The Squalestatins: Inhibitors of Squalene Synthase. Enzyme Inhibitory Activities and *in Vivo* Evaluation of C3-Modified Analogues

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Squalestatin analogues modified at C3 were prepared and evaluated for their ability to inhibit rat liver microsomal squalene synthase *in vitro*. While the 4,6-dimethyloctenoate ester group at C6 was maintained, a number of modifications to the C3 carboxylic acid were well tolerated. However, in the absence of the C6 ester group, similar modifications to the C3 carboxyl group caused loss of activity. Selected compounds were evaluated for their ability to inhibit cholesterol biosynthesis *in vivo* in rats 1 and 6 h postadministration. Analogues of squalestatin 1 (S1) modified at C3 were found to possess a shorter duration of effect *in vivo* which is reflected in their substantially reduced ability to lower serum cholesterol levels in marmosets. Significant cholesterol lowering (up to 62%) for the C3 hydroxymethyl analogue **1b** was observed only when this compound was dosed three times a day for 3 days.

High levels of blood cholesterol and blood lipids are conditions which are implicated in the onset of vessel wall disease. Methods for effective reduction of serum cholesterol levels are therefore of major interest. Currently, the most effective approach to lowering serum cholesterol concentrations is by inhibiting sterol biosynthesis, and a number of therapeutic agents are available which work by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the enzyme which catalyzes the biosynthesis of mevalonic acid. Mevalonic acid, however, is a common precursor of all isoprenyl derivatives, including in animals the ubiquinones, heme A, the dolichols, isopentenyl *t*-RNA, and isoprenylated proteins. A more selective inhibition of cholesterol biosynthesis may be achieved by inhibiting steps beyond the branch in the pathway. The first biosynthetic step which leads exclusively to sterols, the head-to-head condensation of two farnesyl diphosphate (FPP) molecules to give squalene *via* the intermediate presqualene diphosphate (PSPP), is catalyzed by squalene synthase[1] (SQS) (farnesyldiphosphate:farnesyldiphosphate farnesyl transferase, EC 2.5.1.21). Agents which inhibit this enzyme are therefore potential drugs for the regulation of cholesterogenesis.

We have recently described the isolation[2,3] and structure elucidation[4] of the squalestatins, a novel group of fungal metabolites isolated from a previously unknown *Phoma* species (Coelomycetes). Squalestatins S1 (**1a**) and H1 (**2a**) are selective inhibitors of both rat and *Candida* SQS; 50% inhibition of rat liver microsomal SQS activity is observed *in vitro* at a concentration of 12 and 26 nM, respectively. Furthermore, when S1 is administered orally to marmosets for 7 days, a 50% reduction in serum cholesterol levels is observed at a dose of 10 mg/kg/day.[5] Moreover when S1 is administered iv for 7 days at 1 mg/kg/day, an 86% reduction in serum cholesterol is observed; under the same dosing regime H1 showed a 56% reduction. Further studies in marmosets revealed that S1 has a profound and extended effect on lipids (50−60% decrease in serum cholesterol levels during 7 days after a single iv dose of 1 mg/kg).[6] A group at Merck has published the isolation of zaragozic acids, the structure of zaragozic acid A[7,8] being identical with that of S1. More recently a group at Tokyo Noko University−Mitsubishi[9] has also reported the isolation of S1 from another organism, *Setosphaeria khartoumensis* L1685.

As a part of our chemical program aimed at the modification of the complex squalestatin structure and the identification of the key structural features responsible for the biological activity, we have reported on the C1 chain-length requirements;[10] on the role of the tricarboxylic acid moiety;[11] on C6 and C7 modifications;[12] on the C6,C7 dideoxy,[13] C3 decarboxy,[14] C4 deoxy,[15] monocyclic,[16] acyclic,[17,18] C3 hydroxymethyl[19] and C3 heterocyclic[20] analogues; and on modifications at the allylic center.[21] In addition a detailed review bringing together all the published SAR of the squalestatins and the zaragozic acids has just been completed.[22] Our earlier studies have shown that the carboxylic acid at C3 in S1 is not essential. Thus, esterification[11] of the C3 carboxylic acid to the 3-methyl ester, decarboxylation,[14] or reduction to the hydroxymethyl[19] derivative at this position provide analogues with potent SQS inhibitory activity. Furthermore substitution of the C3 carboxylic acid in S1 by a variety of heterocyclic substituents can be well tolerated.[20] In addition researchers at Merck have reported[23] the preparation of the C3 methyl analogue of zaragozic acid A which retains SQS inhibitory activity. In this paper we report further studies at this position on squalestatins **1a** and **2a** and, in particular, the effect on biological activity of replacing the carboxylic acid moiety at C3 by neutral and basic groups.

Chemistry

Esterification of **1a** with excess methyl iodide in the presence of sodium bicarbonate in DMF gave the trimethyl ester **3** which was selectively saponified to the 4,5-dimethyl ester **4**.[14] Reduction of the 3-carboxylate function of **4** was achieved by activation as its 3-*N*-

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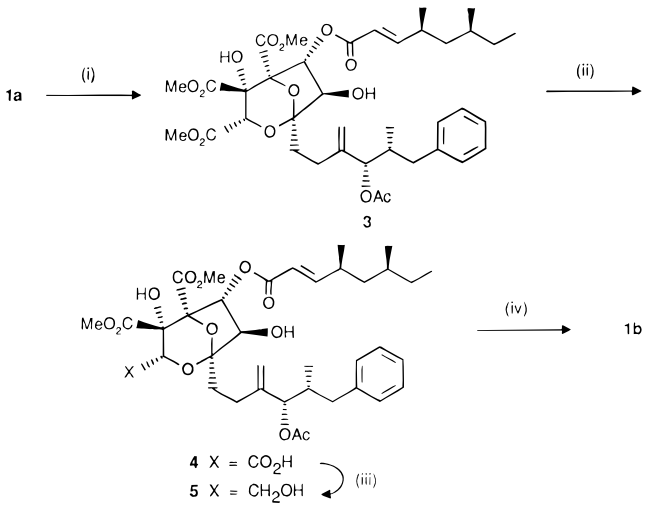
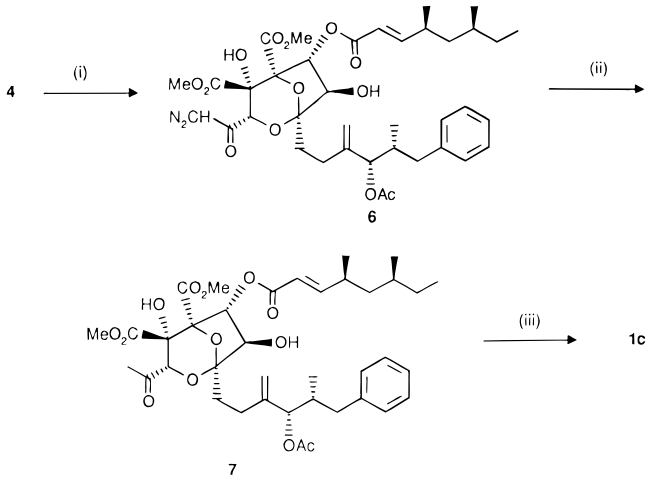
Scheme 1[a]



[a] Conditions: (i) MeI, $NaHCO_3$, DMF; (ii) 0.1 M NaOH, THF, H_2O; (iii) (a) *N*-hydroxysuccinimide, 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate, (b) $NaBH_4$, THF; (iv) LiI, 2,4,6-collidine, 45 °C.

Scheme 2[a]



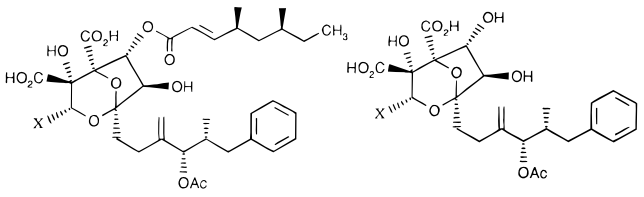
[a] Conditions: (i) (a) *i*-BuOCOCl, *N*-methylmorpholine, CH_2Cl_2; (b) CH_2N_2, Et_2O; (ii) HI, $CHCl_3$; (iii) LiI, 2,4,6-collidine, 45 °C.

hydroxysuccinimidyl ester followed by *in situ* reaction with sodium borohydride[24] to provide the 3-hydroxymethyl analogue **5**. Treatment of the derived product with lithium iodide in 2,4,6-collidine at 45 °C under a stream of nitrogen[25] resulted in selective cleavage of the methyl esters to give the dicarboxylic acid **1b** (Scheme 1).[19] An alternative synthesis of this compound has been published by the Merck group.[23] The synthesis of the C3 acetyl analogue **1c** is shown in Scheme 2. Activation of the 4,5-dimethyl ester **4** followed by treatment with diazomethane and subsequent reduction of the derived diazo ketone[20] **6** with hydrogen iodide gave the desired C3 acetyl derivative **7** from which the dicarboxylic acid **1c** was obtained using procedures similar to those described above.

The methoxymethyl analogue **1d** was synthesized from the trimethyl ester[4] **3** as outlined in Scheme 3. Protection of the C4 and C7 hydroxyl groups as (methoxyethoxy)methyl (MEM) ethers gave the bis-MEM-protected compound **8**. Selective base-catalyzed hydrolysis of the 3-methyl ester afforded the related carboxylic acid[18] **9** which was reduced to the C3 hydroxymethyl derivative **10**. Alkylation of freshly prepared[26] **10** provided the methoxymethyl compound **11** from which the MEM groups were cleaved with aqueous formic acid to give the diol **12**. Selective cleavage of the methyl esters gave the C3 methoxymethyl analogue **1d**.

The preparation of compounds incorporating basic groups at C3 utilized the 3-methyl-4,5-di-*tert*-butyl triester **13** (Scheme 4).[19] Protection of the C7 hydroxyl group as the TBDMS ether gave **14** from which the 3-methyl ester was hydrolyzed selectively with base to give the 3-carboxylic acid **15**. Reduction of the carboxylic acid group *via* the *N*-succinimidoyl ester provided the hydroxymethyl analogue **16**. Reaction of **16** with triflic anhydride provided the triflate **17**, displacement of which with azide gave the C3 azidomethyl analogue **18**. Cleavage of the TBDMS group with fluoride provided the alcohol **19** from which the aminomethyl derivative **20** was derived by reduction of the azido function with triphenylphosphine. Cleavage of the *tert*-butyl ester groups with 6.5 M HCl in dioxane provided the dicarboxylic acid **1e**. Reductive amination of formaldehyde with amine **1e** using sodium triacetoxyborohydride as the reducing agent afforded the corresponding dimethylamino analogue **1f**.

With a route to C3 aminomethyl-substituted analogues established, neutral and acidic derivatives of this functionality were readily accessible. Thus, reaction of the C3 aminomethyl dicarboxylic acid **1e** with phenyl carbamate and triethylamine in THF at reflux provided the C3 ureidomethyl analogue **1g** whereas reaction of **1e** with aminoiminomethanesulfonic acid[27] (**21**) and triethylamine in methanol at 20 °C provided the C3 guanidinomethyl analogue **1h**. The acidic trifluoromethanesulfonamide **1i** was derived as shown in Scheme 5. Reduction of the azidomethyl derivative **18** with triphenylphosphine gave the aminomethyl analogue **22** which on treatment with triflic anhydride and 2,4,6-collidine provided the sulfonamido analogue **23**. Removal of the TBDMS group with tetrabutylammonium fluoride gave the alcohol **24**, and cleavage of the *tert*-butyl esters with 6.5 M HCl in dioxane provided the required sulfonamide **1i**.



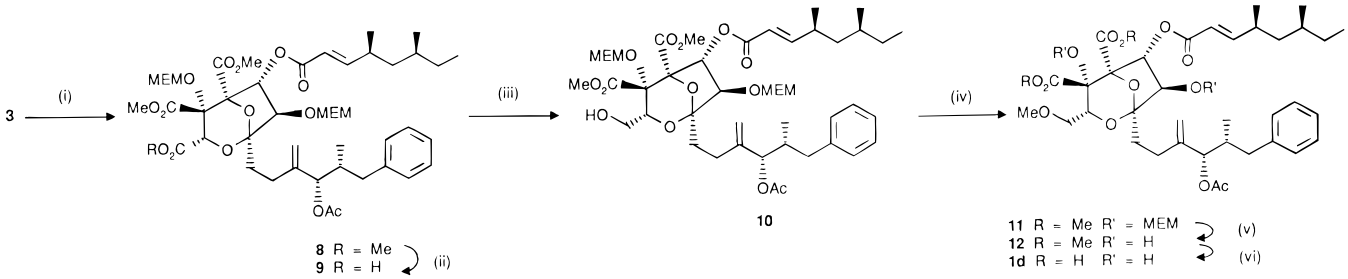
	X		X
a	HO_2C	f	Me_2NCH_2
b	$HOCH_2$	g	$H_2NCONHCH_2$
c	CH_3CO	h	$H_2NC(NH)NHCH_2$
d	CH_3OCH_2	i	$CF_3SO_2NHCH_2$
e	H_2NCH_2		

The C6 ester side chain was selectively removed from compounds **1c**−**e**, **1g**, **1i** by reaction with *N*-methylhydroxylamine according to our previously published procedure[13] to give derivatives **2c**−**e**, **2g**, **2i**.

Results and Discussion

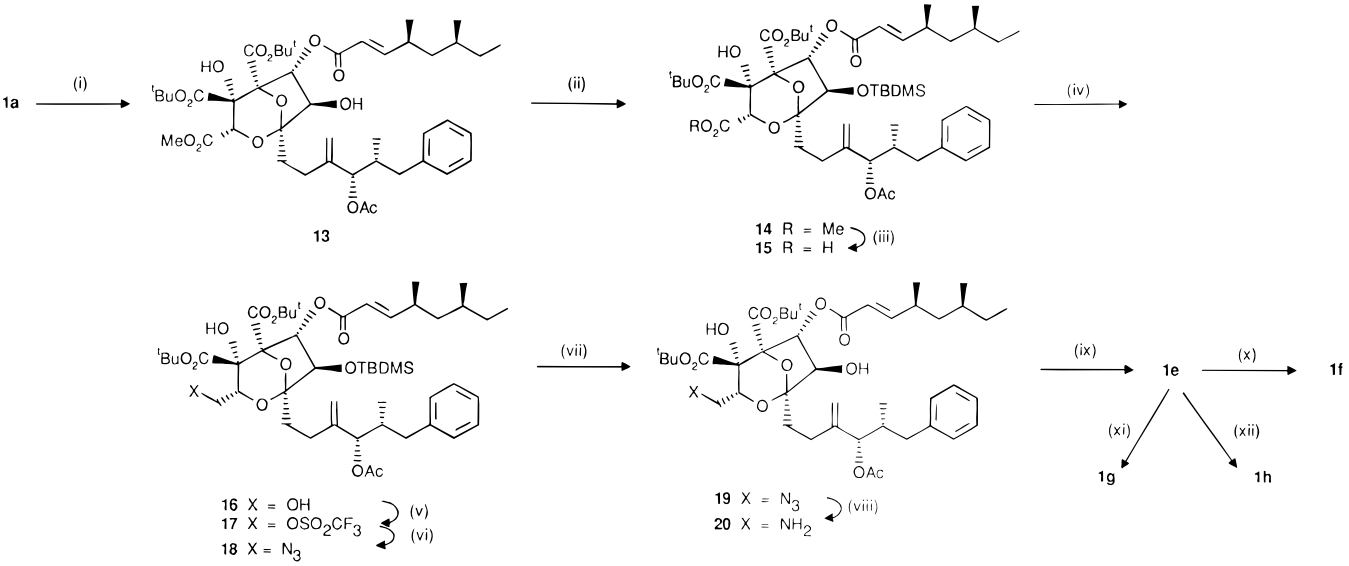
The compounds listed in Table 1 were evaluated for their inhibitory activity against SQS using the same

Scheme 3[a]



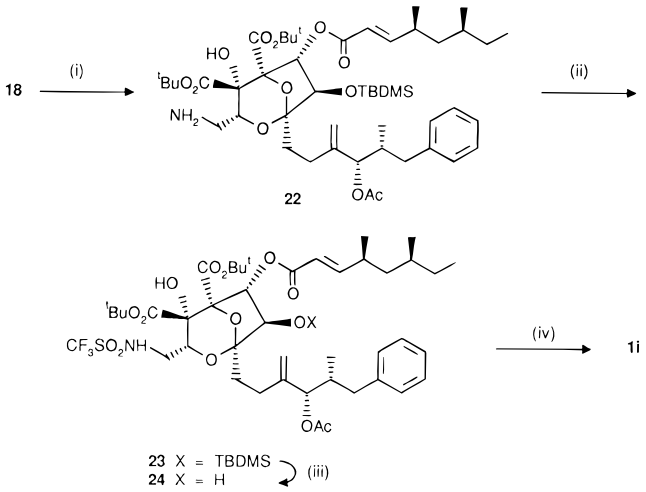
[a] Conditions: (i) (a) MEM-Cl, *i*-Pr_2NEt, $ClCH_2CH_2Cl$, reflux; (b) NaH, DMF, MEM-Cl; (ii) 0.1 M NaOH, THF; (iii) (a) $(COCl)_2$, DMF, CH_2Cl_2; (b) $NaBH_4$, THF, CH_3CN; (iv) NaH, MeI, DMF; (v) HCO_2H, H_2O; (vi) LiI, 2,4,6-collidine, 45 °C.

Scheme 4[a]



[a] Conditions: (i) (a) MeOH, 12 M HCl; (b) $Me_2NCH(O\text{-}t\text{-}Bu)_2$, toluene; (ii) TBDMS-Cl, imidazole; (iii) 0.1 M NaOH, THF, H_2O; (iv) (a) *N*-hydroxysuccinimide, DCC, CH_2Cl_2; (b) $NaBH_4$, THF; (v) $(CF_3SO_2)_2O$, 2,4,6-collidine, CH_2Cl_2; (vi) NaN_3, DMF; (vii) TBAF, THF; (viii) Ph_3P, THF, H_2O; (ix) 6.5 M HCl, dioxane; (x) HCHO, AcOH, $NaBH(OAc)_3$, CH_3CN; (xi) $PhOCONH_2$, Et_3N, THF; (xii) $H_2NC(NH)SO_3H$ (**21**), Et_3N, CH_3CN.

Scheme 5[a]



[a] Conditions: (i) Ph_3P, THF, H_2O; (ii) $(CF_3SO_2)_2O$, 2,4,6-collidine, CH_2Cl_2; (iii) TBAF, THF; (iv) 6.5 M HCl, dioxane.

enzyme preparations and assays as described in our earlier publications.[2,5,28] The data in Table 1 clearly show that potent SQS inhibitory activity is exhibited by those analogues which retain the C6 dimethyloctenoate side chain, although some loss of activity is apparent in the case of the guanidinomethyl and sulfonamido analogues **1h** and **1i**. In contrast, the corresponding analogues of squalestatin H1 (**2a**) which incorporate a C6 hydroxyl group have greatly reduced SQS inhibitory activities. Furthermore, in a series of squalestatin H1 analogues incorporating heterocyclic groups directly attached to C3, only the analogue incorporating a C3 tetrazol-5-yl group, a known carboxylic acid mimetic, retains potent SQS inhibitory activity.[20] These results, together with those previously reported[11] for the squalestatin methyl ester derivatives, suggest that an acidic group must be present at C3 for significant SQS inhibitory activity to be retained in compounds lacking the 4,6-dimethyloctenoate ester

Table 1. *In Vitro* SQS Inhibitory Activity[a]

compd no.	formula	IC_{50} (nM)	compd no.	formula	IC_{50} (nM)
1a	$C_{35}H_{46}O_{14}$	12	**2a**	$C_{25}H_{30}O_{13}$	26
1b	$C_{35}H_{48}O_{13}$	15	**2b**	$C_{25}H_{32}O_{12}$	395[b]
1c	$C_{36}H_{48}O_{13}$	11	**2c**	$C_{26}H_{32}O_{12}$	500
1d	$C_{36}H_{50}O_{13}$	13	**2d**	$C_{26}H_{34}O_{12}$	>1000
1e	$C_{35}H_{49}NO_{12}$	4	**2e**	$C_{25}H_{33}NO_{11}$	>1000
1f	$C_{37}H_{53}NO_{12}$	10	**2f**	np[c]	np[c]
1g	$C_{36}H_{50}N_2O_{13}$	3	**2g**	$C_{26}H_{34}N_2O_{12}$	>1000
1h	$C_{36}H_{51}N_3O_{12}$	65	**2h**	np[c]	np[c]
1i	$C_{36}H_{48}F_3NO_{14}S$	60	**2i**	$C_{26}H_{32}F_3NO_{13}S$	>1000

[a] IC_{50} values were determined at least on two different occasions with a minimum of five and a maximum of eight dose levels of each inhibitor at least in duplicate and are expressed as mean values, using S1 as a reference. [b] Reference 19. [c] np = not prepared.

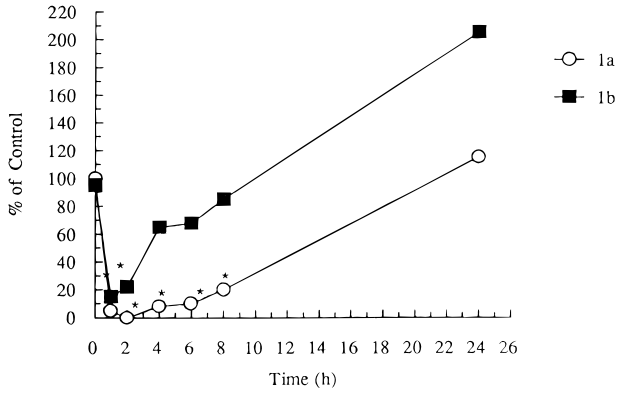


Figure 1. Time-course studies for inhibition of cholesterol biosynthesis in rats ($n = 8$) using **1a** (1 mg/kg iv) and **1b** (10 mg/kg iv). * Significantly ($p < 0.05$) below control.

group at C6. However, as is apparent from the lack of SQS inhibitory activity shown by **2i** which contains the acidic (trifluoromethyl)sulfonamido group at C3, the nature of this acidic group is clearly important. We have suggested previously[6,11,19,20,21] that squalestatin S1 and H1 analogues are presqualene diphosphate and farnesyl diphosphate mimetics, respectively, and that this may account for the observed differences in SAR between C3-modified analogues in each series. It is noteworthy that not only can the carboxylic acid group at C3 be replaced by neutral groups in squalestatins possessing a C6 ester group to provide dianionic compounds which retain potent SQS inhibitory activity, but by incorporating a basic group at C3 as in the aminomethyl and (dimethylamino)methyl analogues (**1e** and **1f**, respectively), compounds with a net charge of −1 can retain potent SQS inhibitory activity.

We have shown previously[5] that S1 inhibits cholesterol biosynthesis from [1-^{14}C]acetate when administered to rats, with an ED_{50} of 0.1 mg/kg iv. Time-course studies for inhibition of cholesterol biosynthesis in rats using S1 (1 mg/kg iv) and **1b** (10 mg/kg iv) revealed that **1b** has a shorter duration of effect (Figure 1). In the light of these data, analogues with further modifications to the C3 carboxylic acid were tested at two time points (1 and 6 h postadministration) at a dose of 10 mg/kg iv using **1a** (1 mg/kg iv) and **1b** (10 mg/kg iv) as controls. The squalestatins **1c**−**f** showed maximal inhibition of hepatic cholesterol biosynthesis *in vivo* in rats only at 1 h postdose; no significant inhibition was observed at the later time point.

We have reported previously[6] that a profound and extended lipid-lowering effect was observed in marmosets following a single iv dose of 1 mg/kg S1. Thus a maximum effect was seen after 3 days, and 7 days postdose serum cholesterol was still reduced by 50%. In similar studies, analogues **1b**,**d**,**e** did not significantly reduce serum cholesterol concentrations even at 10 mg/kg iv. Squalestatin **1b** was examined further in single-dose lipid-lowering studies at doses of 30 and 50 mg/kg iv. Lipid levels were only reduced marginally even at the higher dose tested. When **1b** was dosed at 16.7 mg/kg iv three times a day for 3 days, lipids were significantly reduced by 62% 3 days postadministration and returned toward baseline 7 days postdose (Table 2). These data clearly suggested a shorter elimination half-life for **1b** and studies in perfused marmoset liver using [^{3}H]-S1[29] and [^{3}H]-**1b** established that the latter was abstracted by the liver more quickly than **1a** but was cleared more rapidly into the bile providing thus an explanation for their different lipid lowering properties.[30]

Table 2. Effect of **1b** on Serum Cholesterol Levels in Marmosets ($n = 6$) after a Single iv Dose

	cholesterol reduction (%)		
dose (mg/kg)	day 2	day 3	day 7
10	5 ± 5	2 ± 4	14 ± 6
30	30 ± 7	20 ± 10	16 ± 4
50	31 ± 5	21 ± 3	28 ± 8
16.7[a]	47 ± 4	62 ± 4	32 ± 5

[a] Animals were dosed three times a day for 3 days.

Conclusion

Squalestatin analogues modified at C3 were prepared and evaluated for their ability to inhibit rat SQS *in vitro*. Analogues incorporating neutral and basic groups at C3 were well tolerated while maintaining the C6 ester group found in the naturally occurring squalestatins. In the absence of the C6 ester group, however, similar modifications to the C3 carboxylic acid caused substantial reduction of inhibitory activity. Selected compounds were tested in rats for their ability to inhibit cholesterol biosynthesis 1 and 6 h postadministration. At a dose of 1 mg/kg iv S1 inhibits cholesterol biosynthesis in rats at least 8 h postadministration. Squalestatins **1b**−**f** possess inhibitory activities closely similar to S1 but contrasting abilities to inhibit cholesterol biosynthesis *in vivo*. Thus while these analogues showed complete inhibition of hepatic cholesterol biosynthesis at 1 h postadministration, none showed significant inhibition 6 h postadministration, even when dosed at 10 mg/kg iv. These data together with time-course studies with **1b** for inhibition of cholesterol biosynthesis clearly indicated that these C3-modified analogues of S1 possess shorter duration of effect *in vivo* which is reflected in their substantially reduced ability to lower serum cholesterol levels in marmosets. Thus compounds **1b**,**d**,**e** were ineffective at lowering serum cholesterol levels at a dose of 10 mg/kg iv. Signifigant cholesterol lowering for **1b** was observed only when this compound was dosed three times a day for 3 days. The C3-decarboxylated analogue[14] also has a much reduced ability to lower serum cholesterol levels in marmosets. It is clear that the C3 carboxylic acid plays a minor role in securing enzyme inhibitory activity in S1 analogues that have been modified at C3. However this functional group critically influences the *in vivo* profile of this series.

Experimental Section

Organic solutions were dried over $MgSO_4$, and column chromatography was performed on silica gel 60 (Merck, Art no. 9385). TLC was performed on Merck Kieselgel 60 F_{254} glass-backed plates; compounds were detected by spraying the plates with an ammonium molybdate−sulfuric acid solution and baking the plates. Analytical HPLC was performed on a Spherisorb 5 ODS-2 column (25 cm × 0.46 cm) using CH_3CN−H_2O containing 0.15 mL/L concentrated H_2SO_4 or 1 mL/L TFA as eluent, at a flow rate of 1.5 mL/min and detecting at 210 nm. Preparative HPLC was conducted on a Spherisorb 5 ODS-2 column (25 cm × 2 cm i.d.) using CH_3CN−H_2O containing 0.15 mL/L concentrated H_2SO_4 as eluent, at a flow rate of 15 mL/min and detecting at 210 nm. The appropriate fractions from each run were combined, the CH_3CN removed *in vacuo* (bath temperature <40 °C), and the remainder extracted with EtOAc. The combined extracts were washed with brine and evaporated, the residue was dissolved in H_2O/

dioxane and freeze-dried. IR spectra were recorded on a Nicolet 5SXC FTIR spectrometer. NMR spectra were recorded on Bruker AM 250 or Varian VXR 400 spectrometers using standard pulse sequences. Chemical shifts are expressed as parts per million downfield from internal tetramethylsilane. Negative ion fast-atom-bombardment mass spectrometry [MS-(FAB-ve)] was performed on a Finnigan MAT TSQ70B spectrometer and high-resolution negative ion liquid secondary ion mass spectrometry [HRMS(LSI-ve)] was performed on a VG Autospec spectrometer. Positive ammonia chemical ionisation (CI) and positive FAB mass spectrometry was conducted on a Hewlett-Packard Engine instrument. In order to increase aqueous solubility of the squalestatins tested *in vivo* the carboxylic acids were converted to their potassium salts.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(hydroxymethyl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]-octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Dimethyl Ester (5).** The acid **4** (500 mg, 0.69 mmol) and *N*-hydroxysuccinimide (88 mg, 0.76 mmol) in dry THF (7 mL) were treated at 20 °C with 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate (324 mg, 0.76 mmol). After 2 h further quantities of *N*-hydroxysuccinimide (40 mg, 0.35 mmol) and 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (147 mg, 0.35 mmol) were added. The mixture was stirred for a further 1.5 h when sodium borohydride (132 mg, 0.35 mmol) was added, followed after a further hour by 10% aqueous citric acid (50 mL). The mixture was extracted with ethyl acetate (3 × 50 mL), and the organic phase was washed with brine (4 × 30 mL), dried, and chromatographed on silica, eluting with EtOAc−cyclohexane (3:7, 1:1, 11:9) to give **5** as a white solid (270 mg, 55%): NMR ($CDCl_3$) δ includes 1.05 (d, 3H, J = 7 Hz, =CHCHCH_3), 2.10 (s, 3H, AcO), 2.69 (dd, 1H, J = 14 and 6 Hz, CH_2Ph), 3.74 (br, 2H, CH_2OH), 3.80 and 3.88 (2s, 3H each, $COOCH_3$), 4.03 (br, 1H, 7-H), 4.64 (t, 1H, J = 5 Hz, 3-H), 4.94 and 4.96 (2s, 1H each, =CH_2), 5.10 (d, 1H, J = 5 Hz, C*H*OAc), 5.74 (d, 1H, J = 16 Hz, OCOC*H*=CH), 5.83 (d, 1H, J = 2 Hz, 6-H), 6.85 (dd, 1H, J = 16 and 9 Hz, OCOCH=C*H*), 7.1−7.4 (m, 5H, Ph). Anal. ($C_{37}H_{52}O_{13}$) C, H.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(hydroxymethyl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]-octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate) (1b).** A solution of **5** (200 mg, 0.28 mmol) in 2,4,6-trimethylpyridine (10 mL) was heated to 45 °C under a slow stream of nitrogen in the presence of lithium iodide (759 mg, 5.67 mmol). After 7 d the solvent was evaporated under reduced pressure, brine (50 mL) was added, and the mixture was extracted with ether (3 × 50 mL). The combined organic phases were dried and purified by reverse-phase HPLC (eluting with 40−95% CH_3CN-H_2O containing 0.15 mL/L H_2SO_4) to give **1b** (90.2 mg, 48%) as a white solid: NMR (CD_3OD) δ includes 0.8−0.9 (m, 9H, CH_3), 1.05 (d, 3H, J = 7 Hz, =CHCHCH_3), 2.10 (s, 3H, AcO), 2.65 (dd, 1H, J = 14 and 6 Hz, CH_2Ph), 3.65 (br, 2H, CH_2OH), 4.0 (br, 1H, 7-H), 4.64 (br, 1H, 3-H), 4.94 and 4.96 (2s 1H each, =CH_2), 5.07 (d, 1H, J = 5 Hz, C*H*OAc), 5.80 (d, 1H, J = 16 Hz, OCOC*H*=CH), 6.34 (br, 1H, 6-H), 6.85 (dd, 1H, J = 16 and 9 Hz, OCOCH=C*H*), 7.1−7.3 (m, 5H, Ph); MS(FAB-ve) m/z 675 ($M - H)^-$. Anal. ($C_{35}H_{48}O_{13}\cdot 0.5H_2O$) C, H.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-3-Acetyl-1-[4-(acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Dimethyl Ester (7).** To a stirred solution of **6**[20] (1.0 g, 1.35 mmol) in $CHCl_3$ (3 mL) at 0 °C was added dropwise 66% hydriodic acid (1 mL). After the addition, the mixture was kept at 0 °C for 3 min, then diluted with water (50 mL), and extracted with ether (50 mL). The organic phase was washed successively with water, aqueous sodium thiosulfate, and brine, then dried, and evaporated. The residue was purified by chromatography on silica gel, eluting with EtOAc−petroleum ether (2:3) to give **7** (820 mg, 85%) as a pale foam: NMR ($CDCl_3$) δ includes 1.02 (d, 3H, J = 7.5 Hz, =CHCHCH_3), 2.10 (s, 3H, AcO), 2.28 (s, $COCH_3$), 3.25 (d, 1H, J = 2.5 Hz, 7-OH), 3.80 and 3.95 (2s, 3H each, $COOCH_3$), 4.05 (m, 1H, 7-H), 4.92 (s, 1H, 3-H), 4.98, 5.00 (2s, =CH_2), 5.62 (d, 1H, J = 5 Hz, C*H*OAc), 5.73 (m, 1H, 6-H), 5.76 (d, 1H, J = 16 Hz, OCOC*H*=CH), 6.86 (dd, 1H, J = 16 and 9 Hz, OCOCH=C*H*), 7.10−7.30 (m, 5H, Ph). Anal. ($C_{38}H_{52}O_{13}\cdot 0.5H_2O$) C, H.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-3-Acetyl-1-[4-(acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate) (1c).** To a stirred solution of **7** (760 mg, 1.06 mmol) in 2,4,6-collidine (38 mL) was added lithium iodide (1.52 g, 11.4 mmol). The mixture was stirred and heated at 40 °C under a stream of nitrogen for 24 h, then diluted with water (100 mL), acidified to pH1 with hydrochloric acid, and extracted with ether (2 × 100 mL). The organic extracts were washed with water, dried, and evaporated. The residue was purified by reverse-phase HPLC eluting with 60% CH_3CN-H_2O to give **1c** (200 mg, 27%) as a white solid: NMR (CD_3OD) δ includes 1.03 (d, 3H, J = 7.5 Hz, =CHCHCH_3), 2.10 (s, 3H, AcO), 2.21 (s, 3H, $COCH_3$), 4.01 (d, 1H, J = 2 Hz, 7-H), 5.00 (s, 1H, 3-H), 4.98, 5.01 (2s, =CH_2), 5.09 (d, 1H, J = 5 Hz, C*H*OAc), 5.8 (d, J = 16 Hz, OCOC*H*=CH), 6.28 (d, 1H, J = 2 Hz, 6-H), 6.85 (dd, 1H, J = 16 and 9 Hz, OCOCH=C*H*), 7.10−7.30 (m, 5H, Ph). Anal. ($C_{36}H_{48}O_{13}\cdot H_2O$) C, H.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-6-hydroxy-3-(hydroxymethyl)-4,7-bis[(2-methoxyethoxy)-methoxy]-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Dimethyl Ester (10).** To a solution of dry DMF (1.2 mL) in dry CH_2Cl_2 (15 mL) under nitrogen at 0 °C was added dropwise *via* syringe oxalyl chloride (1.46 mL, 16.7 mmol). Considerable gas evolution occurred, and a white solid was precipitated. Stirring at 0 °C was continued for 0.5 h before addition of **9** (7.5 g, 8.38 mmol) in dry THF (30 mL) and dry CH_3CN (15 mL). Stirring at 0 °C was continued for 1.5 h before addition of sodium borohydride (636 mg, 16.8 mmol) and dry DMF (20 mL). After further stirring at 0 °C for 1.5 h the mixture was allowed to warm to 20 °C and remain at this temperature for 18 h before dilution with water (250 mL), acidification with 2 M hydrochloric acid, and extraction with ether (2 × 250 mL). The extracts were washed with water (2 × 100 mL), dried, and chromatographed on silica gel eluting with EtOAc−petroleum ether (1:1) to give **10** (2.6 g, 35%): NMR ($CDCl_3$) δ includes 0.80−0.90 (m, 9H, CH_3), 1.04 (d, 3H, J = 7.5 Hz, =CHCHCH_3), 2.10 (s, 3H, AcO), 3.32 (s, 6H, CH_2OCH_3), 3.45−3.55 (m, 4H, CH_2OCH$_3$), 3.70 (s, 3H, $COOCH_3$), 3.82 (s, 3H, $COOCH_3$), 4.05 (d, 1H, J = 2.5 Hz, 7-H), 4.58−4.66 (m, 1H, 3-H), 5.70 (d, 1H, J = 15 Hz, OCOC*H*=CH), 6.65 (d, 1H, J = 2.5 Hz, 6-H), 6.82 (dd, 1H, J = 15 and 7 Hz, OCOCH=C*H*), 7.10−7.35 (m, 5H, Ph). Anal. ($C_{45}H_{68}O_{17}\cdot 0.5H_2O$) C, H.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-6-hydroxy-4,7-bis[(2-methoxyethoxy)methoxy]-3-(methoxymethyl)-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Dimethyl Ester (11).** To a stirred solution of **10** (2.6 g, 2.95 mmol) in dry DMF (36 mL) under nitrogen at 20 °C was added sodium hydride (60% oil dispersion; 195 mg, 4.87 mmol). After 15 min methyl iodide (3.4 mL) was added, and stirring under nitrogen at 20 °C was continued for 6 h before dilution with water (200 mL), acidification with 2 M hydrochloric acid, and extraction with ether (2 × 200 mL). The organic solution was washed with water, dried, evaporated, and purified by chromatography on silica gel eluting with EtOAc−petroleum ether (3:2) to afford **11** (1.9 g, 72%) as a pale gum: NMR ($CDCl_3$) δ includes 0.80−0.90 (m, 9H, CH_3), 1.03 (d, 3H, J = 7.5Hz, =CHCHCH_3), 2.10 (s, 3H, AcO), 3.31 (s, 3H, CH_2OCH_3), 3.35 (s, 6H, CH_2OCH_3), 3.69 (s, 3H, $COOCH_3$), 3.82 (s, 3H, $COOCH_3$), 4.08 (d, 1H, J = 2.5 Hz, 7-H), 4.60−4.70 (m, 1H, 3-H), 5.70 (d, 1H, J = 15 Hz, OCOC*H*=CH), 6.68 (d, 1H, J = 2.5 Hz, 6-H), 6.82 (dd, 1H, J = 15 and 7 Hz, OCOCH=C*H*), 7.10−7.35 (m, 5H, Ph).

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(methoxymethyl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]-octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate),**

4,5-Dimethyl Ester (12). A solution of **11** (345 mg, 0.39 mmol) in formic acid−water (3:1) was heated at 65 °C for 4 h. The solvents were removed *in vacuo*, and the residue was dissolved in toluene (50 mL) and re-evaporated to give **12** (270 mg, 97%) as a pale foam: NMR δ ($CDCl_3$) includes 0.80−0.90 (m, 9H, CH_3), 1.02 (d, 3H, J = 7.5 Hz, =CHCHCH_3), 2.08 (s, 3H, AcO), 3.30 (s, 3H, CH_2OCH_3), 3.80 (s, 3H, $COOCH_3$), 3.87 (s, 3H, $COOCH_3$), 4.00 (s, 1H, 7-H), 4.65−4.75 (m, 1H, 3-H), 4.95 and 4.99 (2s, 1H each, $=CH_2$), 5.10 (d, 1H, J = 5 Hz, C*H*OAc), 5.75 (d, 1H, J = 15 Hz, OCOC*H*=CH), 5.85 (s, 1H, 6-H), 6.85 (dd, 1H, J = 15 and 7 Hz, OCOCH=C*H*), 7.10−7.35 (m, 5H, Ph).

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(methoxymethyl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]-octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate) (1d).** To a stirred solution of **12** (230 mg, 0.32 mmol) in dry collidine (12 mL) under nitrogen was added lithium iodide (430 mg, 3.21 mmol). The mixture was heated under nitrogen at 45 °C for 18 h; then diluted with ether (75 mL); washed consecutively with 2 M hydrochloric acid (2 × 100 mL), water (20 mL), and saturated aqueous sodium thiosulfate (5 mL); then dried; evaporated; and purified by reverse phase column chromatography on silica gel (Whatman partisil prep P40; 100 mL) eluting with CH_3CN-H_2O (2:3) containing H_2SO_4 (0.15 mL/L) to afford **1d** (124 mg, 56%) as a colorless foam: NMR (CD_3OD) δ includes 0.80−0.90 (m, 9H, CH_3), 1.02 (d, 3H, J = 7.5 Hz, =CHCHCH_3), 2.10 (s, 3H, AcO), 3.45−3.55 (m, 2H, CH_2OCH$_3$), 3.99 (d, 1H, J = 2 Hz, 7-H), 4.75−4.82 (m, 1H, 3-H), 4.95 and 4.99 (2s, 1H each, $=CH_2$), 5.06 (d, 1H, J = 5 Hz, C*H*OAc), 5.80 (d, 1H, J = 15 Hz, OCOC*H*=CH), 6.30 (d, 1H, J = 2 Hz, 6-H), 6.85 (dd, 1H, J = 15 and 7 Hz, OCOCH=C*H*), 7.10−7.35 (m, 5H, Ph). Anal. ($C_{36}H_{50}O_{13}\cdot 0.5H_2O$) C, H.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-3,4,5-tricarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Bis(1,1-dimethylethyl) Ester, 3-Methyl Ester (13).** A stirred suspension of **1a** (15.6 g, 19.4 mmol) in methanol (1 L) was treated dropwise with concentrated hydrochloric acid (13 mL). The resulting clear solution was stirred at room temperature for 24 h. It was then treated with solid sodium bicarbonate (13.2 g), and most of the solvent was evaporated under reduced pressure. The residue was acidified with 2 M hydrochloric acid (500 mL) and extracted with EtOAc (1 L × 3). The organic extract was washed with water (1 L), dried, filtered, and evaporated. The residue was dissolved in dry toluene (130 mL), heated to 80 °C under nitrogen, and then treated dropwise with *N*,*N*-dimethylformamide di-*tert*-butyl acetal (38 mL, 0.15 mol) over 30 min. The reaction mixture was stirred at 80 °C for 3.25 h and then allowed to cool. It was diluted with ether (700 mL) and washed with brine (600 mL). The organic layer was dried, and the solvent was evaporated under reduced pressure. The residue subjected to flash column chromatography on silica gel eluting with EtOAc−cyclohexane (1:5, 1:1). The appropriate fractions were combined, and the solvent was evaporated to give **13** (5.93 g, 37%) as a foam: IR ν_{max} 3580, 3450, 1729, 1250, 1023 cm^{-1}; NMR ($CDCl_3$) δ includes 0.8−0.9 (m, 9H, $3CH_3$), 1.05 (d, 3H, J = 6.2 Hz, =CHCHCH_3), 1.48 and 1.6 (2s, 9H each, *t*-BuO), 2.1 (s, 3H, AcO), 2.71 (dd, 1H, J = 14 and 5 Hz, CH_2Ph), 2.96 (d, 1H, J = 2 Hz, 7-OH), 3.73 (s, 3H, $COOCH_3$), 4.1 (s, 1H, 4-OH), 4.05 (t, 1H, J = 2 Hz, 7-H), 4.97 (s, 2H, $=CH_2$), 5.1 (d, 1H, J = 5 Hz, C*H*OAc), 5.26 (s, 1H, 3-H), 5.77 (d, 1H, J = 16 Hz, OCOC*H*=CH), 5.97 (d, 1H, J = 2 Hz, 6-H), 6.91 (dd, 1H, J = 16 and 9 Hz, OCOCH=C*H*), 7.1−7.3 (m, 5H, Ph). Anal. ($C_{44}H_{64}O_{14}$) C, H.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-7-[[dimethyl(1,1-dimethylethyl)silyl]oxy]-4,6-dihydroxy-2,8-dioxabicyclo[3.2.1]octane-3,4,5-tricarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Bis(1,1-dimethylethyl) Ester, 3-Methyl Ester (14).** A solution of **13** (10.38 g, 12.7 mmol), *tert*-butyldimethylsilyl chloride (19.6 g, 130 mmol), and imidazole (17.7 g, 260 mmol) in dry DMF (26 mL) was stirred at 65 °C under nitrogen for 16.5 h and then partitioned between EtOAc (200 mL) and 2 M hydrochloric acid (200 mL). The aqueous phase was extracted with EtOAc (200 mL). The combined organic extracts were washed with 2 M hydrochloric acid (100 mL), water, and brine (2 × 100 mL each), dried, and evaporated to an oil. This was chromatographed on silica gel eluting with EtOAc−cyclohexane (1:4). The required fractions were combined and evaporated to give **14** (9.01 g, 76%) as a colorless gum: NMR ($CDCl_3$) δ includes 1.02 (d, 3H, J = 6 Hz, =CHCHCH_3), 1.4 and 1.65 (2s, 9H each, *t*-BuO), 2.1 (s, 3H, AcO), 3.73 (s, $COOCH_3$), 4.02 (s, 1H, 4-OH), 4.12 (d, 1H, J = 2 Hz, 7-H), 4.98 and 5.00 (2s, 1H each, $=CH_2$), 5.12 (d, 1H, J = 5 Hz, C*H*OAc), 5.28 (s, 1H, 3-H), 5.8 (d, 1H, J = 16 Hz, OCOC*H*=CH), 6.38 (d, 1H, J = 2 Hz, 6-H), 6.93 (dd, 1H, J = 16 and 9 Hz, OCOCH=C*H*), 7.1−7.3 (m, 5H, Ph).

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-7-[[dimethyl(1,1-dimethylethyl)silyl]oxy]-4,6-dihydroxy-2,8-dioxabicyclo[3.2.1]octane-3,4,5-tricarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Bis(1,1-dimethylethyl) Ester (15).** A solution of **14** (9.01 g, 9.7 mmol) in THF (450 mL) was treated with 0.1 M sodium hydroxide (116 mL) with stirring at room temperature. After 0.5 h the solution was evaporated to low volume and then partitioned between EtOAc (250 mL) and 2 M hydrochloric acid (500 mL). The aqueous phase was extracted with further EtOAc (2 × 250 mL). The combined extracts were washed with water and brine (2 × 250 mL each), dried, and evaporated to give **15** (8.7 g, 98%) as a white foam: IR ν_{max} ($CHBr_3$) 3444, 1776, 1730 cm^{-1}; NMR ($CDCl_3$) δ includes 1.02 (d, 3H, J = 6 Hz, =CHCHCH_3), 1.4 and 1.65 (2s, 9H each, *t*-BuO), 2.1 (s, 3H, AcO), 4.13 (d, 1H, J = 2 Hz, 7-H), 5.02 and 5.05 (2s, 1H each, $=CH_2$), 5.11 (d, 1H, J = 5 Hz, C*H*OAc), 5.22 (s, 1H, 3-H), 5.78 (d, 1H, J = 16 Hz, OCOC*H*=CH), 6.34 (d, 1H, J = 2 Hz, 6-H), 6.95 (dd, 1H, J = 16 and 9 Hz, OCOCH=C*H*), 7.1−7.3 (m, 5H, Ph); MS(FAB+ve) m/z 917 $(M + H)^+$; HRMS(FAB+ve) found 917.5082, calcd for $C_{49}H_{77}O_{14}Si$ 917.5073.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-7-[[dimethyl-(1,1-dimethylethyl)silyl]oxy]-4,6-dihydroxy-3-hydroxymethyl-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Bis(1,1-dimethylethyl) Ester (16).** A solution of **15** (5.89 g, 6.4 mmol), *N*-hydroxysuccinimide (0.8 g, 6.95 mmol), and *N*,*N*′-dicyclohexylcarbodiimide (1.46 g, 7.08 mmol) in THF (60 mL) was stirred at room temperature for 17 h. The resulting suspension was filtered, and the filtrate was evaporated to a white foam. This was dissolved in DMF (60 mL), stirred at room temperature, and treated with sodium borohydride (242 mg, 6.4 mmol). After 55 min the suspension was filtered. The filtrate was partitioned between EtOAc (500 mL) and 2 M hydrochloric acid (500 mL). The organic phase was washed with water (500 mL), saturated aqueous sodium bicarbonate, and brine (2 × 500 mL each), dried, and evaporated. The residue was chromatographed on silica gel eluting with EtOAc−cyclohexane (1:3) to give **16** (2.55 g, 44%) as a white foam: NMR ($CDCl_3$) δ includes 1.02 (d, 3H, J = 6 Hz, =CHCHCH_3), 1.4 and 1.61 (2s, 9H each, *t*-BuO), 2.1 (s, 3H, AcO), 3.58 and 3.76 (m, 2H, CH_2OH), 3.86 (s, 1H, 4-OH), 4.11 (d, 1H, J = 2 Hz, 7-H), 4.65 (dd, 1H, J = 4 and 6 Hz, 3-H), 4.98 and 5.0 (2s, $=CH_2$), 5.12 (d, 1H, J = 5 Hz, C*H*OAc), 5.8 (d, 1H, J = 16 Hz, OCOC*H*=CH), 6.32 (d, 1H, J = 2 Hz, 6-H), 6.92 (dd, 1H, J = 16 and 9 Hz, OCOCH=C*H*), 7.1−7.3 (m, 5H, Ph).

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-7-[[dimethyl(1,1-dimethylethyl)silyl]oxy]-4,6-dihydroxy-3-[[[(trifluoromethyl)sulfonyl]oxy]methyl]-2,8-dioxabicyclo-[3.2.1]octane-4,5-tricarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Bis(1,1-dimethylethyl) Ester (17).** A solution of **16** (1.19 g, 1.32 mmol) in dry CH_2Cl_2 (20 mL) was stirred under nitrogen in an ice bath and treated with 2,4,6-collidine (0.225 mL, 1.7 mmol) and then trifluoromethanesulfonic anhydride (0.28 mL, 1.7 mmol). After 45 min the yellow solution was diluted with CH_2Cl_2 (150 mL) and washed with 2 M hydrochloric acid (2 × 50 mL), water (100 mL),

saturated aqueous sodium bicarbonate (2 × 100 mL), and brine (2 × 100 mL), dried, and evaporated to give **17** (1.296 g, 95%) as a foam: IR ν_{max} ($CHBr_3$) 1729 cm^{-1}; NMR ($CDCl_3$) δ includes 1.02 (d, 3H, J = 6 Hz, =CHCHCH_3), 1.41 and 1.65 (2s, 9H each, *t*-BuO), 2.1 (s, 3H, AcO), 3.88 (s, 1H, 4-OH), 4.13 (br s, 1H, 7-H), 4.32 (dd, 1H, J = 2.5 and 11 Hz, CH_2OSO_2), 4.57 (dd, 1H, J = 7.5 and 11 Hz, 3-H), 4.95−5.05 (m, 3H, =CH_2 and one of CH_2OSO_2), 5.11 (d, 1H, J = 5 Hz, C*H*OAc), 5.80 (d, 1H, J = 16 Hz, OCOC*H*=CH), 6.30 (br s, 1H, 6-H), 6.95 (dd, 1H, J = 9 and 16 Hz, OCOCH=C*H*), 7.1−7.3 (m, 5H, Ph). Anal. ($C_{50}H_{77}F_3O_{15}SSi$) C, H, S.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(azidomethyl)-7-[[dimethyl(1,1-dimethylethyl)silyl]oxy]-4,6-dihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Bis(1,1-dimethylethyl) Ester (18).** A solution of **17** (584 mg, 0.56 mmol) in dry DMF (6 mL) was stirred at 20 °C and treated with sodium azide (112 mg, 1.72 mmol). After 4 h the mixture was partitioned between EtOAc (100 mL) and 2 M hydrochloric acid (50 mL). The organic phase was washed with 2 M hydrochloric acid (50 mL), then water, and brine (2 × 50 mL each), dried, and evaporated to give **18** (475 mg, 91%) as a colorless gum: IR ν_{max} ($CHBr_3$) 2104, 1728 cm^{-1}; NMR ($CDCl_3$) δ includes 1.02 (d, 3H, J = 6 Hz, =CHCHCH_3), 1.42 and 1.64 (2s, 9H each, *t*-BuO), 2.09 (s, 3H, AcO), 2.91 and 3.52 (2dd, 1H each, J = 2.5, 13 and 7.5, 13 Hz, CH_2N_3), 3.81 (s, 1H, 4-OH), 4.12 (br s, 1H, 7-H), 4.78 (dd, 1H, J = 2.5 and 7.5 Hz, 3-H), 4.97 and 5.0 (2s, 1H each, =CH_2), 5.11 (d, 1H, J = 5 Hz, C*H*OAc), 5.8 (d, 1H, J = 16 Hz, OCOC*H*=CH), 6.32 (br s, 1H, 6-H), 6.94 (dd, 1H, J = 9 and 16 Hz, OCOCH=C*H*), 7.1−7.35 (m, 5H, Ph). Anal. ($C_{49}H_{77}N_3O_{12}Si\cdot0.75C_4H_8O_2$) C, H, N.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(azidomethyl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Bis(1,1-dimethylethyl) Ester (19).** A solution of **18** (453 mg, 0.49 mmol) in THF (10 mL) was stirred at room temperature and treated with tetrabutylammonium fluoride (1 M, 0.5 mL). After 70 min the solution was evaporated to dryness. The residue was partitioned between EtOAc (100 mL) and water (50 mL). The organic phase was washed with water (50 mL) and brine (2 × 50 mL), dried, and evaporated to a colorless gum. This was chromatographed on silica gel eluting with EtOAc−cyclohexane (1:5). The required fractions were combined and evaporated to give **19** (363 mg, 91%) as a colorless gum: IR ν_{max} ($CHBr_3$) 2106, 1727 cm^{-1}; NMR ($CDCl_3$) δ includes 0.8−0.9 (m, 9H, CH_3), 1.05 (d, 3H, J = 6 Hz, =CHCHCH_3), 1.5 and 1.58 (2s, 9H each, *t*-BuO), 2.1 (s, 3H, AcO), 2.98 (d, 1H, J = 2 Hz, 7-OH), 3.12 and 3.55 (2dd, 1H each, J = 5, 12.5 and 7.5, 12.5 Hz, CH_2N_3), 3.85 (s, 1H, 4-OH), 4.06 (d, 1H, J = 2 Hz, 7-H), 4.65 (dd, 1H, J = 5 and 7.5 Hz, 3-H), 4.95 and 4.98 (2s, 1H each, =CH_2), 5.09 (d, 1H, J = 5 Hz, C*H*OAc), 5.78 (d, 1H, J 16 Hz, OCOC*H*=CH), 5.92 (d, 1H, J 2 Hz, 6-H), 6.91 (dd, 1H, J = 16 and 9 Hz, OCOCH=C*H*), 7.1−7.3 (m, 5H, Ph); MS(FAB+ve) m/z 814 $(M + H)^+$; HRMS-(FAB+ve) found 814.4457, calcd for $C_{43}H_{64}N_3O_{12}$ 814.4490.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl)-3-(aminomethyl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Bis(1,1-dimethylethyl) Ester (20).** A solution of **19** (283 mg, 0.35 mmol) in THF (3 mL) and triphenylphosphine (100 mg, 0.38 mmol) was stirred and treated with water (0.6 mL). Stirring was continued for 23 h at 44 °C, and then the solution was evaporated. The residue was dissolved in EtOAc (100 mL), and the solution was washed with saturated aqueous sodium bicarbonate (2 × 25 mL) and brine (2 × 25 mL), then dried, and evaporated to a foam which was chromatographed on silica eluting with MeOH−$CHCl_3$ (1:19, 1:9). The required fractions were combined and evaporated to give **20** (180 mg, 66%) as a colorless gum: NMR ($CDCl_3$) δ includes 0.8−0.9 (m, 9H, CH_3), 1.04 (d, 3H, J = 6 Hz, =CHCHCH_3), 1.50 and 1.56 (2s, 9H each, *t*-BuO), 2.1 (s, 3H, AcO), 2.92 (m, 2H, CH_2NH_2), 4.02 (d, 1H, J = 2 Hz, 7-H), 4.4 (t, 1H, J 4 Hz, 3-H), 4.96 and 4.97 (2s, 1H each, =CH_2), 5.09 (d, 1H, J = 5 Hz, C*H*OAc), 5.78 (d, 1H, J = 16 Hz, OCOC*H*=CH), 5.95 (d, 1H, J = 2 Hz, 6-H), 6.90 (dd, 1H, J = 16 and 9 Hz, OCOCH=C*H*), 7.1−7.3 (m, 5H, Ph).

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(aminomethyl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate) (1e).** A solution of **20** (170 mg, 0.22 mmol) in 6.5 M hydrogen chloride in dioxane (10 mL) was kept at room temperature for 8.25 h and then evaporated to a pale yellow solid. This was redissolved in 6.5 M hydrogen chloride in dioxane (5 mL), and the solution was kept at room temperature for 4 h and then evaporated. The residue was dissolved in ether, and the solution was evaporated to a pale yellow solid which was purified by preparative HPLC eluting with 70% CH_3CN-H_2O containing 0.1% TFA. The required fractions were combined and concentrated by rotary evaporation. A white solid precipitated out which was collected by filtration, washed with water, and dried under vacuum to provide **1e** (61 mg, 38%): NMR (DMSO-d_6) δ includes 0.75−0.88 (m, 9H, CH_3), 0.98 (d, 3H, J = 6 Hz, =CHCHCH_3), 2.1 (s, 3H, AcO), 2.85 (m, 2H, CH_2NH_2), 3.82 (dd, 1H, J = 2 and 5 Hz, 7-H), 4.58 (dd, 1H, J = 4 and 6 Hz, 3-H), 4.9 (br s, 2H, =CH_2), 4.98 (d, 1H, J = 5 Hz, C*H*OAc), 5.75 (d, 1H, J = 16 Hz, OCOC*H*=CH), 6.12 (d, 1H, J = 2 Hz, 6-H), 6.72 (dd, 1H, J = 9 and 16 Hz, OCOCH=C*H*), 7.12−7.3 (m, 5H, Ph); MS(CI) m/z 676 $(M + H)^+$. Anal. ($C_{35}H_{49}NO_{12}\cdot0.1C_2HF_3O_2\cdot1.2H_2O$) C, H, N.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-[(dimethylamino)methyl]-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate) (1f).** A suspension of **1e** (250 mg, 0.37 mmol) in CH_3CN (12 mL) was stirred at 20 °C under nitrogen and treated with glacial acetic acid (21 μL, 0.37 mmol), aqueous 40% formaldehyde (280 μL, 3.7 mmol), and sodium triacetoxyborohydride (235 mg, 1.1 mmol) to give a clear solution. After 2 h the solution was evaporated to dryness. The residue was stirred with EtOAc (10 mL) and filtered. The filtrate was evaporated to a yellow foam which was purified by preparative HPLC eluting with 70% CH_3CN/H_2O containing 0.1% TFA to give **1f** (189 mg, 56%) as a white solid: NMR (DMSO-d_6) δ includes 0.75−0.9 (m, 9H, CH_3), 0.99 (d, 3H, J = 6 Hz, =CHCHCH_3), 2.1 (s, 3H, AcO), 2.81 (s, 6H, $(CH_3)_2N$), 3.99 (br d, 1H, J = 3 Hz, 7-H), 4.7 (d, 1H, J = 9 Hz, 3-H), 4.92 (br s, 2H, =CH_2), 4.97 (d, 1H, J = 5 Hz, C*H*OAc), 5.8 (d, 1H, J = 16 Hz, OCOC*H*=CH), 6.2 (br s, 6-H), 6.77 (dd, 1H, J = 16 and 9 Hz, OCOCH=C*H*), 7.1−7.3 (m, 5H, Ph). Anal. ($C_{37}H_{53}NO_{12}\cdot1.5C_2HF_3O_2\cdot2H_2O$) C, H, N, F.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-[[(aminocarbonyl)amino]methyl]-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate) (1g).** A solution of **1e** (93 mg, 0.14 mmol), phenyl carbamate (123 mg, 0.9 mmol), and triethylamine (0.086 mL, 0.6 mmol) in THF (5 mL) was refluxed for 27 h and then evaporated to dryness. The residue was partitioned between EtOAc (25 mL) and 2 M hydrochloric acid (25 mL). The organic phase was washed with further acid (10 mL), water, and brine (2 × 25 mL each), dried, and evaporated to give a white solid which was purified by HPLC eluting with 60% CH_3CN-H_2O containing 0.15 mL/L H_2SO_4 to give **1g** as a white solid (38 mg, 38%): NMR (DMSO-d_6) 0.75−0.9 (m, 9H, CH_3), 0.98 (d, 3H, J = 7 Hz, =CHCHCH_3), 2.09 (s, 3H, AcO), 2.95 (m, 2H, CH_2N), 3.85 (dd, 1H, J = 2 and 5 Hz, 7-H), 4.38 (dd, 1H, J = 5 and 7 Hz, 3-H), 4.91 (br s, 2H, =CH_2), 4.96 (d, 1H, J = 5 Hz, C*H*OAc), 5.77 (d, 1H, J = 16 Hz, OCOC*H*=CH), 6.21 (br s, 1H, 6-H), 6.73 (dd, 1H, J = 16 and 8 Hz, OCOCH=C*H*), 7.12−7.32 (m, 5H, Ph); MS(FAB+ve) m/z 719 $(M + H)^+$. Anal. ($C_{36}H_{50}N_2O_{13}\cdot0.5H_2O$) C, H, N.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-[[(aminoiminomethyl)amino]methyl]-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), Hydrochloride Salt (1h).** A solution of **1e** (61.3 mg, 0.09 mmol) in MeOH (1 mL) was treated with

triethylamine (0.051 mL, 0.36 mmol), followed by aminoiminomethanesulfonic acid **21** (17 mg, 0.13 mmol), and the mixture was stirred at 20 °C for 18 h. The solvent was removed under reduced pressure, and the residue was partitioned between EtOAc and 2 M hydrochloric acid and brine, dried, and evaporated to dryness to give **1h** (62 mg, 91%) as a glass: NMR (DMSO-d_6) 0.75−0.9 (m, 9H, CH_3), 0.99 (d, 3H, J = 7 Hz, =CHCHCH_3), 2.10 (s, 3H, AcO), 3.18 (m, 2H, NCH_2), 3.89 (br d, 1H, J = 6 Hz, 7-H), 4.52 (br t, 1H, J = 4 Hz, 3-H), 4.90 (s, 2H, =CH_2), 4.96 (d, 1H, J = 5 Hz, CHOAc), 5.79 (d, 1H, J = 15 Hz, OCOCH=CH), 6.04 (d, 1H, J = 6 Hz, 7-OH), 6.08 (br s, 1H, 6-H), 6.75 (dd, 1H, J =15 and 8 Hz, OCOCH=CH), 7.15 (m, 5H, Ph). Anal. ($C_{36}H_{51}N_3O_{12}$·0.5HCl·H_2O) C, H, N, Cl.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl)-3-(aminomethyl)-7-[[dimethyl(1,1-dimethylethyl)silyl]oxy]-4,6-dihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Bis-(1,1-dimethylethyl) Ester (22).** A stirred solution of the azide **18** (655 mg, 0.71 mmol) in THF (7 mL) was treated with triphenylphosphine (202 mg, 0.77 mmol) and water (1.2 mL) at 45 °C. Aqueous sodium bicarbonate was added (25 mL), and the mixture was extracted with EtOAc (3 × 50 mL). The organic phase was washed with sodium bicarbonate (50 mL) and brine (2 × 50 mL), dried, and chromatographed eluting with MeOH−$CHCl_3$ (1:49) to give **22** (518 mg, 81%) as a white foam: ν_{max} ($CHBr_3$) 3400, 3250, 1728 cm^{-1}; NMR ($CDCl_3$) 0.99 (d, 3H, J = 7 Hz, =CHCHCH_3), 1.36 and 1.57 (2s, 9H each, t-BuO), 2.07 (s, 3H, AcO), 2.84 (br t, 2H, CH_2N), 4.07 (d, 1H, J = 2 Hz, 7-H), 4.45 (t, 1H, J = 4 Hz, 3-H), 4.93 and 4.97 (2s, =CH_2), 5.08 (d, 1H, J = 5 Hz, CHOAc), 5.75 (d, 1H, J = 16 Hz, OCOCH=CH), 6.29 (d, 1H, J = 2 Hz, 6-H), 6.90 (dd, 1H, J = 16 and 9 Hz, OCOCH=CH), 7.1−7.3 (m, 5H, Ph); MS-(FAB+ve) m/z 902 $(M + H)^+$; HRMS(FAB+ve) found 902.5482, calcd for $C_{49}H_{80}NO_{12}Si$ 902.5449.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl)-7-[[dimethyl(1,1-dimethylethyl)silyl]oxy]-4,6-dihydroxy-3-[[(trifluoromethyl)sulfonyl]aminomethyl]-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Bis(1,1-dimethylethyl) Ester (23).** A solution of the amine **22** (164 mg, 0.18 mmol) in CH_2Cl_2 (4 mL) was treated with 2,4,6-collidine (0.234 mL, 1.8 mmol) and then trifluoromethanesulfonic anhydride (0.2 mL, 1.2 mmol) with stirring in an ice bath under nitrogen. After 20 min the solution was diluted with EtOAc (100 mL), washed with 2 M hydrochloric acid (100, 50 mL) and brine (100, 50 mL), dried, and chromatographed eluting with EtOAc−cyclohexane (1:6). The required fractions were combined and evaporated to a colourless gum (87 mg, 46%): ν_{max} ($CHBr_3$) 1727, 1200, 1187 cm^{-1}; NMR ($CDCl_3$) δ includes 1.02 (d, 3H, J = 7 Hz, =CHCHCH_3), 1.39 and 1.63 (2s, 9H each, t-BuO), 2.11 (s, 3H, AcO), 3.11−3.36 (m, 2H, CH_2NH), 3.80 (s, 1H, 4-OH), 4.11 (d, 1H, J = 2 Hz, 7-H), 4.68 (dd, 1H, J = 4 and 8 Hz, 3-H), 4.99 and 5.00 (2s, 1H each, =CH_2), 5.12 (d, 1H, J = 5 Hz, CHOAc), 5.80 (m, 2H, NH and OCOCH=CH), 6.29 (d, 1H, J = 2 Hz, 6-H), 6.92 (dd, 1H, J = 16 and 9 Hz, OCOCH=CH), 7.1−7.3 (m, 5H, Ph); MS(CI) 1052 $(M + NH_4)^+$; HRMS(FAB+ve) found 1034.4932, calcd for $C_{50}H_{79}F_3NO_{14}SSi$ 1034.4942.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl)-3-[[[(trifluoromethyl)sulfonyl]amino]methyl]-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Bis(1,1-dimethylethyl) Ester (24).** A solution of the silyl ether **23** (80 mg, 0.08 mmol) in THF (2 mL) was stirred at 20 °C and treated with a THF solution of Bu_4NF (1 M, 0.077 mL). After 30 and 60 min further quantities of Bu_4NF (2 × 0.077 mL) were added. After 3 h the reaction mixture was evaporated to dryness, and the residue was dissolved in EtOAc. The solution was washed with water and brine, dried, and chromatographed eluting with EtOAc−cyclohexane (1:4). The required fractions were combined and evaporated to a white foam (58 mg, 79%): ν_{max} ($CHBr_3$) 1726, 1186 cm^{-1}; NMR ($CDCl_3$) δ includes 1.03 (d, 3H, J = 7 Hz, =CHCHCH_3), 1.48 and 1.58 (2s, 9H each, t-BuO), 2.11 (s, 3H, AcO), 2.88 (br, 1H, 7-OH), 3.25−3.40 (m, 2H, CH_2NH), 3.83 (s, 1H, 4-OH), 4.01 (br s, 1H, 7-H), 4.60 (dd, 1H, J = 12 and 6 Hz, 3-H), 4.98 and 5.00 (2s, 1H each, =CH_2), 5.09 (d, 1H, J = 5 Hz, CHOAc), 5.77 (d, 1H, J = 16 Hz, OCOCH=CH), 5.88 (m, 2H, 6-H, NH), 6.92 (dd, 1H, J = 16 and 9 Hz, OCOCH=CH), 7.1−7.3 (m, 5H, Ph). Anal. ($C_{44}H_{64}F_3NO_{14}S$) C, H, N, S.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α(2*E*,4*R**,6*R**),7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl)-3-[[[(trifluoromethyl)sulfonyl]amino]methyl]-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate) (1i).** A solution of the diester **24** (51 mg, 0.06 mmol) in 6.5 M hydrogen chloride in dioxane (3 mL) was left to stand at 20 °C for 8 h and then evaporated to dryness. The residue was purified by HPLC 65% CH_3CN−H_2O containing 0.15 mL/L H_2SO_4 to give **1i** (20 mg, 41%) as a white solid: NMR (CD_3OD) δ 1.02 (d, 3H, J = 7 Hz, =CHCHCH_3), 2.10 (s, 3H, AcO), 2.69 (dd, 1H, J = 14 and 6 Hz, CH_2Ph), 3.1−3.5 (m, 2H, CH_2NH), 4.00 (d, 1H, J = 2 Hz, 7-H), 4.65 (dd, 1H, J = 6 and 2 Hz, 3-H), 4.93 and 5.00 (2s, 1H each, =CH_2), 5.05 (d, 1H, J = 5 Hz, CHOAc), 5.80 (d, 1H, J = 16 Hz, OCOCH=CH), 6.30 (d, 1H, J = 2 Hz, 6-H), 6.85 (dd, 1H, J = 16 and 9 Hz, OCOCH=CH), 7.1−7.3 (m, 5H, Ph). Anal. ($C_{36}H_{48}F_3NO_{14}S$) C, H, N, S.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α,7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(aminomethyl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid (2e).** A solution of **1e** (100 mg, 0.15 mmol) in DMF (0.5 mL) was treated with *N*-methylhydroxylamine hydrochloride (28 mg, 0.33 mmol) and triethylamine (110 μL, 0.76 mmol). The mixture was stirred for 7 h at 20 °C, and then the solvent was removed by rotary evaporation. The resulting white solid was purified by preparative HPLC eluting with 35% CH_3CN−H_2O containing 0.1% TFA to give **2e** as a white solid (57 mg, 57%): NMR (DMSO-d_6) δ includes 0.78 (d, 3H, J = 6 Hz, CHCH_3), 2.1 (s, 3H, AcO), 3.9 (br d, 1H, J = 4 Hz, 7-H), 4.48 (br d, 1H, J = 6 Hz, 3-H), 4.85 and 4.91 (2s, 3H, =CH_2 and 6-H), 4.99 (d, 1H, J = 5 Hz, CHOAc), 7.1−7.3 (m, 5H, Ph); MS(FAB+ve) m/z 524 $(M + H)^+$. Anal. ($C_{25}H_{33}NO_{11}$·$C_2HF_3O_2$·1.5 H_2O) C, H, N.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α,7*β*]]-3-Acetyl-1-[(4-acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid (2c). 2c** was similarly prepared from **1c** (50 mg, 0.072 mmol) to give after HPLC purification (30−90% CH_3CN−H_2O containing 0.1% TFA) a white solid (16.6 mg, 43%): NMR (CD_3OD) δ includes 0.88 (d, 3H, J = 6.3 Hz, =CHCHCH_3), 2.10 (s, 3H, AcO), 2.20 (s, 3H, $COCH_3$), 4.04 (d, 1H, J = 2 Hz, 7-H), 4.96 (s, 1H, 3-H), 4.99 and 5.02 (2s, 1H each, =CH_2), 5.10 (m, 2H, CHOAc and 6-H), 7.1−7.3 (m, 5H, Ph); MS(FAB-ve) m/z 535 $(M - H)^-$; MS(TSP+ve) m/z 554 $(M + NH_4)^+$. Anal. ($C_{26}H_{32}O_{12}$·2H_2O) C, H.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α,7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(methoxymethyl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid (2d).** Similarly, **2d** was prepared from **1d** (41 mg, 0.06 mmol) to give after HPLC purification (35% CH_3CN−H_2O containing 0.1% TFA) a white solid (13.9 mg, 43%): NMR (D_2O) δ includes 0.89 (d, 3H, J = 7 Hz, =CHCHCH_3), 2.18 (s, 3H, AcO), 3.32 (s, 3H, CH_2OCH_3), 3.4−3.6 (m, 2H, CH_2OCH$_3$), 4.08 (d, 1H, J = 2 Hz, 7-H), 4.55−4.6 (m, 1H, 3-H), 4.93 (d, 1H, J = 5 Hz, CHOAc), 5.00 and 5.04 (2s, 1H each, =CH_2), 5.18 (d, 1H, J = 2 Hz, 6-H), 7.2−7.4 (m, 5H, Ph); MS(FAB-ve) m/z 537 $(M - H)^-$. Anal. ($C_{26}H_{34}O_{12}$·2H_2O) C, H.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α,7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-[[(aminocarbonyl)-amino]methyl]-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]-octane-4,5-dicarboxylic Acid (2g). 2g** was similarly prepared from **1g** (25 mg, 0.03 mmol) to give after HPLC purification (35% CH_3CN−H_2O containing 0.1% TFA) a white solid (2 mg, 12%): NMR (CD_3OD) δ includes 0.85 (d, 3H, J = 6 Hz, CHCH_3), 2.1 (s, 3H, AcO), 2.7 (m, 2H, CH_2N), 4.02 (d, 1H, J = 2 Hz, 7-H), 4.98 (s, 2H =CH_2), 5.07 (d, 1H, J = 5 Hz, CHOAc), 5.1 (br s, 1H, 6-H), 7.1−7.3 (m, 5H, Ph); MS(LSI-ve) m/z 565 $(M - H)^-$.

[1*S*-[1α(4*R,5*S**),3α,4*β*,5α,6α,7*β*]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl)-3-[[[(trifluoromethyl)sulfonyl]amino]methyl]-4,6,7-trihydroxy-2,8-dioxabicyclo-[3.2.1]octane-4,5-dicarboxylic Acid (2i).** Similarly, **2i** was prepared from **1i** (59 mg, 0.073 mmol) to give after HPLC purification (48% CH_3CN-H_2O containing 0.1% TFA) a white solid (25 mg, 52%): NMR (CD_3OD) includes 0.83 (d, 3H, J = 7 Hz, $CHCH_3$), 2.70 (dd, 1H, J = 14 and 6 Hz, CH_2Ph), 3.1−3.45 (m, 2H, CH_2NH), 4.01 (d, 1H, J = 2 Hz, 7-H), 4.55 (d, 1H, J = 10 Hz, 3-H), 4.95 and 4.99 (2s, 1H each, $=CH_2$), 5.07 (d, 1H, J = 5 Hz, $CHOAc$), 5.29 (d, 1H, J = 2 Hz, 6-H), 7.1−7.3 (m, 5H, Ph). Anal. ($C_{26}H_{32}F_3NO_{13}S\cdot 1.5H_2O$) C, H, N.

Animals. The rats used in these studies were juvenile, male CD (Charles River) weighing ∼150 g, fed on standard laboratory chow, and allowed access to water *ad libitum*. The marmosets used were adults between 15 and 28 months of age, and fed a standard primate diet supplemented with fruit.

Rat SQS Assay. The enzyme assay procedures measured the conversion of [2-^{14}C]FPP to [^{14}C]squalene. The assay methodology is described in detail in our earlier publication.[28]

Measurement of Cholesterol Biosynthesis *in Vivo* in Rats. Squalestatins were dosed iv to rats (n = 8 per group) followed immediately by intraperitoneal administration of [1-^{14}C]acetate (250 μCi/kg). One group of rats were killed after 1 h, and another after 6 h, the livers removed, and a sample of 0.5 g was saponified in alcoholic KOH at 80 °C for 1 h. After extraction [^{14}C]cholesterol was separated by HPLC using a Spherisorb ODS-2 column eluted with methanol−2-propanol (4:1). The [1-^{14}C]cholesterol was quantified using the procedure described previously.[5]

Time Course Studies in Rats. Compounds were administered iv at time 0, and the rats sacrificed 1, 2, 4, and 7 h later. One hour prior to sacrifice [1-^{14}C]acetate (250 μCi/kg) was administered ip. Rats were killed by CO_2 asphyxiation and the liver dissected free. A 0.5 g sample of liver was saponified and the [1-^{14}C]cholesterol separated and quantified as above.

Effect on Serum Cholesterol Levels in Marmosets. Marmosets of mixed sex (n = 6) were allocated to treatment groups on the basis of their fasting serum cholesterol levels such that the mean and distribution of serum cholesterol levels were similar for each group. The assay is described in detail in our earlier publication.[6] Squalestatins were converted to their potassium salts and dissolved in water before administration. One control group of animals (n = 6) were dosed with vehicle, and another group (n = 6) were dosed with S1 (1 mg/kg). Serum cholesterol levels were determined at days 0, 2, 3, and 7 postdosing. All results are expressed as the mean of the change in serum cholesterol levels from predose values (±SEM).

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