Steroidal Glycoside Cholesterol Absorption Inhibitors

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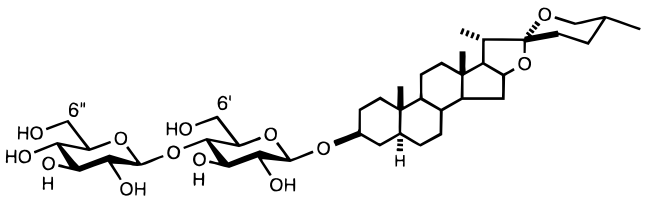
We have explored the use of steroidal glycosides as cholesterol absorption inhibitors which act through an unknown mechanism. The lead for this program was tigogenin cellobioside (**1**, tiqueside) which is a weak inhibitor (ED_{50} = 60 mg/kg) as measured in an acute hamster cholesterol absorption assay. Modification of the steroid portion of the molecule led to the discovery of 11-ketotigogenin cellobioside (**5**, pamaqueside) which has an ED_{50} of 2 mg/kg. Replacement of the cellobiose with other sugars failed to provide more potent analogs. However, large improvements in potency were realized through modification of the hydroxyl groups on the cellobiose. This strategy ultimately led to the 4″,6″-bis[(2-fluorophenyl)carbamoyl]-β-D-cellobiosyl derivative of 11-ketotigogenin (**51**) with an ED_{50} of 0.025 mg/kg in the hamster assay, as well as the corresponding hecogenin analog **64** (ED_{50} = 0.07 mg/kg).

Introduction

The treatment of hypercholesterolemia through the inhibition of dietary and biliary cholesterol absorption has been a popular area of research in recent years. Specific approaches have involved both known molecular targets (e.g., ACAT[1] and CEH[2]) as well as optimizing the activity of compounds with unknown mechanisms of action.[3−6] Our work on the latter class of agents has focused on the steroidal glycoside series of cholesterol absorption inhibitors. As a class, these compounds were attractive because they had extremely low systemic exposure and were assumed to be lumenally active but lacked robust *in vivo* activity. One of these compounds, tiqueside (**1,** CP-88,818),[6] had progressed through phase II clinical trials where it demonstrated adequate efficacy but inadequate potency[7] (4 g/day was required to achieve >20% reduction of LDL cholesterol).



1 (tiqueside)

As part of our discovery program, modifications to the steroid and sugar portions of the lead agent **1** were explored seeking improvements in potency. Modification of both halves of the molecule resulted in potency improvements which were additive, leading to some highly potent cholesterol absorption inhibitors.

Biology

Since the molecular mechanism of action of **1** was unknown, we relied on the *in vivo* screening of analogs. Hamsters seemed to be well suited for our needs since they have a gall bladder and their bile acid metabolism has been well studied.[8] Their small size would also limit compound requirements. A published procedure[6d,9] for assaying ACAT inhibitors proved useful; however, it was modified[10] to increase throughput. Thus, compounds were tested for their ability to inhibit the intestinal absorption of [^{3}H]cholesterol in cholesterol-fed hamsters. The compounds were tested at four concentrations with $N = 4$ per group. The ED_{50} was determined by comparing liver radioactivity of control animals with that of hamsters given experimental compounds and generating a dose−response curve. The ED_{50} of **1** in this protocol was 60 mg/kg.

Chemistry

The spirostane glycosides were prepared using one of two glycosidation procedures. The preferred method utilized an acetobromo sugar as the glycosyl donor and the sterol under ZnF_2 catalysis in acetonitrile.[11] Under these conditions high yields of the β-glycosides were obtained. In some instances (e.g., where the sterol was insoluble in acetonitrile), the more typical Konigs−Knorr[12] conditions (i.e., $HgBr_2$, $Hg(CN)_2$, CH_2Cl_2) were employed. Deprotection using sodium methoxide in methanol afforded compounds **5**−**12**.

The synthesis of the carbohydrate-modified targets proceeded through the coupled spirostane glycosides. Two routes were used to prepare the 6′- and 6″-substituted analogs as shown in Schemes 1 and 2. Compounds with carbamoyl substituents could be prepared from the unprotected glycoside by reaction with an isocyanate in pyridine. From this reaction, a nonselective acylation occurs, and often the 6′-mono-, 6″-mono-, and 6′,6″-di-substituted analogs were isolated from the same reaction. A more selective preparation of the latter series was achieved through the use of protecting groups. Thus, the 6′- and 6″-hydroxyls can be protected as their silyl ethers using *tert*-butyldiphenylsilyl chloride. The remaining hydroxyls are then protected in the same pot with acetic anhydride to give the fully protected derivative **20**. The silyl groups can be selectively cleaved with HF/pyridine to afford the diol **21**. This intermediate was also used to prepare the dihalogenated and dideoxy analogs **29**−**33** by standard methods.

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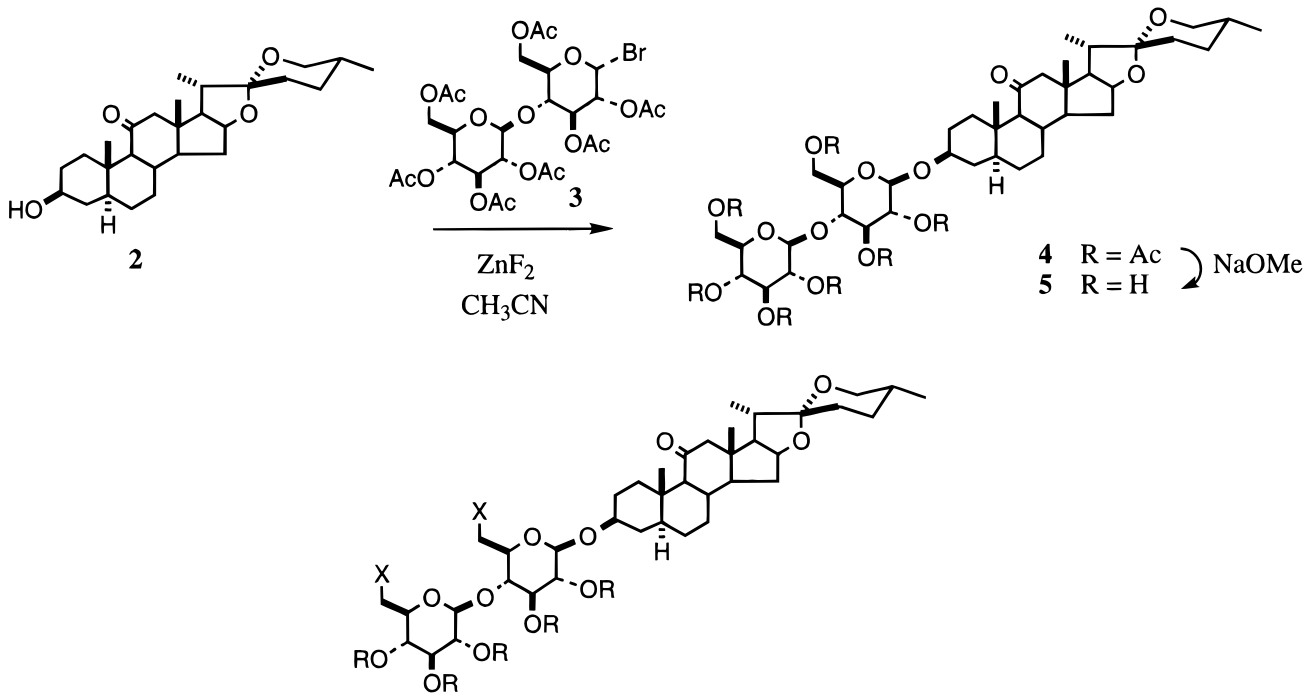
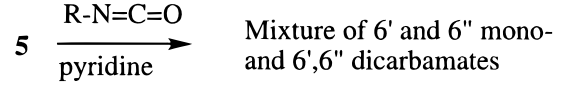
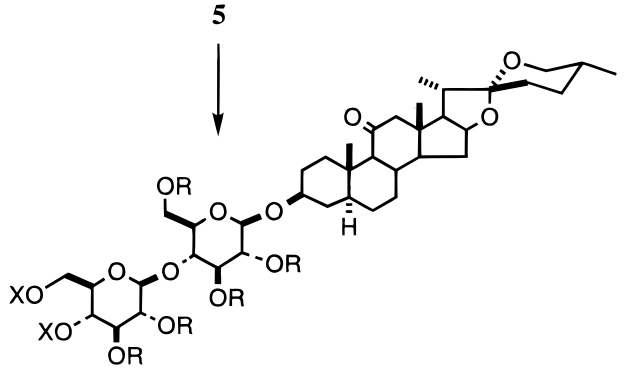
Scheme 1



Scheme 2



Scheme 3

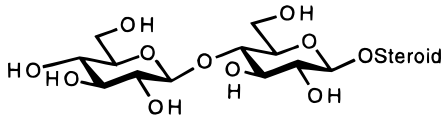


The 4″,6″-dicarbamoyl derivatives **52**−**64** were synthesized from intermediate **49** in which the 4″- and 6″-hydroxyls were uniquely freed (Scheme 3). This compound was prepared by first protecting the 4″,6″-hydroxyls as a *p*-methoxybenzylidene followed by exhaustive acylation with chloroacetic anhydride to furnish the fully protected derivative **48**. The benzylidene was then cleaved with acid in high yield to provide the diol **49**. In general, the carbamates were prepared by reacting the alcohols with the appropriate isocyanate using copper(I) chloride catalysis.[13] The acetates were cleaved with sodium methoxide to furnish the final products. Because of the complex NMR spectra of these compounds, we relied heavily on FAB MS for structure confirmation.

Results and Discussion

With virtually no prior structure−activity relationship (SAR) to guide us, we divided the molecule into lipophilic and hydrophilic sectors and began to make modifications. We first replaced tigogenin with other commercially available steroids, and the results are shown in Table 1. Complete removal of the E and F rings (compound **9**) or use of steroids with hydrocarbon side chains (**10**, **11**) was highly detrimental for activity, indicating that the spiroketal was an important component. Attempts to add oxygen functionality to that region of the steroid led to the epiandrosterone derivative **12** which contained a carbonyl at C17 and showed improved activity. However, aromatization of the A ring (**13**) was not tolerated. All of the spirostane cellobiosides (**1**, **5**−**8**) were active, with the most potent being those containing a carbonyl in the C12 or C11 position.

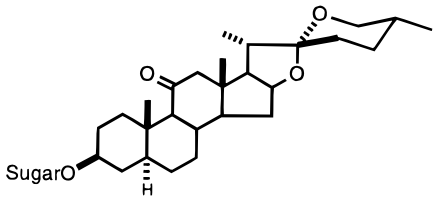
Table 1. Steroidal Cellobiosides



compd[a]	steroid	ED_{50} (mg/kg)[b]
1	tigogenin	60
6	diosgenin	100
7	sarsasapogenin	75
8	hecogenin	10
5	11-ketotigogenin	2.0
9	androstane	37% @ 200 mg/kg
10	cholestanol	150
11	stigmastanol	23% @ 200 mg/kg
12	3-β-epiandrosterone	25
13	estrone	14% @ 100 mg/kg

[a] All new compounds gave satisfactory C,H or C,H,N combustion analyses. [b] Values represent the ED_{50} or the percent inhibition at the highest dose tested. The ED_{50} was determined from a four-point dose-down with 4 hamsters/dose group. Reproducibility is generally ±20% of the average value.

Table 2. Cellobiose Replacements



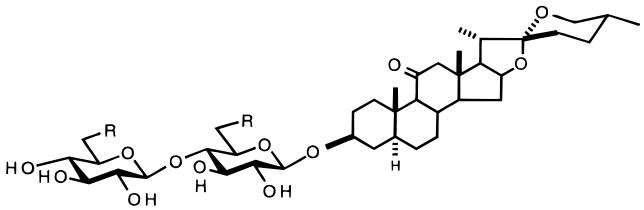
compd	sugar	glycos procedure	ED_{50} (mg/kg)
5	β-D-cellobioside	A	2.0
14	β-D-glucoside	A	16
15	β-D-galactoside	A	12
16	β-D-lactoside	A	4.0
17	β-D-maltoside	B	20
18	β-D-maltotrioside	B	25
19	α-D-cellobioside		23% @ 10 mg/kg

Compound **5** (pamaqueside, CP-148,623) was the most potent with an ED_{50} of 2 mg/kg in the acute hamster assay and was the subject of an earlier communication.[14]

Although simple steroids such as sitosterol have been shown to have lipid-lowering properties,[15] none of the spirostane aglycones we examined inhibited cholesterol absorption at 200 mg/kg. We then set out to investigate the role of the cellobioside by replacing it with other common sugars (Table 2) on the 11-ketotigogenin backbone. Although the potency of the lactoside derivative **16** was comparable to the cellobioside, none of the compounds provided any advantage over compound **5**.

Replacing the sugar had only limited success, so we opted to make modifications to the cellobiose itself. We began by modifying the primary hydroxyl groups which were substituted or replaced with other functional groups, and the results are shown in Table 3. Although several of these analogs lost potency, the fluoro (**31**) and chloro (**32**) derivatives provided some potency improvements with compound **32** being the most potent of this series (ED_{50} = 0.5 mg/kg). The carbamate **34** lost potency, but this result was nevertheless exciting as it opened a series which allowed for the preparation of a large number of analogs. We therefore synthesized a number of mono- and dicarbamates at the 6′- and 6″-positions (Table 4). In addition to providing improvements in potency, it was clear from the data that only the 6″-position needed to be substituted (e.g., see

Table 3. 6′,6″-Modified Cellobiose Analogs



compd	R	ED_{50} (mg/kg)
5	OH	2.0
29	H	6.5
30	$OCH_2OCH_2CH_3$	3.0
31	F	0.7
32	Cl	0.5
33	I	1.5
34	(phenylcarbamoyl)oxy	5.0

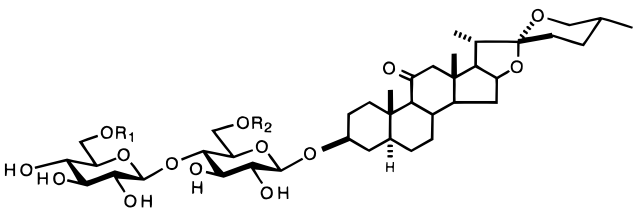
compounds **38**−**40**). Substitution at 6′, in fact, appeared to be detrimental. One of the most potent compounds was the 6″-mono 2,6-dichlorophenyl carbamate **42** with an ED_{50} of 0.2 mg/kg. Further exploration in this series could not break this potency barrier. Analogs containing carbamates derived from non-aniline amines (**45**−**47**) were much less active.

The success of the approach to modify the cellobiose led us to explore other sites on the sugar from which to append carbamates. Another accessable site was the 4″-position which along with the 6″-hydroxyl could be selectively freed using protecting group chemistry (*vide supra*). The diol intermediate **49** was used to synthesize a series of 4″,6″-dicarbamates listed in Table 5. In contrast to the 6″-monocarbamates, the 2,6-dichlorophenyl carbamate derivative **53** was not among the most potent compounds in this series. The 2-fluorophenyl analog **51,** which had an ED_{50} of 0.025 mg/kg, was 2 orders of magnitude more active than the unmodified cellobioside **5**. Once again, carbamates derived from nonaromatic amines were much less active. Even within the substituted phenyl carbamate series, small changes (e.g., F to Cl) led to dramatic effects on activity. This tight SAR and the high levels of potency uncovered in this series have helped to further define the mechanism of action of these agents, which has yet to be fully elucidated. The results provided here have effectively ruled out a simple cholesterol sequestration mechanism that was thought possible with compound **1**.

The 4″,6″-bis[(2-fluorophenyl)carbamoyl] substitution pattern was optimal in this series so the corresponding tigogenin and hecogenin cellobioside analogs were also prepared. The potencies of these compounds (**63**, ED_{50} = 1.0 mg/kg; **64**, ED_{50} = 0.07 mg/kg) were as predicted based on the data in Table 1 indicating that the SAR for both ends of the molecule was independent and additive.

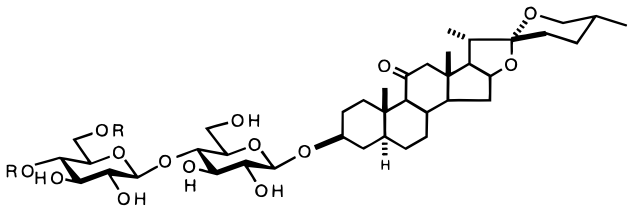
To determine whether the increases in potency realized in the acute cholesterol absorption assay would translate to improved potency upon multiple dosing, we selected several compounds and administered them to hamsters by diet admixture. Hamsters fed a cholesterol-containing diet have increased hepatic and plasma cholesterol levels compared to animals fed a cholesterol-free diet. As shown in Figure 1, the administration of **1**, **5**, or **64** to cholesterol-fed hamsters prevented the accumulation of hepatic cholesterol in a dose−responsive fashion. The incremental increases in potency in

Table 4. 6′- and 6″-Carbamoylcellobiose Analogs



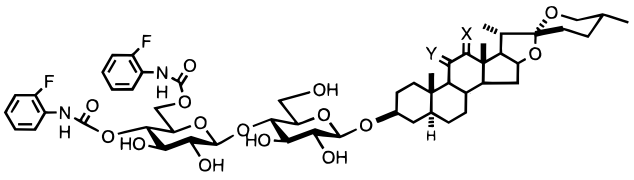
compd	R_1	R_2	ED_{50} (mg/kg)
5	H	H	2.0
35	(4-chlorophenyl)carbamoyl	(4-chlorophenyl)carbamoyl	5.0
36	(4-chlorophenyl)carbamoyl	H	1.0
37	H	(4-chlorophenyl)carbamoyl	37% @ 5 mg/kg
38	(2-fluorophenyl)carbamoyl	(2-fluorophenyl)carbamoyl	0.8
39	(2-fluorophenyl)carbamoyl	H	0.2
40	H	(2-fluorophenyl)carbamoyl	7.0
41	(2,6-dichlorophenyl)carbamoyl	(2,6-dichlorophenyl)carbamoyl	0.4
42	(2,6-dichlorophenyl)carbamoyl	H	0.2
43	(2,4-difluorophenyl)carbamoyl	H	0.25
44	(2-methylphenyl)carbamoyl	H	0.5
45	benzylcarbamoyl	H	5.0
46	phenethylcarbamoyl	H	5.0
47	1-pyrrolidenecarbonyl	H	1.5

Table 5. 4″,6″-Dicarbamoylcellobiose Analogs



compd	R	ED_{50} (mg/kg)
5	H	2.0
52	phenylcarbamoyl	0.1
53	(2,6-dichlorophenyl)carbamoyl	40% @ 5 mg/kg
54	(2,4-difluorophenyl)carbamoyl	0.04
51	(2-fluorophenyl)carbamoyl	0.025
55	(3-fluorophenyl)carbamoyl	0.1
56	(4-fluorophenyl)carbamoyl	0.2
57	(2-chlorophenyl)carbamoyl	1.0
58	benzylcarbamoyl	0.3
59	(2-furylmethyl)carbamoyl	0.3
60	[(ethoxycarbonyl)methyl]carbamoyl	0.6
61	1-morpholinocarbonyl	4.0
62	phenylthiocarbamoyl	0.04

this model mirror those discussed in the acute cholesterol absorption protocol. Similarly, these compounds prevented the elevation of plasma cholesterol levels due to cholesterol feeding.[16] Compounds **5** and **64** were both progressed into clinical development. Details of these studies will be presented elsewhere.



63 X = H,H; Y = H,H: ED50 = 1.0 mg/kg
64 X = O; Y = H,H: ED50 = 0.07 mg/kg
51 X = H,H; Y = O: ED50 = 0.025 mg/kg

Summary

Through iterative modifications to a weak cholesterol absorption inhibitor lead (**1**), we have succeeded in improving the potency by over 3 orders of magnitude.

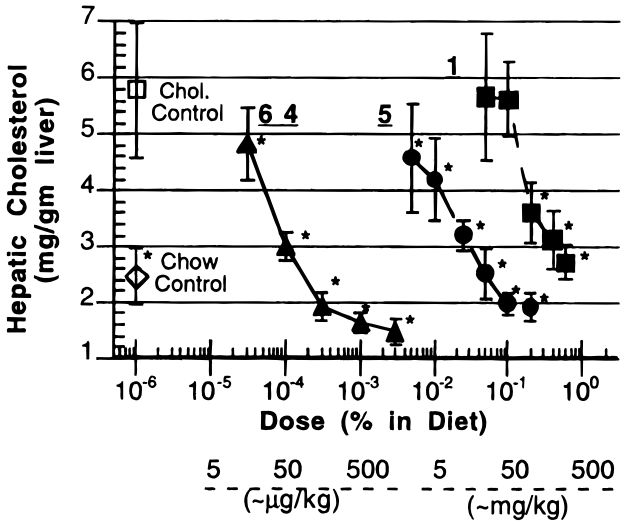


Figure 1. Hamsters ($n = 6$/group) were fed a 0.2% cholesterol, 0.1% cholic acid diet or a cholesterol-free diet for 4 days with **1** (■), **5** (●), or **64** (▲) mixed in the chow at the indicated percentages. The approximate dosage in mg/kg or μg/kg is also included for reference. Livers were excised and saponified, and the cholesterol content was expressed per gram of liver. The data are a compilation of two separate experiments. Values are mean ± SD. *$p < 0.05$ versus cholesterol-fed controls (Student−Newman Keul's test).

Lacking an *in vitro* screen, we relied on a fairly high throughput *in vivo* acute hamster model to rapidly access the activity of the analogs. The spirostane, tigogenin, contained in **1** appeared to be optimal; however, incorporation of carbonyl groups at the C11 or C12 position led to improved activity. The cellobiose unit in **1** was also found to be superior to other mono- or disaccharides. Additional gains in potency were registered upon modification of the cellobiose, preferably with carbamoyl substituents at the 4″- and 6″-positions. The availability of these highly potent inhibitors may help to unravel some of the mysteries associated with the process of cholesterol absorption. Lipid-lowering, pharmacokinetic, and further SAR studies of these spirostane glycosides will be the subject of future publications.

Experimental Section

General. Melting points were obtained on a Buchi melting point apparatus and are uncorrected. Reactions were run in flame- or oven-dried glassware under nitrogen. Anhydrous solvents were purchased from Aldrich Chemical Co. Silica gel chromatography was performed using EM Science silica gel 60 (230−400 mesh). FAB mass spectra were run on a Kratos Concept instrument. Proton NMR were recorded on a Bruker AC300 or AC250 spectrometer. Elemental analyses were obtained from Schwarzkopf Microanalytical Laboratory, Woodside, NY.

Glycosidation Procedure: Method A. (3*β*,5α,25*R*)-3-[(Heptaacetyl-*β*-D-cellobiosyl)oxy]spirostan-11-one (4). A suspension of (3*β*,5α,25*R*)-3-hydroxyspirostan-11-one (**2**) (3.0 g, 6.97 mmol) and anhydrous zinc fluoride (2.88 g, 27.9 mmol) in dry MeCN (175 mL) was dried by removal of 75 mL of MeCN by distillation. The suspension was allowed to cool, heptaacetyl-*β*-D-cellobiosyl bromide (9.75 g, 13.9 mmol) was added, and the resulting suspension was heated to 65 °C for 3 h. After cooling to room temperature, CH_2Cl_2 (150 mL) was added; the suspension was stirred for 10 min and filtered. The filtrate was concentrated *in vacuo* to give 10 g of crude product. This material was dissolved in 8:2 chloroform:methanol, preadsorbed on silica gel, and purified by flash chromatography (eluent: 1:1 ethyl acetate:hexane followed by pure ethyl acetate) to give 6.81 g (93% yield) of **4**: mp 210−212 °C; ^{1}H NMR ($CDCl_3$) δ 5.11 (complex, 2H), 5.04 (t, J = 9 Hz, 1H), 4.90 (t, J = 9 Hz, 1H), 4.83 (t, J = 8 Hz, 1H), 4.49 (complex, 4H), 4.34 (dd, J = 4.5, 12.5 Hz, 1H), 4.04 (t, J = 13 Hz, 1H), 4.03 (t, J = 11 Hz, 1H), 3.72 (t, J = 9.5 Hz, 1H), 3.65 (m, 1H), 3.56 (m, 1H), 3.45 (m, 1H), 2.47 (m, 1H), 2.22 (s, 2H), 2.08 (s, 3H), 2.06 (s, 3H), 2.00 (s, 6H), 1.99 (s, 6H), 1.96 (s, 3H), 2.00−1.00 (m, 22H), 0.98 (s, 3H), 0.92 (d, J = 7 Hz, 3H), 0.77 (d, J = 7 Hz, 3H), 0.68 (s, 3H); FAB MS m/z 1049 (M + H)$^+$. Anal. Calcd for $C_{53}H_{76}O_{21}\cdot H_2O$: C, 59.65; H, 7.37. Found: C, 59.86; H, 7.25.

Method B: Step 1. (3*β*,5α,25*R*)-3-[(Trimethylsilyl)oxy]-spirostan-11-one. Trimethylsilyl chloride (3.27 mL, 25.8 mmol) was added to a solution of (3*β*,5α,25*R*)-3-hydroxyspirostan-11-one (4.0 g, 9.3 mmol) and triethylamine (6.5 mL, 46 mmol) in CH_2Cl_2 (60 mL) at room temperature. One gram of 4-(dimethylamino)pyridine was added, and the reaction mixture was stirred at room temperature for 12 h. The reaction was quenched with MeOH (1 mL), diluted with ethyl acetate, washed with water (5×) and brine (1×), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was triturated with MeOH, filtered, and dried to afford 3.94 g (85%) of product as a white solid: ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.5 (q, 1H, J = 6 Hz), 3.45 (m, 2H), 2.35 (t, 1H, J = 10 Hz), 2.4 (dt, 1H, J = 12, 2 Hz), 2.2 (s, 2H), 2.1−1.1 (m, 12H), 1.02 (s, 3H), 0.9 (d, 3H, J = 7.0 Hz), 0.78 (d, 3H, J = 7 Hz), 0.69 (s, 3H), 0.1 (s, 9H).

Step 2. (3*β*,5α,25*R*)-3-[(Heptaacetyl-*β*-D-maltosyl)oxy]-spirostan-11-one. Powdered 4Å molecular sieves (4 g) were added to a solution of 3-[(trimethylsilyl)oxy]-(3*β*,5α,25*R*)-spirostan-11-one (3.90 g, 7.76 mmol) and acetobromomaltose (8.15 g, 11.7 mmol) in CH_2Cl_2 (60 mL) at room temperature. After the mixture stirred for 15 min, $Hg(CN)_2$ (7.85 g, 31 mmol) and $HgBr_2$ (11.2 g, 31 mmol) were added, and the mixture stirred at room temperature for 7 h. The mixture was diluted with ethyl acetate, washed with 1 N HCl (3×) and brine (1×), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The product was purified by flash chromatography (30−60% EtOAc/hexanes) and afforded 3.5 g (43%) of the title product: ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.4 (d, 1H, J = 3 Hz), 5.3 (dd, 1H, J = 9, 9 Hz), 5.2 (dd, 1H, J = 8, 8 Hz), 5.05 (dd, 1H, J = 9, 9 Hz), 4.88 (dd, 1H, J = 11, 3 Hz), 4.7 (dd, 1H, J = 10, 8 Hz), 4.4 (m, 2H), 4.2 (m, 2H), 4.0 (m, 3H), 3.5 (m, 4H), 2.5 (m, 1H), 2.2 (s, 2H), 2.16 (s, 3H), 2.1 (s, 3H), 2.02 (s, 3H), 2.0 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.95 (s, 3H), 1.95−1.0 (m, 23H), 1.0 (s, 3H), 0.9 (d, 3H, J = 7 Hz), 0.65 (d, 3H, J = 7 Hz), 0.55 (s, 3H); FAB MS 1049 (M + H$^+$).

(3*β*,5α,25*R*)-3-[(Heptaacetyl-α-D-cellobiosyl)oxy]-spirostan-11-one. Hydrobromic acid (30% in acetic acid, 1.2 mL) was added to a room temperature solution of compound **4** (2.0 g) in CH_2Cl_2 (35 mL), and the resulting mixture was stirred at room temperature for 94 h. The reaction was quenched by slow addition of saturated aqueous $NaHCO_3$ (20 mL). The organic layer was separated, dried over magnesium sulfate, and concentrated *in vacuo* to give 1.637 g of a black solid. Purification by repeated flash chromatography (1:1 hexane:ethyl acetate eluent) provided 651 mg (33% yield) of the title compound: mp 248−249 °C; FAB MS m/z 1049 (M + H$^+$), 1071 (M + Na$^+$). Anal. Calcd for $C_{53}H_{76}O_{21}\cdot H_2O$: C, 59.65; H, 7.37. Found: C, 59.66; H, 7.00.

(3*β*,5α,25*R*)-3-[(*β*-D-Cellobiosyl)oxy]spirostan-11-one (5). A mixture of compound **4** (6.57 g, 6.26 mmol), NaOMe (68 mg, 1.25 mmol), MeOH (35 mL), and THF (75 mL) was heated to reflux for 1 h followed by stirring at room temperature for 12 h. A white precipitate formed within 30 min. The final suspension was concentrated *in vacuo* to give 6.0 g of crude product. This material was purified by flash chromatography (chloroform followed by 8:2 chloroform:MeOH) to give 2.71 g (57% yield) of **5**: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 5.22 (d, 1H, J = 5 Hz), 5.00 (m, 3H), 4.64 (s, 1H), 4.58 (t, 1H, J = 5 Hz), 4.54 (t, 1H, J = 6 Hz), 4.34 (q, 1H, J = 8 Hz), 4.27 (d, 1H, J = 8 Hz), 4.23 (d, 1H, J = 8 Hz), 3.68−2.94 (m, 15H), 2.34 (m, 2H), 2.08−0.81 (m, 23H), 0.92 (s, 3H), 0.86 (d, 3H, J = 7 Hz), 0.72 (d, 3H, J = 6 Hz), 0.59 (s, 3H); FAB MS m/z 777 (M + H$^+$). Anal. Calcd for $C_{39}H_{62}O_{14}\cdot 2H_2O$: C, 59.22; H, 8.41. Found: C, 59.48; H, 8.48.

(3*β*,5α,25*R*)-3-[[6′,6″-Bis(*tert*-butyldiphenylsilyl)-2′,2″,3′,3″,4″-pentaacetyl-*β*-D-cellobiosyl]oxy]spirostan-11-one (20). A mixture of compound **5** (25 g, 0.03 mol), imidazole (15.78 g, 0.23 mol), 4-(dimethylamino)pyridine (2.0 g), and DMF (400 mL) was cooled to 0 °C. *tert*-Butyldiphenylsilyl chloride (34.45 mL, 0.13 mol) was added; the mixture was warmed to room temperature and stirred for 5 h. Pyridine (53.57 mL, 0.66 mol) and acetic anhydride (54.87 mL, 0.50 mol) were added, and the reaction mixture was stirred overnight. The reaction was then quenched with water, diluted with ethyl acetate, washed with water (2×), 1 N HCl (3×), and brine (1×), dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford **20** as a foam (66.05 g, >100%): mp 135 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.85−7.3 (m, 20H), 5.3−3.3 (m, 18H), 2.6−1.0 (m, 61H), 0.90 (d, 3H, J = 7 Hz), 0.8 (d, 3H, J = 7 Hz), 0.7 (s, 3H); FAB MS m/z 1441 (M + H$^+$).

(3*β*,5α,25*R*)-3-[(2′,2″,3′,3″,4″-Pentaacetyl-*β*-D-cellobiosyl)oxy]spirostan-11-one (21). A mixture of compound **20** (15.04 g, 0.01 mol) and pyridine (100 mL) was cooled to 0 °C. Hydrogen fluoride in pyridine (36.64 mL) was added, and the reaction mixture was gradually warmed to room temperature and allowed to stir for 4 h. The reaction was cooled and quenched with water. The mixture was then dissolved in ethyl acetate, washed with water (1×), 1 N HCl (4×), and brine (1×), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residual material was triturated with hexanes, filtered, washed with hexanes, and dried to afford 7.22 g (74.8%) of **21**: mp 233−234 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.25−3.3 (m, 20H), 2.65−1.05 (m, 40H), 1.0 (s, 3H), 0.95 (d, 3H, J = 8 Hz), 0.8 (d, 3H, J = 8 Hz), 0.7 (s, 3H); FAB MS m/z 965 (M + H$^+$). Anal. Calcd for $C_{49}H_{72}O_{19}$: C, 60.98; H, 7.52. Found: C, 60.78; H, 7.54.

(3*β*,5α,25*R*)-3-[(6′,6″-Dideoxy-6′,6″-difluoro-2′,2″,3′,3″,4″-pentaacetyl-*β*-D-cellobiosyl)oxy]spirostan-11-one (22). DAST (0.34 mL, 2.59 mmol) was added to a solution of compound **21** (250 mg, 0.259 mmol) in anhydrous dimethoxyethane (5 mL), at 0 °C. After 20 min, the reaction mixture was warmed to room temperature for 30 min and then to 40 °C for 1.5 h. The reaction mixture was cooled to 0 °C, diluted with ethyl acetate (50 mL), and poured into ice water. The organic layer was washed with 1 N HCl (1×), $NaHCO_3$ solution (1×), and brine (1×), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (40% ethyl acetate/hexanes) to afford 190 mg (76%) of **22** as a white solid: mp 267−269 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.15 (dd, 1H, J = 8, 8 Hz), 5.05 (dd, 1H, J = 9, 9 Hz), 4.9 (dd, 1H, J = 9, 8 Hz), 4.85 (dd, 1H, J = 9, 8 Hz), 4.7 (m, 1H), 4.5 (m, 4H), 3.85 (dd, 1H, J = 9.0, 9.0 Hz), 3.6 (m, 1H), 3.5 (m, 1H), 3.35 (dd, 1H, J = 10.0, 11.0 Hz), 2.5 (m, 1H), 2.2 (s, 2H), 2.01 (s, 3H), 2.0 (s, 6H), 1.98 (s, 3H), 1.97 (s, 3H),

1.9−1.0 (m, 27H), 1.0 (s, 3H), 0.92 (d, 3H, J = 7 Hz), 0.75 (d, 3H, J = 7 Hz), 0.7 (s, 3H); FAB MS m/z 969 (M + H^+). Anal. Calcd for $C_{49}H_{70}F_2O_{17}$: C, 60.73; H 7.28. Found: C, 61.06; H, 7.19.

(3*β*,5α,25*R*)-3-[(6′,6″-Dideoxy-6′,6″-diiodo-2′,2″,3′,3″,4″-pentaacetyl-*β*-D-cellobiosyl)oxy]spirostan-11-one (25). A mixture of compound **21** (2.00 g, 2.08 mmol), imidazole (0.85 g, 12.0 mmol), and triphenylphosphine (3.26 g, 12.0 mmol) was dissolved in toluene (40 mL). Iodine (2.10 g, 8.30 mmol) was added, and the reaction mixture was heated at reflux temperature overnight. The reaction mixture was cooled, diluted with ethyl acetate, washed with 1 N HCl (1×), sodium bicarbonate solution (1×), and brine (1×), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (10% ethyl acetate/methylene chloride) to afford 1.78 g (72.3%) of **25** as a white solid: ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.25−3.0 (m, 18H), 2.6−1.05 (m, 42H), 0.95 (d, 3H, J = 7 Hz), 0.80 (s, 3H), 0.75 (d, 3H, J = 7 Hz), 0.60 (s, 3H); FAB MS m/z 1171 (M + H^+).

(3*β*,5α,25*R*)-3-[(6′,6″-Dideoxy-2′,2″,3′,3″,4″-pentaacetyl-*β*-D-cellobiosyl)oxy]spirostan-11-one (26). Azoisobutyrylnitrile (10 mg) was added to a solution of compound **25** (150 mg, 0.128 mmol) and tri-*n*-butyltin hydride (0.105 mL, 0.39 mmol) in anhydrous toluene (5 mL) at room temperature. The reaction mixture was heated at reflux temperature for 3 h, cooled and concentrated *in vacuo*. The residual material was triturated with hexanes, filtered, dried, and concentrated. The crude product was purified by flash chromatography (5% ethyl acetate/methylene chloride) to afford 0.1 g (82%) of **26** as a white solid: ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.1 (m, 2H), 4.8 (m, 2H), 4.5 (m, 2H), 4.1 (q, 1H, J = 7 Hz), 3.4 (m, 4H), 2.45 (bd, 1H, J = 14 Hz), 2.25 (s, 2H), 2.12 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 1.98 (s, 3H), 1.9−1.3 (m, 25H), 1.3 (d, 3H, J = 6 Hz), 1.22 (d, 3H, J = 6 Hz), 1.0 (s, 3H), 0.9 (d, 3H, J = 7 Hz), 0.8 (d, 3H, J = 7 Hz), 0.7 (s, 3H); FAB MS m/z 956 (M + Na^+). Anal. Calcd for $C_{49}H_{72}O_{17}$: C, 63.07; H, 7.78. Found: C, 63.11; H, 7.84.

(3*β*,5α,25*R*)-3-[(6′,6″-Dimesyl-2′,2″,3′,3″,4″-pentaacetyl-*β*-D-cellobiosyl)oxy]spirostan-11-one (23). A solution of compound **21** (1.00 g, 1.04 mmol) and triethylamine (1.50 mL, 10.40 mmol) in CH_2Cl_2 (10 mL) was cooled to 0 °C. Mesyl chloride (0.48 mL, 6.22 mmol) and 4-(dimethylamino)pyridine (0.02 g) were added, and the reaction mixture was stirred at 0 °C for 2 h. The reaction mixture was diluted with ethyl acetate (50 mL), washed with 1 N HCl (2×), sodium bicarbonate solution (1×), and brine (1×), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residual material was dissolved in CH_2Cl_2 (10 mL), and hexanes were added (20 mL). The CH_2Cl_2 was removed *in vacuo*, and the resulting precipitate was filtered, washed with hexanes, and dried under vacuum to afford 1.10 g (94%) of **23** as a white solid: ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.25−3.3 (m, 18H), 3.1 (d, 6H, J = 7 Hz), 2.6−1.1 (m, 40H), 1.0 (s, 3H), 0.95 (d, 3H, J = 7 Hz), 0.77 (d, 3H, J = 7 Hz), 0.7 (s, 3H).

(3*β*,5α,25*R*)-3-[(6′,6″-Dideoxy-6′,6″-dichloro-2′,2″,3′,3″,4″-pentaacetyl-*β*-D-cellobiosyl)oxy]spirostan-11-one (24). A mixture of compound **23** (0.10 g, 0.09 mmol), lithium chloride (0.15 g), and DMF (2 mL) was heated to 85 °C and stirred for 3 h. The reaction mixture was then cooled, diluted with ethyl acetate (20 mL), washed with water (2×) and brine (1×), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residual material was triturated with hexanes, filtered, and dried to afford 0.079 g (88%) of **24** as a white solid: mp >275 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.2−3.3 (m, 18H), 2.6−1.1 (m, 40H), 1.0 (s, 3H), 0.90 (d, 3H, J = 7 Hz), 0.75 (d, 3H, J = 7 Hz), 0.70 (s, 3H); FAB MS m/z 1001 (M + H^+). Anal. Calcd for $C_{49}H_{70}Cl_2O_{17}$: C, 58.74; H, 7.04. Found: C, 58.71; H, 7.12.

(3*β*,5α,25*R*)-3-[[6′,6″-Bis(ethoxymethyl)-2′,2″,3′,3″,4″-pentaacetyl-*β*-D-cellobiosyl)oxy]spirostan-11-one (27). A mixture of compound **21** (0.50 g, 0.52 mmol), dichloroethane (5 mL), diisopropylethylamine (1 mL), and ethoxymethyl chloride (0.196 g, 2.07 mmol) was stirred at room temperature for 4 h. The reaction mixture was warmed to 50 °C for 2 h and cooled and the reaction quenched with MeOH. The mixture was then diluted with ethyl acetate and washed with water (1×), 1 N HCl (2×), and brine (1×). The organic layer was then dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (60% ethyl acetate/40% hexanes) to afford 0.505 g of **27** (46.7%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.15 (dd, 1H, J = 8, 8 Hz), 5.1 (m, 1H), 4.85 (m, 2H), 4.7 (s, 1H), 4.6 (s, 1H), 4.5 (dd, 1H, J = 18, 7 Hz), 4.45 (m, 1H), 4.1 (q, 2H, J = 6 Hz), 3.8 (m, 2H), 3.5 (m, 10H), 2.5 (m, 1H), 2.2 (s, 2H), 2.01 (s, 3H), 2.0 (s, 6H), 1.98 (s, 3H), 1.97 (s, 3H), 1.9−1.0 (m, 26H), 1.2 (m, 6H), 1.0 (s, 3H), 0.92 (d, 3H, J = 7 Hz), 0.75 (d, 3H, J = 7 Hz), 0.7 (s, 3H); FAB MS m/z 1104 (M + Na^+). Anal. Calcd for $C_{55}H_{84}O_{21}$: C, 61.10; H, 7.83. Found: C, 61.05; H, 7.92.

(3*β*,5α,25*R*)-3-[[6′,6″-Bis(phenylcarbamoyl)-2′,2″,3′,3″,4″-pentaacetyl-*β*-D-cellobiosyl]oxy]spirostan-11-one (28). A mixture of compound **21** (0.50 g, 0.52 mmol), CH_2Cl_2 (5 mL), triethylamine (0.50 mL, 3.62 mmol), and 4-(dimethylamino)-pyridine (0.10 g) was cooled to 0 °C. Phenyl isocyanate (0.34 mL, 3.12 mmol) was added, and the reaction mixture was stirred at 0 °C for 15 min and then at room temperature for 30 min. The reaction was quenched with MeOH, diluted with ethyl acetate, washed with water (1×), 1 N HCl (3×), saturated $NaHCO_3$ solution (2×), and brine (1×), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residual material was triturated with hexanes, filtered, washed with hexanes, and dried to afford >100% yield of compound **28**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.55−7.0 (m, 10H), 6.85 (s, 1H), 6.65 (s, 1H), 5.3−3.52 (m, 17H), 3.45 (t, 1H, J = 10 Hz), 2.5−1.05 (m, 40H), 1.0 (s, 3H), 0.95 (d, 3H, J = 8 Hz), 0.8 (d, 3H, J = 8 Hz), 0.7 (s, 3H). Anal. Calcd for $C_{63}H_{82}N_2O_{21}$: C, 62.88; H, 6.87; N, 2.33. Found: C, 62.51; H, 6.91; N, 2.30.

Nonselective Carbamoylation. A mixture of compound **5** (0.50 g, 0.66 mmol), pyridine (3 mL), and 4Å molecular sieves (0.50 g) was stirred for 10 min at room temperature. The reaction mixture was then cooled to −40 °C, and 4-chlorophenyl isocyanate (0.122 g, 0.8 mmol) was added. The reaction mixture was gradually warmed to room temperature and stirred for 1.5 h. The reaction was quenched with MeOH, filtered, and concentrated *in vacuo* twice with toluene in order to remove the pyridine. The crude material was purified by gradient flash chromatography (2−10% MeOH:chloroform). The products were concentrated *in vacuo*, triturated with methanol/water, filtered, and washed with water and dried to afford the products listed below in order of elution.

(3*β*,5α,25*R*)-3-[[6′,6″-Bis[(4-chlorophenyl)carbamoyl]-*β*-D-cellobiosyl]oxy]spirostan-11-one (35): 250 mg (36%); FAB MS m/z 1061 (M + H^+). Anal. Calcd for $C_{53}H_{70}Cl_2N_2O_{16}\cdot 1.5H_2O$: C, 58.51; H, 6.76; N, 2.57. Found: C, 58.55; H, 6.54; N, 2.66.

(3*β*,5α,25*R*)-3-[[6″-[(4-Chlorophenyl)carbamoyl]-*β*-D-cellobiosyl]oxy]spirostan-11-one (36): 93 mg (15%); FAB MS m/z 930 (M + Na^+). Anal. Calcd for $C_{46}H_{66}ClNO_{15}$: C, 60.82; H, 7.32; N, 1.54. Found: C, 60.71; H, 7.24; N, 1.33.

(3*β*,5α,25*R*)-3-[[6′-[(4-Chlorophenyl)carbamoyl]-*β*-D-cellobiosyl]oxy]spirostan-11-one (37): 90 mg (15%); FAB MS m/z 908 (M + H^+). Anal. Calcd for $C_{46}H_{66}ClNO_{15}$: C, 59.63; H, 7.40; N, 1.51. Found: C, 59.54; H, 7.24; N, 1.33.

(3*β*,5α,25*R*)-3-[[6′,6″-Bis(1-pyrrolidinylcarbonyl)-*β*-D-cellobiosyl]oxy]spirostan-11-one (47). Step 1: A mixture of compound **21** (1.0 g, 1.04 mmol), carbonyldiimidazole (0.42 g, 2.6 mmol), and diisopropylethylamine (0.9 mL, 5.2 mmol) in dichloroethane (7 mL) was stirred at room temperature for 2 h. Pyrrolidine (0.185 g, 2.6 mmol) was added, and the mixture was stirred for 5 h at room temperature. The mixture was diluted with ethyl acetate, washed with 1 N HCl (2×), saturated $NaHCO_3$ solution (1×), and brine (1×), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was triturated from EtOAc/hexanes, filtered, and dried to afford 800 mg (88%) of product as a colorless solid: FAB MS m/z 1183 (M + Na^+).

Step 2: Deprotection as described for compound **5** afforded **47** as a colorless solid: 400 mg (78%); FAB MS m/z 852 (M + H^+). Anal. Calcd for $C_{44}H_{69}NO_{15}$: C, 61.38; H, 8.19; N, 1.63. Found: C, 61.10; H, 8.05; N, 1.70.

(3*β*,5α,25*R*)-3-[[4″,6″-(4-Methoxybenzylidene)-2′,2″,3′,3″,6′-pentakis(chloroacetyl)-*β*-D-cellobiosyl]oxy]spirostan-11-one (48). Camphorsulfonic acid (3 g) was added to a mixture of compound **5** (50 g, 0.066 mol) and anisaldehyde dimethyl

acetal (50 mL, 0.29 mol) in 1,2-dichloroethane (1500 mL). The suspension was heated to reflux temperature, and 200 mL of solvent was distilled off. After 4 h at reflux temperature, the dark, gelatinous mixture was cooled to 0 °C and treated with pyridine (160 mL, 1.99 mol) and chloroacetic anhydride (170 g, 1 mol). The reaction mixture was allowed to warm to room temperature and after 2 h was washed with 1 N HCl (3×), $NaHCO_3$ (1×), and brine (1×), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was dissolved in a minimum amount of ethyl acetate, and the product was precipitated with hexanes. The solid was filtered, washed with hexanes, and dried to afford 77 g of **48** as a white solid (93%): mp 256−257 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.35 (d, 2H, J = 9.0 Hz), 6.88 (d, 2H, J = 9.0 Hz), 5.45 (s, 1H), 5.3 (m, 2H), 5.0 (m, 2H), 4.7 (d, 1H, J = 7.0 Hz), 4.6 (m, 2H), 4.5 (dd, 1H, J = 8.0, 8.0 Hz), 4.38 (dd, 1H, J = 11.0, 6.0 Hz), 4.2−3.5 (m, 15H), 3.8 (s, 3H), 3.4 (dd, 1H, J = 11.0, 10.0 Hz), 2.5 (m, 1H), 2.25 (s, 2H), 2.1−1.0 (m, 25H), 1.0 (s, 3H), 0.94 (d, 3H, J = 7.0 Hz), 0.78 (d, 3H, J = 7.0 Hz), 0.7 (s, 3H); FAB MS m/z 1277 ($M + Na^+$).

(3β,5α,25*R*)-3-[[2′,2″,3′,3″,6′-Pentakis(chloroacetyl)-β-D-cellobiosyl]oxy]spirostan-11-one (49). Trifluoroacetic acid (19 mL) was added to a solution of compound **48** (23.7 g, 0.019 mol) in CH_2Cl_2 (150 mL) and MeOH (50 mL). After 4 h, the mixture was washed with water (3×), $NaHCO_3$ (2×), and brine (1×), dried (Na_2SO_4), filtered, and concentrated. The residue was dissolved in a minimal amount of ethyl acetate and precipitated with hexanes. The solid was filtered, washed with hexanes, and dried to afford 19.7 g of **49** as a white solid (92%): mp 228−229 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.2 (dd, 1H, J = 9.0, 9.0 Hz), 5.1 (dd, 1H, J = 9.0, 9.0 Hz), 4.95 (m, 2H), 4.6 (m, 3H), 4.5 (ddd, 1H, J = 8.0, 7.0, 7.0 Hz), 4.2−3.4 (m, 20H), 3.35 (dd, 1H, J = 9.0, 9.0 Hz), 2.9 (d, 1H, J = 6.0 Hz), 2.45 (m, 1H), 2.2 (s, 2H), 2.1−1.1 (m, 22H), 1.0 (s, 3H), 0.94 (d, 3H, J = 7.0 Hz), 0.77 (d, 3H, J = 7.0 Hz), 0.7 (s, 3H); FAB MS m/z 1159 ($M + Na^+$).

(3β,5α,25*R*)-3-[[4″,6″-Bis[(2-fluorophenyl)carbamoyl]-2′,2″,3′,3″,6′-pentakis(chloroacetyl)-β-D-cellobiosyl]oxy]-spirostan-11-one (50). Cuprous chloride (1.74 g, 18 mmol) was added to a solution of compound **49** (5.0 g, 4.4 mmol) and 2-fluorophenyl isocyanate (1.98 mL, 18 mmol) in dry DMF (30 mL) at room temperature. After 2 h, the mixture was diluted with ethyl acetate (100 mL), washed with 1 N HCl (2×) and brine (1×), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (50 mL), and MeOH (50 mL) was added. The CH_2Cl_2 was removed *in vacuo*, and a solid precipitated from the MeOH. The solid was filtered, washed with MeOH, and dried to afford 4.82 g of **50** as a white solid (78%): mp 234−234.5 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.9 (m, 2H), 7.05 (m, 8H), 5.35 (dd, 1H, J = 8.0, 7.0 Hz), 5.28 (dd, 1H, J = 9.0, 8.0 Hz), 5.15 (dd, 1H, J = 9.0, 9.0 Hz), 5.05 (dd, 1H, J = 9.0, 8.0 Hz), 4.98 (dd, 1H, J = 8.0, 7.0 Hz), 4.72 (d, 1H, J = 9.0 Hz), 4.65−3.4 (m, 21H), 3.35 (dd, 1H, J = 10.0, 9.0 Hz), 2.45 (m, 1H), 2.2 (s, 2H), 2.1−1.1 (m, 22H), 1.0 (s, 3H), 0.9 (d, 3H, J = 7.0 Hz), 0.8 (d, 3H, J = 7.0 Hz), 0.7 (s, 3H); FAB MS m/z 1433 ($M + Na^+$).

Pharmacology. 1. Acute Cholesterol Absorption Measurement in Hamsters. Cholesterol absorption was assessed using a modification of the method reported by Kelly and Tsai[9] as reported by Harwood et al.[6d] Male golden Syrian hamsters (~90−110 g) were fed AIN 76C semipurified diet containing 1% cholesterol and 0.5% cholic acid (ICN Biomedicals, Costa Mesa, CA) for 3 days and then fasted overnight. The following morning, they were administered various doses of test compounds suspended in a 10% aqueous ethanol solution containing 0.25% methyl cellulose and 0.6% Tween 80 as suspending agents. Dosing volume was 0.5 mL/100 g of body weight. Immediately afterwards, hamsters received an oral bolus (1.5 mL/hamster) of hamster liquid diet (Bioserve, Frenchtown, NJ) containing [^{3}H]cholesterol (~0.5 μCi), unlabeled cholesterol (15 mg), and cholic acid (7.5 mg). The degree of cholesterol absorption was assessed by measuring the hepatic content of [^{3}H]cholesterol 24 h later. Livers were excised and saponified in 5 mL of 2.5 M KOH for 2 h at 70°C. Aliquots (200 μL) of the saponified livers were decolorized with H_2O_2 (200 μL of 30%), neutralized with 3 N HCl (200 μL), and then counted in a liquid scintillation counter. Total hepatic radioactivity was calculated from the radioactivity in each aliquot after adjusting for the total volume of the saponified liver. The percent inhibition of cholesterol absorption was determined by comparing the hepatic radioactivity in hamsters receiving cholesterol absorption inhibitors to that in hamsters receiving vehicle.

2. Subchronic Assay. Male golden Syrian hamsters (LVG strain; 100−120 g) (Charles Rivers Laboratory, Wilmington, MA), housed 6/cage, were acclimated to an altered light cycle (lights on from 3 p.m. to 3 a.m.) for 1 week and then placed on a powdered AIN 76C semipurified diet (ICN Biomedicals, Costa Mesa, CA) for 3 days to become accustomed to eating a powdered diet. At the start of the study, food was switched to AIN 76C plus 0.2% cholesterol/0.1% cholic acid that contained various concentrations of powdered test compounds. Body weights and food jar weights were recorded daily, and doses of compound (mg/kg) were estimated from mean body weight, food consumed per day, and percent compound in the feed. Hamsters were euthanized on study day 4, and liver was excised for the determination of hepatic cholesterol levels. Livers were saponified in 5 mL of 2.5 M KOH for 2 h at 70 °C. Aliquots (200 μL) of the saponified liver (or homogenate) were added to 1 mL of ethanol containing [^{14}C]cholesterol (~100 000 dpm; used as an internal recovery standard). Tubes were mixed and placed at −20 °C for 15 min to facilitate precipitation of protein before the addition of 4 mL of hexane. After vigorous vortexing, samples were centrifuged at ~1500*g* for 5 min to separate phases; 3 mL of the hexane layer was evaporated to dryness under N_2 before being redissolved in 300 μL of 1% (v/v) Triton X-100 in ethanol. Cholesterol levels were analyzed using a commercial assay kit (Single Vial, Boehringer Mannheim, Indianapolis, IN). Triplicate 15 μL aliquots were analyzed in 96-well microtiter plates; 200 μL of reagent was added, and the plates were allowed to incubate at room temperature for 1 h. Absorbance was read at 490 nm using a microtiter plate spectrophotometer (Molecular Devices, Menlo Park, CA) and compared to a standard curve to calculate cholesterol mass. Radioactivity in the 15 μL aliquots was analyzed by liquid scintillation counting to correct for cholesterol extraction efficiency.

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