The Squalestatins: Synthesis and Biological Activity of Some C3-Modified Analogues; Replacement of a Carboxylic Acid or Methyl Ester with an Isoelectronic Heterocyclic Functionality

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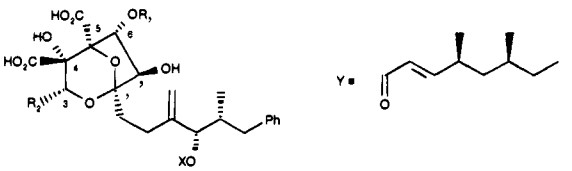
A series of squalestatins modified at the C3-position with a heterocyclic functionality was prepared and evaluated *in vitro* as inhibitors of squalene synthase (SQS). Structure–activity relationships for compounds with the 4,6-dimethyloctenoate at C6(S1 analogues) were different from those for analogues lacking the C6 ester (H1 analogues), with a greater dependence on the nature of the C3-substituent for the H1 series. Potent SQS inhibitory activity equivalent to that of H1 is retained by a C3-(tetrazol-5-yl) analogue, i.e., a carboxylic acid mimetic. The C3-methyl ester derivative is 10-fold less active than H1, and SQS inhibitory activity similar to that of the methyl ester was retained only in those C3-heterocycle-substituted H1 analogues for which electrostatic potential maps of the C3-substituent were closely similar to that of a methyl ester.

Introduction

Hypercholesterolemia is recognized as a major risk factor in the progression of coronary heart disease.[1,2] In humans 70% of body cholesterol is derived from *de novo* synthesis, and currently the most effective approach to lowering cholesterol levels is by inhibition of cholesterol biosynthesis. Inhibitors of the enzyme HMG-CoA reductase, such as lovastatin,[3] are effective therapeutic agents. Squalene synthase (SQS) (farnesyl-diphosphate:farnesyl-diphosphate farnesyltransferase, EC 2.5.1.21) is another key enzyme in the cholesterol biosynthetic pathway and catalyzes the two-step conversion of two molecules of farnesyl diphosphate (Fpp), via presqualene diphosphate, to squalene, which is the first biosynthetic step leading exclusively to sterols.[4] Agents which inhibit this enzyme are, therefore, particularly attractive, since nonsteroidal pathways should be minimally affected. Such inhibitors include synthetic substrate analogues of Fpp,[5] for which binding is strongly dependent on the presence of the diphosphate moiety or a close mimetic, and to a lesser extent on relatively nonspecific lipophilic interactions of the hydrocarbon chain.[6] In our own laboratories a recent screening program found potent activity in fermentation broths of the newly isolated *Phoma* sp. C2932 and resulted in the discovery of the novel family of squalestatins[7] **1**–**3** (Figure 1; denoted S1, S2, and H1[8]). These are potent inhibitors of mammalian SQS (IC_{50} = 12, 5, and 26 nM, respectively). Furthermore, when S1 is administered orally to marmosets for 7 days, 50% reduction in serum cholesterol levels is observed at a dose of 10 mg/kg/day.[7c] Equally, when S1 is administered i.v. for 7 days at 1 mg/kg/day, 86% reduction in serum cholesterol is observed; under the same dosing regime, S2 and H1 showed[9a] 59% and 56% reductions, respectively.



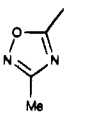
	X	R_1	R_2
1 (S1)	Ac	Y	HO_2C
2 (S2)	H	Y	HO_2C
3 (H1)	Ac	H	HO_2C
4a	Ac	Y	MeO_2C
4b	Ac	H	MeO_2C
5	Ac	H	
6	Ac	H	

Figure 1. Some of the squalestatins reported to date and C3-heterocycle-substituted derivatives initially targeted for synthesis herein.

The inhibition of SQS by S1 has been shown[10] to be competitive with respect to [^{3}H]Fpp, and S1 has been shown[10] to inhibit both partial reactions effected by the enzyme complex. It has been recognized[9b,10,11a] that there is a close structural similarity of S1 and H1 with presqualene diphosphate (the product of the first catalytic step) and Fpp, respectively.

Following our own publication on the squalestatins, the zaragozic acids were reported,[11] one of which (zaragozic acid A) is identical with S1. Some of our extensive studies[9] on the structure–activity relationships (SAR) in series derived from S1 and H1 have centered on the role of the carboxylic acids at the 3-, 4-, and 5-positions

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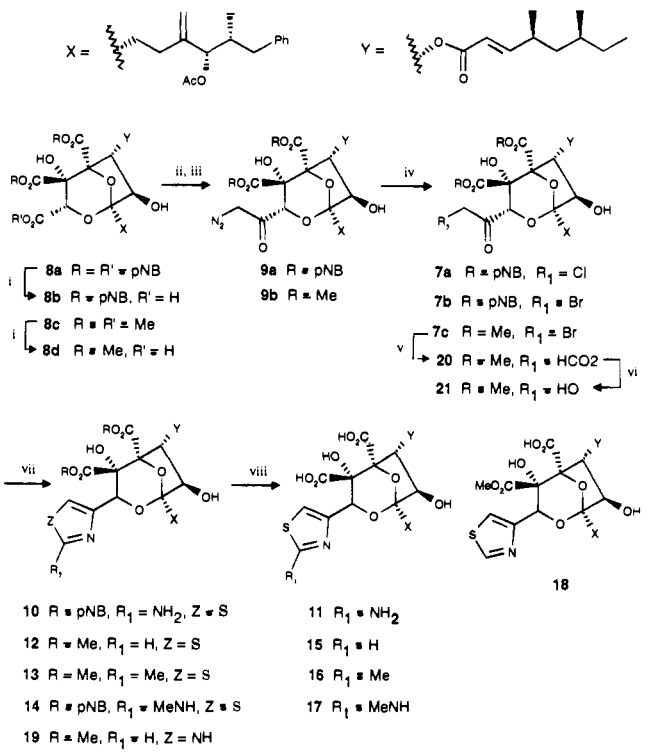
of the bicyclic core.[9b-e] It was demonstrated with series derived from S1 that, while modification of the acid at C5 was not tolerated, the acids at C4 and C3 were not essential for the retention of good SQS inhibitory activity.[9b] For example, the 3-methyl ester derivative **4a** of S1 retains potent SQS inhibitory activity. In addition, a wide range of neutral and basic functionality could be incorporated at C3.[9d] However, in series derived from H1, while reasonable activity could be retained for certain C3 substitutions (for example, the 3-methyl ester derivative **4b**, IC_{50} = 220 nM), activity was generally compromised and in many cases eradicated at all concentrations tested. In our search for novel inhibitors of mammalian SQS of the H1 type,[9f] we were looking to incorporate functionality which would be metabolically robust. In this paper we develop further chemistry for the selective derivatization of these complex molecules and describe the synthesis of a series of C3-heterocycle-substituted analogues of S1 and H1. Initial targets included the C3-(tetrazol-5-yl) and the C3-(3-methyl-1,2,4-oxadiazol-5-yl) H1 derivatives **5** and **6**, respectively, since these functionalities are reported[12,13] to mimic a carboxylic acid and methyl ester, respectively. The scope was widened subsequently to include more diverse unsubstituted and substituted heterocycles. We demonstrate that significant SQS inhibitory activity can be retained in such H1 derivatives and propose a rationalization of the SAR based on an examination of the electrostatic potential maps of the functionalities involved.

Chemistry

Heterocycles containing two heteroatoms were synthesized as shown in Scheme 1. The key suitably protected intermediate C3-halomethyl ketone derivatives **7a-c** were obtained[9d] by firstly activation of the 3-acids **8b,d** (obtained from the respective triesters **8a,c** by selective[9e] saponification) followed by treatment with diazomethane to give the diazomethyl ketones **9a,b**, respectively. Activation of the acid **8b** was effected with oxalyl chloride in the presence of catalytic DMF; the liberated HCl reacted *in situ* with **9a** to give the chloromethyl ketone **7a** as the major product, and **9a** was isolated only as the minor component. **9a** was converted into **7b** using 48% hydrobromic acid. For the synthesis of **7c**, activation of **8d** was effected with isobutyl chloroformate in the presence of *N*-methylmorpholine; this allowed efficient isolation of **9b**, which was converted into the desired **7c** using 48% hydrobromic acid. **7a-c** proved useful intermediates in heterocycle synthesis. Thus, reaction of **7a** with thiourea in ethanol gave the 3-(2-aminothiazol-4-yl) derivative **10**, which was deprotected with zinc/aqueous HCl in THF (method A) to give the S1 analogue **11** as its TFA salt following preparative HPLC. Analogous reaction[14] of **7c** with thioformamide[15] or thioacetamide, or of **7a** with *N*-methylthiourea afforded the methyl ester-protected forms of the 3-(thiazol-4-yl) derivative **12** and the 3-(2-methylthiazol-4-yl) derivative **13** or the pNB ester-protected form of the 3-[2-(methylamino)thiazol-4-yl] derivative **14**, respectively. Deprotection of **12** and **13** with lithium iodide in warm 2,4,6-collidine (method B) gave the corresponding dicarboxylic acids **15** and **16**, respectively, and deprotection of **14** (method A) gave **17**. Incomplete deprotection of **12** resulted in the 3-thiazole 4-methyl ester **18** also being isolated. Inverse HMBC studies to assign the positional isomeric identity of the

Scheme 1[a]

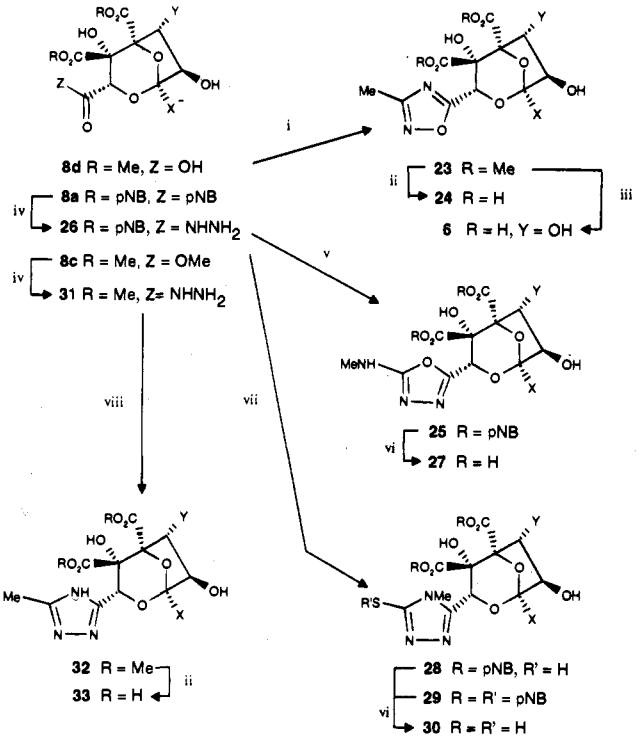


[a] Conditions (i) 0.1 M NaOH, THF; (ii) R = pNB, $(COCl)_2$, DMF; R = Me, iBuOCOCl, NMM, CH_2Cl_2; (iii) CH_2N_2, Et2O; (iv) for **7a** HCl generated *in situ* under conditions of step ii, for **7b,c** 48% HBr, $CHCl_3$; (v) $NaHCO_2$, $(EtO)_3CH$, Bu_4NBr, EtOAc/H_2O; (vi) SiO_2 chromatography; (vii) for thiazole synthesis $R_1C(S)NH_2$, EtOH; for imidazole synthesis HCO_2NH_4, EtOH; (vii) R = pNB; Zn/H_2O/THF, 2 M HCl (method A); R = Me, LiI, collidine (method B).

methyl ester were not successful, but by comparison with previous compounds,[9b] the methyl group in **18** could be confidently assigned as being at the 4-position. Access to the 3-(imidazol-4-yl) derivative **19** was not achieved by the literature method of heating **7c** with formamide[16a] but was prepared via 3-[(formyloxy)methyl ketone] analogue **20**, obtained by reaction of **7c** with sodium formate in the presence of triethyl orthoformate and tetrabutylammonium bromide under phase transfer conditions. The crude product[16b] was reacted directly with ammonium formate in refluxing ethanol. Under such conditions one may have expected the formation of the corresponding oxazol-5-yl derivative;[17] a formyl shift from O to N in the intermediate imino derivative of **20** accounts for the observed product **19**. The yield of **19** was poor (14%), and a number of unisolated minor side products were also produced in this reaction. The 3-(imidazol-4-yl) compound **19** was deprotected in two stages to its H1 derivative **22** (see below). The related S1 dicarboxylic acid analogue was not isolated.

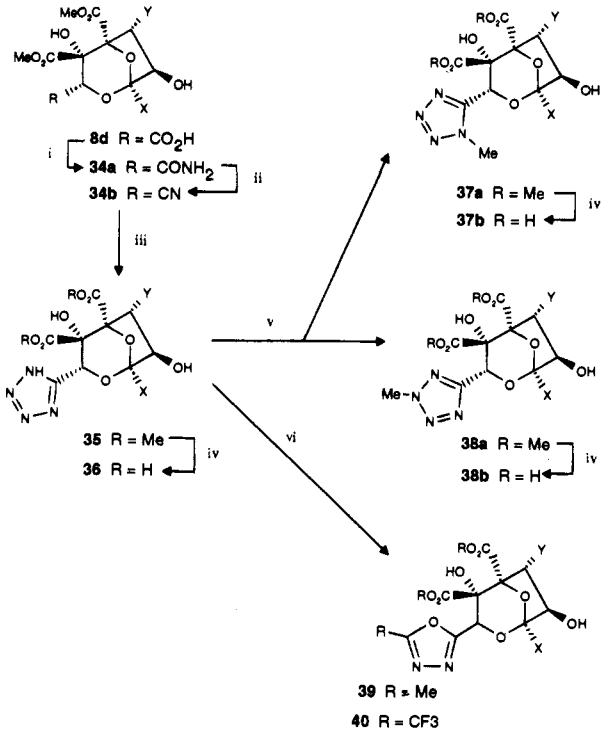
The recognized methyl ester-mimicking 3-methyl-1,2,4-oxadiazol-5-yl functionality[12b] was introduced[13] as shown in Scheme 2. Activation of the C3-carboxylic acid in diester **8d** under Vilsmeier conditions, followed by reaction with *N*-hydroxyacetamidine and subsequent cyclization in refluxing toluene, gave the oxadiazole **23** which was deprotected (method B) to the required diacid **24**; deprotection at a higher temperature provided the H1 analogue **6** in good yield. The 3-[2-(methylamino)-1,3,4-oxadiazol-5-yl]-S1 analogue **25** was obtained[18] (Scheme 2) in 23% yield from the 3-hydrazide **26** (obtained in turn from the 3-ester **8a** by reaction with

Scheme 2[a]



[a] Conditions: (i) $CH_3C(NOH)NH_2$, Et_3N, EtOH; (ii) method B; (iii) LiI, collidine, 65 °C, 16 h; (iv) H_2NNH_2, THF; (v) MeNCS, DCC, toluene; (vi) method A; (vii) MeNCS, DBU, nPrOH; (viii) $CH_3C(NH)OEt$·HCl, Et_3N, EtOH.

Scheme 3[a]



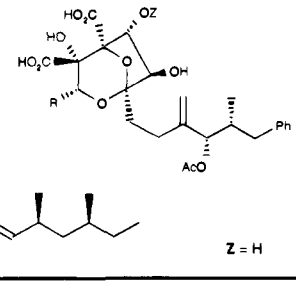
[a] Conditions: (i) 1. DMF, $(COCl)_2$, CH_2Cl_2, 2. NH3, THF, CH_3CN, CH_2Cl_2; (ii) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2; (iii) NaN_3, Et_3N·HCl, DMF; (iv) method B; (v) MeI, Na_2CO_3, DMF; (vi) $(R_2CO)_2O$, CH_2Cl_2.

hydrazine) by treating with methyl thiocyanate in toluene in the presence of *N,N'*-dicyclohexylcarbodiimide;[18] deprotection (method A) gave the S1 analogue **27**. Altering the conditions of this reaction by using DBU in *n*-propanol with methyl thiocyanate allowed conversion[19] of **26** into **28** (11%) and **29** (20%). **29** may arise as a result of an intermolecular *p*-nitrobenzyl group migration from an acid to the sulfur of the heterocycle. Deprotection (method A) of either of these gave the desired 3-(4-methyl-3-thiotriazol-5-yl)-S1 derivative **30** in good yield. Selective reaction of **8c** with hydrazine (Scheme 2) gave 3-hydrazide derivative **31**; this was converted[20] using ethyl acetimidate in refluxing ethanol into the 3-(2-methyl-1,2,4-triazol-5-yl) derivative **32**, which was deprotected (method B) to give target compound **33**.

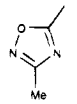
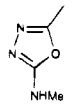
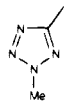
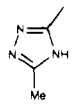
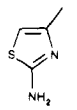
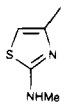
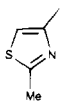
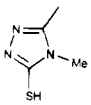
The well-known[12a] carboxylic acid mimetic 1*H*-tetrazole was introduced into the S1 molecule at C3 as shown in Scheme 3. Activation of **8d** and reaction with ammonia gave 3-carboxamido derivative **34a**; dehydration of this with trifluoroacetic anhydride gave the required 3-nitrile intermediate **34b**. This was then reacted with sodium azide in hot DMF in the presence of triethylammonium chloride to give the desired protected 3-(tetrazol-5-yl) compound **35**, which was subjected to selective removal of 4- and 5-carboxyl protecting groups (method B) to provide the S1 analogue **36** in the usual way. Reaction of tetrazole **35** with methyl iodide gave the isomeric protected 1- and 2-methyltetrazol-5-yl derivatives **37a** (37%) and **38a** (22%). The 1- and 2-methyltetrazole isomers were readily distinguished[12a,21] by comparison of their ^1H NMR spectra; the signal for the *N*-Me protons in the 2-methyl isomer is at lower field by 0.15−0.2 ppm than the corresponding signal in the 1-methyl isomer. Each of these was then subjected to deprotection (method B) to give the S1 derivatives **37b** and **38b**, respectively. Access[22] to the 2-methyl- and 2-(trifluoromethyl)-1,3,4-oxadiazole systems (**39** and **40**, respectively; Scheme 3) in protected form was achieved by reaction of 3-(tetrazol-5-yl) diester **35** with either acetic or trifluoroacetic anhydride, respectively. However, their poor chemical stability during deprotection of the carboxylic esters prevented isolation of the corresponding dicarboxylic acids.

Each of the C3-heterocyclic S1 analogues obtained above was converted to its H1 counterpart by reaction with *N*-methylhydroxylamine hydrochloride and triethylamine in DMF (method C);[9f] thus, tetrazole **36**, thiazole **15**, methylthiazole **16**, (methylamino)thiazole **17**, (methylamino)oxadiazole **27**, and 4-methyl-3-thio-1,2,4-triazole **30** S1 analogues were converted to H1 derivatives **5**, and **41**−**45**, respectively (Table 1). Attempted deprotection in this manner of the aminothiazole **11** gave an intractable residue which could not be purified. Exceptions to this direct methodology were the H1 analogues **46**−**48** and **22**, substituted at C3 with 1-methyltetrazole, 2-methyltetrazole, 3-methyltriazole, and imidazole, respectively; these were obtained (Scheme 4) from the respective protected S1 intermediates **37a**, **38a**, **32**, and **19** by initial deprotection at C6 (method C) to give dimethyl esters **49**−**52**, respectively. De-esterification (method B) of 1-methyltetrazole intermediate **49** gave predominantly the monomethyl ester **53** (by analogy with previous studies,[9b] substituted at C4), which required further reaction under more forcing conditions to give the required **46**. Removal of methyl ester protecting groups (method B) from the 2-methyltetrazole intermediate **50** gave a mixture of three components identified as the required compound **47** together with the monomethyl derivative **54** and the desacetyl analogue **55** (Figure 2). Selective de-esteri-

Table 1. *In Vitro* Inhibitory Activities vs Rat SQS for the S1 and H1 Compounds Substituted with a Heterocyclic Moiety at C3[d]



Z = H

R	Compound	IC_{50} (nM)	Compound	IC_{50} (nM)
HO_2C	1	12[a,8]	3	26[a,8]
MeO_2C	4a	7[8]	4b	220[8]
	36	4	5	25
	24	72	6	310
	27	10	44	147
	38a	43	47	156
	33	nt[b]	48	312
	15	92	41	505
	11	63	np[c]	np[c]
	np[c]	np[c]	22	785
	17	146	43	1181
	16	357	42	2875
	30	172	45	1940
	37a	57	46	442

[a] IC_{50} values quoted for H1 and S1 are those obtained in parellel assays with the C3-modified derivatives quoted in the table. [b] nt = not tested. [c] np = not prepared. [d] 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.

fication of **51** gave **48** and of **52** gave a mixture of H1 derivative **22** together with its 4-methyl ester **56**.

Electrostatic Potential (ESP) Maps. Structures were built in Spartan[23a] and minimized. To simplify calculations, the bulk of the bicyclic ketal was replaced by a methyl group. ESP maps were generated on the van der Waals surface using AM1 geometrical optimization[23b] and color coded from blue to red for the energy range of −10−10 kcal/mol (Figures 4−6). When appropriate, calculations were carried out for all the tautomers. To check whether the replacement of the bicyclic ketal with a methyl group was a valid approach, ESP maps for the bicyclic ketal having a carboxylic acid and a 2-methyltetrazole at C3 were also generated. When compared with the corresponding simplified ESP maps, these showed a similar pattern at the C3 position, hence verifying the approach (results not shown).

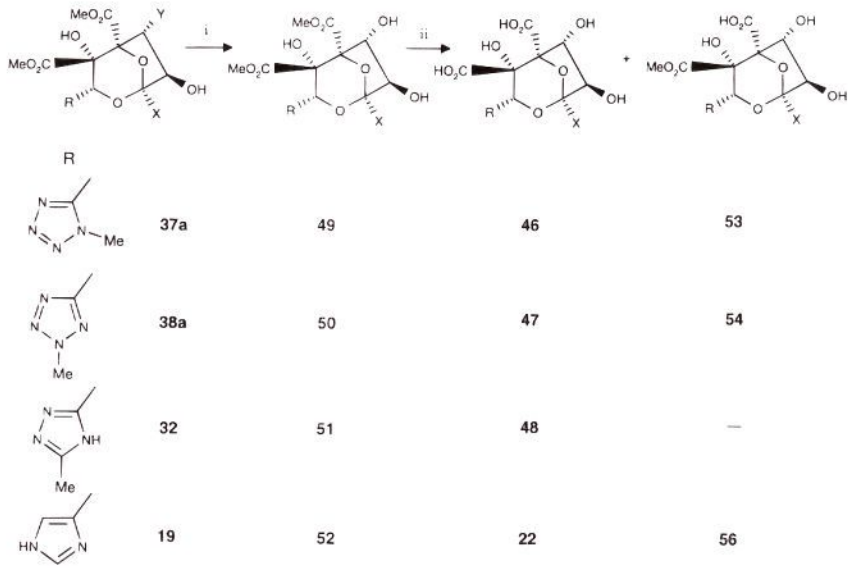
Results and Discussion

The *in vitro* inhibitory activities vs rat SQS for the S1 and H1 compounds substituted with a heterocyclic moiety at C3 are shown in Table 1. The enzyme preparation and assay procedures used in this study were the same as those described in our earlier publication.[7c] The most notable compounds in these series are the 3-(tetrazol-5-yl) derivatives **5** and **36** (IC_{50} = 25 and 4 nM), respectively, which are equipotent to H1 (3) and S1 (**1**), respectively, presumably due to the well-recognized similarity of the tetrazole group to a carboxylic acid. In addition, substitution of the 3-carboxylic acid in S1 by a variety of heterocyclic substituents can be well-tolerated, which is in agreement with previous findings[9b−e] with other C3 modifications. In contrast to previous observations,[9b−e] however, a wider spread of IC_{50}'s is observed among S1 analogues substituted at C3 with a heterocyclic moiety.

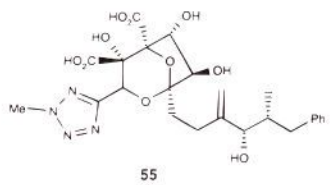
These studies established that significant SQS inhibitory activity could be retained in certain C3-heterocyclic derivatives of H1 but that there is a particularly strong dependence of activity on the nature of the C3-heterocyclic substituent. For example, substituting nitrogen for oxygen in the heterocycle of the oxadiazole **6** (IC_{50} = 310 nM) gave the equipotent 3-(3-methyl-1,2,4-triazol-5-yl) derivative **48** (IC_{50} = 312 nM), while additional modifications to the triazole moiety to give the 1-methyl-3-thio analogue **45** resulted in eradication of significant activity against rat SQS. Results in the 3-(thiazol-4-yl) series indicated that while the parent **41** possessed some activity (IC_{50} = 505 nM), those thiazoles bearing a 2-methyl- or 2-methyl amino-substituent (**42** and **43**) possessed reduced activity (IC_{50}'s > 1 μM). The presence of a substituent on a heterocyclic ring, however, does not preclude activity *per se*, as the above active substituted heterocyclic analogues (e.g., **6** and **48**) indicate. Results in the 3-(thiazol-4-yl) series prompted greater attention on the synthesis of parent heterocyclic systems of this type. However, the imidazol-4-yl compound **22** proved somewhat less active (IC_{50} = 785 nM), and oxazol-4-yl and oxazol-2-yl systems proved synthetically elusive.

In an attempt to understand the key role of the heterocyclic substituent in determining activity in the H1 series, the electrostatic potential map of each substituent was examined. As a result we can propose a possible model for the mode of interaction of H1 with SQS in the region of the C3-substituent (Figure 3). All ESP maps generated for parent and substituted heterocycles were compared with those for acetic acid (Figure 4) and methyl acetate (Figure 5), both of which characteristically show a negative region on either side

Scheme 4[a]



[a] Conditions: (i) MeNHOH·HCl, Et_3N, DMF; (ii) method B.



55

Figure 2. Desacetyl analogue **55** obtained as a component of the mixture due to deprotection of 2-methyltetrazol-3-yl intermediate **50**.

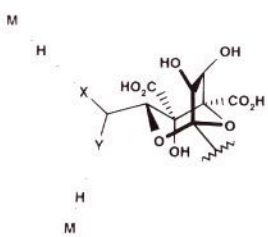


Figure 3. Possible model for the mode of interaction of H1 with SQS in the region of the C3-substituent.

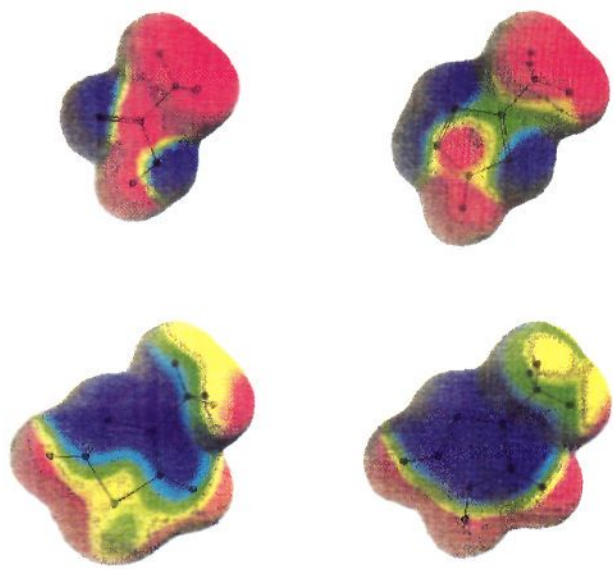


Figure 4. Electrostatic potential maps (ESP's) of model functionality for the highly active compounds H1 (**3**) and **5** (represented by acetic acid, top left, and 5-methyl-2*H*-tetrazole, top right, respectively) and two compounds, **41** and **22** (represented by 4-methylthiazole, bottom left, and 4-methylimidazole, bottom right, respectively), having greatly reduced activities, demonstrating the key features of the ESP required for good activity vs rat SQS.

of the van der Waals surface. The carboxylic acid in its unionized form can thus form two (H-bond) interactions at the enzyme active site (Figure 3). The methyl ester can also form two similar interactions (Figure 3), but the overall binding is diminished. The latter may be due to the inability of the ester to ionize; the deprotonated acid may be just a very good bidentate acceptor as would be the ionized tetrazole. Alternatively, the steric bulk of the methyl group in the ester may adversely affect the approach of the functional group to form the two interactions in Figure 3.

The ionizable nature of the tetrazole group and the nature of the ESP map of its 3*H* tautomer (Figure 4) possessing the two negative regions show, as expected, that this is a very good mimic of the carboxylic acid which is reflected in an IC_{50} value for **5** similar to that for H1. The ESP maps for 4-methylthiazole and -imidazole (Figure 4) have only one negative region, so compounds **41** and **22**, respectively, can only form one of the two interactions in Figure 3, and this is reflected in their reduced activities relative to methyl ester **4b**. This activity is further reduced by incorporation of steric bulk as present in compounds **42** and **43**. Compound **45** can be considered in the same way. Those compounds which show similar levels of activity to **4b** (i.e, **6**, **44**, **47**, and **48**), however, have heterocycles at C3 with ESP maps closely similar (Figure 5) to that for methyl acetate and so can, therefore, form the two interactions depicted in Figure 3.

The ESP map for 1,5-dimethyltetrazole (Figure 6) has an extensive negative region, encompassing those negative regions present in methyl acetate. Thus, 1-methyltetrazol-5-yl derivative **46** retains the capacity to form the two interactions with the enzyme, while the reduced activity relative to carboxylic acid **3**, methyl ester **4b**, and 2-methyltetrazol-5-yl analogue **47** may reflect an adverse steric effect of the 1-methyl substituent, pos-

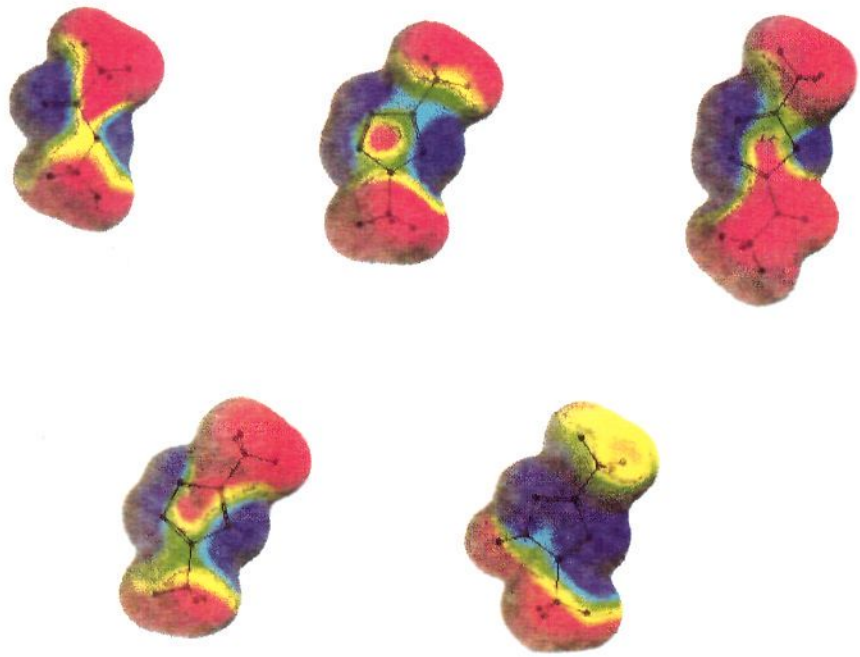


Figure 5. ESP maps for heterocyclic functionality (clockwise from top center, 3,5-dimethyl-1,2,4-oxadiazole, 2-(methylamino)-5-methyl-1,3,4-oxadiazole, 3,5-dimethyl-1,2,4-triazole, and 2,5-dimethyltetrazole) representing the C3-substituent of compounds **6**, **44**, **47**, and **48**, respectively, possessing similar levels of activity to **4b** (represented by methyl acetate, top left), capable of forming the two interactions depicted in Figure 3.

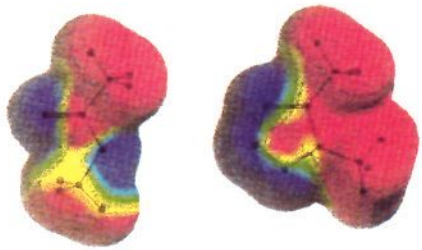


Figure 6. ESP map for 1,5-dimethyltetrazole (right), showing the extensive negative region encompassing those negative regions present in methyl acetate (left).

sibly on the torsional angle between the aromatic heterocycle and the bicyclic core.

A similar replacement of carboxylic acid functionality (of *N*-arylanthranilic acid anti-inflammatory agents) with acidic heterocyclic groups has been recently published[12b] showing good retention of biological activity. Bioisosteric replacement of esters with oxadiazole or certain triazoles has also been reported[12c,13] and rationalized in terms of electrostatic potential maps. Our own results deal with the ESP maps of a different class of heterocycles and provide evidence of a generalized predictor for mimetics of carboxylic acids and methyl esters.

A correlation between ESP map and activity in the C3-heterocycle-substituted S1 analogues is not apparent. Compounds **16**, **17**, and **30** are somewhat less active than **1** and **4a**, in contrast to our previous publications[9b-e] where a wide range of substituents (neutral, acidic, and basic) is well-tolerated at C3 of S1, and dependence of good SQS inhibitory activity on the C3-substituent is therefore not as great.

That good activity is only retained in those C3-heterocycle-substituted analogues of H1 possessing a C3-substituent which closely mimics an acid or its methyl ester suggests that, in such potential mimetics of Fpp, there is a key contribution to tight binding with SQS by the C3-substituent. This is in good agreement with the finding[5] that binding of substrate analogues of Fpp to SQS is particularly strongly dependent on the nature of the diphosphate mimetic. That there is a lesser dependence on the nature of the C3-heterocyclic group in presqualene diphosphate-like[10] S1 derivatives supports[9] a more dominant role of the lipophilic side chains in this molecule, in its interaction with SQS.

Conclusion

A series of analogues of the novel fungal metabolites S1 and H1 have been synthesized possessing heterocyclic functionality at C3 in place of the carboxylic acid. A number of these show potent inhibition of rat SQS *in vitro*. There is, as in previous studies, a greater dependence of activity on the C3-substituent in the H1 series than in the S1 series. The electrostatic potential maps of these heterocyclic substituents have been generated, and we can therefore propose the 3-methyl-1,2,4-oxadiazol-5-yl, 2-(methylamino)-1,3,4-oxadiazol-5-yl, 3-methyl-1,2,4-triazol-5-yl, and 2-methyltetrazol-5-yl groups as potentially suitable replacements for a methoxycarbonyl group. In the C3-heterocycle-substituted H1 series, potent activity similar to that for H1 vs rat SQS is retained by the tetrazole analogue, which closely mimics the parent carboxylic acid. Inhibitory activity similar to that for the methyl ester derivative (which is 10-fold less active than the parent acid) was retained only in those C3-heterocycle-substituted H1 analogues for which ESP maps of the C3-substituent were closely similar to that of a methyl ester.

Experimental Section

^{1}H NMR spectra were recorded on a Bruker AM250 Fourier transform spectrometer with Me_4Si as internal standard; chemical shifts are reported in parts per million (δ), and signals are quoted as s, d, t, m, or br. UV spectra were obtained on a Hewlett Packard 8452A diode array spectrophotometer, and a Nicolet 5SXC FTIR spectrometer was used

to obtain IR spectra. Mass spectra were recorded using either a Finnegan 4600, Finnegan TSQ-700, Hewlett Packard Engine, or VG Autospec instrument for electron impact (EI), chemical ionization (CI), or thermospray (TSP) modes of ionization or using a Bio-Ion 20 instrument for plasma desorption time of flight (TOF); liquid secondary ion mass spectra (LSIMS) were recorded using the VG Autospec spectrometer. Organic solutions were dried over $MgSO_4$. TLC was performed on Merck Kieselgel 60 F_{254} glass-backed plates; compounds were detected by dipping plates in ammonium molybdate–sulfuric acid solution and heating the wet plates. Merck Kieselgel 9385 silica gel was used for preparative column chromatography with flash elution. HPLC was performed on Spherisorb 5 μm ODS-2, 25 cm × 20 mm (preparative) or 25 cm × 4.6 mm (analytical), with a mobile phase consisting of gradient mixtures of A = H_2O containing H_2SO_4 or TFA (150 μL/L) and B = acetonitrile containing 5% A, detection at 210 nm. Flow rates were 15 mL/min for preparative and 1.5 mL/min for analytical HPLC. Appropriate fractions from preparative HPLC were combined, CH_3CN was removed *in vacuo* (bath $T \leq 40$ °C), and the remaining aqueous phase was saturated with ammonium chloride and extracted with EtOAc. Extracts were evaporated; the residue was dissolved in water/dioxane and freeze-dried. Capillary zone electrophoresis (CZE) analysis was performed using a 50 μm fused silica column, 72 cm total length, UV detector at 210 nm, 20 kV at 30 °C, pH 7 (attained by 50 mM phosphate + 50 mM borate buffer). To increase solubility in water, the S1 derivatives described were converted to their dipotassium salts (or tripotassium salt in the case of the tetrazole **36**) prior to testing in the *in vitro* assay.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-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), 3,4,5-Tris(4-nitrobenzyl) Ester (8a).** Squalestatin 1 (6.9 g, 10 mmol) in DMF (100 mL) and triethylamine (5.6 mL, 40 mmol) was treated with 4-nitrobenzyl bromide (7.65 g, 35 mmol). A further portion of DMF (20 mL) was added and the mixture stirred at 20 °C for 18 h. A further portion of 4-nitrobenzyl (pNB) bromide (1.08 g, 5 mmol) was added and stirring continued for a further 66 h. The mixture was then diluted with EtOAc and washed with 2 M HCl. The aqueous layer was extracted with EtOAc (3×), and combined organics were washed with 2 M HCl (3×) and then dried and evaporated. The residue was purified by flash column chromatography with a gradient of EtOAc/cyclohexane (3:7–1:1), and appropriate fractions were combined and evaporated to give **8a** (6.24 g, 57%) as a white foam: IR ($CHBr_3$) 1772, 1732, 1608, 1523, 1347 cm^{-1}; ^1H NMR δ 8.2–8.1 (6 H, m, $NO_2C_6H_4$), 7.6–7.4 (6 H, m, $NO_2C_6H_4$), 7.28–7.07 (5 H, m, Ph), 6.87 (1 H, m, J = 15, 9 Hz, OCOCH=C*H*), 5.87 (1 H, d, J = 2 Hz, H6), 5.49 (1 H, d, J = 15 Hz, OCOC*H*=CH), 5.36 (1 H, s, H3), 5.3–5.1 (6 H, m, $NO_2C_4H_6CH_2$), 5.06 (1 H, d, J = 5 Hz, C*H*OAc), 5.00, 4.98 (2 H, 2s, $=CH_2$), 4.05 (1 H, br, H7), 3.91 (1 H, s, OH-4), 3.28 (1 H, d, J = 2.5 Hz, OH-7), 2.66 (1 H, dd, J = 14, 6 Hz, $PhCH_2$), 2.09 (3H, s, OAc), 1.02 (3 H, d, J = 7 Hz, $CHCH_3$). Anal. ($C_{56}H_{61}N_3O_{20}$) C, H, N.

[1*S*-[1α(4*R,5*S**),3α,4β,5β,6β(2*E*,4*R**,6*R**),7β]]-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(4-nitrobenzyl) Ester (8b).** To a stirred solution of **8a** (105 mg, 0.096 mmol) in THF (1 mL) was added dropwise over 5 min aqueous NaOH (0.1 M, 0.097 mL). The mixture was stirred for 21 h and then diluted with water (10 mL), acidified with 2 M HCl (2 mL), and extracted with EtOAc (2 × 30 mL). Combined extracts were washed with brine (2 × 10 mL), dried, filtered, and evaporated. The residue was chromatographed with $CHCl_3$/MeOH (9:1) to give **8b** (81 mg, 89%)as a white solid: ^1H NMR (CD_3OD) δ 8.12–8.03 (4 H, m, $NO_2C_6H_4$), 7.60 (2 H, d, J = 10 Hz, $NO_2C_6H_4$), 7.48 (2 H, d, J = 10 Hz, $NO_2C_6H_4$), 7.15 (5 H, m, Ph), 6.75 (1 H, dd, J = 16, 9 Hz, OCOCH=C*H*), 6.35 (1 H, d, J = 2 Hz, H6), 5.60 (1 H, d, J = 16 Hz, OCOC*H*=CH), 5.3–4.6 (8 H, m, $=CH_2$, C*H*OAc, H3, $NO_2C_6H_4CH_2$), 4.05 (1 H, d, J = 2 Hz, H7), 2.65 (1 H, m, $PhCH_2$), 2.05 (3 H, s, OAc), 0.95 (3 H, d, J = 7 Hz, $CHCH_3$); MS (CI) m/z 964 $(MH)^+$. Anal. ($C_{49}H_{56}N_2O_{18}$·2.25H_2O) C, H, N.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(diazoacetyl)-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 (9b).** To a stirred solution of [1*S*-[1α(4*R**,5*S**),3α,4β,5α,6α (2*E*,4*R**,6*R**),7β]]-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-6-octenoate), 4,5-dimethyl ester (**8d**)[9e] (15 g, 20.87 mmol) in dry THF (85 mL) cooled to 0 °C in ice was added *N*-methylmorpholine (2.78 mL, 22.7 mmol) followed by isobutyl chloroformate (3.42 mL, 22.7 mmol). A white solid was deposited. The mixture was then filtered under dry nitrogen. To this at 21 °C in a dropwise fashion was then added an ethereal diazomethane solution (300 mL, from 74 mmol of Diazald). This was kept with occasional swirling at 21 °C for 2.5 h. Acetic acid (1.5 mL) was added. After a further 10 min the whole was diluted to 700 mL with EtOAc and washed with water (2 × 700 mL). The organic phase was then dried, filtered, and evaporated *in vacuo* to a pale yellow foam. This was chromatographed, eluting with EtOAc/petroleum ether (40–60 °C) (1:1), to give **9b** (7.11 g, 46%) as a white foam: ^1H NMR ($CDCl_3$) δ 7.30–7.10 (5 H, m, Ph), 6.85 (1 H, dd, J = 17, 7 Hz, OCOCH=C*H*), 5.65–5.80 (3 H, m, CHN_2, H6, OCOC*H*=CH), 5.10 (1 H, d, J = 5 Hz, C*H*OAc), 5.08 (1 H, s, H3), 5.03, 4.98 (2 H, 2s, $=CH_2$), 4.01 (1 H, s, H7), 3.92, 3.80 (6 H, 2s, 2 CO_2CH_3), 3.22 (1 H, br, OH-7), 2.10 (3 H, s, OAc), 1.05 (3 H, d, J = 7.5 Hz, CH=CHC*H*$_3$).

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(chloroacetyl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Bis(4-nitrobenzyl) Ester, (7a) and [1*S*-[1α(4*R**,5*S**),-3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(bromoacetyl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Bis(*p*-nitrobenzyl) Ester (7b).** To an ice cold solution of **8b** (4.8 g, 50 mmol) in dry dichloromethane (40 mL) was added dry dimethylformamide (39 μL, 0.5 mmol) followed by the gradual addition over 10 min of oxalyl chloride (2.18 mL, 25 mmol). The mixture was allowed to warm to 20 °C, stirring was continued until effervescence ceased (approximately 0.5 h), and then the mixture was concentrated to dryness. The residue was digested with THF (40 mL) and filtered under nitrogen, and the filtrate was added to an ice cold ethereal solution of diazomethane (60 mL from Diazald, 4.2 g, 17 mmol). The reaction mixture was kept at 0 °C for 0.75 h and then the reaction quenched by the cautious addition of glacial acetic acid (5 mL). When effervescence had ceased, the mixture was diluted with ether (100 mL) and poured into saturated aqueous sodium bicarbonate (400 mL). The mixture was extracted with ether (2 × 150 mL); the organic extract was washed with saturated aqueous $NaHCO_3$ and then brine and then dried, filtered, and evaporated *in vacuo* at 20 °C. The residue was chromatographed eluting with cyclohexane/EtOAc (3:1) to afford **7a** (1.0 g, 20%) from early fractions as a white foam: ^1H NMR ($CDCl_3$) δ 8.20 (4 H, 2d, J = 7.5 Hz, $NO_2C_6H_4$), 7.51 (4 H, 2d, J = 7.5 Hz, $NO_2C_6H_4$), 6.86 (1 H, dd, J = 16, 9 Hz, OCOCH=C*H*), 5.75 (1 H, d, J = 2.5 Hz, H6), 5.46 (1 H, d, J = 16 Hz, OCOC*H*=CH), 5.5–5.1 (4 H, m, CH_2Ph), 5.18 (1 H, s, H3), 5.07 (1 H, d, J = 5 Hz, C*H*OAc), 4.98, 5.02 (2 H, 2s, $=CH_2$), 4.58 (1 H, d, J = 17.5 Hz, CH_2Cl), 4.46 (1 H, d, J = 17.5 Hz, CH_2Cl), 4.01 (1 H, dd, J = 2.5, 2.5 Hz, H7), 3.91 (1 H, s, OH-4), 3.18 (1 H, d, J = 2.5 Hz, OH-7), 2.10 (3 H, s, OAc), 1.01 (3 H, d, J = 7 Hz, CH=CHC*H*$_3$). Anal. ($C_{50}H_{57}ClN_2O_{17}$) C, H, N, Cl.

From later fractions was obtained a foam (600 mg) which was dissolved in chloroform (4 mL) and stirred at 20 °C for 1 h with aqueous hydrobromic acid (48%, 2 mL). The mixture was then diluted with water (10 mL) and extracted with ether. The ether extracts were washed with aqueous $NaHCO_3$, dried, and evaporated. The residue was purified by chromatography eluting with petroleum ether (40–60 °C)/EtOAc (3:1) to afford

7b (280 mg, 5.4%) as a white foam: IR ($CHBr_3$) 3551, 2960, 1734, 1523, 1347, 1245, 1016 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.00 (2 H, d, J = 9 Hz, $NO_2C_6H_4$), 7.97 (2 H, d, J = 9 Hz, $NO_2C_6H_4$), 7.52 (2 H, d, J = 9 Hz, $NO_2C_6H_4$), 7.48 (2 H, d, J = 9 Hz, $NO_2C_6H_4$), 6.86 (1 H, dd, J = 16, 9 Hz, OCOCH=C*H*), 5.78 (1 H, d, J = 2 Hz, H6), 5.46 (1 H, d, J = 16 Hz, OCOC*H*=CH), 5.46–5.16 (5 H, m, $CH_2C_6H_4NO_2$, H3), 5.07 (1 H, d, J = 5 Hz, C*H*OAc), 4.98, 5.02 (2 H, 2s, $=CH_2$), 4.36 (1 H, d, J = 15 Hz, CH_2Br), 4.22 (1 H, d, J = 15 Hz, CH_2Br), 4.02 (1 H, m, H7), 3.22 (1 H, d, J = 2 Hz, OH-7), 2.10 (3 H, s, OAc), 1.02 (3 H, d, J = 7 Hz, CH_3); MS (CI) m/z 1054 $(M + NH_4)^+$, 974 $(M + NH_4^+ - Br)$. Anal. ($C_{50}H_{57}BrN_2O_{17}$) C, H, N.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(bromoacetyl)-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 (7c).** To a solution of **9b** (3.67 g, 4.8 mmol) in chloroform (7.4 mL) at 0 °C was added dropwise aqueous hydrobromic acid (48%, w/w, solution, 7.4 mL). This was stirred at 21 °C for 2 h and then diluted with water (350 mL) and extracted with ether (200 + 100 mL). Combined ethereal extracts were washed with water (200 mL), dried, filtered, and evaporated to a crispy yellow foam (3.5 g). This was chromatographed, eluting with EtOAc/petroleum ether (40–60 °C) (1:2), to give **7c** (2.10 g, 55%) as a white foam: ^1H NMR ($CDCl_3$) δ 7.30–7.10 (5 H, m, Ph), 6.85 (1 H, dd, J = 16, 9 Hz, OCOCH=C*H*), 5.75 (1 H, d, J = 16 Hz, OCOC*H*=CH), 5.72 (1H, d, J = 2.5 Hz, H6), 5.19 (1 H, s, H3), 5.10 (1 H, d, J = 5 Hz, C*H*OAc), 4.99, 5.02 (2 H, 2s, $=CH_2$), 4.39 (1 H, d, J = 15 Hz, CH_2Br), 4.25 (1 H, d, J = 15 Hz, CH_2Br), 4.05 (1 H, br, H7), 3.96, 3.80 (6 H, 2s, CO_2CH_3), 3.91 (1 H, s, OH-4), 3.21 (1 H, d, J = 2.5 Hz, OH-7), 2.10 (3 H, s, OAc), 1.05 (3 H, d, J = 7 Hz, CH_3); HRMS (LSIMS, +ve) m/z 817/819 (MNa^+) (1 Br present) found 817.2399, calcd for $C_{38}H_{51}{}^{79}BrNaO_{13}$ 817.2411, found 819.2379, calcd for $C_{38}H_{51}{}^{81}BrNaO_{13}$ 819.2390; MS (FAB, +ve) m/z 817/819 (MNa^+). Anal. ($C_{38}H_{51}BrO_{13}$) C, H.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(thiazol-4-yl)-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).** To a solution of **7c** (0.5 g, 0.628 mmol), in ethanol (2.5 mL), and diethyl ether (1.5 mL) at 0 °C was added dropwise a solution of thioformamide[15] in ethanol (1 mL). This was then stirred at 21 °C for 18 h. The mixture was diluted with ether (75 mL), washed with saturated aqueous $NaHCO_3$ and brine, dried, filtered, and evaporated. The residue was chromatographed, eluting with EtOAc/petroleum ether (40–60 °C) (1:2), to give **12** (0.446 g, 94%) as a white foam: ^1H NMR ($CDCl_3$) δ includes 8.70 (1 H, d, J = 2.5 Hz, thiazole H2), 7.44 (1 H, d, J = 2.5 Hz, thiazole H5), 7.30–7.10 (5 H, m, Ph), 6.85 (1 H, dd, J = 17, 7 Hz, OCOCH=C*H*), 6.01 (1 H, d, J = 2 Hz, H6), 5.90 (1 H, s, H3), 5.78 (1 H, d, J = 17 Hz, OCOC*H*=CH), 5.10 (1 H, d, J = 5 Hz, C*H*OAc), 4.99, 5.02 (2 H, 2s, $=CH_2$), 4.10 (1 H, m, H7), 3.90, 3.80 (6 H, 2s, CO_2CH_3), 3.30 (1 H, d, J = 2.5 Hz, OH-7), 2.10 (3 H, s, OAc), 1.05 (3 H, d, J = 7 Hz, $CHCH_3$); MS (CI) m/z 758 (MH^+), 816 $(M + OAc)^+$. Anal. ($C_{39}H_{51}NO_{12}S$) C, H, N.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(1,3-thiazol-4-yl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]-octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate) (15) and [1*S*-[1α(4*R**,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(1,3-thiazol-4-yl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]-octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4-Methyl Ester (18) (Method B).** A solution of **12** (0.44 g, 0.57 mmol) in distilled 2,4,6-collidine (20 mL) was treated with lithium iodide (0.758 g, 5.69 mmol) and the suspension stirred and heated at 45–50 °C under nitrogen for 45 h. The whole was then partitioned between ether (100 mL) and 2 M HCl (30 mL). The HCl layer was back-extracted with ether (40 mL), and the combined ether extracts washed sequentially with 50 mL portions each of 2 M HCl, water, saturated sodium thiosulfate, water, and brine and then dried, filtered, and evaporated. The brown residue was purified by preparative HPLC. Appropriate more polar fractions were combined, evaporated to remove organic solvent, and then extracted with EtOAc. The aqueous layer was saturated with sodium chloride and then re-extracted. Combined extracts were dried, filtered, and evaporated to give **15** (0.134 g, 32%) as a glassy solid: ^1H NMR ($CDCl_3$) δ 8.90 (1 H, d, J = 2 Hz, thiazole H2), 7.73 (1 H, d, J = 2 Hz, thiazole H5), 7.30–7.10 (5 H, m, Ph), 6.95 (1 H, dd, J = 17, 7 Hz, OCOCH=C*H*), 6.32 (1 H, s, H3), 6.28 (1 H, s, H6), 5.83 (1 H, d, J = 17 Hz, OCOC*H*=CH), 5.19 (1 H, d, J = 5 Hz, C*H*OAc), 5.00 (2 H, 2s, $=CH_2$), 4.55 (1 H, br, OH-4), 4.22 (1 H, s, H7), 4.20 (1 H, br, OH-7), 2.12 (3 H, s, OAc), 1.02 (3 H, d, J = 7 Hz, $CHCH_3$); MS (TSP, +ve) m/z 730 (MH^+). Anal. ($C_{37}H_{47}NO_{12}S{\cdot}H_2O$) C, H, N.

A less polar HPLC fraction afforded **18** (31 mg, 7.3%) as a white solid: ^1H NMR ($CDCl_3$) δ includes 8.70 (1 H, d, J = 2 Hz, thiazole H2), 7.46 (1 H, d, J = 2 Hz, thiazole H5), 7.30–7.10 (5 H, m, Ph), 6.90 (1 H, dd, J = 17, 7 Hz, OCOCH=C*H*), 5.97 (1 H, d, J = 1 Hz, H6), 5.90 (1 H, s, H3), 5.83 (1 H, d, J = 17 Hz, OCOC*H*=CH), 5.20 (1 H, d, J = 5 Hz, C*H*OAc), 5.00 (2 H, 2s, $=CH_2$), 4.10 (1 H, d, J = 1 Hz, H7), 3.90 (3 H, s, CO_2CH_3), 2.12 (3 H, s, OAc), 1.08 (3 H, d, J = 6 Hz, $CHCH_3$); MS (TSP, +ve) m/z 744 $(MH)^+$. Anal. ($C_{38}H_{49}NO_{12}S{\cdot}H_2O$) C, H, N.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α,7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(thiazol-4-yl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid** (41) **(Method C).** A solution of **15** (77 mg, 0.106 mmol) in anhydrous DMF (0.5 mL) was treated with *N*-methylhydroxylamine hydrochloride (30 mg, 0.325 mmol) and triethylamine (50 μL, 0.651 mmol) and stirred at 21 °C for 18 h. Solvent was removed *in vacuo*, and the residue purified by preparative HPLC. The appropriate peak was collected, organic solvent removed *in vacuo* and the resulting aqueous solution freeze-dried to give **41** (47 mg, 77%) as a fluffy white solid: CZE, 99.1% pure; ^1H NMR (CD_3OD) δ 8.87 (1 H, d, J = 2 Hz, thiazole H2), 7.55 (1 H, d, J = 2 Hz, thiazole H5), 7.30–7.10 (5 H, m, Ph), 5.88 (1 H, s, H3), 5.40 (1 H, d, J = 2 Hz, H6), 5.10(1 H, d, J = 5 Hz, C*H*OAc), 5.00, 4.95 (2 H, 2s, $=CH_2$), 4.10 (1 H, d, J = 2 Hz, H7), 2.70 (1 H, m, $PhCH_2$), 2.10 (3 H, s, C*H*OAc), 0.83 (3 H, d, J = 7 Hz, $CHCH_3$); MS (TSP, +ve) m/z 578 $(MH)^+$, 560 $(M - H_2O)^+$, (TSP, −ve) m/z 576 $(M - H)^-$, 558 $(M - H_2O)^-$. Anal. ($C_{27}H_{31}NO_{11}S{\cdot}2TFA{\cdot}2H_2O$) H, N; C: calcd, 44.23; found, 43.70.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-[(formyloxy)acetyl]-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 (20).** A solution of **7c** (1.36 g, 1.71 mmol) in EtOAc (13.6 mL) was treated with water (13.6 mL) and sodium formate (1.09 g, 15.84 mmol). To this were added triethyl orthoformate (0.86 mL, 5.14 mmol) and tetra-*n*-butylammonium bromide (0.167 g, 0.53 mmol). The mixture was stirred rapidly and heated at 50 °C for 24 h. The whole was partitioned between EtOAc and water (150 mL each) and the organic layer washed with water (2 × 100 mL) and then dried, filtered, and evaporated to give **20** (1.20 g) as a white foam: ^1H NMR ($CDCl_3$) δ includes 8.12 (1 H, s, OCHO), 7.30–7.10 (5 H, m, Ph), 6.85 (1 H, dd, J = 17, 7 Hz, OCOCH=C*H*), 5.75 (1 H, d, J = 17 Hz, OCOC*H*=CH), 5.72 (1 H, d, J = 2 Hz, H6), 5.17–4.98 (6 H, m, $=CH_2$, H3, C*H*OAc, $COCH_2OCHO$), 4.03 (1 H, br, OH-7), 4.00 (1 H, s, OH-4), 3.97, 3.80 (6 H, 2s, CO_2CH_3), 3.26 (1 H, d, J = 2 Hz, H7), 2.10 (3 H, s, OAc), 1.05 (3 H, d, J = 7 Hz, $CHCH_3$); MS (CI) m/z 778 $(MNH_4)^+$; (LSI, −ve) m/z 759 $(M - H)^-$.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(imidazol-4-yl)-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 (19).** A solution of crude **20** (1.1 g, 1.51 mmol) and ammonium formate (0.252 g, 4.00 mmol) in ethanol (12 mL) was refluxed under nitrogen for 90 min. The whole was partitioned between EtOAc and water (200 mL of each), and the organic layer was washed with water (200 mL), dried, filtered, and evaporated. The residue was chromatographed eluting with $CHCl_3$/MeOH (19:1) to give **19** (77 mg, 14%) as a glassy solid: ^1H NMR (CD_3OD) δ 7.60 (1 H, s, imidazole H2), 7.25–7.10 (6 H, m, Ph, imidazole H5), 6.85 (1 H, dd, J = 17, 7 Hz, OCOCH=C*H*), 6.40 (1 H, d, J = 2 Hz, H6), 5.80 (1 H, d, J = 17 Hz, OCOC*H*=CH), 5.70 (1 H, s, H3), 5.07 (1 H, d, J =

5 Hz, C*H*OAc), 4.99, 4.95 (2 H, 2s, =CH_2), 4.10 (1 H, d, J = 2 Hz, H7), 3.75, 3.68 (6 H, 2s, CO_2CH_3), 2.65 (1 H, m, PhCH_2), 2.09 (3 H, s, OAc), 1.05 (3 H, d, J = 8 Hz, CH=CHCH_3); MS (TSP, +ve) m/z 741 $(MH)^+$.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α,7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(imidazol-4-yl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 4,5-Dimethyl Ester (52).** Removal of the C6-side chain from **19** (77 mg, 0.104 mmol) according to method C gave, following chromatography with MeOH/$CHCl_3$ (1:19), compound **52** (62 mg, 80%) as a white foam: ^1H NMR ($CDCl_3$) δ 7.52 (1 H, s, imidazole H2), 7.30–7.10 (6 H, m, Ph, imidazole H5), 5.58 (1 H, s, H3), 5.24 (1 H, br, H6), 4.96 (1 H, d, J = 5 Hz, C*H*OAc), 4.89 (2 H, 2s, =CH_2), 4.19 (1 H, br, H7), 3.75, 3.68 (6 H, 2s, CO_2Me), 2.08 (3 H, s, OAc); MS (FAB, +ve) m/z 589 $(MH)^+$, (TSP, +ve) m/z 589 $(MH)^+$, (TSP, −ve) m/z 587 $(M - H)^-$.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α,7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(imidazol-4-yl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid (22) and [1*S*-[1α(4*R**,5*S**),3α,4β,5α,6α,7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(imidazol-4-yl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 4-Methyl Ester (56).** De-esterification of **52** (57 mg, 0.097 mmol) (method B) followed by preparative HPLC allowed isolation of the more polar **22** (10 mg, 18%) as a white solid after freeze-drying: analytical HPLC, 97% pure; ^1H NMR (D_2O) δ 8.69 (1 H, s, imidazole H2), 7.45 (1 H, s, imidazole H5), 7.35–7.20 (5 H, m, Ph), 5.68 (1 H, s, H3), 5.30 (1 H, s, H6), 5.05, 5.01 (2 H, 2s, =CH_2), 4.90 (1 H, d, J = 5 Hz, C*H*OAc), 4.20 (1 H, s, H7), 2.62 (2 H, m, PhCH_2), 2.20 (3 H, s, OAc), 0.90 (3 H, d, C*H*CH_3); MS (TOF) m/z 584.1 $(MNH_4)^+$, 561.9 $(MH)^+$, 502.8 $(M - OAc)^+$; HRMS (LSI, +ve) found 561.2094, calcd for $C_{27}H_{32}N_2O_{11}$ 561.2084.

Preparative HPLC also gave the less polar **56** (30 mg, 54%) as a white solid after freeze-drying: IR (DMSO) 3423, 3267, 1732, 1691cm^{-1}; ^1H NMR (D_2O) δ 8.71 (1 H, s, imidazole H2), 7.49 (1 H, s, imidazole H5), 7.38–7.20 (5 H, m, Ph), 5.67 (1 H, s, H3), 5.09 (1 H, d, J = 1 Hz, H6), 5.05, 5.02 (2 H, 2s, =CH_2), 4.89 (1 H, d, J = 5 Hz, C*H*OAc), 4.21 (1 H, d, J = 1 Hz, H7), 3.30 (3 H, s, CO_2CH_3), 2.60 (2 H, m, PhCH_2), 2.20 (3 H, s, OAc), 0.89 (3 H, d, J = 9 Hz, CHCH_3); MS (TOF) m/z 597.7 $(MNa)^+$, 575.1 $(MH)^+$. Anal. ($C_{28}H_{34}N_2O_{11}$·0.9TFA·$2H_2O$) C, H, N.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(3-methyl-1,2,4-oxadiazol-5-yl)-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 (23).** Oxalyl chloride (28 μL, 0.32 mmol) was added to a solution of DMF (37 μL, 0.48 mmol) in dichloromethane (0.5 mL). After 5 min a solution of **8d** (200 mg, 0.28 mmol) in chloroform (1 mL) was added and the mixture stirred for a further 5 min. Acetamide oxime (41.5 mg, 0.56 mmol) was added and the mixture heated to reflux. After 2.5 h toluene (3 mL) was added and refluxing continued overnight. After cooling to 21 °C, the solution was diluted with EtOAc and then washed with water, dried, filtered, and evaporated. The residue was chromatographed on silica gel, eluting with cyclohexane/EtOAc (2:1), to give **23** (77 mg, 36%) as a foam: IR ($CHBr_3$) 3542, 1770, 1732, 1648, 1247cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.10–7.30 (5 H, m, Ph), 6.86 (1 H, dd, J = 16, 9 Hz, OCOCH=C*H*), 6.00 (1 H, s, H3), 5.90 (1 H, d, J = 2 Hz, H6), 5.77 (1 H, d, J = 16 Hz, OCOC*H*=CH), 5.10 (1 H, d, J = 6Hz, C*H*OAc), 4.98, 5.00 (2 H, 2s, =CH_2), 4.12 (1 H, br, H7), 3.94, 3.82 (6 H, 2s, CO_2CH_3), 3.91 (1 H, s, OH-4), 3.41 (1 H, d, J = 2 Hz, OH-7), 2.70 (1 H, dd, J = 14, 6 Hz, PhCH_2), 2.39 (3 H, s, ArCH_3), 2.10 (3 H, s, OAc), 1.05 (3 H, d, J = 7 Hz, CH=CHCHCH_3). Anal. ($C_{39}H_{52}N_2O_{13}$) C, H, N.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α,7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(3-methyl-1,2,4-oxadiazol-5-yl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid (6).** A solution of **23** (181 mg, 0.24 mmol) in collidine (8 mL) was treated with LiI (320 mg, 2.4 mmol) and heated at 65 °C for 16 h. Solvent was removed under reduced pressure and the residue taken up in EtOAc, washed sequentially with 2 M HCl, aqueous $NaHSO_3$, and brine, and then dried, filtered, and evaporated. The residue was purified by preparative HPLC giving **6** (81 mg, 58%) as a white solid after freeze-drying: IR (DMSO) 3430, 1733, 1662 (br), 1243 cm^{-1}; ^1H NMR (CD_3OD) δ 7.10–7.30 (5 H, m, Ph), 5.94 (1 H, s, H3), 5.20 (1 H, d, J = 2 Hz, H6), 5.09 (1 H, d, J = 6 Hz, C*H*OAc), 4.99, 5.03 (2 H, 2s, =CH_2), 4.12 (1 H, d, J = 2 Hz, H7), 2.70 (1 H, dd, J = 14, 6 Hz, PhCH_2), 2.34 (3 H, s, ArCH_3), 2.10 (3 H, s, OAc), 0.86 (3 H, d, J = 7 Hz, CHCH_3); MS (TSP, +ve) m/z 577 $(MH)^+$, 599 $(MNa)^+$. Anal. ($C_{27}H_{32}N_2O_{12}$·1.5H_2O) C, H, N.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-hydrazido-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Bis(4-nitrobenzyl) Ester (26).** A stirred solution of **8a** (3.41 g, 3.11 mmol) in dry THF (50 mL) was treated with anhydrous hydrazine (110 mg, 0.107 mL, 3.42 mmol). The resulting solution was stirred at 21 °C for 15 h. Solvent was removed *in vacuo* and the residue chromatographed on silica gel with EtOAc/cyclohexane (4:1) as solvent to give **26** (2.02 g, 67%) as a white solid: ^1H NMR (DMSO-d_6) δ 8.75 (1 H, br, NHCO), 8.13 and 8.12 (4 H, 2d, J = 9 Hz, $NO_2C_6H_4$), 7.63 (4 H, 2d, J = 9 Hz, $NO_2C_6H_4$), 7.31–7.10 (5 H, m, Ph), 6.65 (1 H, dd, J = 16, 9 Hz, OCOCH=C*H*), 6.31 (1 H, d, J = 6 Hz, OH-7), 6.25 (1 H, s, H6), 6.08 (1 H, s, OH-4), 5.59 (1 H, d, J = 16 Hz, OCOC*H*=CH_2), 5.32–5.10 (4 H, m, C$H_2C_6H_4NO_2$), 5.06 (1 H, s, H3), 5.01 (1 H, d, J = 4 Hz, C*H*OAc), 4.97 and 4.92 (2 H, 2s, =CH_2), 4.30 (2 H, br, NH_2), 3.97 (1 H, s, H7), 2.63 (1 H, dd, J = 14 Hz, 6 Hz, PhCH_2), 2.09 (3 H, s, OAc), 0.89 (3 H, d, J = 7 Hz, CHCH_3). Anal. ($C_{49}H_{58}N_4O_{17}$) C, H, N.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-[2-(methylamino)-1,3,4-oxadiazol-5-yl]-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Bis(4-nitrobenzyl) Ester (25).** To a suspension of **26** (100 mg, 0.103 mmol) in toluene (5 mL) was added methyl isothiocyanate (10 mg, 0.137 mmol). This was heated under reflux for 30 min. *N,N'*-Dicyclohexylcarbodiimide (32 mg, 0.154 mmol) was added and refluxing continued for a further 6 h, whereupon the mixture was allowed to cool to 21 °C, stirred for 16 h, and then applied directly to a silica gel column. Chromatography with cyclohexane/EtOAc (1:1–1:3) as eluent afforded **25** (28 mg, 27%) as a cream-colored solid: ^1H NMR ($CDCl_3$) δ 8.19, 8.13 (4 H, 2d, J = 9 Hz, $NO_2C_6H_4$), 7.52,7.44 (4 H, 2d, J = 9 Hz, $NO_2C_6H_4$), 7.29–7.05 (5 H, m, Ph), 6.86 (1 H, dd, J = 16, 9 Hz, OCOCH=C*H*), 5.92 (1 H, d, J = 2 Hz, H6), 5.75 (1 H, s, H3), 5.49 (1 H, d, J = 16 Hz, OCOC*H*=CH), 5.36–5.03 (4 H, m, C$H_2C_6H_4NO_2$), 4.98, 4.93 (2 H, 2s, =CH_2), 4.72 (1 H, s, OH-4), 4.10 (1 H, br, H7), 3.49 (1 H, br, OH-7), 2.99 (3 H, d, J = 5Hz, CH_3NH), 2.66 (1 H, dd, J = 13, 6 Hz, PhCH_2), 2.08 (3 H, s, OAc), 1.00 (3 H, d, J = 7 Hz, CH=CHCHCH_3); MS (TOF) m/z 1037.0 $(MNa)^+$, 1015.7 $(MH)^+$, 956.2 $(MH - OAc)^+$. Anal. ($C_{51}H_{59}N_5O_{17}$) C, H, N.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(4-methyl-3-thio-1,2,4-triazol-5-yl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Bis(4-nitrobenzyl) Ester (28) and [1*S*-[1α(4*R**,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-[4-methyl-3-[(4-nitrobenzyl)thio)]1,2,4-triazol-5-yl]-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate), 4,5-Bis(4-nitrobenzyl) Ester (29).** A suspension of **26** (1.5 g, 1.538 mmol) in *n*-propanol (30 mL) was treated with methyl isothiocyanate (150 mg, 2.055 mmol) and stirred at reflux for 1.5 h. DBU (0.15 mL) was added and the mixture stirred for a further 1.5 h at reflux, before cooling to 21 °C. Glacial acetic acid (2 mL) was added and the whole poured into water (300 mL). This was extracted with EtOAc (200 mL) and the extract washed with brine and then dried, filtered, and evaporated *in vacuo*. The foam residue was chromatographed eluting with cyclohexane/EtOAc (3:2). Early fractions were combined and evaporated to give **28** (172 mg, 11%) as a cream-coloured foam: ^1H NMR ($CDCl_3$) δ 11.84 (1 H, s, SH), 8.22, 8.13 (4 H, 2d, J = 7 Hz, $NO_2C_6H_4$), 7.54, 7.40 (4 H, 2d, J = 7 Hz, $NO_2C_6H_4$), 7.25–7.06 (5 H, m, Ph), 6.88 (1 H, dd, J = 13, 7

Hz, OCOCH=C*H*), 5.89 (1 H, s, H6), 5.75 (1 H, s, H3), 5.49 (1 H, d, J = 13 Hz, OCOC*H*=CH), 5.44–4.82 (7 H, m, CH_2C$_6$H$_4$-NO$_2$, =CH$_2$, C*H*OAc), 4.42 (1 H, s, OH-4), 4.11 (1 H, s, H7), 3.69 (3 H, s, N-CH$_3$), 3.40 (1 H, br, OH-7), 2.65 (1 H, dd, J = 11, 5 Hz, PhCH_2), 2.09 (3 H, s, OAc), 1.02 (3 H, d, J = 5 Hz, CH=CHCHCH_3); MS (TOF) m/z 1031.2 (MH)$^+$. Anal. (C$_{51}$H$_{59}$N$_5$O$_{16}$S) C, H, N, S.

Later fractions were also combined and evaporated to give **29** (355 mg, 20%) as a white solid: ^{1}H NMR (CDCl$_3$) δ 8.23–8.09, 7.59–7.42, 7.28–7.04 (17 H, 3m, NO$_2$C$_6$H$_4$, Ph), 6.85 (1 H, dd, J = 14, 6 Hz, OCOCH=C*H*), 5.88 (1 H, d, J = 2 Hz, H6), 5.71 (1 H, s, H3), 5.60 (1 H, s, OH-4), 5.49 (1 H, d, J = 14 Hz, OCOC*H*=CH), 5.40–5.04 (4 H, m, CH_2C$_6$H$_4$NO$_2$), 5.01 (1 H, d, J = 4 Hz, C*H*OAc), 4.97, 4.91 (2 H, 2s, =CH$_2$), 4.56–4.50 (2 H, d, J = 10 Hz, CH_2C$_6$H$_4$NO$_2$), 4.08 (1 H, s, H7), 3.45 (3 H, s, *N*-CH$_3$), 3.29 (1 H, d, J = 2 Hz, OH-7), 2.63 (1 H, dd, J = 11, 5 Hz, PhCH_2), 2.06 (3 H, s, OAc), 1.00 (3 H, d, J = 6Hz, CH=CHCHCH_3); MS (TOF) m/z 1166.7 (MH)$^+$. Anal. (C$_{58}$H$_{64}$N$_6$O$_{18}$S) H, N, S; C: calcd, 59.78; found, 60.31.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(4-methyl-3-thio-1,2,4-triazol-5-yl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-2-octenoate) (30) (Method A).** A solution of **28** (152 mg, 0.148 mmol) in THF (8 mL) and water (4 mL) was treated with zinc dust (700 mg) followed by 2 M HCl to adjust the pH to 1. The mixture was stirred at 21 °C for 30 min with further additions of 2 M HCl to maintain pH. The whole was then diluted with 2 M HCl (150 mL) and extracted with EtOAc (150 + 100 mL). Combined extracts were washed with brine (100 mL), dried, filtered, and evaporated. The residue was purified by preparative HPLC. The eluent was evaporated to remove organic solvent, and then the remaining aqueous solution freeze-dried to give **30** (49 mg, 44%) as an off-white solid: ^{1}H NMR (CD$_3$OD) δ 7.34–7.10 (5 H, m, Ph), 6.87 (1 H, dd, J = 16, 8 Hz, OCOCH=C*H*), 6.40 (1 H, s, H6), 5.89 (1 H, s, H3), 5.82 (1 H, d, J = 16 Hz, OCOC*H*=CH), 5.05 (1 H, d, J = 5 Hz, C*H*OAc), 5.00, 4.97 (2 H, 2s, =CH$_2$), 4.11 (1 H, s, H7), 3.72 (3 H, s, *N*-CH$_3$), 2.65 (1 H, dd, J = 13, 7 Hz, PhCH_3), 2.10 (3 H, s, OAc), 1.04 (3 H, d, J = 6 Hz, CH=CHCHCH_3); MS (TSP, +ve) m/z 760 (MH)$^+$. Anal. (C$_{37}$H$_{49}$N$_3$O$_{12}$S·1.5H$_2$O) C, H, N, S.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-hydrazido-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 (31).** Trimethyl ester **8c** (0.93 g, 1.27 mmol) was dissolved in dry THF (3 mL) and stirred at 21°C under nitrogen. Anhydrous hydrazine (42 μL, 1.339 mmol) was added and the mixture stirred for 24 h. The whole was then partitioned between EtOAc (50 mL) and water (25 mL). The organic phase was washed with brine (25 mL) and then dried, filtered, and evaporated. The residue was chromatographed on silica gel with CHCl$_3$/MeOH (15:1) as eluent to give **31** (0.58 g, 63%) as a white foam: IR (CHBr$_3$) 2923, 1772, 1728 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.70–7.61 (1 H, br, NHCO), 7.32–7.00 (5 H, m, Ph), 6.86 (1 H, dd, J = 16, 9 Hz, OCOCH=C*H*), 5.78 (1 H, s, H6), 5.76 (1 H, d, J = 16 Hz, OCOC*H*=CH), 5.17 (1 H, s, H3), 5.09 (1 H, d, J = 5 Hz, C*H*OAc), 5.04, 5.00 (2 H, 2s, =CH$_2$), 4.02 (1 H, s, H7), 3.97 (4 H, s, CO$_2$CH$_3$, OH-4), 3.82 (2 H, br, H$_2$N), 3.81 (3 H, s, CO$_2$CH$_3$), 3.32 (1 H, s, OH-7), 2.64 (1 H, dd, J = 13, 6 Hz, PhCH_2), 2.11 (3 H, s, OAc), 1.04 (3 H, d, J = 6 Hz, CH=CHCHCH_3); MS (TOF) m/z 733 (MH)$^+$. Anal. (C$_{37}$H$_{52}$N$_2$O$_{13}$·H$_2$O) C, H, N.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(3-methyl-1,2,4-triazol-5-yl)-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 (32).** A solution of **31** (10.02 g, 13.7 mmol) in ethanol (300 mL) was treated with triethylamine (9.55 mL, 0.685 mol) and ethyl acetimidate hydrochloride (2.54 g, 20.6 mmol) and refluxed for 15 h. Solvent was removed *in vacuo* and the residue chromatographed on silica gel, eluting with EtOAc/cyclohexane (1:1). Evaporation of appropriate fractions gave **32** (7.46 g, 72%) as a foam: ^{1}H NMR (CDCl$_3$) δ 7.35–7.10 (5 H, m, Ph), 6.86 (1 H, dd, J = 16, 9 Hz, OCOCH=C*H*), 5.95 (1 H, d, J = 2 Hz, H6), 5.84 (1 H, s, H3), 5.77 (1 H, d, J = 16 Hz, OCOC*H*=CH), 5.06 (1 H, d, J = 5 Hz, C*H*OAc), 4.98, 4.94 (2 H, 2s, =CH$_2$), 4.11 (1 H, br, H7), 3.86, 3.84 (6 H, 2s, CO$_2$CH$_3$), 2.67 (1 H, dd, J = 14, 6 Hz, PhCH_2), 2.38 (3 H, s, ArCH_3), 2.09 (3 H, s, OAc), 1.04 (3 H, d, J = 7 Hz, CHCH_3); MS (TSP, +ve) m/z 756 (MH)$^+$. Anal. (C$_{39}$H$_{53}$-N$_3$O$_{12}$·H$_2$O) C, H, N.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-carbamoyl-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 (34a).** To a solution of dry DMF (1.84 mL, 25 mmol) in dry dichloromethane (30 mL) cooled to 0 °C under nitrogen was added oxalyl chloride (2.2 mL, 25 mmol) dropwise with stirring. This was stirred at 0 °C for 5 min before dropwise addition of **8d** (6 g, 8.36 mmol) as a solution in dry THF (44 mL) and dry acetonitrile (26 mL). Stirring at 0 °C was continued for 2 h, precipitate gradually dissolving. Ammonia gas was then bubbled in with ice bath cooling (initial exotherm to 40 °C). Copious white precipitate was deposited. Ammonia bubbling was continued for 2 h at 0 °C. The mixture was then allowed to warm to 21 °C and stirring continued for a further 2 h. The mixture was diluted with ether (100 mL) and washed with successive portions (100 mL) of water, 2 M HCl, and water. The organic phase was dried, filtered, and evaporated. The yellow, gummy residue was chromatographed on silica gel (500 g) with EtOAc/petroleum ether (40–60 °C) (5:1) to give **34a** (3.64 g, 61%) as a white foam: IR (CHBr$_3$) 1735, 1698, 1247 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.30–7.10 (5 H, m, Ph), 6.85 (1 H, dd, J = 17, 7 Hz, OCOCH=C*H*), 6.49 (1 H, br, NH), 5.79 (1 H, br, H6), 5.76 (1 H, d, J = 17 Hz, OCOC*H*=CH), 5.49 (1 H, br, NH), 5.10 (1 H, s, H3), 5.09 (1 H, d, J = 5 Hz, C*H*OAc), 5.02, 4.98 (2 H, 2s, =CH$_2$), 4.04 (1 H, s, H7), 3.95, 3.80 (6 H, 2s, CO$_2$CH$_3$), 3.26 (1 H, d, OH-7), 2.69 (1 H, dd, PhCH_2), 2.10 (3 H, s, OAc), 1.07 (3 H, d, J = 8 Hz, CH=CHCHCH_3), 0.85 (9 H, m, 3 CH$_3$). Anal. (C$_{37}$H$_{51}$NO$_{13}$) C, H, N.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-cyano-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 (34b).** Compound **34a** (2.79 g, 3.88 mmol) was dissolved in dichloromethane (83 mL), and the solution was treated with triethylamine (TEA) (1.59 mL, 11.4 mmol) and trifluoroacetic anhydride (TFAA) (1.09 mL, 7.75 mmol). This was then stirred at 21 °C for 3 h. Further portions of TEA (0.4 mL) and TFAA (0.27 mL) were added, and the mixture was stirred for a further 2 h. The mixture was diluted with dichloromethane (100 mL) and washed with 2 M HCl (200 mL) and then with saturated aqueous NaHCO$_3$ (200 mL). The organic phase was dried, filtered, and evaporated *in vacuo*. The residue was chromatographed on silica gel (100 g) eluting with cyclohexane/EtOAc (3:1) to give **34b** (2.30 g, 85%) as a white foam: IR (CHBr$_3$) 3546, 1772, 1732 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.33–7.10 (5 H, m, Ph), 6.85 (1 H, dd, J = 16, 8 Hz, OCOCH=C*H*), 5.74 (1 H, d, J = 16 Hz, OCOC*H*=CH), 5.66 (1 H, d, J = 2.5 Hz, H6), 5.50 (1 H, s, H3), 5.07 (1 H, d, J = 5 Hz, C*H*OAc), 5.00, 4.95 (2H, 2s, =CH$_2$), 4.05 (1 H, s, OH-4), 4.03 (1 H, d, J = 2.5 Hz, H7), 3.96, 3.81 (6 H, 2s, CO$_2$CH$_3$), 3.26 (1 H, d, J = 2.5 Hz, OH-7), 2.68 (1 H, dd, J = 14, 5 Hz, PhCH_2), 2.09 (3 H, s, OAc), 1.04 (3 H, d, J = 6 Hz, CH=CHCHCH_3). Anal. (C$_{37}$H$_{49}$NO$_{12}$) C, H, N.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(1*H*-tetrazol-5-yl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]-octane-4,5-dicarboxylic Acid, 6-(4,6-Dimethyl-6-octenoate), 4,5-Dimethyl Ester (35).** Compound **34b** (1 g, 1.429 mmol) was dissolved in *N*,*N*-dimethylformamide (40 mL). Sodium azide (650 mg, 10 mmol) and triethylamine hydrochloride (1.375 g, 10 mmol) were added, and the mixture was stirred, under nitrogen, at 120 °C for 2 h. The mixture was allowed to cool to room temperature, poured into aqueous sodium nitrite solution, acidified with 2 N HCl, and extracted with EtOAc (2 × 150 mL). The organic extracts were combined, washed with water (4 × 100 mL), dried, filtered, and evaporated to give a yellow oil (1.877 g). This was dissolved in ether (50 mL) and washed with water (3 × 30 mL). The organic phase was dried, filtered, and evaporated to give a colorless

foam (898 mg). The bulk was used crude in the next stage, while a sample was purified by preparative HPLC to give **35** as a colorless solid: ^{1}H NMR (DMSO-d_6) δ 7.30–7.11 (5 H, m, Ph), 6.92 (1 H, dd, J = 17.5, 8.75 Hz, OCOCH=C*H*), 6.41 (1 H, d, J = 5 Hz, OH-7), 6.12 (1 H, d, J = 2 Hz, H6), 6.21–6.13 (1 H, br, s, NH or OH-4), 5.98 (1 H, s, H3), 5.82 (1 H, d, J = 17.5 Hz, OCOC*H*=CH), 5.02 (1 H, d, J = 4 Hz, C*H*OAc), 4.96, 4.92 (2 H, 2s, =CH_2), 4.05 (3 H, dd, J = 5Hz, CO_2CH_3), 2.64 (1 H, dd, J = 12.5, 6 Hz, PhCH_2), 2.10 (3 H, s, OAc), 1.00 (3 H, d, J = 7 Hz, CH=CHCHCH_3); MS (DCI, NH_3, +ve) m/z 743 $(MH)^+$, 760 $(MNH_4)^+$, (TOF) m/z 766 $(MNa)^+$, 742.3 $(MH)^+$, (FAB, +ve) m/z 743 $(MH)^+$, 765 $(MNa)^+$. Anal. ($C_{37}H_{50}N_4O_{12}$·0.5H_2O) C, H, N.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(1-methyl-1*H*-tetrazol-5-yl)-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 (37a) and [1*S*-[1α(4*R**,5*S**),-3α,4β,5α,6α(2*E*,4*R**,6*R**),7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(2-methyl-2*H*-tetrazol-5-yl)-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 (38a).** **35** (0.4 g, 0.539 mmol) was dissolved in *N*,*N*-dimethylformamide (5 mL). $NaHCO_3$ (0.48 g, 5.7 mmol) and iodomethane (0.24 mL, 3.86 mmol) were added, and the mixture was stirred for 16 h. The mixture was then poured into brine (50 mL) and extracted with EtOAc (100 mL). The organic layer was washed with water (3 × 50 mL), dried, and evaporated. The residue (0.41 g) was chromatographed on silica gel, eluting with a gradient of cyclohexane/EtOAc (3:1–1:1). Appropriate less polar fractions were combined and evaporated to give **37a** (0.153 g, 37%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 7.30–7.06 (5 H, m, Ph), 6.88 (1 H, dd, J = 16, 8 Hz, OCOCH=C*H*), 6.18 (1 H, s, H3), 5.87 (1 H, d, J = 2.5 Hz, H6), 5.78 (1 H, d, J = 16 Hz, OCOC*H*=CH), 5.05 (1 H, d, J = 5 Hz, C*H*OAc), 5.00, 4.95 (2 H, 2s, =CH_2), 4.18 (3 H, s, *N*-CH_3), 4.14 (1 H, d, J = 2.5 Hz, H7), 3.96, 3.84 (6 H, 2s, CO_2CH_3), 3.32 (1 H, d, J = 2.5 Hz, OH-7), 2.68 (1 H, dd, J = 14, 5 Hz, PhCH_2), 2.08 (3 H, s, OAc), 1.05 (3

Appropriate slower running fractions were combined and evaporated to give **38a** (0.077 g, 19%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 7.30–7.08 (5 H, m, Ph), 6.88 (1 H, dd, J = 16, 8 Hz, OCOCH=C*H*), 6.08 (1 H, s, H3), 5.96 (1 H, d, J = 2.5 Hz, H6), 5.78 (1 H, d, J = 16 Hz, OCOC*H*=CH), 5.10 (1 H, d, J = 5 Hz, C*H*OAc), 4.98, 4.97 (2 H, 2s, =CH_2), 4.30 (3 H, s, *N*-CH_3), 4.14 (1 H, br, H7), 3.88, 3.82 (6 H, 2s, CO_2CH_3), 3.35 (1 H, d, J = 2.5 Hz, OH-7), 2.70 (1 H, dd, J = 14, 5 Hz, PhCH_2), 2.07 (3 H, s, OAc), 1.04 (3 H, d, J = 6 Hz, CH=CHCHCH_3). Anal. ($C_{38}H_{52}N_4O_{12}$) C, H, N.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α,7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(1-methyl-1*H*-tetrazol-5-yl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid (46).** **49** (401 mg, 0.663 mol) was treated in 2,4,6-collidine (15 mL) with anhydrous lithium iodide (887 mg, 6.630 mmol) (method B) and purified by HPLC to yield [1*S*-[1α(4*R**,5*S**),3α,4β,5α,6α,7β]]-1-[4-(acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-4,6,7-trihydroxy-3-(1-methyl-1*H*-tetrazol-5-yl)-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic acid, 4-methyl ester (**53**) (29 mg, 7%) as an off-white foam: ^{1}H NMR (CD_3OD) δ 7.29–7.08 (5 H, m, Ph), 6.15 (1 H, s, H3), 5.18 (1 H, d, J = 2 Hz, H6), 5.08 (1 H, d, J = 5 Hz, C*H*OAc), 5.03, 5.01 (2 H, 2s, =CH_2), 4.22 (3 H, s, *N*-CH_3), 4.17 (1 H, d, J = 2 Hz, H7), 3.81 (**3** H, s, CO_2CH_3), 2.09 (3 H, s, OAc), 0.88 (3 H, d, J = 6 Hz, CHCH_3).

This was retreated under the above conditions for a further 48 h at 40 °C and purified by preparative HPLC to give **46** (10 mg, 34%) as a white foam following freeze-drying: analytical HPLC, 96% pure; ^{1}H NMR (CD_3OD) δ 7.27–7.06 (5 H, m, Ph), 6.20 (1 H, s, H3), 4.19 (3 H, s, *N*-CH_3), 4.14 (1 H, s, H7), 2.12 (3 H, s, OAc), 0.84 (3 H, d, J = 6 Hz, CHCH_3); HRMS (LSI, +ve) found 575.2013, calcd for $C_{26}H_{31}N_4O_{11}$ 575.1989.

[1*S*-[1α(4*R,5*S**),3α,4β,5α,6α,7β]]-1-(4-Hydroxy-5-methyl-3-methylene-6-phenylhexyl)-3-(2-methyl-2*H*-tetrazol-5-yl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid (55), [1*S*-[1α(4*R**,5*S**),3α,4β,5α,6α,7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(2-methyl-2*H*-tetrazol-5-yl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid (47), and [1*S*-[1α(4*R**,5*S**),3α,4β,5α,6α,7β]]-1-[4-(Acetyloxy)-5-methyl-3-methylene-6-phenylhexyl]-3-(2-methyl-2*H*-tetrazol-5-yl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-4,5-dicarboxylic Acid, 4-Methyl Ester (54).** **50** (184 mg, 0.304 mmol) was treated with anhydrous lithium iodide (407 mg, 3.04 mmol) (method B) and purified by preparative HPLC. The most polar product was **55** (10 mg, 6%): analytical HPLC, 95% pure; ^{1}H NMR (CD_3OD) δ 7.29–7.06 (5 H, m, Ph), 6.02 (1 H, s, H3), 5.38 (1 H, br s, H6), 4.32 (3 H, s, *N*-CH_3), 4.15 (1 H, br s, H7), 3.94 (1 H, d, J = 5 Hz, C*H*OH), 2.78 (1 H, dd, J = 14, 5 Hz, PhCH_2), 0.80 (3 H, d, J = 7 Hz, CHCH_3); HRMS $(MNa)^+$ found 557.1894, calcd for $C_{24}H_{30}N_4O_{10}Na$ 557.1860.

The middle running fraction gave **47** (68 mg, 39%) as a white solid: analytical HPLC, 100% pure; IR (DMSO) 3435, 1733, 1691, 1647, 1243, 1197 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.29–7.09 (5 H, m, Ph), 6.00 (1 H, s, H3), 5.42 (1 H, d, J = 2 Hz, H6), 5.10 (1 H, d, J = 5 Hz, C*H*OAc), 5.01, 4.95 (2 H, 2s, =CH_2), 4.30 (3 H, s, *N*-CH_3), 4.12 (1 H, d, J = 2 Hz, H7), 2.71 (1 H, dd, J = 7, 14 Hz, PhCH_2), 2.09 (3 H, s, OAc), 0.83 (3 H, d, J = 6 Hz, CHCH_3); HRMS (LSI, +ve) found 577.2148, calcd for $C_{26}H_{33}N_4O_{11}$ 577.2146. Anal ($C_{26}H_{32}N_4O_{11}$·1.5CF_3CO_2H·2H_2O) H, N; C: calcd, 44.45; found, 44.93.

The least polar fraction gave **54** (93 mg, 52%) as an off-white solid: analytical HPLC, 98% pure; ^{1}H NMR (CD_3OD) δ 7.29–7.08 (5 H, m, Ph), 5.95 (1 H, s, H3), 5.10 (2 H, br, C*H*OAc, H6), 5.02, 4.96 (2 H, 2s, =CH_2), 4.32 (3 H, s, *N*-CH_3), 4.12 (1 H, d, J = 2 Hz, H7), 3.78 (3 H, s, CO_2CH_3), 2.72 (1 H, dd, J = 14, 6 Hz, PhCH_2), 2.08 (3 H, s, OAc), 0.84 (3 H, d, J = 6 Hz, CHCH_3); HRMS (LSI, +ve) found 591.2324, calcd for $C_{27}H_{35}N_4O_{11}$ 591.2302.

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Supporting Information Available: Synthesis and characterization of compounds **10**, **11**, **13**, **16**, **42**, **14**, **17**, **43**, **21**, **24**, **27**, **44**, **45**, **33**, **48**, **36**, **5**, **37b**, **38b**, **49**, and **50** (9 pages). Ordering information is available on any current masthead page.

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