Pergamon

Tetrahedron, Vol. 53, No. 32, pp. 11007-11020, 1997
© 1997 Elsevier Science Ltd
All rights reserved. Printed in Great Britain
0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00361-X

The ^{14}C Radiolabelled Synthesis of the Cholesterol Absorption Inhibitor CP-148,623. A Novel Method for the Incorporation of a ^{14}C Label in Enones.‡

Michael P. DeNinno and Keith E. McCarthy

Pfizer Inc, Central Research Division, Groton CT 06340

Abstract

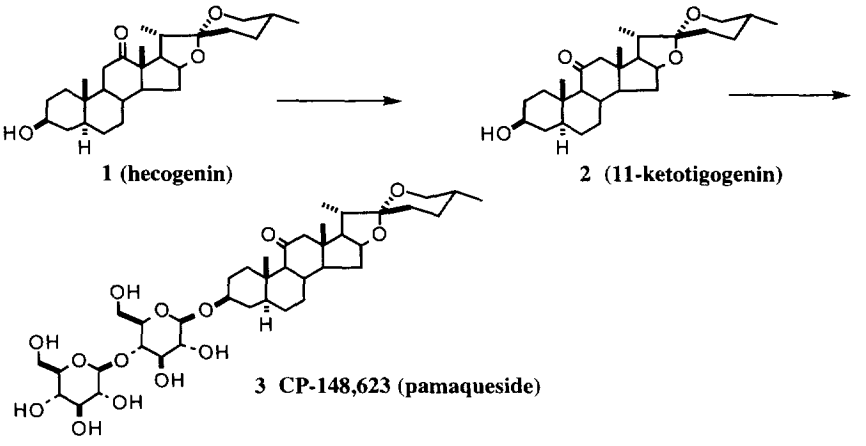
Isotopically labelled compounds play important roles in pharmaceutical research. New strategies for the synthesis of these compounds, carbon isotopes in particular, are needed due to the limited availability of labelled reagents. We have developed a novel method for the ^{14}C labelling of enones and have applied it to the synthesis of a ^{14}C radiolabelled version of the cholesterol absorption inhibitor CP-148,623. The key steps involve the 1,2 addition of [^{14}C]methyl Grignard to a cyclic enone followed by the "anomalous" ozonolysis of the resulting allylic alcohol utilizing a non-traditional basic work-up. In this two step procedure, the original enone was regenerated with a ^{14}C carbon atom at the alpha position. After several standard chemical steps, the synthesis of [^{14}C]CP-148,623 was completed. The method has also been applied to a series of other enones demonstrating the generality of the procedure for the introduction of carbon isotopes in enone systems. © 1997 Elsevier Science Ltd.

INTRODUCTION

Radiolabelled compounds play important roles in the pharmaceutical industry. Labeled drugs are useful for radioligand binding assays, mechanistic studies and complete pharmacokinetic (PK) profiling. Although iodine or tritium isotopes are usually preferred for *in vitro* studies due to their high specific activity, drugs containing a ^{14}C atom are often required for PK analysis because of their metabolic stability. We recently needed to prepare a ^{14}C derivative of CP-148,623 (**3**, pamaqueside), a cholesterol absorption inhibitor in clinical development.[1] Because the glycosidic linkage was potentially labile *in vivo*, the label needed to be located on the spirostane portion of the molecule. This steroid, 11-ketotigogenin **2**, is prepared from hecogenin **1** without any C-C bond-forming reactions, and thus there is no direct opportunity for the introduction of the label. Therefore, a degradation-rebuilding strategy was required.

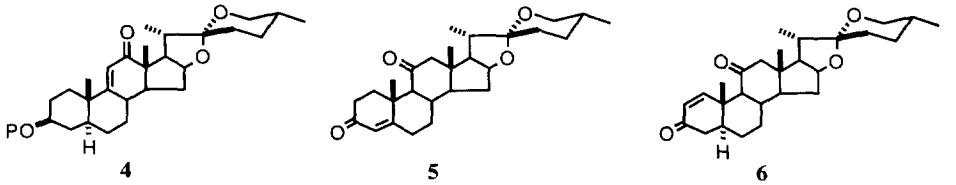
‡Dedicated to Samuel J. Danishefsky who instilled in those of us who worked with him an excitement for the challenge and elegance of organic synthesis that still remains today.

Scheme 1



One possibility for labelling the spirostane was to employ the Fujimoto-Belleau (F-B) procedure as it has been successfully applied to a number of ^{14}C-labelled steroids.[2] The key steps involve the degradation of a steroidal enone to an enol-lactone, followed by ^{14}C-methyl Grignard addition. The resulting diketone is then condensed to afford the labelled enone. One could envision the application of this process to either the A or C ring of the present system. Initial attempts to label the C-ring via the enol-lactone derived from intermediate **4**[3] were hampered by a lack of reactivity which directed our efforts to the sterically less demanding A-ring. However, the synthesis of the typical F-B substrate **5** from 11-ketotigogenin was not precedented and was predicted to be difficult in light of the trans A-B ring juncture.

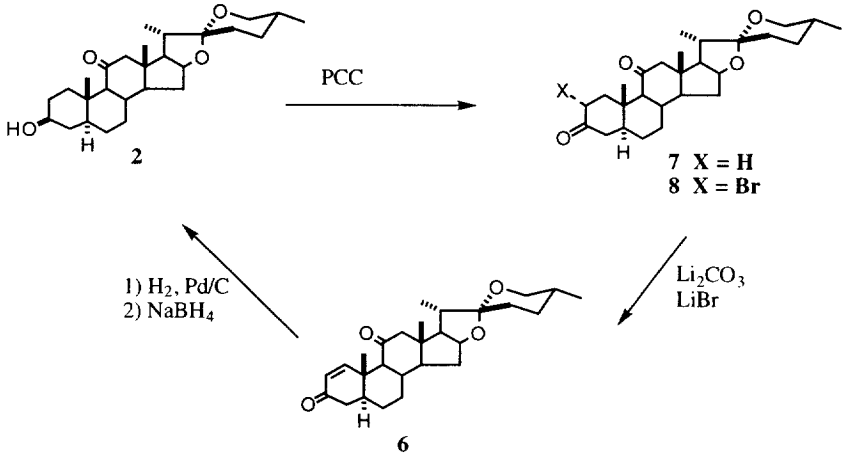
The more readily available (25R)spirost-1-en-3,11-dione **6** would not be a candidate for the F-B procedure due to the presence of a γ-hydrogen, however it could prove viable for labeling if the methyl Grignard was added prior to oxidative degradation. Not only was this plan productive, but the solution also involved the employment of a novel key step: the anomalous ozonolysis of a cyclic allylic alcohol. This method provided for a concise label incorporation resulting in an efficient synthesis of [^{14}C]CP-148,623 (**3**). The scope of this labelling procedure was evaluated on a series of enones, focusing on steroidal enones.



RESULTS AND DISCUSSION

The success of the A-ring labelling strategy relied on a ready access to the enone **6**. Fortunately, the synthesis of **6** was straightforward, requiring only three steps from 11-ketotigogenin **2** (11-KT). 11-KT was available to us in large quantities from the bulk campaigns of CP-148,623.[4] Thus, 11-KT was oxidized to the diketone **7** in 90 % yield with PCC in CH_2Cl_2 (Scheme 2). The diketone was selectively brominated using bromine in THF.[5] The outcome of this reaction was not without concern since both the C-12 and C-23 positions are also susceptible to bromination. The bromine in compound **8** was eliminated under standard conditions (Li_2CO_3, LiBr, DMF 120°C) in 76% yield. The sequence required no chromatography and afforded the crystalline enone **6** in 55% overall yield.

Scheme 2

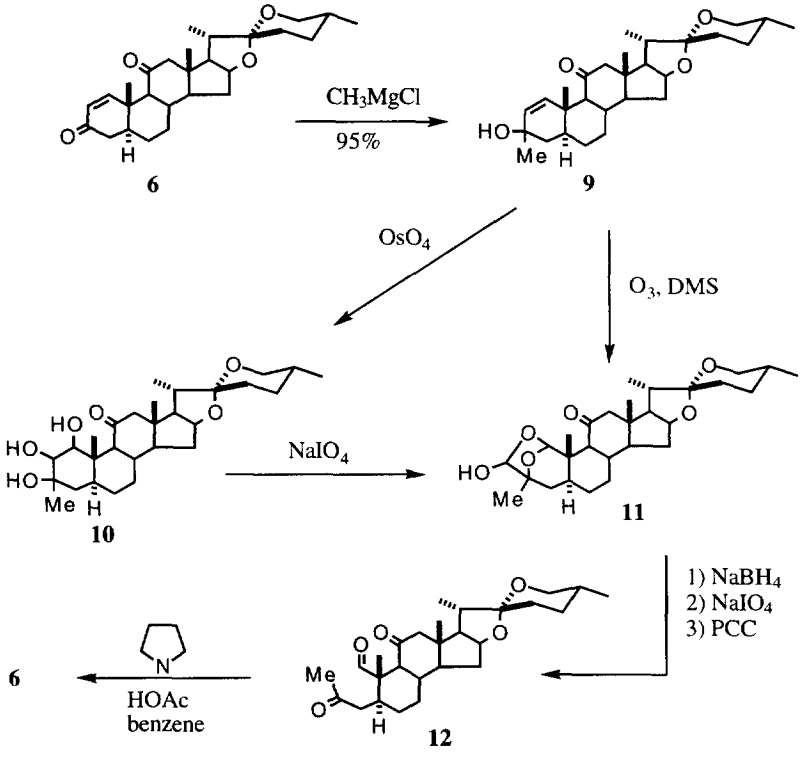


With enone **6** in hand, its conversion back to 11-KT could now be investigated. This transformation was accomplished without event. First, the double bond was quantitatively hydrogenated using Pd/C catalyst, followed by a regiospecific and stereoselective reduction of compound **7** with sodium borohydride (80% overall yield).

With these initial experiments successfully completed, we focused on the incorporation of the ^{14}C label in enone **6**. Although this task initially seemed readily achievable, it soon became problematic. The early strategies are shown in Scheme 3. The first step in this sequence, the 1,2-addition of methyl Grignard to the enone, worked well, to give the allylic alcohol **9** as a 10:1 mixture of isomers in 95 % yield. Once again, the C-11 ketone was unaffected. Two methods for the synthesis of ketoaldehyde **12** from **9** were initially explored. The first strategy involved osmylation of the olefin followed by periodate treatment, whereas the second route used ozone as the primary oxidant. Both of these methods provided the same product, which

unfortunately was not the desired ketoaldehyde but the lactol **11**. This lactol was very stable and resisted reaction with a number of glycol cleaving agents, including $NaIO_4$, HIO_4 and $Pb(OAc)_4$. Ultimately, compound **11** was converted to aldehyde **12** through a lengthy and low yielding sequence of reduction ($NaBH_4$), sodium periodate treatment and oxidation with PCC (~ 25% overall). As expected, the keto-aldehyde, upon treatment with pyrrolidine and acetic acid in benzene, afforded the enone in good yield.

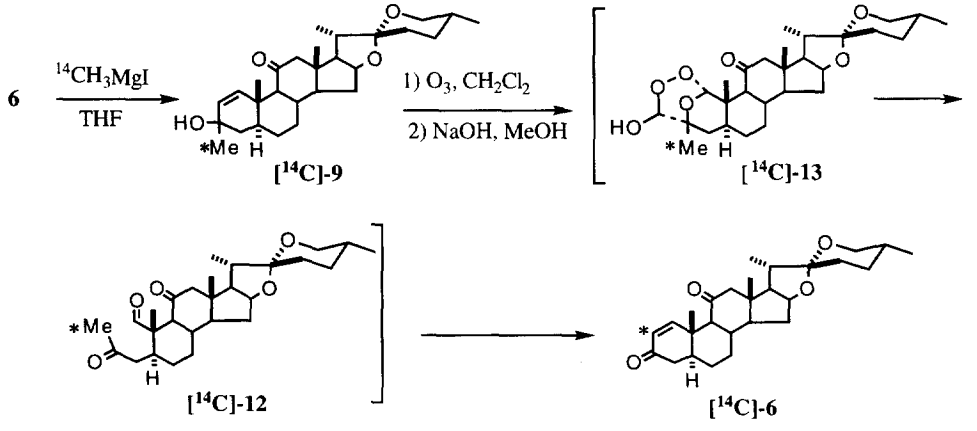
Scheme 3



Although the procedure described above provided a reasonable solution for the preparation of **6** from **9**, the low overall yield and number of "hot" steps prompted the exploration of alternate strategies. Several options were considered with the most attractive being the reinvestigation of the ozonolysis reaction. The ozone reaction described in Scheme 3 afforded the normal dialdehyde product; however, there is precedent for allylic alcohols to follow an "anomalous" ozonolysis pathway.[6] This abnormal ozonolysis results in the cleavage of both the double bond and an adjacent single bond. Such a process would lead to the direct formation of aldehyde **12** from **9**. This strategy indeed was successful and the details of this work have been published elsewhere.[7] The key to favoring the anomalous ozonolytic pathway was to use a non-reductive, basic work-up. Thus, the allylic alcohol **9** was treated with ozone at -78°C in dichloromethane. Sodium hydroxide solution

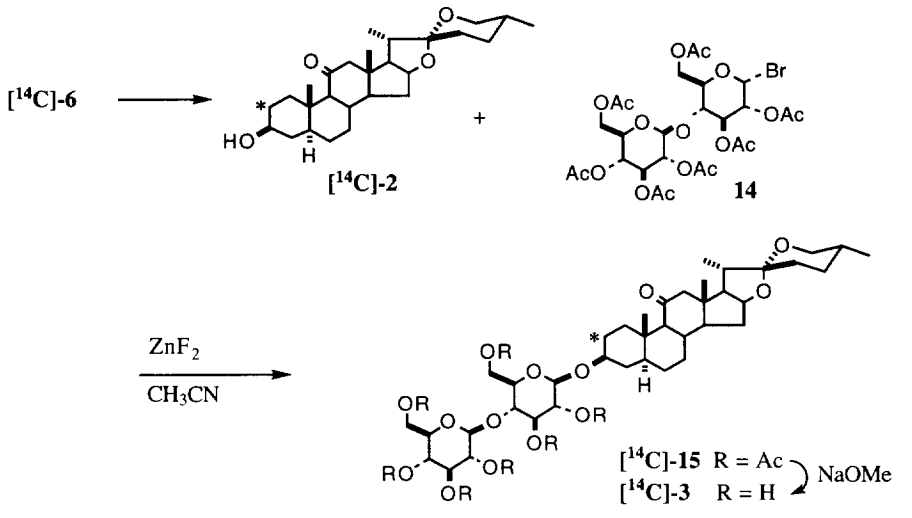
was added to the reaction at room temperature after diluting with methanol. Within a few hours, the reaction was complete, and the enone **6** was isolated in 40% yield. The reaction was shown to proceed through the 1,2,4 trioxane intermediate **13** (Scheme 4).[7]

Scheme 4



Application of this new method using [^{14}C]methyl iodide proceeded relatively uneventfully. Whereas Grignard addition using freshly prepared [^{14}C]methymagnesium iodide afforded the labelled allylic alcohol **9** in only 33% yield, the conversion of [^{14}C]-**9** to [^{14}C]-**6** proceeded in 53% yield.[8] The higher yield seen in the conversion of radiolabelled **9** to **6**, is most likely a result of the ability to quantitatively monitor the progress of this transformation by radio-TLC.[9] To complete the synthesis, [^{14}C]-**6** was converted to the labelled 11-ketotigogenin **2** by the two-step process of hydrogenation and reduction described earlier for the unlabeled enone. This ^{14}C derivative of 11-KT was prepared in seven steps without the need for any protecting groups. In the final two steps, the sugar unit was coupled to [^{14}C]-**2** using the cellobiosyl bromide **14** under ZnF_2 catalysis[1], and the acetate protecting groups cleaved with sodium methoxide to afford the target compound [^{14}C]-**3**, [^{14}C]CP-148,623 in 55% yield from [^{14}C]-**2**. Purification of [^{14}C]CP-148,623 could be effected by silica gel chromatography, however in order to obtain ultra-pure material predicted to be necessary because of its low bioavailability, [^{14}C]CP-148,623 was recrystallized from EtOAc then triturated with 3% aqueous methanol to >99.9% radiochemical purity.

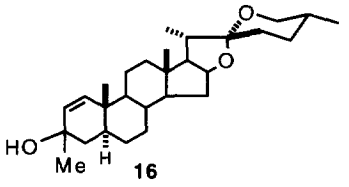
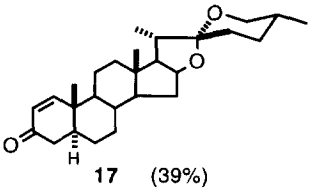
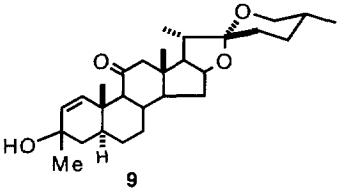
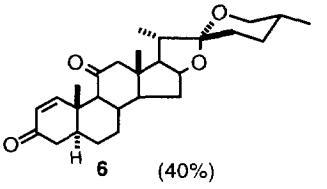
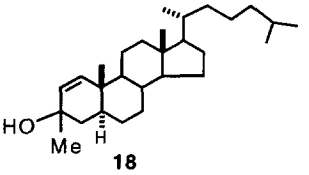
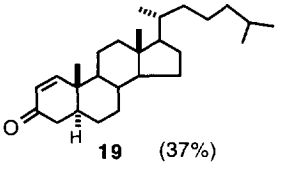
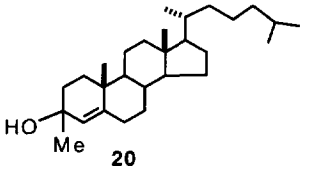
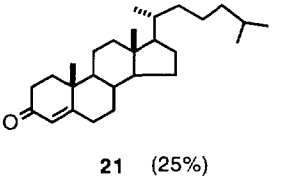
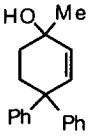
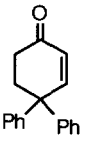
Scheme 5



The newly described procedure of Grignard addition and ozonolysis is the shortest method known for the labeling of cyclic enones. Furthermore, cyclic enones can be readily prepared from cycloalkanones and cycloalkanols, making these templates potential candidates for this method. Examples of how this method can be applied are shown in the Table. Although the unoptimized yields are modest, the brevity of the procedure is attractive, particularly when working with radioactive compounds.

In summary, we have developed a concise nine step synthesis of [^{14}C]CP-148,623 (**3**). The key steps involve the 1,2-addition of [^{14}C]methyl magnesium iodide to a cyclic enone followed by an anomalous ozonolysis of the resulting allylic alcohol. This two-step procedure replaced the alpha carbon of the enone with a ^{14}C label. This method has been shown to be useful for the synthesis of a variety of labelled enones not readily accessible by present methods. The protocol provides modest to good yields of isotopically labelled material directly from the unlabelled enone. Armed with a greater understanding of the mechanism, efforts to improve the yield of the ozonolysis reaction are underway.

Table: Anomalous Ozonolysis of Cyclic Allylic Alcohols

Allylic Alcohol	Product (% yield)
 **16**	 **17** (39%)
 **9**	 **6** (40%)
 **18**	 **19** (37%)
 **20**	 **21** (25%)
 **22**	 **23** (42%)

EXPERIMENTAL

(5α,25R)-spirostan-3,11-dione (7).

Pyridinium chlorochromate (40 g, 0.186 mol) was added to a solution of (3β,5α,25R)-3-hydroxy-spirostan-11-one **2** (20 g, 0.0464 mol) in dichloromethane (500 mL) containing 30 g of dry Celite® at 0°C. The mixture was warmed to room temperature and after 4 hours, was diluted with ether (1L) and filtered through an 8x10 cm plug of silica gel. The silica gel was flushed with 2L of 30% CH_2Cl_2/Et_2O and the combined filtrates were concentrated in vacuo. The residue was triturated with diisopropyl ether, filtered and dried to afford 18 g product as colorless crystals (90%). m.p. 238-241 °C (hexanes-ethyl acetate); CI Mass Spect. 429 $(M+H)^+$; ^1H NMR (250 MHz, $CDCl_3$) δ 4.5 (q, 1H, J = 7.3 Hz); 3.5 (m, 1H); 3.34 (dd, 1H, J = 10.8, 10.5 Hz); 2.78 (m, 1H); 2.5-1.2 (m, 24H); 1.2 (s, 3H); 0.92 (d, 3H, J = 7.0 Hz); 0.79 (d, 3H, J = 7.0 Hz); 0.71 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.4, 209.7, 109.2, 80.5, 66.9, 63.9, 60.7, 57.6, 55.5, 46.9, 44.3, 44.2, 41.8, 37.9, 37.0, 36.9, 35.2, 32.4, 31.3, 31.2, 30.2, 28.7, 28.2, 17.2, 17.1, 14.2, 11.1.

(2α,5α,25R)-2-bromo-spirostan-3,11-dione (8).

Bromine (2.56 mL, 0.05 mol) was added dropwise to a solution of (5α,25R)-spirostan-3,11-dione (15 g, 0.035 mol) in tetrahydrofuran (150 mL) at -10°C. When the addition was complete, the reaction was warmed to room temperature over one hour. Excess bromine was quenched by the addition of methanol (0.5 mL) and the mixture was diluted with ethyl acetate (500 mL) and washed with aq. $NaHCO_3$ (2 x 200 mL), and brine (1 x 200 mL), dried (Na_2SO_4) filtered and concentrated to ~20 mL *in vacuo*. Hexane was added (100 mL) and the solid was collected by vacuum filtration to afford 12 g product as colorless crystals. m.p. 202-204 °C(dec). CI Mass Spect. 429 $(M+H)^+$; ^1H NMR (250 MHz, $CDCl_3$) δ 4.78 (dd, 1H, J = 12.5, 6.5 Hz); 4.5 (q, 1H, J = 7.3 Hz); 3.5 (m, 2H); 3.34 (dd, 1H, J = 10.8, 10.5 Hz); 2.5-1.3 (m, 22H); 1.28 (s, 3H); 0.92 (d, 3H, J = 7.0 Hz); 0.78 (d, 3H, J = 7.0 Hz); 0.71 (s, 3H).

(5α,25R)-spirost-1-en-3,11-dione (6).

(2α,5α,25R)-2-bromo-spirostan-3,11-dione (10 g, 0.0197 mol) was added to a suspension of Li_2CO_3 (3 g) and LiBr (2.5 g) in DMF (75 mL) at 90°C. The temperature was raised to 120°C and after 90 minutes, the mixture was cooled and diluted with ethyl acetate (300 mL) and washed with water (3x200 mL), and brine (1 x 200 mL), dried (Na_2SO_4) filtered and concentrated *in vacuo*. The product was recrystallized from EtOAc/hexanes, to afford 6 g product as colorless crystals (71 %). m.p. 225-226°C. $[\alpha]_D$ = +54° (c 1.39, $CHCl_3$). CI Mass Spect. 429 $(M+H)^+$; ^1H NMR (250 MHz, $CDCl_3$) δ 7.5 (d, 1H, J = 10.5 Hz); 5.82 (d, 1H, J = 10.5 Hz); 4.5 (q, 1H, J = 7.3 Hz); 3.5 (m, 1H); 3.34 (dd, 1H, J = 10.8, 10.5 Hz); 2.5-1.3 (m, 20H); 1.27 (s, 3H); 0.95 (d, 3H, J = 7.0 Hz); 0.78 (d, 3H, J = 7.0 Hz); 0.74 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 209.4, 199.4, 159.3, 127.5, 109.2, 80.4, 66.9,

60.7, 59.8, 59.7, 57.3, 55.3, 44.3, 44.2, 41.8, 40.6, 38.0, 36.9, 32.1, 31.2, 30.2, 28.7, 27.0, 17.3, 17.1, 14.2, 13.7. Analysis calc. for $C_{27}H_{38}O_4$: C 76.02 H 8.98; Found: C 76.06 H 8.96.

Conversion of enone 6 to 11-ketotigogenin 2:

Step 1: (5α,25R)-spirost-1-en-3,11-dione was hydrogenated using 10 % Pd on carbon catalysis in 1:1 EtOAc:EtOH solvent under 40 psi hydrogen. After 3 hours, the system was purged with nitrogen, the catalyst filtered off, and the filtrate concentrated to afford a near quantitative yield of of (5α,25R)-spirostan-3,11-dione, identical in all respects to that prepared above.

Step 2: Sodium borohydride was added to a solution of (5α,25R)-spirostan-3,11-dione in ethanol at 0°C. After one hour, the mixture was quenched with water, the ethanol was removed in vacuo and the residue was extracted with ethyl acetate (3X). The combined extracts were washed with 1N HCl, and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. This afforded a 95% yield of a ~10:1 mixture of beta:alpha C3 alcohol isomers. The mixture was purified by flash chromatography (25% EtOAc/hexanes) to afford the desired isomer, (3β,5α,25R)-3-hydroxy-spirostan-11-one in 80% overall yield from **9** which was identical to an authenic sample.

(3R,5α,25R)-3-Methyl-3-hydroxy-spirost-1-en-11-one (9).

Methyl magnesium chloride (0.82 mL of a 3M solution in THF, 2.4 mmol) was added dropwise to a solution of (5α,25R)spirost-1-en-3,11-dione (1 g, 2.35 mmol) in THF (20 mL) at -20°C. The mixture was allowed to warm to room temperature over 1 hour. The mixture was diluted with ethyl acetate (100 mL) and washed with 1N HCl sol (2 x 50 mL), brine (1 x 50 mL) dried (Na_2SO_4) filtered and concentrated in vacuo to give 0.98 g (95 %) of a ~10:1 mixture of α:β addition products. Generally, the mixture can be used without purification, but to obtain a pure sample, the mixture was chromatographed on silica gel (25 % EtOAc/hex eluant) to afford the major product (alpha addition) as a waxy solid.

CI Mass Spect. 425 $(M+H)^+$; ^1H NMR (250 MHz, $CDCl_3$) δ 6.22 (d, 1H, J = 10.2 Hz); 5.35 (d, 1H, J = 10.2 Hz); 4.5 (q, 1H, J = 7.3 Hz); 3.5 (m, 1H); 3.35 (dd, 1H, J = 10.8, 10.5 Hz); 2.4-1.3 (m, 24H); 1.25 (s, 3H); 1.12 (s, 3H); 0.93 (m, 3H); 0.78 (d, 3H, J = 7.0 Hz); 0.71 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 209.8, 136.6, 132.6, 109.2, 80.6, 70.6, 66.9, 61.4, 60.7, 57.4, 55.7, 44.4, 43.5, 41.8, 41.5, 37.3, 36.9, 32.6, 31.2, 31.2, 30.2, 29.1, 28.7, 27.5, 17.2, 17.1, 15.4, 14.2. HRMS calc. for $C_{28}H_{43}O_4$: 443.3161. Found: 443.3149.

Further eluting affords the minor beta addition product.

CI Mass Spect. 425 $(M+H)^+$; ^1H NMR (250 MHz, $CDCl_3$) δ 6.3 (d, 1H, J = 10.2 Hz); 5.4 (dd, 1H, J = 10.2, 2.0 Hz); 4.5 (q, 1H, J = 7.3 Hz); 3.5 (m, 1H); 3.35 (dd, 1H, J = 10.8, 10.5 Hz); 2.3-1.3 (m, 24H); 1.24 (s, 3H); 1.12 (s, 3H); 0.91 (m, 3H); 0.76 (d, 3H, J = 7.0 Hz); 0.71 (s, 3H).

(3R,5α,25R)-1,2,3-trihydroxy-3-methyl-spirostan-11-one (10).

OsO_4 (7.6 mL of a 0.1 g/mL solution in THF, 3.0 mmol) was added to a solution of **9** (1.0 g, 2.26 mmol) in THF (15 mL) at room temperature. Pyridine (0.5 mL) was added and the mixture stirred at RT. After 24 hours, additional THF was added (20 mL) and 1 mL of H_2O followed by the addition of solid Na_2SO_3 (1 g). Florisil (5 g) was added and the mixture stirred vigorously for 1 h, then the mixture was filtered through a plug of silica gel (10% MeOH/EtOAc eluant). The filtrate was concentrated to afford essentially pure (3R,5α,25R)-1,2,3-trihydroxy-3-methyl-spirostan-11-one (1.05 g). CIMS 459 $(M - 17(OH))^+$. ^1H NMR (250 MHz, $CDCl_3$) δ 4.5 (m, 2H); 3.75 (d, 1H, J = 2 Hz); 3.5 (m, 1H); 3.35 (dd, 1H, J = 10.8, 10.5 Hz); 2.75 (bs, 3H); 2.3 (m, 2H); 2.15 (m, 1H); 2.05 (m, 1H); 1.9-1.05 (m, H); 1.29 (s, 3H); 1.03 (s, 3H); 0.92 (d, 3H, J = 7 Hz); 0.77 (d, 3H, J = 7 Hz); 0.69 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 211, 109.2, 80.6, 75.2, 74.6, 73.6, 67.0, 66.9, 60.8, 57.6, 57.3, 55.5, 44.3, 41.9, 41.3, 39.9, 36.7, 36.0, 32.2, 31.4, 30.2, 28.7, 27.4, 23.0, 17.2, 17.1, 14.1, 12.7.

Preparation of bicyclic lactol 11.

Periodic acid (3.78 mmol) was added to a solution of triol **10** (600 mg, 1.26 mmol) in THF (5 mL) and H_2O (0.5 mL) at room temperature. After 1 hour the reaction was complete. The mixture was diluted with EtOAc (20 mL) and washed with water (1x), $NaHCO_3$ sol (1x), brine (1x), dried (Na_2SO_4) filtered and concentrated to give a colorless solid (420 mg, 70%). m.p. 249-250 °C (dec). CI MS 475 $(M+H)^+$. ^1H NMR (250 MHz, $CDCl_3$) δ 6.0 (s, 1H); 5.0 (s, 1H); 4.5 (q, 1H, J = 7.0 Hz); 3.5 (m, 1H); 3.34 (dd, 1H, J = 10.8, 10.5 Hz); 2.21 (s, 2H); 2.1-1.2 (m, 20H); 1.22 (s, 3H); 1.05 (s, 3H); 0.91 (d, 3H, J = 7.0 Hz); 0.78 (d, 3H, J = 7.0 Hz); 0.69 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 210, 109.2, 105.1, 97.7, 89.5, 81.4, 80.5, 66.9, 60.9, 60.7, 57.8, 56.9, 55.3, 44.2, 41.8, 39.6, 37.0, 36.7, 35.5, 32.1, 31.2, 30.2, 28.7, 25.8, 19.8 17.3, 14.2, 11.5. HRMS calc. for $C_{28}H_{43}O_6$: 475.3059. Found: 475.3016.

Ozonolysis of (3R,5α,25R)-3-methyl-3-hydroxy-spirost-1-en-11-one

Ozone was bubbled through a solution of (3R,5α,25R)-3-methyl-3-hydroxy-spirost-1-en-11-one (200 mg, 4.5 mmol) in 5:1 dichloromethane:methanol (15 mL) at -78°C. When the blue color persisted for 3 minutes, the excess ozone was purged with nitrogen for ten minutes and triphenyl phosphine (400 mg) was added. The reaction was allowed to warm to room temperature and stirred overnight. The mixture was concentrated and purified by flash chromatograpy (10 % EtOAc/hexanes) to afford 243 mg (87%) of product **12** as a colorless solid, identical in all respects to the compound prepared above.

Preparation of keto-aldehyde 12.

Step 1: Sodium borohydride (300 mg) was added to a solution of lactol **11** (300 mg, 0.63 mmol) in dichloromethane (6 mL) and methanol (3 mL) at room temperature. After 14 h, the mixture was diluted

with EtOAc and washed with 0.1 N HCl (2x), and brine (1x). The solution was dried (Na_2SO_4), filtered and concentrated *in vacuo*. This material was used directly in the next step.

Step 2: Sodium periodate (100 mg) was added to a solution of the crude product from above in THF (5 mL) and H_2O (0.5 mL) at room temperature. After 30 min, the mixture was diluted with EtOAc and washed with water (1x) brine (1x) dried (Na_2SO_4), filtered and concentrated. This crude product was used directly in the next reaction.

Step 3: PCC (100 mg) was added to a solution of the crude product from the above reaction in dichloromethane (3 mL) containing 500 mg of dry celite. After 24 h at room temperature, the mixture was diluted with ether (10 mL) and filtered through a plug of silica gel. The filtrate was concentrated and the residue was purified by flash chromatography (gradient elution 10 to 25% EtOAc/hexanes) afforded the keto-aldehyde **12** as a colorless solid (90 mg, 28 % from **11**). ^1H NMR (400 MHz, $CDCl_3$) δ 9.4 (s, 1H); 4.5 (q, 1H, J = 7.3 Hz); 3.5 (m, 1H); 3.35 (dd, 1H, J = 10.8, 10.5 Hz); 2.4-1.2 (m, 21 H); 2.05 (s, 3H); 1.08 (s, 3H); 0.93 (d, 3H, J= 7 Hz); 0.78 (d, 3H, J = 7 Hz); 0.69 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.0, 206.8, 205.2, 109.2, 80.5, 66.9, 61.6, 60.6, 55.4, 53.9, 49.0, 44.4, 44.3, 43.0, 41.9, 36.0, 35.2, 31.5, 31.3, 31.2, 30.2, 28.7, 27.2, 17.6, 17.1, 14.1, 10.1. HRMS calc. for $C_{27}H_{41}O_5$: 445.2943. Found: 445.2993.

(3R,5α,25R)-3-hydroxy-3-[^{14}C]methyl-spirost-1-en-11-one ([^{14}C]-9).

A 50 mL round bottom flask was charged with magnesium (0.243 g, 10.0 mmole) and anhydrous ether (10 mL). A reflux condenser was attached to the flask which was connected to a vacuum manifold. The flask was cooled to -195°C and placed under vacuum. [^{14}C]methyl iodide (300 mCi, 5.50 mmole) was vacuum transferred into the flask, then the flask was vented to argon and warmed to room temperature. After 15 minutes the reaction mixture started to reflux then subsided after an additional 10 minutes. The mixture was stirred an additional 90 minutes at room temperature to give a gray colored solution containing [^{14}C]methylmagnesium iodide.

The enone (**6**) (1.79 g, 4.20 mmole) was dissolved in anhydrous THF (50 mL), cooled to -78°C and treated with the above solution of [^{14}C]methylmagnesium iodide via cannula. The mixture was slowly warmed to room temperature, stirred for 4 hours then quenched by addition of 30 ml of sat. NH_4Cl and 50 mL of Et_2O. The aqueous was extracted with Et_2O and the organics concentrated and purified by flash chromatography (1:1 hexane-ethyl acetate) to give 99.6 mCi of the allylic alcohol **13** (33%).

(5α,25R)-spirost-1-en-[2-^{14}C]-3,11-dione ([^{14}C]-6).

(5α,25R)-3-[^{14}C]-methyl-3-hydroxy-spirost-1-en-11-one **9** (99.3 mCi, 1.83 mmol) was dissolved in 3:2 dichloromethane-methanol (40 mL), cooled to -78°C and treated with ozone until a blue color persisted. The solution was stirred for an additional 30 minutes, then purged with argon and warmed to room temperature. A 15% NaOH (10 mL) solution was added and the reaction mixture vigorously stirred. When the progress of the reaction ceased (~4 hours), the mixture was diluted with water and extracted with $CHCl_3$. The combined organic layers were washed with 1N HCl, brine (1 x 50 mL),

dried (Na_2SO_4) filtered and concentrated in vacuo. Flash chromatography (30 % EtOAc/hexanes) afforded 0.46 grams of the enone **[^{14}C]-6**. The product was further purified by suspending in methanol, heating to reflux then cooling to O°C. The crystalline product was filtered to give 0.416 g (53 mCi) of >98% radiochemically pure enone having a specific activity of 54.1 mCi / mmol.

(5α,25R)-spirost-[2-^{14}C]-3,11-dione ([^{14}C]-7).
A 100 mL round bottom flask was charged with a stir bar and 200 mg of 10% Pd/C. Under an atmoshpere of N_2, 8 mL of a 1:1 THF/ethanol solution was added followed by 52 mCi of **[^{14}C]-6** in 20 mL of THF / ethanol. After degassing, hydrogen was introduced and the reaction was stirred at room temperature for 20 hours. The reaction mixture was filtered through Celite and concentrated to give 51.5 mCi of >98% radiochemically pure 3,11-dione which co-eluted with authentic standard **7**. Radio-TLC: R_f (3 / 1 hexane / EtOAc) 0.37.

(3β,5α,25R)-3-hydroxy-spirost-[2-^{14}C]-11-one ([^{14}C]-2). Sodium borohydride (16.9 mg, 0.445 mmols) was added to a solution of (5α,25R)-spirostan-[2-^{14}C]-3,11-dione (51.5 mCi, 1.08 mmols) in methanol at room temperature. After 30 minutes, the mixture was quenched with dilute HCl and extracted with EtOAc, dried (Na_2SO_4), filtered and concentrated to afford a 51 mCi of an ~10:1 mixture of β : α alcohols. The mixture was initially purified by crystyallization from EtOAc to give 23 mCi (45%) of radiochemically pure [2-^{14}C]-(3β,5α,25R)-3-hydroxy-spirostan-11-one. The mother liquors (ca. 3:1 β : α) were purified by flash chromatography (7% EtOAc/$CHCl_3$) to afford an additional 19.5 mCi (38%) of 3β alcohol. Radio-TLC: R_f (2/1 hexanes/EtOAc) 0.37.

[^{14}C]CP-148,623 ([^{14}C]-3). [^{14}C]-**2** (6.8 mCi, 0.13 mmol) was azeotropically dried from acetonitrile (10 mL), suspended in 10 ml acetonitrile and treated with ZnF_2 (51 mg, 0.49 mmol). The mixture was concentrated to a volume of ca. 3 mL and solid heptaacetylcellobiosyl bromide (359 mg, 0.693 mmol) was added and the mixture warmed to 81°C. After 2 hours, progress of the reaction ceased (as judged by radio-TLC) and the mixture was filtered through Celite and concentrated in vacuo to give 6.3 mCi (93%) of a mixture containing 85% **15**, ca. 7% unreacted starting material **2** and ca. 8% acetylated **2**. Although this mixture could be purified by chromatography, it was used without further purification.
Sodium methoxide (185 mg, 3.42 mmol) was added to the solution of [^{14}C](3β,5α,25R)-3-[(hepta-O-acetyl-β-D-cellobiosyl)oxy]-spirostan-11-one (6.3 mCi, 0.12 mmol) in 4 mL MeOH. The mixture was heated to 60°C for two hours, cooled to room temperature and filtered. The filtrate was concentrated to a yellow solid then recrystallized from EtOAc followed by trituration with 3% aqueous methanol to afford 3.77 mCi (60%) of [^{14}C]CP-148,623 as a colorless solid. The radiochemical purity was established at >99.9% by HPLC. HPLC: Microsorb C18, 5μ, 250 x 4.6 mm; 70 : 30 CH_3OH / water with 2% hydroxypropylcyclodextrin; 1.2 mL / min.; 204 nm; 250 μl YtSi solid cell (IN/US Systems Inc.).

References and Data for Compounds in the Table.

(3R,5α,25R)-3-Hydroxy-3-methyl-spirost-1-ene (16). CI Mass Spect: 429 $(M+H)^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 5.82 (d, 1H, J = 10.2 Hz); 5.35 (d, 1H, J = 10.2 Hz); 4.4 (q, 1H, J = 7.3 Hz); 3.48 (m, 1H); 3.39 (dd, 1H, J = 10.8, 10.5 Hz); 2.0-1.1 (m, 24H); 1.3 (s, 3H); 0.95 (d, 3H, J = 7.0 Hz); 0.9 (s, 3H); 0.79 (d, 3H, J = 7.0 Hz); 0.78 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.2, 132.4, 109.6, 80.3, 70.6, 66.8, 62.2, 56.2, 51.1, 44.0, 42.1, 41.6, 40.3, 39.9, 35.2, 32.1, 31.7, 31.4, 30.4, 29.4, 28.8, 28.0, 21.0, 17.4, 17.3, 16.7, 15.0, 14.3.

(5α,25R)-Spirost-1-en-3-one (17). CI Mass Spect. 413 $(M+H)^+$; ^1H NMR (250 MHz, $CDCl_3$) δ 7.12 (d, 1H, J = 10.5 Hz); 5.83 (d, 1H, J = 10.5 Hz); 4.4 (q, 1H, J = 7.3 Hz); 3.45 (m, 1H); 3.35 (dd, 1H, J = 10.8, 10.5 Hz); 2.38 (dd, 1H, J = 17.8, 15.3 Hz); 2.22 (dd, 1H, J = 17.8, 5.5 Hz); 2.0-1.1 (m, 22H); 1.05 (s, 3H); 0.95 (d, 3H, J = 7.0 Hz); 0.8 (s, 3H); 0.78 (d, 3H, J = 7.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.2, 158.3, 127.4, 109.4, 80.7, 66.9, 62.2, 56.2, 50.0, 44.3, 41.6, 40.9, 40.6, 39.8, 39.0, 35.3, 31.7, 31.5, 31.4, 30.3, 28.8, 27.5, 21.0, 17.0, 16.2, 14.2, 13.1.

(3R) 3-methyl-cholestan-1-en-3-ol (18). See: Nickon, A.; DiGiorgio, J.B.; Daniels, P.J.L. *J. Org. Chem.* **1973**, *38*, 533-539.

Cholestan-1-en-3-one (19). See: Sharpless, K.B.; Laver, R.F.; Teranishi, A.Y. *J. Am. Chem. Soc.* **1973**, *95*, 6137-6139.

(3S) 3-Methyl-cholestan-4-en-3-ol (20). See: Lichtfouse, E.; Albrecht, P. *Tetrahedron* **1994**, *50*, 1731-1744.

Cholestan-4-en-3-one (21): Commercially available from the Aldrich Chemical Co.

4,4-Diphenyl-1-methyl-cyclohex-2-en-1-ol (22).

Methyl magnesium chloride (4 mL of a 3M solution in THF, 12.1 mmol) was added dropwise to a solution of 4,4-diphenyl-cyclohex-2-en-1-one (2 g, 8.1 mmol, commercially available from the Aldrich Chemical Co.) in THF (20 mL) at -20°C. The mixture was allowed to warm to room temperature over 1 hour. The mixture was diluted with ethyl acetate (100 mL) and washed with 1N HCl sol (2 x 50 mL), brine (1 x 50 mL) dried (Na_2SO_4) filtered and concentrated in vacuo to give 1.95 g (93 %) of the title compound as a waxy solid. CI Mass Spect. 265 $(M+H)^+$; ^1H NMR (250 MHz, $CDCl_3$) δ 7.2 (m, 10H); 6.15 (d, 1H, J = 10.5 Hz); 5.88 (d, 1H, J = 10.5 Hz); 2.5 (m, 1H); 2.3 (m, 1H); 1.7 (m, 2H); 1.35 (s, 3H).

4,4-Diphenyl-cyclohex-2-en-1-one (23). Commercially available from the Aldrich Chemical Co.

REFERENCES AND NOTES

1. McCarthy, P.A.; DeNinno, M.P.; Morehouse, L.A.; Chandler, C.E.; Bangerter, F.W.; Wilson, T.C.; Urban, F.J.; Walinsky, S.W.; Cosgrove, P.G.; Duplantier, K.C.; Etienne, J.B.; Fowler, M.A.; Lambert, J.F.; O'Donnell, J.P.; Pezzullo, S.L.; Watson Jr.,H.A.; Wilkins, R.W.; Zaccaro, L.M.; Zawistoski, M.P. *J. Med. Chem.* **1996**, *39*, 1935-1937.
2. Turner, R. B.; *J. Am. Chem. Soc.*, **1950**, *72*, 579-585. Fujimoto, G. I.; Prager, J., *J. Am. Chem. Soc.*, **1953**, *75*, 3259-3261. Belleau, B. *J. Am. Chem. Soc.* **1951**, *73*, 5441-5443. Henrick, C. A.; Bohme, E.; Edwards, J. A.; Fried, J. H., *J. Am. Chem Soc.*, **1968**, *90*, 5926-5927. Rao, P. N.; Axelrod, L. R., *J. Chem Soc.*, **1965**, 1356-1359.
3. Engal, Ch. R.; Chowdhury, M.N.; *Tetrahedron Lett.* **1968**, *17*, 2107-2111. Kutney, J.P.; Vlattas, I.; Rao, G.V. *Can. J. Chem.*, **1963**, *41*, 958-965.
4. The large scale preps of CP-148,623 were used for toxicology and clinical studies. 11-Ketotigogenin is also available from Steraloids Inc., Wilton, N.H.
5. Baran, J.S.; Langford, D.D.; Laos, I.; Liang, C.D. *Tetrahedron* **1977**, *33*, 609-616.
6. Bailey, P.S., "Ozonation in Organic Chemistry", Academic: New York, **1978**; Vol. 1. Bailey, P.S. *Chem. Rev.* **1958**, *58*, 925-1010. Everest, D.J.; Grant, P.K.; Slim, G.C.; Yeo, I.K.L. *Aust. J. Chem.* **1988**, *41*, 1025-1035. Cargill, R.L.; Wright, B.W. *J. Org. Chem.* **1975**, *40*, 120-122. Wadt, W.R.; Goddard III, W.A. *J. Am. Chem. Soc.* **1975**, *97*, 3004-3021.
7. DeNinno, M.P. *J. Am. Chem. Soc.* **1995**, *117*, 9927-9928.
8. We would like to thank Dr. David Leuck of Chemsyn Science Laboratories for carrying out this transformation.
9. Extended reaction times leads to decomposition of the enone. The reaction was monitored by radio-TLC until the maximum amount of enone **6** was formed, then the reaction was worked-up.

(*Received* 9 *August* 1996; *revised* 25 *October* 1996; *accepted* 29 *October* 1996)