** and ***8a***

A mixture of 416 mg of ***6*** (1.0 mmol), 20 mg of 5% palladium on carbon (Aldrich), 15 ml of dioxane, and 0.05 ml of TEA was stirred vigorously under deuterium gas for 15 hours at room temperature, during which 35 ml of deuterium gas was consumed. After flushing with nitrogen, the reaction mixture was filtered to remove the catalyst and the resulting filtrate was concentrated under reduced pressure. The crude residue was dissolved in 10 ml of methylene chloride and vigorously stirred with 2 ml of 12N HCl for 30 min at room temperature. The mixture was adjusted to pH 6 with 1N NaOH, and concentrated at reduced pressure to ~10 ml. The concentrate was extracted with 2 x 25 ml of methylene chloride. The combined extracts were washed with brine, dried over anhydrous sodium sulfate, and concentrated. The residue was purified on a column of 80 g of silica gel packed in and eluted with 20% v/v acetone in methylene chloride to give 270 mg of ***9a*** (67%). HPLC analysis (Supelcosil LC-18, 5μ analytical column, mobile phase C) showed the material to be >99% pure as determined by UV detection at 210 nm; ^{1}H-NMR, chloroform-d: δ 0.95 (s, 3H, C_{18} methyl), 1.1 (d, 3H, C_6 methyl), 1.4 (s, 3H, C_{19} methyl), 2.2 (s, 3H, C_{24} methyl), 2.8 (s, 1H, C_{11} hydroxyl), 4.5 (bt, 1H, C_{11} methine), 4.85, 5.05 (2d, 2H, C_{21} methylene), 5.7 (d, 1H, J = 1Hz, C_4 vinyl, no deuterium detectable by integration). ^{13}C-NMR, chloroform-d: ppm 205.1 (C_{20}), 200.1 (C_3), 175.9 (C_{22}), 170.9 (C_5), 119.7 (C_4, no deuterium by peak height comparison to standard), 89.7 (C_{17}), 68.4

(C_{11}), 68.0 (C_{21}), 55.8 (C_9), 51.8 (C_{14}), 47.7 (C_{13}), 42.0 (C_7, deuterium coupled, peak obscured by baseline noise), 39.7 (C_{12} or C_{16}), 39.5 (C_{10}), 35.0 (C_{12} or C_{16}), 34.6 (C_1), 33.6 (C_2), 33.1 (C_6), 31.1 (C_8), 23.6 (C_{15}), 22.2 (C_6 methyl), 20.6 (C_{19}), 18.2 (C_{23}), 17.1 (C_{18}).

11β,17α,21-Trihydroxy-6α-methyl [7-^{3}H]pregna-4-ene-3,20-dione 21-Acetate (***9b***) *via* ***7b*** and ***8b***

A mixture of 416 mg of ***6*** (1.0 mmol), 40 mg of 5% palladium on carbon (Aldrich), 15 ml of dioxane, and 0.05 ml of TEA was prepared for reduction. The semi-micro hydrogenation apparatus (5) was fitted with a glass cell filled with nominally 10.0 Curies of tritium gas (DuPont/NEN). The cell, which had break seals at both ends, was opened at one end to the evacuated apparatus and the mixture was stirred at room temperature for 2 hours. Reduction was completed by opening the opposite end of the cell, allowing hydrogen to flush through the cell to the reaction mixture. After stirring for a total of 4 hours, a slurry of 40 mg of palladium catalyst in 1 ml of dioxane was added to the reaction mixture with a syringe. Stirring was continued for an additional 4 hours until a total of 26 ml of gas had been consumed by the reaction. After purging the apparatus with nitrogen, the reaction mixture was filtered to remove the catalyst and the filtrate was mixed with 2 ml of 12N HCl and stirred for 35 min at room temperature. The solution was adjusted to apparent pH 6.0 with 1N NaOH, and concentrated under reduced pressure at 40°C to ~10 ml. This concentrate was extracted twice with 25 ml of methylene chloride. The combined extract was washed with 25 ml water, 25 ml of brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was redissolved in 30 ml of dioxane and 2 ml of 12N HCl and the solution stirred for 20 min at room temperature. The solution was adjusted to apparent pH 6.0 with 1N sodium hydroxide and concentrated. The residue was triturated with 25 ml of water, and the remaining solids were collected by filtration, washed with water and dried under high vacuum at room temperature to give 191 mg of ***9b*** (45.7%). The specific activity was found to be 6.68 mCi/mg (total activity 1.28 Ci) and HPLC analysis with Supelcosil LC-18 column and mobil phase C showed the material was 95.7% pure as determined by UV detection at 210 nm.

[7-^{2}H]Methylprednisolone 21-Acetate (***10a***)

A mixture of 270 mg of ***9a*** (0.64 mmol), 343 mg of selenium dioxide (3.1 mmol, Aldrich), and 25 ml of *t*-butanol was refluxed under a nitrogen atmosphere for 41 hours. The mixture was concentrated under reduced pressure at 40°C to remove the *t*-butanol, and the residue was redissolved in 25 ml of methanol, and 8 ml of 25% lead tetraacetate solution was added. The resulting gray precipitate was removed by filtration. The filtrate was reduced in volume by concentrating under reduced pressure, and partitioned between 20 ml of methylene chloride and 8 ml of 25% lead tetraacetate solution. The aqueous phase was extracted with 20 ml of methylene chloride and the combined extracts were washed with 20 ml of brine, and dried over anhydrous sodium sulfate. The dry extract was concentrated and the residue chromatographed on a column of 60 g of silica gel packed in and eluted with 20% acetone in methylene chloride. Fractions containing ***10a*** were pooled and concentrated. The residue was crystallized from acetone-water, and dried under high vacuum at room temperature to give 151 mg of ***10a*** (56.1%). HPLC analysis (Supelcosil LC-18, 5μ analytical column, mobile phase C) showed the material was >99% pure as determined by UV detection at 210 nm; ^{1}H-NMR, chloroform-d: δ 0.95 (s, 3H, C_{18} methyl), 1.1 (d, 3H, C_6 methyl), 1.4 (s, 3H, C_{19} methyl), 2.15 (s, 3H, C_{24} methyl), 3.0 (S, 1H, C_{11} hydroxyl), 4.5 (bs, 1H, C_{11} methine), 4.85, 5.05 (2d, 2H, C_{21} methylene), 6.0 (s, 1H, C_4 vinyl), 6.25 (d, 1H, J = 10.4 Hz, C_2 vinyl), 7.3 (d, 1H, J = 10.4 Hz, C_1 vinyl). ^{13}C-NMR, chloroform-d, ppm: 205.0 (C_{20}), 186.8 (C_3), 173.6 (C_{22}), 170.9 (C_5), 157.2 (C_1), 127.4 (C_2), 119.7 (C_4), 89.8 (C_{17}), 70.2 (C_{11}), 68.1 (C_{21}), 56.0 (C_9), 51.4 (C_{14}), 47.8 (C_{13}), 44.4 (C_{10}), 43.1 (C_7, triplet, deuterium coupling indicating deuterium enrichment), 39.7 (C_{12}), 34.6 (C_{16}), 33.1 (C_6), 31.2 (C_8), 23.9 (C_{15}), 21.5 (C_6 methyl), 20.6 (C_{19}), 17.6 (C_{23}), 17.0 (C_{18}).

[7-^{2}H]Methylprednisolone 21-Acetate (***10b***)

Similarly, from 185 mg of ***9b*** (0.44 mmol), with 266 mg of selenium dioxide (2.4 mmol), there was obtained 109 mg of ***10b*** (59.2%). The material had a specific activity of 6.66 mCi/mg (total activity 726 mCi) and was 98.4% radiochemically pure by HPLC analysis (Supelcosil LC-18 column, mobile phase C).

[7-^{2}H]Methylprednisolone (***11a***)

A solution of 110 mg of ***10a*** (0.26 mmol) in 22 ml of methanol and 3 ml of 0.1N potassium hydroxide solution was stirred at room temperature for 30 min. The solution was adjusted to apparent pH 5.8 by the addition of 0.2N hydrochloric acid. Some insoluble material was removed by filtration and the filtrate was concentrated under reduced pressure at 40°C. The residue was chromatographed on a column of 50 g of silica gel packed in and eluted with 1:9 v/v methanol in methylene chloride. Fractions containing ***11a*** were pooled and concentrated under reduced pressure. The residue was crystallized from 2 ml of acetone and 10 ml of water. The crystals were dried under high vacuum at room temperature to give 66 mg of ***11a*** (66.7%). HPLC analysis (Supelcosil LC-18, 5μ analytical column, mobile phase D) showed the material was 98.3% pure as determined by UV detection at 210 nm.

[7-^{3}H]Methylprednisolone (***11b***)

Similarly, from 100 mg of ***10b*** (0.24 mmol), there was obtained 75.8 mg of ***11b*** (84.2%). The material was found to have a specific activity of 7.4 mCi/mg (total activity, 564 mCi) and a radiochemical purity of 98.5% by HPLC analysis (Supelcosil LC-18 column, mobile phase D).

[7-^{2}H]Methylprednisolone Suleptanate (***13a***)

A solution containing 112 mg of ***12*** (0.35 mol) in 2.0 ml of dry pyridine was cooled to -10°C in an ice/ethanol bath while 46 μl of pivaloyl chloride (0.37 mmol) was added. The reaction temperature was maintained at -10°C for 2.0 hr while stirring under nitrogen to produce the mixed anhydride. A solution of 66 mg of ***11a*** (0.18 mmol) in 1 ml of pyridine was added dropwise. The resulting solution was warmed to 55°C for 3 hr under nitrogen and a second portion of equal amount of the mixed anhydride as above was prepared and added to the reaction mixture. Stirring was continued for 1 hour more. TLC analysis indicated that all of the steroid ***11a*** had been consumed (silica gel, 10% v/v methanol in methylene chloride). Sixty-six mg of unlabeled methylprednisolone (0.18 mmol) was added and the mixture was stirred at 55°C for 1 hour to consume any unreacted mixed anhydride. The reaction mixture was filtered

to remove some insoluble material and the filtrate was concentrated at 40°C under reduced pressure. The residue was dissolved in 5.0 ml of water and extracted with 2 x 5 ml of ethyl acetate (discarded). The aqueous layer was mixed with 5.0 ml of 1.0 M sodium sulfate solution and extracted with 2 x 8 ml of n-butanol. The combined butanol extracts were washed with 2 x 10 ml of 0.5 M sodium sulfate solution and dried over anhydrous sodium sulfate. The solution was concentrated under reduced pressure and the residue was crystallized from 1.5 ml of 10% v/v methanol in methylene chloride and 25 ml of ether to give 210 mg of ***13a*** (88.6%). HPLC analysis (Supelcosil LC-18 5μ column, mobile phase A) showed this material was 99.0% pure as determined by UV detection at 254 nm.

[7-^{3}H]Methylprednisolone Suleptanate (***13b***)

Similarly, from 118 mg of ***12*** (0.37 mmol) in 2.0 ml of dry pyridine, 48 μl of pivaloyl chloride (0.39 mmol), 19.4 mg of ***11b*** (0.052 mmol), and 50.0 mg of unlabeled methylprednisolone (0.13 mmol), there was obtained 144 mg of ***13b***, specific activity 844 μCi/mg (total activity 122 mCi, 84.4% radiochemical yield). HPLC analysis (Supelcosil LC-18 column, mobile phase A,) showed the material to be 99% radiochemically pure.

7-Bromoheptanol (***15***)

Fifty ml of 2M borane-dimethylsulfide (100 mmol) in THF (Aldrich Chemical Co.) was added dropwise over 1 hour, with stirring under a nitrogen atmosphere at 18°C (water bath), to a solution of 19.1 g of 7-bromoheptanoic acid (***14***) (91.4 mmol, Overlook Industries) in 100 ml of dry THF. After initial evolution of gases had subsided, the mixture was stirred at room temperature overnight. Methanol (95 ml) was added and the solution concentrated to an oil. The oil was distilled to give 14.96 g (83.9%) of ***15*** (bp 100-103°C at 2.0 torr); ^{1}H-NMR; chloroform-d: δ 3.6 (t, 2H, J = 6H_z, C$\underline{H}_2$-O), 3.4 (t, 2H, J = 6H_z, C$\underline{H}_2$-Br), 2.4 (s, 1H, $\underline{H}$-O), 1.8-1.2 (m, 10H, C$\underline{H}_2$).

8-[Methyl(2-sulfoethyl)amino]-8-oxo-[8-^{14}C]octanoic Acid Monosodium Salt (***16***)

a) Alcohol Protection with DHP

A solution of 5.0 g of bromoalcohol ***15*** (25.6 mmol) and 5.47 g of 3,4-dihydro-2H-pyran (66.7 mmol, Aldrich Chemical Co.) in 50 ml of methylene chloride was cooled to 0°C under nitrogen. *p*-Toluenesulfonic acid hydrate, (25 mg, 0.13 mmol, Eastman Kodak) was added and the cooling bath was removed. The reaction was stirred at room temperature for 40 min. The solution was concentrated and the remaining oil dissolved in 20 ml of ether, washed with 20 ml of saturated sodium bicarbonate solution, 10 ml of water and 20 ml of brine in that order, and dried over Na_2SO_4. The dry solution was concentrated and chromatographed on a column of 160 g of silica gel packed in and eluted with 20:80 v/v ethyl acetate:hexane, to give 6.13 g (85.6%) of tetrahydropyran (THP) protected alcohol; TLC (25:75 v/v ethyl acetate:hexane) showed a single component, Rf = 0.65; ^1H-NMR,* chloroform-d: δ 4.5 (t, 1H, J = 3Hz, O-C<u>H</u>-O), 3.6 (m, 4H, C<u>H</u>$_2$-O), 3.4 (t, 2H, J = 6Hz, C<u>H</u>$_2$-Br), 1.0-2.1 (m, 16H, C<u>H</u>$_2$).

b) Introduction of ^{14}C

A solution of 240 mg of sodium [^{14}C]cyanide (4.75 mol, nominally 250 mCi, DuPont/NEN), and 1.33 g of the above protected alcohol (4.75 mmol), in 9 ml of dry dimethylsulfoxide (DMSO), was stirred under nitrogen at 70°C for 1.5 hr. The cooled reaction mixture was mixed with 50 ml of 2.5% sodium carbonate solution and extracted with 2 x 30 ml of ether. The combined extracts were washed with 3 x 25 ml of water followed by 25 ml of brine, dried over sodium sulfate, and concentrated to give 1.18 g (~100% yield) of oil; TLC (25:75 v/v ethyl acetate:hexane) showed a single component, RF = 0.35; ^1H-NMR,* chloroform-d: δ 4.5 (bt, 1H, J = 3Hz, O-C<u>H</u>-O), 3.2-4.0 (m, 4H, C<u>H</u>$_2$-O), 2.3 (t, 2H, J = 6Hz, C<u>H</u>$_2$-CN), 1.0-1.9 (m, 16H, C<u>H</u>$_2$).

c) Hydrolysis of Nitrile

The freshly prepared ^{14}C labeled nitrile from above was mixed with 8 ml of

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*These NMR data were obtained with unlabeled samples which were prepared in the same manner as the corresponding ^{14}C labeled materials.

ethanol and 6 ml of 6N sodium hydroxide solution. The two-phase mixture was refluxed with stirring for 16 hr. The cooled mixture was concentrated to remove the ethanol, and extracted with 2 x 25 ml of ether. The aqueous phase was cooled to 0°C and adjusted to pH 3.0 with 6N HCl. The mixture was extracted with 2 x 25 ml of ether, the extracts were washed with 25 ml of brine, dried over sodium sulfate, and concentrated. A minor radioactive component (5%) was removed by column chromatography on 50 g of CC-4 Silic AR (Mallinkrodt Chemicals) packed in and eluted with 4% v/v methanol in methylene chloride. The pooled eluate containing the product was concentrated to give 1.09 g (93%) of crude THP ether of 8-hydroxy-[1-^{14}C]octanoic acid. TLC (95:5:1 v/v, methylene chloride:methanol:acetic acid) showed a single component, Rf = 0.56; ^{1}H-NMR,* chloroform-d: δ 10.6 (s, 1H, $CO_2\underline{H}$), 4.55 (bs, 1H, O-C$\underline{H}$-O), 3.8 (m, 2H, C$\underline{H_2}$-O); 3.4 (m, 2H, C$\underline{H_2}$-O), 2.3 (t, 2H, J = 6H_z, C$\underline{H_2}$-CO), 1.2-1.9 (m, 16H, C$\underline{H_2}$).

d) Amide Formation and Removal of THP Protecting Group

The THP-protected acid, (1.09 g, 4.4 mmol) was dissolved in 15 ml of dry DMF and cooled to 0°C under nitrogen. 1,1'-Carbonyldiimidazole (CDI), 0.823 g (5.07 mmol, Aldrich Chemical Co.) was added and the solution was stirred for 3 hr. To the solution was added 713 mg of NMT-Na (4.4 mmol) and the mixture was stirred under nitrogen for 16 hr at room temperature. The cloudy solution was filtered through a bed of Celite and the clear filtrate was concentrated under vacuum to give the crude NMT-Na amide of THP-protected 8-hydroxy[1-^{14}C]octanoic acid. This crude material** was

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* These NMR data were obtained with unlabeled samples which were prepared in the same manner as the corresponding ^{14}C labeled materials.

**TLC analysis (55:35:5:5 v/v, methylene chloride:methanol:water:ammonium hydroxide) showed a single component, Rf = 0.72; ^{1}H-NMR,* D_2O: δ, 4.6 (S, 1H, O-C$\underline{H}$-O), 3.6 (m, 6H, C$\underline{H_2}$-O, C$\underline{H_2}$-S), 3.1 (m, 2H, C$\underline{H_2}$-N), 3.05 and 2.85 (2S, 3H, C$\underline{H_2}$-N), 2.3 (t, 3H, J = 7H_z, C$\underline{H_2}$-CON), 1.2-1.8 (m, 16H, C$\underline{H_2}$).

dissolved in methanol and eluted through a 30 g column of Dowex 50W-X8 (10-50 mesh) resin preconditioned in the H^+ form. The collected eluate (pH 0.01) was adjusted to pH 5.25 with a few drops of 6N sodium hydroxide. The slightly turbid solution was filtered through a bed of celite and the filtrate concentrated. The residue was dissolved in 1 ml of methanol and 5 ml of THF was added dropwise with stirring followed by 25 ml of ether. The resulting precipitates were filtered, washed with ether, and dried under high vacuum at room temperature, 0.88 g, specific activity 140 μCi/mg. TLC (70:25:5 v/v methylene chloride:methanol:ammonium hydroxide, Rf = 0.34) showed radiochemical purity of >98%. Analysis by HPLC (DuPont Zorbax C-8, mobile phase E, detection by radioactivity) showed radiochemical purity of >99.3%.

e) Oxidation of Alcohol to Acid

A solution of 88 mg of the deprotected alcohol (2.88 mmol) in 10 ml of 5% sodium carbonate was mixed with 0.910 mg of potassium permanganate (5.76 mmol) in 13 ml of water. The solution was stirred at room temperature for 44 hours. The reaction mixture was filtered, acidified to pH 3.3 and concentrated. The residue was triturated with methanol and the insoluble salts removed by filtration. Analysis by HPLC (DuPont Zorbax C-8, mobile phase E) showed the filtrate contained substantial amounts of unoxidized alcohol. The solution was concentrated and the oxidation continued by adding 10 ml of 5% sodium carbonate solution, and a solution of 0.910 mg of potassium permanganate (5.76 mmol) in 10 ml of water. The reaction mixture was stirred for 16 hours at 50°C, cooled to room temperature, and filtered. The filtrate was adjusted to pH 0.75 with 8 ml of 6N sulfuric acid, and rapidly readjusted to pH 3.27 with 5.6 ml of 6N sodium hydroxide. (This procedure was to favor, by mass action, formation of the sodium salt ***16*** in preference to a mixture of sodium and potassium salts). The solution was concentrated and the residue triturated with 15 ml of methanol. The insoluble salts were removed and the crude material was chromatographed on a column of 60 g of silica gel packed in and eluted with 55:35:5:5 v/v methylene chloride:methanol:water:ammonium hydroxide. Fractions were analyzed by TLC and by radioactivity determinations. The fractions containing ***16*** were pooled, adjusted to

pH 3.5, and concentrated. The residue was triturated with methanol and the insoluble salts collected by filtration, 548 mg. The material was dissolved in 5.5 ml of 2:18:0.4 v/v tri-n-butylamine:water:formic acid and purified by preparative HPLC. Injections of 400 µl were made onto a Supelcosil LC-18 column (21.5 mm I.D. x 250 mm) eluted with a step gradient. The initial mobile phase of *35:965:10:2* v/v THF:water:tri-n-butylamine:formic acid) was pumped for 30 min at 30 ml/min, and switched to 70:930:10:2 v/v THF:water:tri-n-butylamine:formic acid which eluted the desired ***16*** detected by UV at 210 nm. The fractions containing eluate ***16*** from several injections were pooled and concentrated at 50°C and 15 torr to remove THF. The aqueous residue was passed through a 75 g Dowex 50W-X 8 ion exchange resin (preconditioned to the H^+ form). The eluate was concentrated to remove water and formic acid. The residual oil was redissolved in water and adjusted to pH 11.0 with 3 ml of 1N sodium hydroxide. The cloudy solution was extracted with 2 x 25 ml of ether which removed tri-n-butylamine. The aqueous phase was adjusted to pH 3.0 with 1N sulfuric acid and concentrated. The residue was triturated with methanol and the insoluble salts removed by filtration. The filtrate was concentrated and triturated with acetone to give 247 mg of ***16***, specific activity 169.5 µCi/mg. HPLC analysis showed a radiochemical purity of >99%. (Supelcosil LC-18 column, mobile phase B, detection by radioactivity).

Methylprednisolone, 21-[8-[Methyl(2-sulfoethyl)amino]-8-oxo[8-^{14}C]octanoate] Monosodium Salt, (Methylprednisolone [carboxamide-^{14}C]Suleptanate) (***17***)

A solution consisting of 195 mg of ***16*** (0.61 mmol), 550 mg of ***3b*** (1.22 mmol) and 79 mg of diisopropylethylamine (0.61 mmol) in 8 ml of dry DMF, was stirred for 24 hours at 78°C. The mixture was concentrated under vacuum to remove DMF, mixed with 10 ml of water and adjusted to pH 5.5 carefully with 5% sodium bicarbonate solution. The solution was extracted with 2 x 25 ml of ethyl acetate and the aqueous phase was salted by the addition of 15 ml of saturated sodium sulfate solution. The mixture was extracted with 2 x 20 ml of n-butanol. The combined extracts were washed with 3 x 15 ml of 0.5 M sodium sulfate solution followed by 10 ml of saturated sodium sulfate solution, and dried over anhydrous sodium sulfate. The dried butanol solution was concentrated, and the residue dissolved in 2 ml of 10% v/v methanol in

methylene chloride, and added dropwise to 40 ml of ether with stirring at room temperature. The resulting precipitates were collected by filtration, washed with ether-hexane, and dried under vacuum at room temperature to give 281 g of ***17*** (87% yield*), specific activity 78.1 μCi/mg, >98% radiochemically pure by TLC (70:25:5 v/v methylene chloride:methanol:ammonium hydroxide) and HPLC (Supelcosil LC-18 column, mobile phase B).

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* The yield accounted for unreacted starting material ***16***, recovered from the butanol-extracted aqueous solution by removing water, triturating the residue with methanol and filtering (to remove the insoluble salts), to give 47 mg (7.9 mCi) of ***16***.

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