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Synthesis Of [2,2,3α,4,4-D5]CP-88,818 (Tiqueside), An Internal Standard For A Quantitative HPLC/MS Assay System

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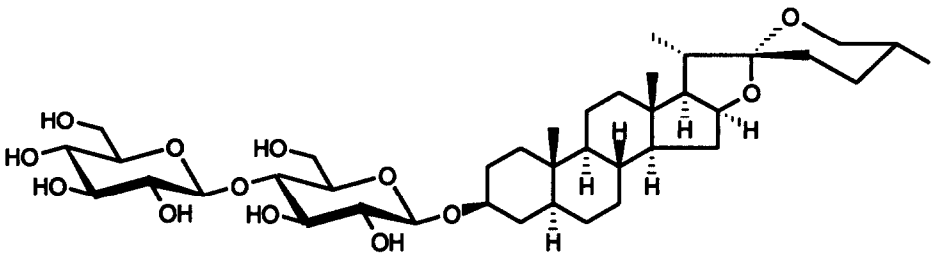
Abstract: A polydeuterated form of CP-88,818 (1, tiqueside) was needed as an internal standard for a quantitative HPLC/MS assay system. [2,2,3α,4,4-D_5]CP-88,818 (11) with undetectable D_0 content was synthesized in five steps from tigogenin (2). The low D_0 content was achieved through two sequential incorporation procedures which gave results superior to those achieved through a single incorporation procedure. A preparatively useful procedure for removing spirostane impurities found in naturally occurring tigogenin was also discovered.

INTRODUCTION

Compounds containing stable isotopes have demonstrated great utility for several types of drug metabolism studies.[1] One powerful example of this utility is the application of compounds containing stable isotopes as internal standards for quantitative HPLC/MS assay systems.[2] Not only does such an internal standard have extraction properties identical to that of the sample compound, but it also has an identical HPLC retention time, thereby avoiding precision errors introduced through ionization fluctuations. So long as the compound containing the stable isotope is essentially free of unlabelled (D_0) material, it provides a clear comparison between sample compound and internal standard needed to normalize assay samples.



1

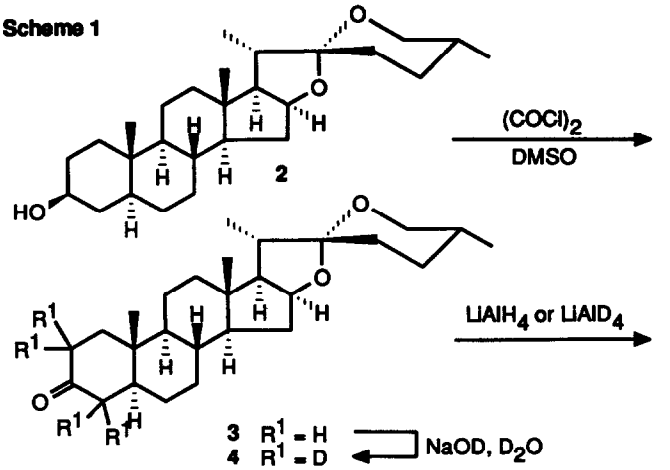
CP-88,818 (1, tiqueside) is a novel hypocholesterolemic agent now under clinical investigation.[3] While developing a quantitative HPLC/MS-based assay system, it became clear that use of CP-88,818 labeled with stable isotopes as an internal standard was mandatory if acceptable precision were to be achieved. After consideration of several alternatives, we selected a pentadeuterated derivative, [2,2,3α,4,4-D_5]CP-88,818 (11) as our target. The basis for this choice was manifold. First of all, most large mass spectrometry fragments of CP-88,818 contained an intact A-ring, thus locating the deuteriums in this ring allowed the option of analyzing daughter particles if necessary. Secondly, the incorporation of multiple deuteriums would allow for adequate separation of the ion plumes in the mass spectrum. Thirdly, the position appeared synthetically accessible, yet unlikely to alter significantly fragmentation rates relative to the unlabeled sample. Finally, the deuteriums could be incorporated into the molecule in two separate synthetic steps. While such a strategy tends to reduce the isotopic purity of the D_5 product, it also reduces the likelihood that any D_0 material will remain. With these considerations in mind, we set out to synthesize [2,2,3α,4,4-D_5]CP-88,818 (11).

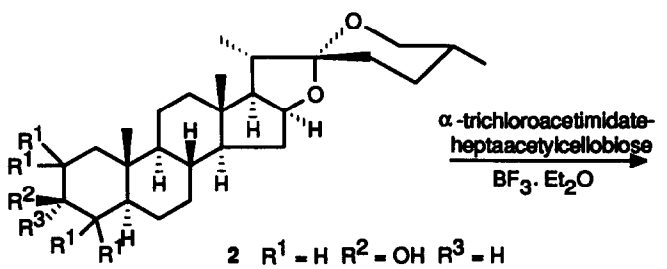
DISCUSSION

Our synthetic route is shown in Scheme I. The final steps would involve coupling of a cellobiose unit with [2,2,3α,4,4-D_5]tigogenin (8). The labeled tigogenin would be derived from unlabeled tigogenin (2) through a three step process of oxidation, deuterium exchange and deuteride reduction.

The first objective was to obtain tigogenin which was free of other naturally occuring spirostane impurities. Our starting lot of tigogenin contained three spirostane impurities: diosgenin (1.5%), hecogenin (0.8%) and an unknown spirostane (2.7%). Although the presence of any of these impurities reduces the quality of the internal standard, olefin-containing impurities such as diosgenin were the biggest problem in that they introduced a starting material

Scheme 1





2 $R^1 = H$ $R^2 = OH$ $R^3 = H$

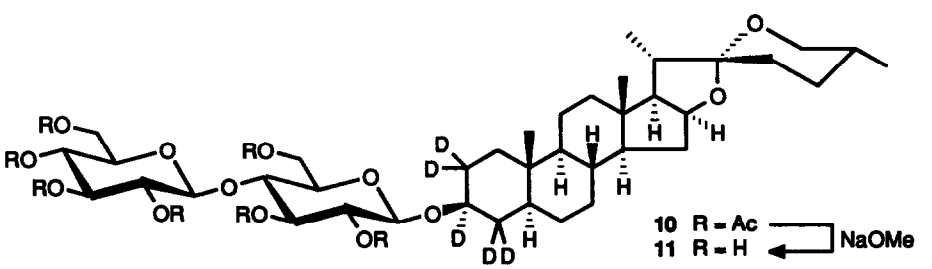
5 $R^1 = H$ $R^2 = H$ $R^3 = OH$

6 $R^1 = D$ $R^2 = OH$ $R^3 = H$

7 $R^1 = D$ $R^2 = H$ $R^3 = OH$

8 $R^1 = D$ $R^2 = OH$ $R^3 = D$

9 $R^1 = D$ $R^2 = D$ $R^3 = OH$



with a molecular weight two mass units lower than that of tigogenin. Recrystallization from 1:3 THF:MeOH slightly altered the content of these impurities (1.9% diosgenin, 0.5% hecogenin and 2.7% unknown spirostane), but not to an acceptable level. HPLC separation of the impurities was achieved but deemed impractical for preparative work. Removal of diosgenin through a classical bromination route[4] gave low yields of pure tigogenin, presumably because of 23-bromide formation.[4a,5] Although satisfactory, the procedure was eventually replaced as described below.

The second objective was to assure that high purity 3β-alcohol was obtainable from 5α-spirostan-3-one (**3**). Achieving 3β-selectivity during hydride reductions in steroidal systems is trivial;[4a] however, we needed to assure that any 3α-alcohol produced could be effectively removed after the reduction. To this end, recrystallized tigogenin was oxidized to **3** using Swern conditions (Scheme I). This material was purified by flash chromatography and reduced with $LiAlH_4$ to give a 6:1 mixture of alcohols. The α-alcohol (**5**) migrated nearly 0.1 R_f unit faster than the β-alcohol (**2**), thus achievement of **2** in high purity was assured. HPLC analysis of **2** also demonstrated that all of the spirostane impurities contained in the starting material were absent from the product. Subsequent investigation showed that the spirostane impurities were removed during chromatography of **3**. This result obviated the bromination procedure as a means of removing the spirostane impurities.

The third objective of this work was to incorporate the deuteriums into tigogenin. Treatment of **3** with NaOD in THF and CD_3OD provided 5α-[2,2,4,4-D_4]spirostan-3-one (**4**). Reduction with $LiAlH_4$ and purification provided [2,2,4,4-D_4]tigogenin (**6**) with the following product distribution as determined by MS (ion, relative intensity): D_0, 2%; D_1, 2%; D_2, 11%; D_3, 21%; D_4, 100%. Reduction of **4** with $LiAlD_4$ and purification provided [2,2,3α,4,4-D_5]tigogenin (**8**) with the following MS product distribution: D_0, 0%; D_1, 0%; D_2, 1%; D_3, 4%; D_4, 23%; D_5, 100%. These results clearly indicate that the strategy of sequential deuterations was successful in minimizing the D_0 content of the sample.

With **8** in hand, conversion into [2,2,3α,4,4-D_5]CP-88,818 (**11**) was achieved in the following manner: Following the procedure of Urban[6], **8** was coupled with α-trichloroacetimidate heptaacetylcellobiose in the presence of $BF_3{\cdot}Et_2O$ to give heptaacetate **10**. Deprotection with NaOMe provided **11** with the following MS product distribution: D_0, 0%; D_1, 0%, D_2, 1%; D_3, 2%, D_4, 18%, D_5, 100%. This product distribution corresponds to 97%/atom deuterium incorporation for **11**.

The LSIMS (liquid secondary ion mass spectrometry) spectra of **1** (Fig. 1) and **11** (Fig. 2) are shown above for comparison of the regions of the protonated molecules. The $(M+H)^+$ ion from the unlabelled compound, at m/z 741, shows two losses of H_2 at m/z 739 and 737. The ^{13}C isotope at 738 and 740 are present at the expected intensities, given the level of chemical

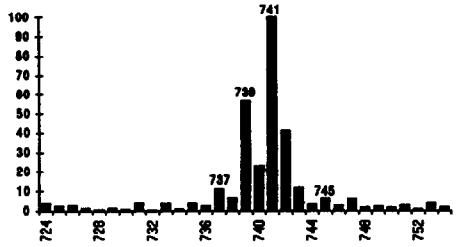


Fig. 1. Mass Spectum of **1**

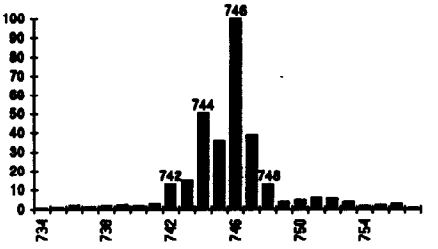


Fig. 2. Mass Spectrum of **11**

noise in the spectrum. The deuterated material exhibits an intense $(M+H)^+$ ion at m/z 746, demonstrating that the material is primarily D_5. This ion also loses $2H_2$, but no loss of H_2 and HD, 2HD or $2D_2$ is observable above chemical noise. These losses should take place from specific locations in the molecule, rather than at random; so we conclude that the losses of H_2 do not involve the protons at the 2, 3 or 4 positions. The peaks at 741-746 in the deuterated material's spectrum are therefore a convolution of the unlabelled compound's spectrum in the region of 737-741, the protonated molecules of the D_0-D_5 compounds, and their ^{13}C isotope peaks. The relative peak intensities from the unlabelled material may be subtracted, and the resultant D_0-D_5 amounts, as reported above, are available by inspection.

In conclusion, we have described a synthesis of [2,2,3α,4,4-D_5]CP-88,818 (**11**). This material has undetectable D_0 content as judged by LSIMS mass spectrometry. The low D_0 content was achieved through two sequential incorporation procedures which gave results superior to those achieved through a single incorporation procedure. We also discovered a preparatively-useful procedure for removing spirostane impurities found in naturally-occurring tigogenin. The [2,2,3α,4,4-D_5]CP-88,818 (**11**) prepared by this route has been successfully used to establish a quantitative HPLC/MS assay system for CP-88,818 (tiqueside, **1**).

ACKNOWLEDGMENT

The authors wish to thank M. M. Andino and M. L. Dumont for kindly providing HPLC analysis of intermediates.

EXPERIMENTAL

General. Mass spectrometric analyses were carried out using a Kratos Concept-1S spectrometer (Kratos Analytical, Ramsey, NJ), operating at 8 kV accelerating potential. Electron ionization

spectra were generated under impact of 70 eV electrons, with the source temperature at 200°C. Samples were introduced via a heated direct insertion probe. Liquid Secondary Ion Mass Spectra (LSIMS, similar to FAB/MS) were generated from a matrix of 1:4 v/v dithioerythritol/dithiothreitol with an impacting Cs^+ ion energy of 16 kV. High resolution results were obtained at a resolving power of 10,000 - 12,000.

Complete carbon NMR data and diagnostic proton NMR and IR data are provided for new compounds. Carbon NMR assignments are based on literature precedence,[7] DEPT and ^1H-^{13}C correlation spectra, and deuterium-induced muting of ^{13}C signals.[8]

(25R)-5a-Spirostan-3-one (3). DMSO (3.7 mL, 51.8 mmoles) was added dropwise with stirring to a solution of oxalyl chloride (3.656 g, 28.8 mmoles) in anhydrous CH_2Cl_2 (20 mL) and anhydrous THF (20 mL) at -78°C under N_2. The resulting solution was stirred at -78°C for 5 min, then it was added dropwise with stirring to a solution of **2** (10.0 g, 24.0 mmoles) in anhydrous THF (200 mL) at -78°C under N_2. The resulting white suspension was stirred at -78°C for 30 min, then Et_3N (17 mL, 122 mmoles) was added. The resulting thick suspension was stirred at -78°C for 40 min, then allowed to warm to rt. The reaction was diluted with CH_2Cl_2 (160 mL) and hexane (640 mL) and this mixture was washed first with 10% aqueous $NaHSO_4$ (250 mL) and then with H_2O (2 X 160 mL). The organic layer was dried ($MgSO_4$) then evaporated *in vacuo* to give 9.71 g of crude product. Repeated purification (2X) by flash chromatography, eluting with 4:1 hexane:EtOAc gave 4.368 g (44% yield) of **3** as a white solid, mp 209-210°C; ^1H NMR ($CDCl_3$) δ ; 2.40-2.25 (m, 2H, $H_{2\alpha}$, $H_{2\beta}$), 2.22 (dd, J = 16 and 14 Hz, 1H, $H_{4\beta}$), 2.03 (ddd, J = 16 Hz, 3 Hz and 2 Hz, 1H, $H_{4\alpha}$); ^{13}C NMR ($CDCl_3$) δ 211.8 (C_3), 109.2 (C_{22}), 80.7 (C_{16}), 66.8 (C_{26}), 62.2 (C_{17}), 56.1 (C_{14}), 53.8 (C_9), 46.6 (C_5), 44.7 (C_4), 41.6 (C_{20}), 40.5 (C_{13}), 39.9 (C_{12}), 38.4 (C_1), 38.1 (C_2), 35.8 (C_{10}), 35.0 (C_8), 31.9 (C_7), 31.8 (C_{15}), 31.4 (C_{23}), 30.3 (C_{25}), 28.8 (C_6), 28.8 (C_{24}), 21.2 (C_{11}), 17.1 (C_{27}), 16.4 (C_{18}), 14.5 (C_{21}), 11.5 (C_{19}); IR (KBr) 2900 (C-H stretch), 1720 (C=O stretch), 1055 (C-O-C asymmetric stretch) cm^{-1}; EI MS, m/z (relative intensity) 414 ($M^{+\cdot}$).

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.22; H, 10.39.

(25R)-2,2,4,4-Tetradeuterio-5α-spirostan-3-one (4). NaOD (30% in D_2O) (2.20 g, 16.1 mmoles) was added to a solution of **3** (2.00 g, 4.82 mmoles) in anhydrous THF (70 mL) and CD_3OD (30.00 g, 832 mmoles) and this solution was stirred at rt under N_2 for 17 h. The reaction mixture was evaporated *in vacuo* to give 3.373 g of **4** as a yellow solid. This was used immediately in the next step.

(25R)-5α-Spirostan-3β-ol (2) and (25R)-5α-spirostan-3α-ol (5). A solution of **3** (0.300 g, 0.7

mmole) in Et_2O (35 mL) was added dropwise with stirring to a suspension of $LiAlH_4$ (0.088 g, 2.3 mmole) in Et_2O (20 mL) at rt under N_2. The reaction mixture was stirred at rt under N_2 for 3 h, then quenched by the successive dropwise addition of H_2O (1.0 mL), 15% aqueous NaOH (1.0 mL) and H_2O (3.0 mL). The resulting suspension was filtered and the filtrate was evaporated *in vacuo* to give 0.282 g of crude mixed alcohols. Purification by flash chromatography, eluting with 6:4 hexane:EtOAc gave 0.050 g (17% yield)[9] of **2** as a white solid, (Rf = 0.44, 1:1 hexane:EtOAc), mp 211-212°C; ^1H NMR ($CDCl_3$) δ 3.55 (dddd, J = 11, 11, 5.5, 5.5 Hz, 1H, $H_{3\alpha}$); ^{13}C NMR[7b] ($CDCl_3$) δ 109.2 (C_{22}), 80.8 (C_{16}), 71.3 (C_3), 66.8 (C_{26}), 62.2 (C_{17}), 56.3 (C_{14}), 54.4 (C_9), 44.8 (C_5), 41.6 (C_{20}), 40.6 (C_{13}), 40.1 (C_{12}), 38.2 (C_4), 37.0 (C_1), 35.6 (C_{10}), 35.1 (C_8), 32.2 (C_7), 31.8 (C_{15}), 31.5 (C_2), 31.4 (C_{23}), 30.3 (C_{25}), 28.8 (C_{24}), 28.6 (C_6), 21.1 (C_{11}), 17.1 (C_{27}), 16.5 (C_{18}), 14.5 (C_{21}), 12.4 (C_{19}); IR (KBr) 3550-3200 (O-H stretch), 2930 (C-H stretch), 1240 (O-H bend), 1175 (C-O stretch) cm^{-1}; EI MS, m/z (relative intensity) 416 ($M^{+\cdot}$).

Anal. Calcd. for $C_{27}H_{44}O_3 \cdot 0.5H_2O$: C, 76.19; H, 10.66. Found: C, 75.89; H, 10.60.

Flash chromatography also gave 0.025 g (8% yield) of **5** as a white solid, (Rf = 0.54, 1:1 hexane:EtOAc), mp 230-232°C; ^1H NMR ($CDCl_3$) δ 4.01 (br s, 1H, $H_{3\beta}$); ^{13}C NMR ($CDCL_3$) δ 109.2 (C_{22}), 80.9 (C_{16}), 66.8 (C_{26}), 66.5 (C_3), 62.2 (C_{17}), 56.4 (C_{14}), 54.3 (C_9), 41.6 (C_{20}), 40.6 (C_{13}), 40.1 (C_{12}), 39.1 (C_5), 36.2 (C_{10}), 35.9 (C_4), 35.1 (C_1), 32.2 (C_7), 32.1 (C_8), 31.7 (C_{15}), 31.4 (C_{23}), 30.3 (C_{25}), 29.0 (C_2), 28.8 (C_{24}), 28.5 (C_6), 20.6 (C_{11}), 17.1 (C_{27}), 16.5 (C_{18}), 14.5 (C_{21}), 11.2 (C_{19}); IR (KBr) 3500-3300 (O-H stretch), 2910 (C-H stretch), 1250 (O-H bend), 1165 (C-O stretch) cm^{-1}; EI MS, m/z (relative intensity) 416 ($M^{+\cdot}$).

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.83; H, 10.32.

(25R)-2,2,4,4-Tetradeuterio-5α-spirostan-3β-ol (6). A solution of crude **4** (0.644 g, assumed to contain 0.404 g, 0.97 mmole of ketone) in anhydrous Et_2O (50 mL) was added slowly with stirring to a suspension of $LiAlH_4$ (0.13 g, 3.4 mmoles) in anhydrous Et_2O (25 mL) at rt under N_2. The reaction was stirred at rt for 30 min, then quenched by the successive dropwise addition of H_2O (1.0 mL), 15% NaOH in H_2O (1.0 mL) and H_2O (3.0 mL). The resulting suspension was filtered and the filtrate was evaporated *in vacuo* to give 2.354 g of crude product. Purification by flash chromatography, eluting with 8:2 hexane:EtOAc gave 0.018 g (4% yield)[9] of **6** as a white solid, mp 198-200°C; ^1H NMR ($CDCl_3$) δ 3.54 (s, 1H, $H_{3\alpha}$); ^{13}C NMR[8] ($CDCl_3$) δ 109.2 (C_{22}), 80.8 (C_{16}), 71.1 (C_3), 66.8 (C_{26}), 62.2 (C_{17}), 56.3 (C_{14}), 54.3 (C_9), 44.7 (C_5), 41.6 (C_{20}), 40.6 (C_{13}), 40.1 (C_{12}), 36.8 (C_1), 35.5 (C_{10}), 35.1 (C_8), 32.2 (C_7), 31.8 (C_{15}), 31.4 (C_{23}), 30.3 (C_{25}), 28.8 (C_{24}), 28.6 (C_6), 21.1 (C_{11}), 17.1 (C_{27}), 16.5 (C_{18}), 14.5 (C_{21}), 12.4 (C_{19}); IR (KBr) 3450-3050 (O-H stretch), 2920 (C-H stretch), 2190, 2120 (C-D stretch), 1250 (O-H bend), 1185 (C-O stretch) cm^{-1}; High Resolution LSIMS: Calcd. for

$C_{27}H_{41}D_4O_3$ $(M+H)^+$: m/z 421.3620. Found: m/z 421.3585.

Anal. Calcd. for $C_{27}H_{40}D_4O_3 \cdot 0.7H_2O$: C, 74.84; H, 10.33. Found: C, 74.80; H, 10.42.

The (25R)-2,2,4,4-tetradeuterio-5α-spirostan-3α-ol (7) which was produced in the reaction was not isolated.

(25R)-2,2,3α,4,4-Pentadeuterio-5α-spirostan-3β-ol (8) and (25R)-2,2,3β,4,4-pentadeuterio-5α-spirostan-3α-ol (9). A solution of crude 4 (3.373 g, assumed to contain 2.018 g, 4.8 mmoles of ketone) in anhydrous Et_2O (250 mL) was added slowly with stirring to a suspension of $LiAlD_4$ (1.79 g, 42.6 mmoles) in anhydrous Et_2O (100 mL) at rt under N_2. The reaction mixture was stirred at rt for 30 min, then quenched by the successive dropwise addition of D_2O (2.0 mL), 30% NaOD in D_2O (1.0 mL) and D_2O (6.0 mL). The resulting suspension was filtered and the filtrate was evaporated *in vacuo* to give 1.537 g of crude mixed alcohols. Purification by flash chromatography, eluting with 8:2 hexane:EtOAc gave 1.266 g (62% recovery) of **8** as a white solid, (Rf = 0.13, 8:2 hexane:EtOAc), mp 205-207°C; ^{13}C NMR[8] ($CDCl_3$) δ 109.2 (C_{22}), 80.8 (C_{16}), 66.8 (C_{26}), 62.2 (C_{17}), 56.3 (C_{14}), 54.4 (C_9), 44.7 (C_5), 41.6 (C_{20}), 40.6 (C_{13}), 40.1 (C_{12}), 36.8 (C_1), 35.5 (C_{10}), 35.1 (C_8), 32.2 (C_7), 31.8 (C_{15}), 31.4 (C_{23}), 30.3 (C_{25}), 28.8 (C_{24}), 28.6 (C_6), 21.1 (C_{11}), 17.1 (C_{27}), 16.5 (C_{18}), 14.5 (C_{21}), 12.3 (C_{19}); IR (KBr) 3450-3100 (O-H stretch), 2930 (C-H stretch), 2200, 2120, 2100 (C-D stretch), 1225 (O-H bend), 1165 (C-O stretch) cm^{-1}; High Resolution LSIMS: Calcd. for $C_{27}H_{40}D_5O_3$ $(M+H)^+$: m/z 422.3682. Found: m/z 422.3722. An X-ray crystal structure of **8** confirmed the 3β configuration.

Anal. Calcd. for $C_{27}H_{39}D_5O_3 \cdot H_2O$: C, 73.75; H, 10.51. Found: C, 73.97; H, 10.89.

Flash chromatography also gave 0.201 g (10% recovery) of **9** as a white solid, (Rf = 0.18, 8:2 hexane:EtOAc), mp 240-242°C; ^{13}C NMR[8] ($CDCl_3$) δ 109.2 (C_{22}), 80.9 (C_{16}), 66.8 (C_{26}), 62.2 (C_{17}), 56.4 (C_{14}), 54.3 (C_9), 41.6 (C_{20}), 40.6 (C_{13}), 40.1 (C_{12}), 38.9 (C_5), 36.1 (C_{10}), 35.1 (C_1), 32.2 (C_7), 31.9 (C_8), 31.7 (C_{15}), 31.4 (C_{23}), 30.3 (C_{25}), 28.8 (C_{24}), 28.4 (C_6), 20.6 (C_{11}), 17.1 (C_{27}), 16.5 (C_{18}), 14.5 (C_{21}), 11.2 (C_{19}); IR (KBr) 3550-3250 (O-H stretch), 2900 (C-H stretch), 2190, 2160, 2130 (C-D stretch), 1245 (O-H bend), 1175 (C-O stretch) cm^{-1}; High Resolution LSIMS: Calcd. for $C_{27}H_{40}D_5O_3$ $(M+H)^+$: m/z 422.3682. Found: m/z 422.3679.

Anal. Calcd. for $C_{27}H_{39}D_5O_3$: C, 76.90; H, 10.53. Found: C, 77.17; H, 10.73.

(25R)-2,2,3α,4,4-Pentadeuterio-5α-spirostan-3β-yl β-cellobioside heptaacetate (10). A solution of **8** (0.200 g, 0.5 mmole) and a-trichloroacetimidate heptaacetylcellobiose (0.370 g, 0.5 mmole) in anhydrous toluene (30 mL) was refluxed under N_2 with a Dean-Stark trap for 30 min,

then cooled to rt. $BF_3{\cdot}Et_2O$ (0.012 mL, 1.0 mmole) was added and the reaction mixture was stirred at rt under N_2 for 1.5 h. The reaction was quenched by the addition of $NaHCO_3$ (0.4 g) in H_2O (7 mL). The resulting mixture was stirred for 10 min then the aqueous layer was separated, and the organic layer was washed with H_2O (7 mL), dried ($MgSO_4$) and evaporated *in vacuo* to give 0.459 g of yellow solid. Purification by flash chromatography, eluting with 7:3 hexane:EtOAc gave 0.210 g (43% yield) of **10** as a white solid, mp 231-233°C; ^1H NMR ($CDCl_3$) δ 2.08 (s, 3H, acetyl CH_3), 2.05 (s, 3H, acetyl CH_3), 2.00 (s, 6H, acetyl CH_3), 1.97 (s, 6H, acetyl CH_3), 1.95 (s, 3H, acetyl CH_3); ^{13}C NMR[8] ($CDCl_3$) δ 170.5 ($C_{6''}Ac$), 170.3 ($C_{6'}Ac$), 170.2 ($C_{3''}Ac$), 169.8 ($C_{2'}Ac$), 169.5 ($C_{3'}Ac$), 169.3 ($C_{4''}Ac$), 169.0 ($C_{2''}Ac$), 109.2 (C_{22}), 100.7 ($C_{1''}$), 99.4 ($C_{1'}$), 80.8 (C_{16}), 76.6 ($C_{4'}$), 73.0 ($C_{3''}$), 72.6 ($C_{5''}$), 72.6 ($C_{2''}$), 71.9 ($C_{5'}$), 71.8 ($C_{3'}$), 71.7 ($C_{2'}$), 67.9 ($C_{4''}$), 66.8 (C_{26}), 62.2 (C_{17}), 62.1 ($C_{6''}$), 61.6 ($C_{6'}$), 56.3 (C_{14}), 54.3 (C_9), 44.6 (C_5), 41.6 (C_{20}), 40.6 (C_{13}), 40.0 (C_{12}), 36.7 (C_1), 35.5 (C_{10}), 35.1 (C_8), 32.2 (C_7), 31.8 (C_{15}), 31.4 (C_{23}), 30.3 (C_{25}), 28.8 (C_{24}), 28.6 (C_6), 21.0 (C_{11}), 20.8, 20.7, 20.6, 20.5 (Ac methyls), 17.1 (C_{27}), 16.5 (C_{18}), 14.5 (C_{21}), 12.3 (C_{19}); IR (KBr) 2930 (C-H stretch), 2200, 2130,2100 (C-D stretch), 1760 (C=O stretch) cm^{-1}; High Resolution LSIMS: Calcd. for $C_{53}H_{74}D_5O_{20}$ $(M+H)^+$: m/z 1040.5479. Found: m/z 1040.5430.

Anal. Calcd. for $C_{53}H_{73}D_5O_{20}$: C, 61.19; H, 7.23. Found: C, 61.55; H, 7.59.

(25R)-2,2,3α,4,4-Pentadeuterio-5α-spirostan-3β-yl β-cellobioside (11, [2,2,3α,4,4-D_5]CP-88,818). A mixture of **10** (0.150 g, 0.15 mmole) and $NaOCH_3$ (0.001g , 0.02 mmole) in MeOH (3.0 mL) and anhydrous THF (3.0 mL) was refluxed under N_2 for 1 h. The THF was removed by distillation and H_2O (2 drops) was added and the mixture was refluxed for 2.5 h, then cooled to rt and stirred at rt overnight. The solid that separated was collected to give 0.087 g (81% yield) of **11** as a white solid, mp > 300°C; ^{13}C NMR[8,10] (DMSO-d_6) δ 108.4 (C_{22}), 103.2 ($C_{1''}$), 100.3 ($C_{1'}$), 80.7 (C_{16}), 80.2 ($C_{4'}$), 76.8 ($C_{3''}$), 76.5 ($C_{5''}$), 75.1 ($_{5'}$), 74.7 ($C_{2''}$), 73.3 ($C_{3'}$), 73.2 ($C_{3'}$), 70.1 ($C_{4''}$), 65.9 (C_{26}), 62.0 (C_{17}), 61.1 ($C_{6''}$), 60.5 ($C_{6'}$),55.7 (C_{14}), 53.7 (C_9), 43.9 (C_5), 41.1 (C_{20}),36.3 (C_1), 35.3 (C_{10}), 34.7 (C_8), 31.9 (C_7), 31.4 (C_{15}), 31.0 (C_{23}), 29.8 (C_{25}), 28.5 (C_{24}), 28.3 (C_6), 20.6 (C_{11}), 17.1 (C_{27}), 16.2 (C_{18}), 14.7 (C_{21}), 12.1 (C_{19}); IR (KBr) 3650-3050 (O-H stretch), 2900 (C-H stretch), 2200, 2120 (C-D stretch); High Resolution LSIMS: Calcd. for $C_{39}H_{60}D_5O_{13}$ $(M+H)^+$: m/z 746.4739. Found: m/z 746.4720.

Anal. Calcd. for $C_{39}H_{59}D_5O_{13}{\cdot}H_2O$: C, 61.31; H, 8.43. Found: C, 61.03; H, 8.63.

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(9) Yields for exploratory $LiAlH_4$ reductions were unoptimized.

(10) C_{12} and C_{13} peaks are obscured by the DMSO peaks.