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Articles

Process Research and Large-Scale Synthesis of 4″,6″-Bis((2-fluorophenyl)carbamoyl)hecogenyl β-*O*-Cellobioside: A Potent Cholesterol Absorption Inhibitor

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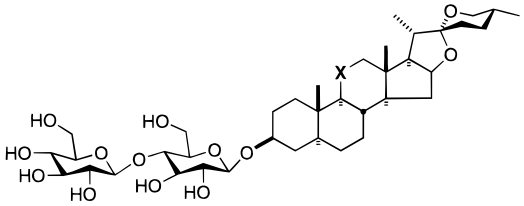
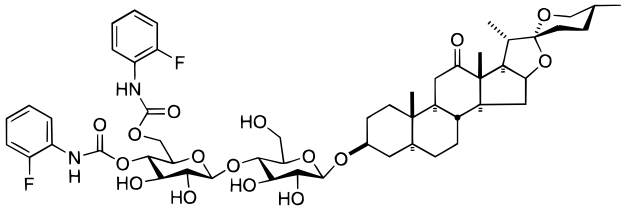
Abstract:

This paper describes process research leading to the successful scale-up of a potent cholesterol absorption inhibitor 4″,6″-bis-((2-fluorophenyl)carbamoyl)hecogenyl β-*O*-cellobioside 3. The synthesis of 3 from hecogenyl β-*O*-cellobioside 4 required five synthetic steps: (1) the selective protection of the 4″,6″-diol group, (2) acylation of the remaining five hydroxyl groups, (3) unmasking of the diol moiety, (4) carbamoylation with 2-fluorophenyl isocyanate, and finally, (5) deacylation. The synthesis by our discovery group utilized chloroacetate protecting groups for five of the sugar alcohols at step two, which led to problems on scale-up due to the instability of this group in solution and the poor crystallinity of the intermediates. Methoxyacetates were identified as the optimal acyl-protecting group. The identification of mild reaction conditions led to an efficient synthesis of bis(carbamate) 3 in very high purity and 42% yield from 4 over five synthetic steps and one recrystallization/polymorph conversion. The process was simple to operate and was carried out to provide 80 kg of 4″,6″-bis(2-fluorophenyl-carbamoyl)hecogenyl β-*O*-cellobioside 3.

Recently, the inhibition of cholesterol absorption by steroid disaccharides has been studied at Pfizer Central Research for the treatment of atherosclerosis. Tiqueside,[1] tigogenyl β-*O*-cellobioside **1**, which was first described by Rene Malinow et al.,[2] reached phase 2 clinical studies. While tiqueside was efficacious and well-tolerated, its relatively weak potency discouraged further development. Structural modification of the steroid nucleus led to the discovery of the more potent pamaqueside, 11-ketotigogenyl β-*O*-cellobioside **2**.[3] Further research by DeNinno and McCarthy on modification of the glycoside moiety afforded additional improvements in potency in primary animal screening and led to the selection of 4″,6″-bis((2-fluorophenyl)carbamoyl)-hecogenyl β-*O*-cellobioside **3** as a development candidate.[4] This paper describes the process research on compound **3** which allowed the preparation of 80 kg of high-purity material.



1, **X** = CH_2, tiqueside
2, **X** = C=O, pamaqueside



3

For this discussion, hecogenyl β-*O*-cellobioside **4** will be considered the starting material. This was available on large scale by using the processes previously described for compounds **1** and **2**.[5] Commercially available hecogenin was coupled with 1-bromocellobiose heptaacetate in acetonitrile in the presence of zinc fluoride. The initial product was

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(1) Harwood, H. J., Jr.; Chandler, C. E.; Pellarin, L. D.; Bangerter, F. W.; Wilkins, R. W.; Long, C. A.; Cosgrove, P. G.; Malinow, M. R.; Marzetta, C. A.; Pettini, J. L.; Savoy, Y. E.; Mayne, J. T. *J. Lipid Res.* **1993**, *34*, 377.
(2) Malinow, M. R.; Gardner, J. O.; Nelson, J. T.; McLaughlin, P.; Upson, B.; Aigner-Held, R. *Steroids* **1986**, *48*, 197. Malinow, M. R. U.S. 4 602 003, **1986**; *Chem. Abstr.* **1986**, *105*: 172980.
(3) 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.; Etienne, J. B.; Fowler, M. A.; Lambert, J. F.; O'Donnell, J. P.; Pezzullo, S. L.; Watson, H. A., Jr.; Wilkins, R. W.; Zaccaro, L. M.; Zawistoski, M. P. *J. Med. Chem.* **1996**, *39*, 1935.
(4) DeNinno, M. P.; McCarthy, P. A.; Etienne, J. B.; Eller, C.; Duplantier, K. C.; Chandler, C. E.; Morehouse, L. A. 20th IUPAC Symposium on the Chemistry of Natural Products, Chicago, IL, Sept 15, 1996; Abstr. BM-12.
(5) (a) Allen, D. J. M.; Busch, F. R.; Lambert, J. E.; Shine, R. J.; Walinsky, S. W. U.S. 5 530 107, **1996**; *Chem. Abstr.* **1993**, *119*, 271616. (b) Urban, F. J.; Moore, B. S.; Breitenbach, R. *Tetrahedron Lett.* **1990**, *31*, 4421 and supporting data for ref 3 of the present paper.

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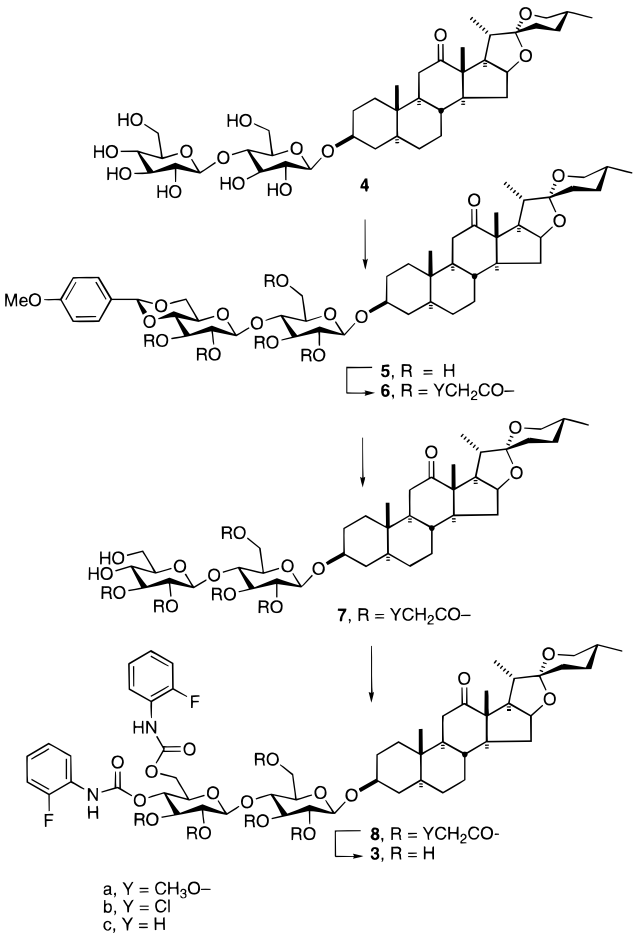
Scheme 1



deacetylated with catalytic sodium methoxide in methanol to provide **4**.

The synthesis of **3** from **4** required five synthetic steps: (1) the selective protection of the 4″,6″-diol group, (2) acylation of the remaining five hydroxyl groups, (3) unmasking of the diol moiety, (4) carbamoylation with 2-fluorophenyl isocyanate, and finally, (5) deacylation (Scheme 1). This sequence was demonstrated first by the discovery lab of DeNinno. The first two steps were combined by treating compound **4** with 4 equiv of 4-methoxybenzaldehyde dimethyl acetal and catalytic camphorsulfonic acid in 1,2-dichloroethane (DCE). This formed acetal **5**, which was acylated in situ with 15 equiv of chloroacetic anhydride and excess pyridine to give acetal pentachloroacetate **6b** as a crude solid. The cyclic acetal in **6b** was deprotected with trifluoroacetic acid in methylene chloride, and diol **7b** was precipitated as a crude solid. The carbamate groups were introduced with 4 equiv of 2-fluorophenyl isocyanate in dimethylformamide (DMF) with 4 equiv of cuprous chloride as catalyst to give **8b**. The final deacetylation was done with catalytic sodium methoxide as before, but the reaction had to be monitored very carefully since these conditions also removed the carbamoyl group, albeit at a slower rate. The lability of the 2-fluorophenyl carbamates to the deacylation conditions had led to the initial selection of chloroacetates over acetates. This process was suitable for <1 kg of final product but was not useful at larger scales. The major issues were the lack of crystalline intermediates for purification, the instability of several intermediates under the reaction conditions, the large excesses of reagents used, and the irritating nature of chloroacetate derivatives. Since we were required to produce multikilogram quantities of **3**, each step was studied to allow efficient large-scale preparation. These are discussed individually.

Acetal Formation. The initial reaction in DCE proceeded with formation of a thick gel, which slowly dissolved as pyridine and chloroacetic anhydride were added. Thin layer chromatographic (TLC) analysis of the acetal reaction showed a major product and a number of minor less polar (lp) materials. It was thought that these lp materials represented either larger ring cyclic acetals such as one incorporating the 3′- and 2″-alcohols or hemiacetal derivatives of the desired **5**.[6] When the reaction was carried out with tetrahydrofuran as solvent, 2 equiv of *p*-methoxybenzaldehyde dimethyl acetal, and pyridinium tosylate[7] as catalyst, no gel formation was seen and the reaction went to completion in several hours at reflux. Acetal **5** was precipitated by addition of water to the cooled reaction mixture. When these conditions were used, the less stable acetal side products hydrolyzed and very clean **5** was isolated. If the initial precipitate contained any less polar impurities by TLC, reslurry of the material in THF/water removed these. The isolation of purified acetal **5** was important since the minor acetal side reactions resulted in numerous impurities later in the synthesis. Replacement of *p*-methoxybenzaldehyde dimethyl acetal with the cheaper benzaldehyde dimethyl acetal was found to require longer reaction times for completion, and the acetal product, as well as later intermediates, was much less crystalline. It was not considered for scale-up.

Acylation Reaction. The selection of the acyl group for protection of the remaining five hydroxyl groups was critical. Chloroacetyl groups were too reactive and tended to either hydrolyze or migrate when the 4″, 6″-acetal group was removed with TFA. Acetates could not be removed at the end of the synthesis without significant loss of carbamate groups. An ideal choice was the methoxyacetate protecting group, which provided crystalline intermediates with good stability and could be removed cleanly without side reactions at the end of the synthesis.[8] Air-dried acetal **5** was treated with 8.8 equiv of methoxyacetyl chloride in methylene chloride solution in the presence of excess pyridine and catalytic 4-(dimethylamino)pyridine. Excess acid chloride was used to ensure complete acylation of all five hydroxyl groups, and it was found that acetal **5** contained 4.75% water even after drying in vacuo. No effort was made at this time to remove this water before the acylation reaction.

Deacetalization. In the process by our discovery group, this was the most problematic step for scale-up. It required large amounts of TFA that could not be partially removed

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(7) Sterzycki, R. *Synthesis* **1979**, 724.
(8) (a) Reese, C. B.; Stewart, J. C. M. *Tetrahedron Lett.* **1968**, 4273. (b) Reese, C. B.; Stewart, J. C. M.; van Boom, J. H.; de Leeuw, H. P. M.; Nagel, J.; de Rooy, J. F. M. *J. Chem. Soc., Perkin Trans. 1* **1975**, 934.

on pilot plant scale before workup as was done in the laboratory by evaporation at reduced pressure. In the large-scale workup of this deprotection, emulsions were seen when the methylene chloride solution of product **7b** was washed with alkaline water. Under these conditions, some chloroacetyl groups were lost to hydrolysis, while some evidence of migration[9] of chloroacetyl groups within **7b** was seen also. Initial scale-up of this process resulted in **3** with a large number of impurities. Using methoxyacetates with the TFA process improved the workup significantly, but more efficient procedures were sought.

Ceric ammonium nitrate has been used to remove benzylidene groups from a glycoside in a presumed oxidative deprotection.[10] This procedure was examined first in aqueous acetonitrile with 2 equiv of reagent. Since the crude, isolated diol **7a** contained a significant amount of anisaldehyde, the reaction was repeated with 25 mol % of ceric ammonium nitrate. This resulted in clean deprotection over several hours to provide **7a**. However, by analytical HPLC, the material was contaminated with a second compound (ca. 4%), which was shown to be an analogue of **7a** with a 9,11-double bond in the steroid moiety. This steroid was a contaminant of the commercial hecogenin derived from natural sources. It was postulated that hydrogenation from the less hindered face would give the desired stereochemistry, and this led us to examine hydrogenation as the deacetalization method.

The first successful hydrogenation conditions used dry, 10% Pd/C in a mixture of methanol and THF at 50 psi of hydrogen pressure. The solvent mixture was chosen for solubility of the starting material **6a** and the polarity of the solution; without a protic solvent no deprotection was seen. On small scale in the lab, this gave very clean **7a**. On scale-up, an equal weight of palladium hydroxide was used (50% water wet). The catalyst was combined with **6a** in THF and methanol. Sodium sulfate was added to remove the water from the catalyst, and the mixture was hydrogenated at 40−50 psi. This protocol worked better than the TFA reaction but would sometimes stop before complete deprotection was achieved. Further experimentation led to the optimized hydrogenation procedure. Palladium hydroxide (50% water wet, 0.2 equiv) was suspended in ethyl acetate, and solvent was distilled at reduced pressure to remove most of the water azeotropically. The dried catalyst slurry was added to a solution of **6a** in ethyl acetate with concd HCl (10 mol %). This mixture was hydrogenated for about 5 h at 40−60 psi. After filtration of the catalyst, the ethyl acetate was removed by distillation in vacuo and replaced by isopropyl ether to give diol **7a** of very high purity as a white solid.

Carbamate Formation. The use of $Cu^{I}Cl$ as a catalyst for formation of carbamates was described by Duggan.[11] The discovery process used 4 equiv each of 2-fluorophenyl isocyanate and $Cu^{I}Cl$ in DMF. Optimization studies showed that as little as 0.5 equiv of $Cu^{I}Cl$ would work, but 1 equiv was used for the scale-up because of the shorter reaction times and to ensure complete reaction. The isocyanate was used in excess (2.8 equiv). If the reaction was found by TLC to be incomplete, additional isocyanate was added. The incompleteness was probably due to insufficient drying of the diol precursor. One attempt to replace DMF with methylene chloride showed that only the 6″-monocarbamate formed in the less polar solvent. This provided an authentic sample of that potential impurity.

Deacylation. As described above, the carbamoyl groups were not stable to sodium methoxide in methanol, so milder conditions were sought.[12] The reaction of aqueous ammonia with acetylated glycosides has been known for a long time.[13] With steroid diglycoside **8a** an organic cosolvent was needed. Bis(carbamate) **8a** was dissolved in THF and treated with a large excess of 28% ammonium hydroxide at room temperature. The reaction was followed by TLC. Over a period of 24 h, the starting penta(methoxyacetate) **8a** was converted through a series of uncharacterized intermediates to **3**. Stability experiments with **3** showed that the two carbamate groups were unaffected under these conditions. The product was precipitated by the addition of water and collected by filtration. Crude **3** was dissolved in THF and filtered to remove trace particulate matter. The THF was distilled off at atmospheric pressure and was replaced with ethyl acetate to precipitate **3**. The final, desired polymorphic form of pure **3** was isolated from this ethyl acetate slurry.

In summary, process research which has identified optimal protecting groups and reaction conditions has led to an efficient synthesis of the drug candidate **3** in very high purity and 42% yield over five synthetic steps from **4** and one recrystallization/polymorph conversion. The process was simple to operate and was carried out to provide 80 kg of 4″,6″-bis((2-fluorophenyl)carbamoyl)hecogenyl *β*-*O*-cellobioside **3**.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and were uncorrected. NMR spectra were obtained on either a Brucker WM 300 (300 MHz): or a Varian Unity 400 (400 MHz): spectrometer in deuteriochloroform or dimethyl sulfoxide-d_6. Since the NMR spectra of the molecules are complex, only the diagnostic, well-resolved peaks in the proton spectra are reported. Copies of the proton and carbon spectra are included in the Supporting Information. Infrared spectra were taken in KBr by diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS). Mass spectra were determined with a Finnigan 4510 mass spectrometer using fast atom bombardment (FAB). Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, NY. The reactions were monitored by TLC on silica gel plates, and isolated compounds were analyzed by HPLC on a C18 column with mixtures of acetonitrile and water as eluant and UV detection.

3-[[4′-*O*-(*β*-D-Glucopyranosyl)-*β*-D-glucopyranosyl]oxy]-(3*β*,5α,25*R*)-spirostan-12-one (4). This was prepared as described in ref 5a. The NMR data is presented for

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(10) Coleman, R. S.; Carpenter, A. J. *J. Org. Chem.* **1992**, *57*, 5813.
(11) Duggan, M, E.; Imagire, J. S. *Synthesis* **1989**, 131.
(12) Bouveng, H. O. *Acta Chem. Scand.* **1961**, *15*, 87.
(13) Deferrari, J. O.; Cadenas, R. A. *J. Org. Chem.* **1963**, *28*, 1070.

reference. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.22 (d, 1), 5.00 (d, 1), 4.97 (d, 2), 4.65 (s, 1), 4.58 (t, 1), 4.53 (t, 1), 0.95 (s, 3), 0.92 (d, 3), 0.83 (s, 3), 0.72 (d, 3). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 213.0, 108.8, 103.6, 100.8, 81.1, 79.2, 77.2, 76.9, 76.7, 75.5. 75.1, 73.7, 73.5, 70.4, 66.4, 61.4, 60.9, 55.4, 55.1, 54.9, 53.6, 44.1, 42.0, 37.8, 36.3, 36.2, 34.3, 34.1, 31.5, 31.3, 30.2, 29.3, 28.9, 28.4, 17.5, 16.0, 13.8, 11.9.

3-[[4′-*O*-[4″,6″-(4-Methoxybenzylidene)-β-D-glucopyranosyl]-β-D-glucopyranosyl]oxy]-(3β,5α, 25R)-spirostan-12-one (5). Compound **4** (45.75 kg, 60.6 mol) was suspended in tetrahydrofuran (220 gal). Pyridinium *p*-toluenesulfonate (2.287 kg, 9.1 mol) was added followed by *p*-methoxybenzaldehyde dimethyl acetal (33.1 kg, 181.8 mol). The mixture was heated to reflux and held there for 2.5 h, at which time TLC (9:1, methylene chloride/methanol, silica gel) showed the reaction to be complete. The solution was cooled to 20 °C, and water (220 gal) was added to precipitate the product. After stirring for several hours, the solid was collected by filtration and the solid was washed with 1:1 tetrahydrofuran and water. The solid was dried in an air dryer at 55 °C to provide 42.15 kg (80% yield) of **5** as a white solid. If the initial solid contained either lp bands by the TLC assay or excess *p*-methoxybenzaldehyde, it was reslurried in 1:1 tetrahydrofuran/water (10 L/kg). Mp: 250−255 °C dec. IR: ν 3461, 2955, 2930, 1707, 1615, 1597, 1518 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.30 (d, 2), 6.86 (d, 2), 5.46 (s, 1), 5.31 (d, 1), 4.96 (d, 1), 4.51 (t, 1), 4.44 (d, 1), 4.32 (s, 1), 4.23 (d, 1), 3.69 (s, 3), 0.92 (s, 3), 0.89 (s, 3), 0.80 (s, 3), 0.68 (d, 3). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 213.0, 159.9, 130.4, 128.0, 113.7, 108.8, 103.5, 101.0, 100.8, 80.7, 79.6, 79.1, 76.7, 75.1, 74.9, 74.7, 73.7, 73.2, 68.0, 66.4, 60.5, 55.5, 55.4, 55.1, 54.9, 53.5, 44.1, 41.9, 37.8, 36.3, 36.2, 34.3, 34.1, 31.5, 31.3, 31.1, 30.2, 29.3, 28.8, 28.4, 17.5, 16.0, 13.8, 11.9. Mass spectrum: *m*/*e* 873 (M$^+$). Karl-Fisher: 4.74% H_2O.

Anal. Calcd for $C_{47}H_{68}O_{15}$ (2.5 H_2O): C, 61.49; H, 8.01. Found: C, 61.51; H, 8.01.

2′,3′,6′,2″,3″-Pentakis(methoxyacetyl)-3-[[4′-O-[4″,6″-(4-methoxybenzylidene)-β-D-glucopyranosyl]-β-D-glucopyranosyl]oxy]-(3β,5α,25*R*)-spirostan-12-one (6a). *p*-Methoxybenzylidene **5** (49 kg, 56.1 mol) was suspended in CH_2Cl_2 (103 gal) with 4-(dimethylamino)pyridine (3.079 kg, 25.2 mol). Pyridine (44.39 kg, 11.3 gal, 561 mol) was added, and the mixture was cooled to 0 °C. Methoxyacetyl chloride (53.472 kg, 492.7 mol) was added neat, with cooling over 3.5 h. The reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was stirred at 20 °C while 1 N HCl (74 gal) was added over 1 h. The layers were separated, and the organics were washed with water (103 gal). The organic layer was stirred with water a second time while the pH of the aqueous layer was adjusted from 2.4 to 7.0−7.5 with 5% sodium carbonate solution. The CH_2Cl_2 layer was diluted with hexanes (77 gal), the CH_2Cl_2 was removed by atmospheric distillation, and the mixture was cooled to room temperature and granulated overnight. Product **6a** was collected, washed with hexanes, and dried under vacuum at 35 °C. The yield for the reaction was 89%, 57.3 kg of a white solid; mp 250−256 °C. IR: ν 2929, 2873, 1767, 1707, 1615, 1589, 1519 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.32 (d, 2), 6.86 (d, 2), 5.41 (s, 1), 5.30 (t, 1), 5.22 (t, 1), 4.99 (t, 1), 4.91 (t, 1), 4.65 (d, 1), 4.58 (m, 2), 3.80 (s, 3), 3.46 (s, 3), 3.43 (s, 3), 3.40 (2s, 6), 3.38 (s, 3), 2.50 (dd, 1), 2.37 (t, 1), 1.05 (d, 3), 1.02 (s, 3), 0.87 (s, 3), 0.79 (d, 3). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 213.0, 170.4, 169.9, 169.4, 160.0, 129.9, 127.8, 113.9, 108.8, 100.7, 100.5, 98.0, 79.2, 78.6, 77.5, 77.0, 72.9, 72.5, 72.1, 71.8, 69.3, 69.2, 69.1, 67.7, 66.4, 65.8, 62.6, 59.0, 58.9, 55.6, 55.4, 55.0, 54.9, 53.6, 44.1, 42.0, 37.8, 36.1, 34.5, 34.1, 31.5, 31.3, 31.1, 30.2, 29.2, 28.9, 28.3, 17.5, 16.0, 13.8, 11.9. $[\alpha]_D$: −26.8° (*c* = 1.01, chloroform).

Anal. Calcd for $C_{62}H_{88}O_{25}$: C, 60.38; H, 7.19. Found: C, 60.51; H, 7.33.

2′,3′, 6′, 2″, 3″-Pentakis(methoxyacetyl)-3-[[4′-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]-(3β,5α,25*R*)-spirostan-12-one (7a). Palladium hydroxide on carbon (10 kg, 50% water wet) was slurried in ethyl acetate (73 gal). The solvent was distilled under vacuum to a volume of 20 gal. Additional ethyl acetate was added to the slurry, and the water content was determined by Karl-Fisher titration to be 0.012%. The catalyst slurry was transferred to a reactor containing *p*-methoxybenzylidene **6a** (50 kg, 40.5 mol), ethyl acetate (140 gal), and concd HCl (325 mL, 3.9 mol). The reactor was pressurized with hydrogen (60 psi), and agitation was started. The hydrogenation required 5 h with the hydrogen pressure maintained between 40 and 60 psi. The temperature increased to ca. 38 °C during the reaction. The catalyst was removed by filtration and was washed with ethyl acetate (26 gal). The ethyl acetate filtrate was evaporated in vacuo to a thick slurry and was diluted with isopropyl ether (100 gal). After stirring overnight, the product was collected by filtration, washed with isopropyl ether (26 gal), and dried under vacuum. The resulting white solid represented a 93% yield, 41.9 kg; mp 227−231 °C. IR: ν 3400, 2991, 2933, 2912, 1761, 1709, 1453 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.20 (t, 1), 5.07 (t, 1), 4.96 (t, 1), 4.85 (t, 1), 4.60 (d, 1), 4.55 (d, 2), 3.45 (2s, 6), 3.39 (2s, 9), 2.50 (dd, 1), 2.38 (t, 1), 1.05 (d, 3), 1.01 (s, 3), 0.85 (s, 3), 0.78 (d, 3). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 213.4, 170.5, 169.7, 169.4, 169.0, 168.9, 109.2, 100.4, 99.2, 79.3, 79.2, 76.4, 76.2, 75.9, 73.4, 72.5, 72.3, 71.8, 69.8, 69.7, 69.4, 68.2, 66.9, 62.1, 59.8, 59.5, 59.4, 59.3, 55.7, 55.4, 55.1, 53.5, 44.5, 42.2, 37.7, 36.4, 36.1, 34.4, 34.3, 31.5, 31.4, 31.1, 30.17, 29.0, 28.8, 28.3, 17.1, 16.0, 13.2, 11.9. Mass spectrum: *m*/*e* 1115 (M$^+$). $[\alpha]_D$: −17.8° (*c* = 0.94, chloroform).

Anal. Calcd for $C_{54}H_{82}O_{24}$: C, 58.16; H, 7.41. Found: C, 58.05; H, 7.31.

2′,3′,6′,2″,3″-Pentakis(methoxyacetyl)-3-[[4′-*O*-[4″,6″-bis[(2-fluorophenyl)carbamoyl]-β-D-glucopyranosyl]-β-D-glucopyranosyl]oxy]-(3β,5α,25*R*)-spirostan-12-one (8a). Diol **7a** (66.45 kg, 59.6 mol) was dissolved in dimethylformamide (78 gal), and the solution was cooled to 0 °C. Copper(I) chloride (5.9 kg, 59.6 mol) was added in one portion. 2-Fluorophenyl isocyanate (22.9 kg, 167 mol) was added to the reaction mixture neat over 1 h. The reaction mixture was warmed to 25 °C and stirred for 2 h. Ethyl

acetate (351 gal) was added to the reactor followed by 1 N HCl (78 gal), and the mixture was stirred for 20 min. The layers were separated, and the organics were washed twice with 1 N HCl (78 gal each). The ethyl acetate solution was washed with brine (78 gal). The ethyl acetate was distilled off at atmospheric pressure to leave a thick slurry, which was cooled to room temperature and diluted with isopropyl ether (175 gal). This was stirred overnight, and the solids were collected by filtration, washed with isopropyl ether (17.5 gal), and dried in vacuo. The dried white solid weighed 65.9 kg, which represented an 80% yield. Mp: 210−212 °C. IR: ν 3323, 2930, 2872, 2830, 1764, 1752, 1705, 1623, 1599, 1543 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 7.95 (m, 3), 7.10−6.89 (m, 7), 5.31 (t, 1), 5.22 (t, 1), 5.09−4.91 (m, 3), 4.70 (d, 1), 3.48 (s, 6), 3.43 (s, 3), 3.40 (s, 3), 3.29 (s, 3), 2.50 (dd, 1), 2.39 (t, 1), 1.07 (d, 3), 1.02 (s, 3), 0.86 (s, 3), 0.79 (d, 3). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 213.4, 169.8, 169.7, 169.5, 169.1, 168.8, 153.6, 152.5, 151.1, 126.3, 126.2, 125.4, 124.6, 124.4, 123.6, 120.6, 115.1, 115.0, 114.9, 114.8, 109.2, 100.3, 99.2, 79.4, 79.1, 76.4, 74.0, 73.0, 72.3, 72.1, 72.0, 69.7, 69.4, 69.3, 69.2, 68.8, 68.4, 66.9, 62.5, 62.2, 69.6, 59.6, 59.44, 59.40, 59.3, 59.2, 55.7, 55.4, 55.1, 53.5, 51.4, 44.4, 42.2, 40.1, 37.7, 36.4, 36.1, 34.4, 34.3, 31.5, 31.4, 31.1, 30.2, 29.0, 28.8, 28.3, 17.1, 16.0, 13.2, 11.9. $[\alpha]_D$: 1.4° (c = 1.02, chloroform).

Anal. Calcd for $C_{68}H_{90}F_2N_2O_{26}$: C, 58.78; H, 6.53; N, 2.02. Found: C, 58.83; H, 6.60; N, 2.03.

3-[[4′-*O*-[4″,6″-Bis[(2-fluorophenyl)carbamoyl]-β-D-glucopyranosyl]-β-D-glucopyranosyl]oxy]-(3β,5α,25*R*)-spirostan-12-one (3). Bis(carbamate) penta(methoxyacetate) **8a** (32.5 kg, 23.4 mol) was dissolved in tetrahydrofuran (172 gal) and the reaction mixture was stirred while 28% ammonium hydroxide (18.3 gal, 1778.4 mol) was added. The mixture was stirred overnight at room temperature. A TLC analysis of an aliquot showed the deprotection to be complete after 24 h (TLC, silica gel, 50% acetone/50% methylene chloride). The reaction mixture was added to water (215 gal) which had been cooled to 10 °C over 1 h. The resulting slurry was then collected by filtration, and the solids were washed with water (17 gal). The material was dried in vacuo at 45 °C for 3 days. The yield was 21 kg, 87.2%. The crude product (80.35 kg, 78.1 mol) was dissolved in tetrahydrofuran (426 gal). The solution was filtered through a 5-μm filter, and half of the solvent was removed by atmospheric distillation. Ethyl acetate was added as the distillation was continued until the tetrahydrofuran was displaced and total of 330 gal of ethyl acetate had been used. The slurry was cooled to room temperature and stirred overnight. The white solid was collected by filtration and dried in vacuo at 65 °C to provide 72.6 kg of the purified product, 90% yield. Mp: 277−280 °C. IR: ν 3578, 3363, 3316, 1753, 1744, 1705, 1620, 1595, 1230 cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ 9.40 (s, 1), 9.30 (s, 1), 7.66 (m, 2), 7.25−7.05 (m, 6), 5.58 (d, 1), 5.46 (d, 1), 5.03 (d, 1), 4.68 (t, 1), 4.60 (t, 1), 4.50 (d, 1), 4.41 (s, 1), 4.30 (d, 1), 0.96 (s, 3), 0.93 (d, 3), 0.87 (s, 3), 0.74 (d, 3). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 213.0, 155.7, 155.6, 154.1, 153.7, 153.3, 126.4, 125.5, 125.0, 124.6, 116.0, 115.8, 108.8, 102.7, 100.8, 79.7, 79.2, 76.7, 75.0, 74.9, 74.1, 73.9, 73.7, 72.2, 71.8, 66.4, 63.6, 60.6, 55.4, 55.1, 54.9, 53.6, 44.1, 42.0, 37.8, 36.3, 36.2, 34.3, 34.1, 31.5, 31.3, 31.1, 30.2, 29.3, 28.9, 28.4, 17.5, 16.0, 13.8, 11.9. Mass spectrum: *m*/*e* 1029 (M^+). $[\alpha]_D$: −13.0° (c = 1.0, *N*,*N*-dimethylacetamide).

Anal. Calcd for $C_{53}H_{70}F_2N_2O_{16}$: C, 61.86; H, 6.86; N, 2.72; F, 3.69. Found: C, 61.72; H, 7.11; N, 2.82; F, 3.80.

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