Synthesis of 7-Aza- and 7-Thiasphingosines, and Evaluation of Their Interaction with Sphingosine Kinases and with T-Cells

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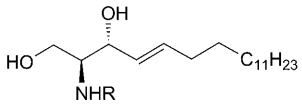
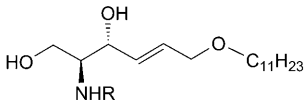
The synthesis of 7-oxasphingosine (**3**) and 7-oxaceramide (**4**) was improved by starting from the 4-methoxybenzyl-protected D-galactal **9**. The sphingosine analogues **5**–**7** and **24** were synthesized *via* the azido alcohol **13**. The 7-thiasphingosine **5** is a poorer substrate for both isoforms of sphingosine kinase (SPHK) than sphingosine, but showed a slight preference for SPHK2. The sulfone **6** and the 7-aza compounds **7** and **24** were not phosphorylated by either SPHK1 or SPHK2, and none of **5**–**7** and **24** activated invariant natural killer T (iNKT) cell clones when presented by human CD1d-transfected antigen-presenting cells (APC) or by plate-bound human CD1d. Only **7** and **24** associated with plate-bound recombinant CD1d prevented stimulation of iNKT cells by α-galactosylceramide (α-GalCer).

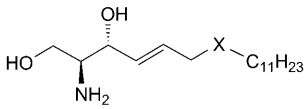
Introduction. – Sphingolipids are structural components of the cell and exert multiple functions in cell signalling, including the role of D-*erythro*-sphingosine (**1**) and ceramide (**2**) in the regulation of apoptosis [1–7], and the role of sphingosine-1-phosphate and ceramide in immune responses [8–13]. These functions are thought to depend on specific interactions between receptors, and both the lipid tail (lipid tails for ceramide) and the polar head group of sphingosine and ceramide [14]. We began exploring these interactions by synthesizing and evaluating analogues, and so far reported on modifications of the polar head group and on the replacement of the $C(7)H_2$ group by an O-atom, as in **3** and **4**, a replacement that did not appear to affect the tested biological properties [15]. This may be different for analogues where the $C(7)H_2$ group is replaced by bulkier, more polar, or charged groups, and we considered the 7-thia and 7-aza analogues **5**–**7** of interest.



Sphingosine (**1**) R = H
Ceramide (**2**) R = $C(O)C_{17}H_{35}$



7-Oxasphingosine (**3**) R = H
7-Oxaceramide (**4**) R = $C(O)C_{17}H_{35}$



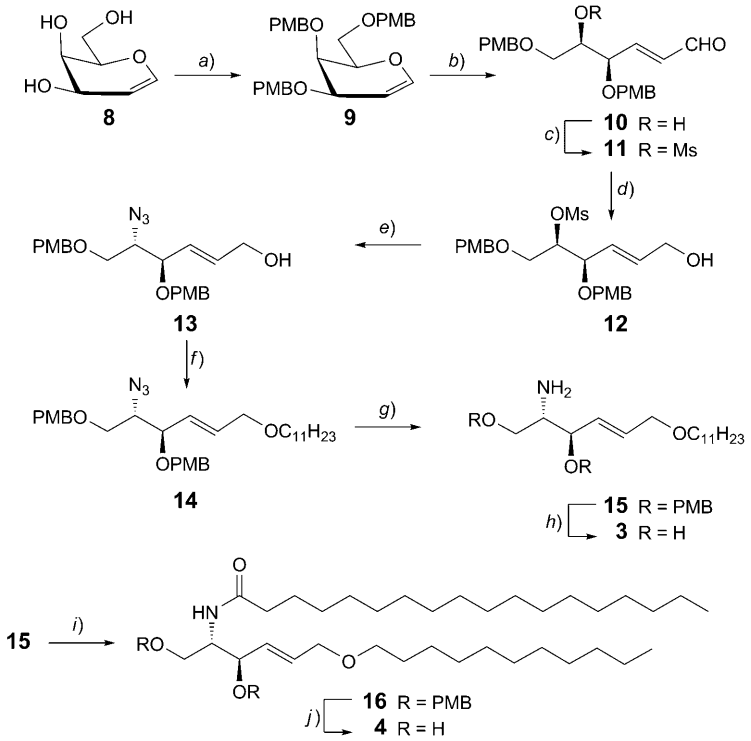
5 X = S
6 X = SO_2
7 X = NH

During the synthesis of 7-oxasphingosines we protected the OH groups by benzylation, deprotecting them by cleaving the benzyl ether moieties with $AlCl_3$ in the

presence of anisole [16]. For some analogues, these conditions led to cleavage of the allylic C–O bond, substantially lowering the overall yield, and suggesting to replace the benzyl (Bn) by 4-methoxybenzyl (PMB) groups that are cleaved under milder conditions, and to otherwise follow the established synthetic route [15]; the expected improvement should facilitate the synthesis of the envisaged analogues **5**–**7**. We now describe the modified synthesis of 7-oxasphingosine and 7-oxaceramide, the synthesis of 7-thia- and 7-azasphingosines, and the evaluation of these analogues as substrates of sphingosine kinases (SPHK), as CD1d ligands, and as activators of iNKT cells.

Synthesis. – To improve the synthesis of 7-oxasphingosine and 7-oxaceramide, we started from PMB-protected D-galactal **9** [17–20] rather than from the benzylated analogue that we used in the past. Galactal **9** was obtained in 90% yield from D-galactal **8**[1]), itself available in an overall yield of 75% from galactose [21][22] (*Scheme 1*).

Scheme 1



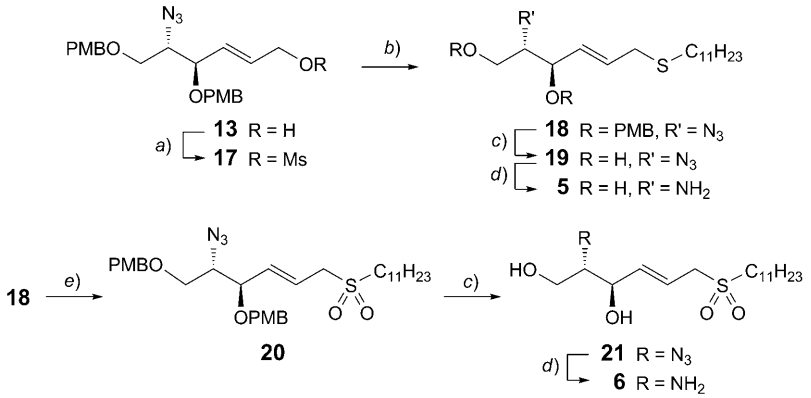
a) NaH, 4-methoxybenzyl (PMB) chloride, Bu_4NI, DMF; 90%. *b*) $HgSO_4$, 0.02N H_2SO_4, 1,4-dioxane; 92%. *c*) MsCl, pyridine, CH_2Cl_2; 96%. *d*) $NaBH_4$, MeOH/CH_2Cl_2 26:4; 93%. *e*) NaN_3, DMF, 140°; 89%. *f*) NaH, $C_{11}H_{23}Br$, DMF; 78%. *g*) $LiAlH_4$, Et_2O; 99%. *h*) CF_3COOH (TFA)/CH_2Cl_2 1:9; 74% of **3**; 75% of **4**. *i*) $C_{17}H_{35}COCl$, pyridine, CH_2Cl_2; 95%.

[1]) Prepared similarly as the D-glucal analogue (see [17][18]).

Treatment of **9** with $HgSO_4$ and dilute H_2SO_4 [23][24] provided the α,β-unsaturated aldehyde **10** (*Scheme 1*). Mesylation of **10** to **11**, followed by reduction with $NaBH_4$, yielded the allylic alcohol **12** in 93% yield. Substitution of the MsO group of **12** with NaN_3 yielded 89% of the azido alcohol **13** that was *O*-alkylated with 1-bromoundecane to the azido ether **14** (78%). Reduction of **14** with $LiAlH_4$ yielded the PMB-protected amino alcohol **15** (99%) that was debenzylated by treatment with 10% CF_3COOH (TFA) in CH_2Cl_2 to afford the 7-oxasphingosine **3** (74%), while *N*-acylation of **15** followed by deprotection provided the 7-oxaceramide **4** in 71% yield. As expected, exchanging the Bn for PMB groups improved the overall yield of **4** from **8**, from 22 to 36%.

To synthesize the 7-thia analogue **5** and the corresponding sulfone **6**, we transformed the allylic alcohol **13** into the methanesulfonate **17** (*Scheme 2*). The methanesulfonate was obtained in a high yield, and reacted smoothly with the *in situ* generated thiolate of 1-sulfanylundecane to yield 87% of the protected 7-thio ether **18**.

Scheme 2



a) Ms_2O, $^iPr_2NEt$, CH_2Cl_2; 99%. *b*) $C_{11}H_{23}SH$, NaH, DMF; 87%. *c*) TFA/CH_2Cl_2 1:9. *d*) PMe_3, THF/H_2O 4:1; 83.5% of **5**; 84% of **6**. *e*) Oxone, MeOH/CH_2Cl_2 1:2; 78%.

Cleavage of the PMB group of **18** by treatment with CF_3COOH/CH_2Cl_2 and *Staudinger* reduction [25] of the azide **19** gave the 7-thiasphingosine **5** (84% yield from **18**). Oxidation of the thio ether **18** with oxone led to the protected sulfone **20** (78%), and debenzylation of **20** (CF_3COOH/CH_2Cl_2), followed by a *Staudinger* reduction, provided the crystalline sulfone **6** in 84% yield. The structure of **6** was established by X-ray crystal structure analysis (*Fig. 1*)[2]).

The 7-azasphingosine **7** was similarly obtained from the methanesulfonate **17** *via* the corresponding *in situ* generated bromide that was treated with *N*-Boc-protected undecylamine to yield the protected 7-azasphingosine **22** (*Scheme 3*). *Staudinger*

[2]) The crystallographic data have been deposited with the *Cambridge Crystallographic Data Centre* (*CCDC*) as deposition No. CCDC-716357. Copies of the data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/data_request/cif (or from the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB21EZ; fax: +44(1223)336033; e-mail: deposit@ccdc.cam.ac.uk).

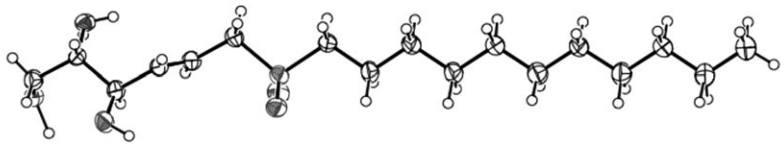
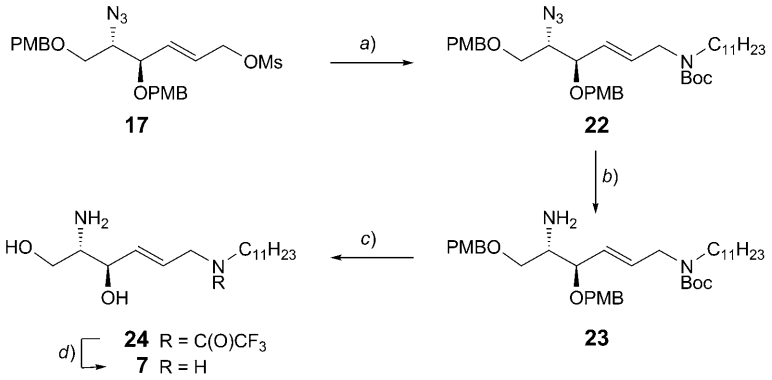


Fig. 1. *X-Ray structure of the sulfone* **6**

reduction of **22** afforded the partially protected amine **23**. To remove the PMB and the Boc groups, we treated **23** with 10% TFA in CH_2Cl_2 to obtain the 7-trifluoroacetamide **24** (60% from **22**), as evidenced by a ^{19}F-NMR signal at -75.5 ppm, two ^{13}C *q*s at 157.58 (^2J(C,F) = 36.7 Hz) and 116.3 ppm (^1J(C,F) = 286.0 Hz), and the HR-ESI-MS peak at *m*/*z* 397.2650. The trifluoroacetamide **24** was hydrolyzed by treatment with *Amberlyst A-26* (OH^- form) [26] to provide the 7-azasphingosine **7** in 95% yield.

Scheme 3



a) LiBr, THF; $BocNHC_{11}H_{23}$, NaH, DMF; 70%. *b*) PMe_3, THF/H_2O 4 : 1. *c*) TFA/CH_2Cl_2 1 : 9; 60% from **22**. *d*) *Amberlyst A-26* (OH^- form), MeOH; 95%.

The ^{1}H-NMR chemical shifts and coupling constants for sphingosine (**1**) and the analogues **3**, **5**, **7**, and **24** in $CDCl_3$, and for **6** in CD_3OD are listed in *Table 1*. The D-*erythro*-configuration of **5**–**7** and **24** is evidenced by $J(2,3) = 3.6–6.0$ Hz, in agreement with $J(2,3)$ for **1** and **3**. *HO*–C(1) and *HO*–C(3) of **1**, **3**, **5**, and **7** resonate at similar fields, but those of **24** are shifted downfield, in agreement with the acceptor effect of the trifluoroacetamido group.

Biological Results. – *Phosporylation by Sphingosine Kinase* (*SPHK*). The relative rates for phosphorylation of the sphingosine derivatives by SPHK1 and SPHK2, as determined in comparison to sphingosine (**1**) [27] are shown in *Table 2*. While sphingosine is phosphorylated with similar efficiency by both SPHK isoforms [28], the 7-oxasphingosine was phosphorylated faster by SPHK1 than by SPHK2. 7-Oxasphingosine (**3**) was phosphorylated by SPHK1 with a similar rate as sphingosine. The 7-thiasphingosine (**5**) proved a poorer substrate than sphingosine for both isoforms, but showed a slight preference for SPHK2. Both the sulfone **6** analogue, and the 7-aza

Table 1. *Selected ^{1}H-NMR Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *of* D-erythro-*Sphingosine* (**1**) *and Its Analogues* **3**, **5**–**7**, *and* **24** (in $CDCl_3$)

	1 [27]	**3** [15]	**5**	**6**[a])	**7**	**24**
H_a–C(1)	3.67	3.71–3.61	3.64	3.66	3.70–3.61	4.04
H_b–C(1)	3.60	3.71–3.61	3.64	3.52	3.70–3.61	3.67
H–C(2)	2.87	2.90	2.83	2.81	2.89	3.92
H–C(3)	4.04	4.14	4.11	4.13	4.11	4.38
H–C(4)	5.46	5.76	5.58	5.98	5.68	5.71
H–C(5)	5.75	5.89	5.77	5.81	5.87	5.93
CH_2(6)	2.04	3.98	3.14	3.92–3.78	3.27	3.25
HO–C(3)	1.95	2.25–1.82	2.52	–	1.88	3.25
HO–C(1)	1.95	2.25–1.82	2.52	–	1.88	3.25
J(1a,2)	5.9	5.1	4.8	4.8	5.3	2.3
J(1b,2)	4.5	[b])	4.8	6.3	5.3	3.3
J(2,3)	5.1	5.1	5.7	5.1	6.0	3.6
J(3,4)	6.9	6.3	6.6	6.3	6.7	5.2
J(4,5)	15.5	15.6	15.3	15.6	15.6	15.5
J(5,6)	7.0	5.1	7.2	7.2	6.0	5.8

[a]) Recorded in CD_3OD. [b]) Not assigned.

Table 2. *Rate of Phosphorylation of* D-erythro-*Sphingosine* (**1**) *and Its Analogues* **3**, **5**–**7**, *and* **24** *by SPHK1 and SPHK2* (values relative to the rate for **1**)

Compound	SPHK1	SPHK2
1	1	1
3	0.86	0.081
5	0.12	0.18
6	not detectable	not detectable
7	not detectable	not detectable
24	not detectable	not detectable

compounds **7** and **24** were not phosphorylated by either SPHK1 or SPHK2. The results clearly show that the electronic environment and/or nature of the groups near C(7) of sphingosine do have an effect on the biological activity.

CD1d Binding. Compounds **5**–**7** and **24** did neither activate iNKT-cell clones when presented by human CD1d-transfected antigen-presenting cells (APC) nor by plate-bound human CD1d (data not shown). Compounds **5**, **7**, and **24** were cytotoxic for APC and iNKT cells above 5 μg/ml as assessed by flow cytometry (data not shown), and, therefore, cytotoxicity could mask their stimulatory capacity. Next, binding to CD1d was evaluated in cell-free competition assays. When T cells were stimulated with plate-bound recombinant CD1d and α-GalCer, only the 7-azasphingosines **7** and **24** were partially inhibitory at a 20-fold molar excess (*Fig. 2*).

The large molar excess needed to compete with α-GalCer could be attributed to the fact that these lipids have only one tail, and thus bind weakly to CD1d, whereas α-GalCer binds with high affinity because it has two lipid tails. The compounds were also tested for inhibition of the response to α-GalCer using living APC. This is a more

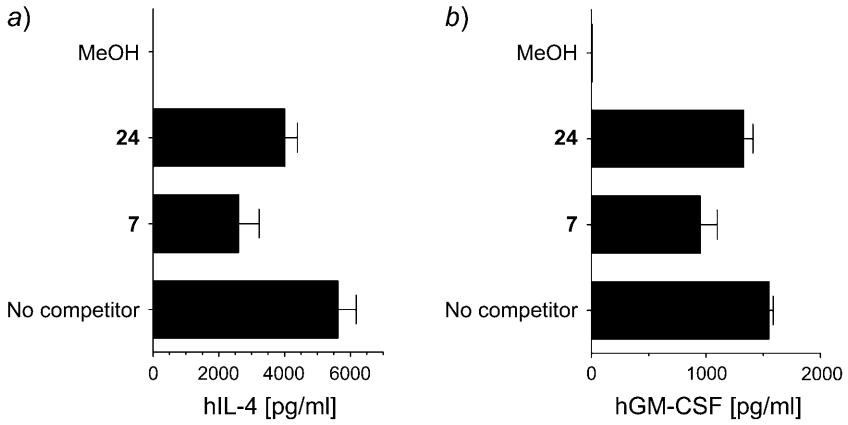


Fig. 2. *The 7-azasphingosines* **7** *and* **24** *are able to compete with α-GalCer for plate-bound human CD1d.* Weak reduction of both *a*) human IL-4 and *b*) GM-CSF release is observed.

sensitive assay than the one using plate-bound CD1d, but could be affected by other types of lipid influence on APC. All compounds were carefully applied at nontoxic doses. As observed with plate-bound assays, also with living APC, compounds **7** and **24** slightly inhibited T-cell response (*Fig. 3*). This was observed with all three tested human cytokines (IL-4, IFN-γ, and TNF-α).

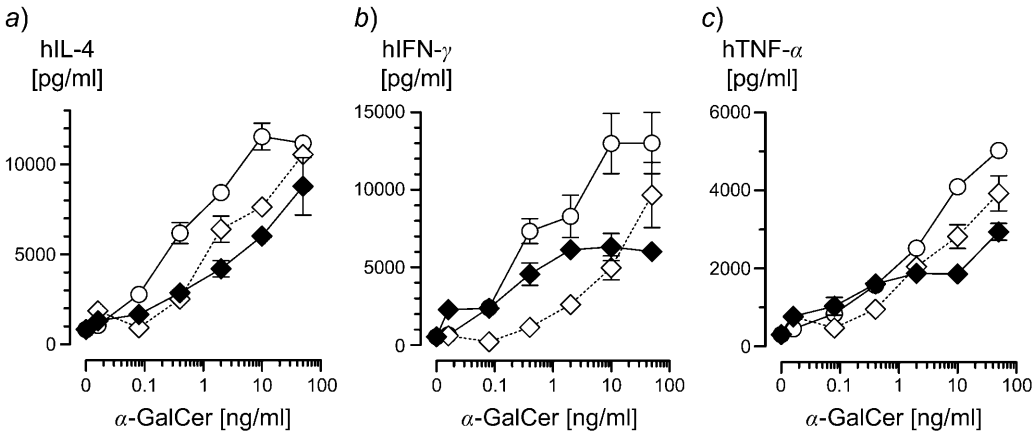


Fig. 3. *The 7-azasphingosines* **7** *and* **24** *are able to compete with α-GalCer for human CD1d on living antigen-presenting cells* (APC). Reduction of iNKT activation is seen by release of *a*) human IL-4, *b*) IFN-γ, and *c*) TNF-α. Compounds **7** and **24** affect potency and/or efficacy of α-GalCer differently regarding the cytokine measured ($\circ$ = no competitor, $\diamond$ = **24**, $\blacklozenge$ = **7**).

In conclusion, both 7-azasphingosines **7** and **24** do not stimulate iNKT cells but bind to human CD1d, and might be used to influence iNKT-cell responses.

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Experimental Part

1. *Synthesis.* – *General.* THF was distilled from Na and benzophenone, CH_2Cl_2 from P_2O_5, and MeOH from CaH_2. Reactions were carried out under N_2, unless stated otherwise. Qual. TLC: precoated silica-gel plates (*Merck* silica gel *60 F_{254}*); detection by heating with 'mostain' (400 ml of 10% H_2SO_4 soln., 20 g of $(NH_4)_6Mo_7O_{24}\cdot 6\,H_2O$, 0.4 g of $Ce(SO_4)_2$). Flash chromatography (FC): silica gel *Fluka 60* (0.04–0.063 mm). M.p.: uncorrected. Optical rotations: 1-dm cell at 25°, 589 nm. FT-IR Spectra: ATR or *ca.* 2% soln. in $CHCl_3$, $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR spectra: chemical shifts δ in ppm rel. to TMS as external standard and coupling constants J in Hz. HR-MALDI-MS: in gentisic acid (=2,5-dihydroxybenzoic acid, DHB) matrix.

*3,4,6-Tris-*O*-(4-methoxybenzyl)-*D*-galactal* (**9**) [18]. A soln. of **8** (1.526 g, 10.44 mmol) in DMF (20 ml) was cooled to 0° and treated with NaH (60% in oil; 2.08 g, 52.21 mmol) in small portions. After the evolution of gas had ceased (90 min), the mixture was treated with 4-methoxybenzyl (PMB) chloride (7.08 ml, 52.21 mmol) and Bu_4NI (200 mg, 5.22 mmol), and stirred at r.t. for 14 h. After dilution with AcOEt and H_2O, the layers were separated. The org. phase was washed with brine (2×30 ml), and the aq. phase was extracted with AcOEt (2×30 ml). The combined org. phases were dried (Na_2SO_4) and evaporated. FC (hexane/AcOEt 7:3) gave **9** (4.88 g, 92%). Pale yellow syrup. R_f (hexane/AcOEt 7:3) 0.42. $[\alpha]_D^{25} = -43.2$ ($c = 2.0$, $CHCl_3$). IR (ATR): 2921*w*, 2853*w*, 1643*w*, 1611*m*, 1585*w*, 1511*s*, 1463*m*, 1442*w*, 1301*m*, 1243*s*, 1171*m*, 1084*s*, 1056*m*, 1030*s*, 909*w*, 816*s*, 730*m*. ^1H-NMR (300 MHz, $CDCl_3$): 7.27–7.20 (*m*, 6 arom. H); 6.89–6.83 (*m*, 6 arom. H); 6.34 (*dd*, J=6.0, 1.5, H–C(1)); 4.83–4.81 (*m*, H–C(2)); 4.79 (*d*, J=11.1), 4.57 (*d*, J=11.7) (ArCH_2); 4.56 (*s*, ArCH_2); 4.43, 4,33 (2*d*, J=11.4, ArCH_2); 4.16–4.12 (*m*, H–C(4), H–C(5)); 3.90–3.87 (*m*, H–C(3)); 3.81, 3.80, 3.79 (3*s*, 3 MeO); 3.71 (*dd*, J=9.9, 2.7, H_a–C(6)); 3.56 (*dd*, J=10.2, 5.1, H_b–C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): 159.05 (2*s*); 158.94 (*s*); 143.96 (*d*, C(1)); 130.51, 130.44, 129.95 (3*s*); 129.70, 129.43, 128.92 (6*d*); 113.66 (4*d*); 113.58 (2*d*); 100.10 (*d*, C(2)); 75.73 (*d*, C(4)); 73.03 (*d*, C(3)); 72.88 (*t*, 3 ArCH$_2$); 70.51 (*d*, C(5)); 68.17 (*t*, C(6)); 55.25 (*q*, 3 MeO). HR-MALDI-MS: 529.2188 (100, $[M+Na]^+$, $C_{30}H_{34}NaO_7^+$; calc. 529.2197). Anal. calc. for $C_{30}H_{34}O_7$ (506.59): C 71.13, H 6.76; found: C 70.95, H 6.79.

*(*E*)-2,3-Dideoxy-4,6-bis-*O*-(4-methoxybenzyl)-*D*-threo-hex-2-enose* (**10**). A soln. of **9** (2 g, 3.947 mmol) in 1,4-dioxane (40 ml) was treated with 0.02N H_2SO_4 (8 ml) and $HgSO_4$ (12 mg, 0.039 mmol), and stirred at 25° for 23 h. The mixture was neutralised by the addition of sat. aq. $NaHCO_3$ soln. (20 ml). After extraction with AcOEt (2×20 ml), the org. phase was dried (Na_2SO_4) and evaporated. FC (pentane/AcOEt 7:3) gave **10** (>92%). Colourless oil. R_f (hexane/AcOEt 7:3) 0.14. IR (ATR): 3452 (br.), 2910*w*, 2863*w*, 2836*w*, 1686*m*, 1611*m*, 1585*w*, 1511*s*, 1463*w*, 1442*w*, 1421*w*, 1364*w*, 1301*m*, 1244*s*, 1173*m*, 1087*s*, 1029*s*, 979*m*, 816*s*, 757*w*. ^1H-NMR (300 MHz, $CDCl_3$): 9.55 (*d*, J=7.8, H–C(1)); 7.21 (*d*, J = 8.7, 4 arom. H); 6.87 (*d*, J=7.8, 4 arom. H); 6.75 (*dd*, J=15.6, 5.7, H–C(3)); 6.32 (*ddd*, J=15.9, 7.8, 1.2, H–C(2)); 4.56, 4.36 (2*d*, J=11.1, ArCH_2); 4.46, 4.40 (2*d*, J=11.4, ArCH_2); 4.25 (*td*, J = 6.3, 1.2, H–C(4)); 3.83–3.80 (*m*, H–C(5)); 3.81 (*s*, 2 MeO); 3.54 (*dd*, J=9.9, 4.8, H_a–C(6)); 3.45 (*dd*, J=9.6, 5.4, H_b–C(6)); 2.58 (*d*, J=5.1, OH). ^{13}C-NMR (75 MHz, $CDCl_3$): 192.93 (*d*, C(1)); 159.39, 159.22 (2*s*); 153.22 (*d*, C(3)); 133.55 (*d*, C(2)); 129.62 (2*d*); 129.54 (2*d*); 129.46, 129.07 (2*s*); 113.87 (2*d*); 113.77 (2*d*); 78.09 (*d*, C(4)); 73.15 (*t*, ArCH$_2$); 72.16 (*d*, C(5)); 71,85 (*t*, ArCH_2); 69.62 (*t*, C(6)); 55.33, 55.28 (2*q*, 2 MeO). HR-MALDI-MS: 409.1616 (100, $[M+Na]^+$, $C_{22}H_{26}NaO_6^+$; calc. 409.1622).

*(*E*)-2,3-Dideoxy-4,6-bis-*O*-(4-methoxybenzyl)-5-*O*-(methylsulfonyl)-*D*-threo-hex-2-enose* (**11**). An ice-cold soln. of **10** (100 mg, 0.258 mmol) in CH_2Cl_2 (1 ml) was treated successively with pyridine (55 µl, 0.68 mmol) and MsCl (34 µl, 0.43 mmol), stirred at 0°, and allowed to warm to 25° over 3.5 h. The mixture was diluted with H_2O. After extraction with CH_2Cl_2 (3×20 ml), the combined org. phases were washed with brine (2×20 ml), dried (Na_2SO_4), evaporated, and dried under high vacuum overnight. Crude **11** (115 mg, 96%) was used for the next step without further purification. R_f (hexane/AcOEt 7:3) 0.24. IR (ATR): 3002*w*, 2921*m*, 2852*w*, 1737*w*, 1689*m*, 1611*m*, 1585*w*, 1512*m*, 1463*w*, 1360*m*, 1302*w*, 1245*s*, 1170*s*, 1096*m*, 1029*s*, 969*m*, 918*s*, 813*s*, 748*m*. ^1H-NMR (300 MHz, $CDCl_3$): 9.55 (*d*, J=7.8, H–C(1)); 7.22–7.19 (*m*, 4 arom. H); 6.87 (*d*, J=8.7, 4 arom. H); 6.73 (*dd*, J=15.6, 5.1, H–C(3)); 6.36 (*ddd*, J=15.9, 7.8, 1.2, H–C(2)); 4.81–4.76 (*m*, H–C(5)); 4.57–4.36 (*m*, H–C(4), 2 ArCH_2); 3.79 (*s*, 2 MeO); 3.67 (*dd*, J=11.1, 3.0, H_a–C(6)); 3.57 (*dd*, J=11.1, 6.6, H_b–C(6)); 2.97 (*s*, MsO). ^{13}C-NMR (75 MHz, $CDCl_3$): 192.58 (*d*, C(1)); 159.46, 159.30 (2*s*); 150.39 (*d*, C(3)); 134.19 (*d*, C(2)); 129.65 (2*d*); 129.48 (2*d*); 128.99,

128.61 (2*s*); 113.87 (2*d*); 113.82 (2*d*); 81.14 (*d*, C(5)); 76.50 (*d*, C(4)); 73.18, 72.09 (2*t*, 2 ArCH$_2$); 68.23 (*t*, C(6)); 55.31, 55.25 (2*q*, 2 MeO); 38.53 (*q*, MsO). HR-MALDI-MS: 487.1389 (100, $[M+Na]^+$, $C_{23}H_{28}NaO_8S^+$; calc. 487.1397).

*(*E*)-2,3-Dideoxy-4,6-bis-*O*-(4-methoxybenzyl)-5-*O*-(methylsulfonyl)-*D*-threo-hex-2-enitol* (**12**). An ice-cold soln. of **11** (115 mg, 0.247 mmol) in MeOH/CH_2Cl_2 26 :4 (3 ml) was treated with $NaBH_4$ (9.4 mg, 0.247 mmol), stirred at 0° for 50 min, diluted with H_2O, and evaporated. A soln. of the residue in $CHCl_3$ was washed with H_2O. The combined org. phases were dried (Na_2SO_4) and evaporated. Crude **12** (108 mg, 93%) was used for the next step without further purification. R_f (hexane/AcOEt 6 :4) 0.24. IR (ATR): 3453 (br.), 3006*w*, 2936*w*, 2867*w*, 1665*w*, 1611*m*, 1585*w*, 1512*s*, 1463*w*, 1420*w*, 1346*m*, 1302*m*, 1244*s*, 1170*s*, 1086*m*, 1029*s*, 968*m*, 915*s*, 816*s*, 756*w*, 663*w*, 636*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.24–7.19 (*m*, 4 arom. H); 6.89–6.30 (*m*, 4 arom. H); 5.96 (br. *dt*, *J* = 15.6, 5.8, H–C(2)); 5.61 (*ddt*, *J* = 15.6, 7.5, 1.5, H–C(3)); 4.71 (*td*, *J* = 5.7, 3.9, H–C(5)); 4.55, 4.27 (2*d*, *J* = 11.4, ArCH_2); 4.46, 4.37 (2*d*, *J* = 11.1, ArCH_2); 4.15 (br. *s*, 2 H–C(1)); 4.11 (br. *t*, *J* ≈ 6.6, H–C(4)); 3.79 (*s*, 2 MeO); 3.66–3.64 (*m*, 2 H–C(6)); 2.95 (*s*, MsO); 2.44 (br. *s*, OH). ^{13}C-NMR (75 MHz, $CDCl_3$): 159.16, 159.10 (2*s*); 135.37 (*d*, C(3)); 129.57 (2*s*); 129.49 (2*d*); 129.41 (2*d*); 125.40 (*d*, C(2)); 113.73 (2*d*); 113.71 (2*d*); 83.22 (*d*, C(5)); 77.61 (*d*, C(4)); 73.03, 70.44 (2*t*, 2 ArCH$_2$); 68.91 (*t*, C(6)); 62.37 (*t*, C(1)); 51.27 (*q*, 2 MeO); 38.64 (*q*, MsO). HR-MALDI-MS: 489.1549 (100, $[M+Na]^+$, $C_{23}H_{30}NaO_8S^+$; calc. 489.1554).

*(*E*)-2-Azido-2,4,5-trideoxy-1,3-bis-*O*-(4-methoxybenzyl)-*D*-erythro-hex-4-enitol* (**13**). A soln. of NaN_3 (464 mg, 7.13 mmol) and **12** (555 mg, 1.13 mmol) in dry DMF (10 ml) was heated to 140° for 4.5 h, cooled to 25°, and diluted with H_2O. The aq. phase was extracted with Et_2O. The combined org. phases were dried (Na_2SO_4) and evaporated to give crude **13** (525 mg, 89%), which was used for the next step without further purification. R_f (hexane/AcOEt 3 :2) 0.30. $[\alpha]_D^{25} = -27.7$ (*c* = 1.9, $CHCl_3$). IR (ATR): 3402 (br.), 2922*w*, 2857*w*, 2097*m*, 1665*m*, 1611*m*, 1585*w*, 1512*s*, 1463*w*, 1386*w*, 1301*m*, 1244*s*, 1173*m*, 1088*m*, 1030*s*, 974*m*, 816*s*, 756*w*, 660*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.31–7.17 (*m*, 4 arom. H); 6.92–6.83 (*m*, 4 arom. H); 5.90 (*dt*, *J* = 15.9, 5.1, H–C(5)); 5.66 (*ddt*, *J* = 15.6, 7.8, 1.5, H–C(4)); 4.64, 4.52 (2*dd*, *J* = 11.7, ArCH_2); 4.47, 4.42 (2*dd*, *J* = 11.4, ArCH_2); 4.19 (*dd*, *J* = 4.8, 1.5, 2 H–C(6)); 3.95 (*dd*, *J* = 7.8, 5.7, H–C(3)); 3.80, 3.79 (2*s*, 2 MeO); 3.65–3.51 (*m*, 2 H–C(1), H–C(2)); 1.90 (br. *s*, OH). ^{13}C-NMR (75 MHz, $CDCl_3$): 159.18, 159.09 (2*s*); 135.14 (*d*, C(4)); 129.84, 129.75 (2*s*); 129.24 (2*d*); 129.20 (2*d*); 127.07 (*d*, C(5)); 113.70 (2*d*); 113.67 (2*d*); 78.30 (*d*, C(3)); 72.91, 70.08 (2*t*, 2 ArCH$_2$); 68.80 (*t*, C(1)); 64.09 (*d*, C(2)); 62.55 (*t*, C(6)); 55.16 (*q*, 2 MeO). HR-MALDI-MS: 436.1850 (100, $[M+Na]^+$, $C_{22}H_{27}N_3NaO_5^+$; calc. 436.1843). Anal. calc. for $C_{22}H_{27}N_3O_5$ (413.20): C 63.91, H 6.58, N 10.16; found: C 63.84, H 6.67, N 10.04.

*(*E*)-2-Azido-2,4,5-trideoxy-1,3-bis-*O*-(4-methoxybenzyl)-6-*O*-undecyl-*D*-erythro-hex-4-enitol* (**14**). A soln. of **13** (500 mg, 1.21 mmol) in DMF (15 ml) was treated with NaH (60% in oil; 145 mg, 3.63 mmol), stirred at r.t. for 15 min, treated with 1-bromoundecane (542 µl, 2.42 mmol), stirred at 25° for 20 h, and treated with MeOH (10 ml). The mixture was diluted with H_2O and extracted with AcOEt. The combined org. phases were washed with H_2O and brine, dried (Na_2SO_4), and evaporated. FC (pentane/AcOEt 95 :5) gave **14** (536 mg, 78%). Colourless oil. R_f (hexane/AcOEt 95 :5) 0.17. $[\alpha]_D^{25} = -25.9$ (*c* = 0.75, $CHCl_3$). IR (ATR): 2923*m*, 2853*m*, 2097*m*, 1739*w*, 1612*m*, 1586*w*, 1512*s*, 1484*w*, 1362*w*, 1301*m*, 1245*s*, 1172*m*, 1095*m*, 1035*s*, 973*w*, 819*m*, 757*w*, 721*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.26–7.16 (*m*, 4 arom. H); 6.91–6.83 (*m*, 4 arom. H); 5.81 (*dt*, *J* = 15.6, 5.1, H–C(5)); 5.67 (*ddt*, *J* = 15.6, 7.8, 1.2, H–C(4)); 4.54, 4.28 (2*d*, *J* = 11.4, ArCH_2); 4.45 (*s*, ArCH_2); 4.01 (*dd*, *J* = 5.4, 1.2, 2 H–C(6)); 3.95 (*dd*, *J* = 7.8, 5.7, H–C(3)); 3.80, 3.79 (2*s*, 2 MeO); 3.65–3.51 (*m*, 2 H–C(1), H–C(2)); 3.42 (*t*, *J* = 6.6, 2 H–C(1′)); 1.64–1.54 (*m*, 2 H–C(2′)); 1.38–1.20 (*m*, 16 H); 0.88 (*t*, *J* = 6.8, Me). ^{13}C-NMR (75 MHz, $CDCl_3$): 159.09, 158.99 (2*s*); 133.00 (*d*, C(4)); 129.83 (*d*, C(5)); 129.74, 128.27 (2*s*); 129.17 (4*d*); 113.70 (2*d*); 113.67 (2*d*); 78.46 (*d*, C(3)); 73.01, 70.60 (2*t*, 2 ArCH$_2$); 70.44 (*t*, C(1)); 70.14 (*t*, C(6)); 68.98 (*d*, C(2)); 64.23 (*t*, C(1′)); 55.26 (*q*, 2 MeO); 31.99 (*t*); 29.83–29.42 (several *t*); 26.31, 22.77 (2*t*); 14.23 (*q*, Me). HR-MALDI-MS: 590.3564 (100, $[M+Na]^+$, $C_{33}H_{49}N_3NaO_5^+$; calc. 590.3564). Anal. calc. for $C_{33}H_{49}N_3O_6$ (567.76): C 69.81, H 8.70, N 7.40; found: C 69.99, H 8.71, N 7.26.

*(*E*)-2-Amino-2,4,5-trideoxy-1,3-bis-*O*-(4-methoxybenzyl)-6-*O*-undecyl-*D*-erythro-hex-4-enitol* (**15**). A suspension of $LiAlH_4$ (133 mg, 3.49 mmol) in dry Et_2O (5 ml) was cooled to 0°, treated dropwise with a soln. of **14** (500 mg, 0.88 mmol) in dry Et_2O (5 ml), allowed to warm to 25°, and stirred for 14 h. The mixture was cooled to 0°, and treated dropwise with H_2O (750 µl), 1N NaOH (1.5 ml), and H_2O (900

μl). The suspension was filtered through *Celite*, and the filtrate was extracted with AcOEt (3 × 50 ml). The combined org. phases were dried ($MgSO_4$) and evaporated to afford crude **15** (475 mg, >99%) which was used for the next step without further purification. R_f (hexane/AcOEt 2:3) 0.15. IR (ATR): 3290*w* (br.), 2922*s*, 2852*m*, 1612*w*, 1586*w*, 1513*s*, 1464*m*, 1376*w*, 1301*w*, 1246*s*, 1172*m*, 1083*m*, 1036*s*, 975*w*, 819*m*, 756*w*, 721*w*, 637*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.32–7.17 (*m*, 4 arom. H); 6.92–6.82 (*m*, 4 arom. H); 5.82 (*dt*, *J* = 15.3, 5.4, H–C(5)); 5.66 (*ddt*, *J* = 15.6, 8.1, 1.5, H–C(4)); 4.51, 4.25 (2*d*, *J* = 11.4, ArCH_2); 4.41 (*s*, ArCH_2); 4.01 (*dd*, *J* = 5.4, 1.2, 2 H–C(6)); 3.80, 3.79 (2*s*, 2 MeO); 3.63 (*t*, *J* = 6.6, 2 H–C(1′)); 3.56 (*dd*, *J* = 9.3, 4.2, H_a–C(1)); 3.47–3.40 (*m*, H_b–C(1), H–C(3)); 3.10–3.04 (*m*, H–C(2)); 1.64–1.54 (*m*, 2 H–C(2′)); 1.38–1.20 (*m*, 16 H); 0.88 (*t*, *J* = 6.8, Me). ^{13}C-NMR (75 MHz, $CDCl_3$): 159.02 (2*s*); 132.46 (*d*, C(4)); 130.42 (2*s*); 129.80 (*d*, C(5)); 129.25 (4*d*); 113.69 (4*d*); 80.76 (*d*, C(3)); 72.97 (*t*, ArCH_2); 71.62 (*t*, C(1)); 70.63 (*t*, ArCH_2); 70.13 (*t*, C(6)); 63.11 (*t*, C(1′)); 55.32 (*q*, 2 MeO); 54.39 (*d*, C(2)); 32.03 (*t*); 29.81–29.46 (several *t*); 26.36, 22.82 (2*t*); 14.27 (*q*, Me). HR-MALDI-MS: 542.3831 (100, $[M+Na]^+$, $C_{33}H_{51}NNaO_5^+$; calc. 542.3840).

*(*E*)-2-Amino-2,4,5-trideoxy-6-*O*-undecyl-*D*-*erythro*-hex-4-enitol* (**3**). A soln. of **15** (60 mg, 0.11 mmol) in CH_2Cl_2 (4.5 ml) was treated dropwise with TFA (0.5 ml, 6.5 mmol), stirred at 25° for 15 min., and poured into sat. aq. $NaHCO_3$ soln. (30 ml). The aq. layer was extracted with AcOEt (2 × 25 ml). The combined org. layers were dried (Na_2SO_4) and evaporated. FC (AcOEt/pentane 1:1) gave **3** (24.7 mg, 74%) whose spectral and physical data are in accordance with those reported in [15].

*(*E*)-1,3-Bis-*O*-(4-methoxybenzyl)-2,4,5-trideoxy-2-(octadecanoylamino)-6-*O*-undecyl-*D*-erythro-hex-4-enitol* (**16**). A soln. of crude **15** (250 mg, 0.46 mmol) in dry CH_2Cl_2 (10 ml) was treated with pyridine (186 μl, 2.31 mmol) and stearoyl chloride (209 mg, 0.69 mmol), stirred at 25° for 6 h, diluted with H_2O, and extracted with CH_2Cl_2 (3 × 100 ml). The combined org. layers were dried (Na_2SO_4) and evaporated. Residual pyridine was removed by azeotropic distillation with toluene. FC (AcOEt/hexane 1:3) gave **16** (353 mg, 95%). White fluffy powder. M.p. 83.9°. R_f (AcOEt/hexane 1:3) 0.21. $[\alpha]_D^{25} = -25.4$ (*c* = 0.6, $CHCl_3$). IR (ATR): 3297*w*, 2953*w*, 2915*s*, 2848*s*, 1703*w*, 1644*s*, 1614*w*, 1586*w*, 1543*m*, 1515*m*, 1469*m*, 1364*w*, 1302*w*, 1253*m*, 1172*w*, 1119*m*, 1093*m*, 1033*m*, 888*w*, 811*m*, 718*w*. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.20–7.16 (*m*, 4 arom. H); 6.87–6.83 (*m*, 4 arom. H); 5.78 (*dt*, *J* = 15.6, 6.0, H–C(5)); 5.72 (*d*, *J* = 9.3, NH); 5.64 (*ddt*, *J* = 15.6, 7.9, 1.2, H–C(4)); 4.52, 4.41, 4.35 (3*d*, *J* = 11.4, 3 ArCH); 4.24–4.18 (*m*, H–C(2)): 4.22 (*d*, *J* = 11.6, ArCH); 3.97 (*dd*, *J* = 5.6, 0.9, 2 H–C(6)); 3.93 (*t*, *J* = 7.6, H–C(3)); 3.80, 3.79 (2*s*, 2 MeO); 3.75 (*dd*, *J* = 9.6, 4.0, H_a–C(1)); 3.48 (*dd*, *J* = 9.6, 4.0, H_b–C(1)); 3.44–3.35 (*AB*, 2 H–C(1′)); 2.32 (*t*, *J* = 7.5, 2 H–C(1″)); 2.13–2.01 (*m*, 2 H–C(2′)); 1.61–1.53 (*m*, 4 H); 1.35–1.30 (*m*, 42 H); 0.89 (*t*, *J* = 6.8, Me); 0.88 (*t*, *J* = 6.8, Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 172.57 (*s*, *C*=O); 159.28, 159.19 (2*s*); 132.17 (*d*, C(4)); 130.36 (*s*); 130.23 (*d*, C(5)); 130.20 (*s*); 129.41 (2*d*); 129.36 (2*d*); 113.78 (2*d*); 113.76 (2*d*); 78.54 (*d*, C(3)); 72.75 (*t*, ArCH_2); 70.66 (*t*, C(6)); 70.51 (*t*, C(1′)); 70.24 (*t*, ArCH_2); 68.07 (*t*, C(1)); 55.24 (*q*, 2 MeO); 51.48 (*d*, C(2)); 36.95 (*t*, C(2″)); 33.86 (2*t*); 31.94 (*t*); 29.81–29.16 (several *t*); 26.26, 25.75 (2*t*); 22.70 (2*t*); 14.11 (*q*, 2 Me). HR-MALDI-MS: 830.6270 (100, $[M+Na]^+$, $C_{51}H_{85}NNaO_6^+$; calc. 830.6275). Anal. calc. for $C_{51}H_{85}NO_6$ (808.22): C 75.79, H 10.60, N 1.73; found: C 75.98, H 10.69, N 1.62.

*(*E*)-2,4,5-Trideoxy-2-[(octadecanoyl)amino]-6-*O*-undecyl-*D*-erythro-hex-4-enitol* (**4**). An ice-cold soln. of **16** (60 mg, 0.074 mmol) in CH_2Cl_2 (4.5 ml) was treated dropwise with TFA (0.5 ml, 6.5 mmol), stirred at 0° for 30 min, and then at 25° for 30 min, and poured into sat. aq. $NaHCO_3$ soln (30 ml). The aq. layer was extracted with AcOEt (2 × 25 ml). The combined org. layers were dried (Na_2SO_4) and evaporated. FC (CH_2Cl_2/MeOH 4:1) gave **4** (31.5 mg, 75%) whose spectral and physical data are in accordance with those reported in [15].

*(*E*)-2-Azido-2,4,5-trideoxy-1,3-bis-*O*-(4-methoxybenzyl)-6-*O*-(methylsulfonyl)-*D*-erythro-hex-4-enitol* (**17**). A soln. of **13** (1.64 g, 3.97 mmol) in CH_2Cl_2 was cooled to 0° and treated successively with Ms_2O (1.036 g, 5.95 mmol) and $EtN(i\text{-}Pr)_2$ (0.98 ml, 5.95 mmol). The mixture was stirred for 2.5 h, diluted with CH_2Cl_2, and washed with H_2O. After separation of the layers, the aq. phase was extracted with CH_2Cl_2 (3 × 100 ml). The combined org. phases were washed with brine, dried (Na_2SO_4), and evaporated to afford crude **17**, which was filtered through a short pad of silica gel (AcOEt/hexane 2:3) to yield anal. pure **17** (1.95 g, >99%). R_f (AcOEt/hexane 3:7) 0.19. IR (ATR): 3010–2835*w*, 2097*w*, 1745*w*, 1715*w*, 1611*m*, 1585*w*, 1512*s*, 1463*w*, 1353*m*, 1301*m*, 1274*m*, 1244*s*, 1171*s*, 1087*m*, 1064*m*, 1030*s*, 972*m*, 927*s*, 816*s*, 750*s*. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.25–7.19 (*m*, 4 arom. H); 6.89–6.86 (*m*, 4 arom. H); 5.92–5.81 (*m*,

H–C(4), H–C(5)); 4.74 (*d*, *J* = 4.5, 2 H–C(6)); 4.52, 4.32 (2*d*, *J* = 11.4, ArC*H*$_2$); 4.46, 4.44 (2*d*, *J* = 11.6, ArC*H*$_2$); 4.00 (*td*, *J* = 5.5, 1.0, H–C(3)); 3.81, 3.80 (2*s*, 2 MeO); 3.63 (*td*, *J* = 6.0, 4.6, H–C(2)); 3.56–3.54 (*m*, 2 H–C(1)); 3.02 (*s*, MsO). ^{13}C-NMR (100 MHz, $CDCl_3$): 159.39 (2*s*); 133.37 (*d*, C(4)); 129.74, 129.70 (2*s*); 129.39 (4*d*); 127.44 (*d*, C(5)); 113.88 (4*d*); 77.71 (*d*, C(3)); 73.08, 70.74 (2*t*, 2 Ar*C*H$_2$); 69.00 (*t*, C(6)); 68.68 (*t*, C(1)); 63.95 (*d*, C(2)); 55.29 (*q*, 2 MeO); 38.06 (*q*, MsO). HR-MALDI-MS: 514.1620 (100, $[M+Na]^+$, $C_{23}H_{29}N_3NaO_7S^+$; calc. 514.1624).

*(*E*)-2-Azido-2,4,5,6-tetradeoxy-1,3-bis-*O*-(methoxybenzyl)-6-(undecylsulfanyl)-*D*-erythro-hex-4-enitol* (**18**). A suspension of NaH, (60% in oil, 112 mg, 2.8 mmol) in DMF (3 ml) was cooled to 0°, treated with a soln. of $C_{11}H_{23}SH$ (263.8 mg, 0.54 mmol) in DMF (2 ml), stirred for 5 min, treated dropwise with a soln. of **17** (459 mg, 0.934 mmol), stirred for 75 min, treated dropwise with ice-cold H_2O (3 ml), diluted with H_2O (20 ml) and brine (5 ml), and extracted with AcOEt (3 × 100 ml). The combined org. phases were washed with brine, dried (Na_2SO_4), and evaporated. FC (hexane/Et_2O 1:0 → 4:1) gave **18** (472 mg, 87%). Pale yellow oil. R_f (Et_2O/hexane 15:85) 0.26. $[\alpha]_D^{25} = -29.3$ ($c = 0.53$, $CHCl_3$). IR ($CHCl_3$): 3002*w*, 2923*s*, 2853*s*, 2095*m*, 1612*w*, 1586*w*, 1512*s*, 1464*w*, 1441*w*, 1419*w*, 1362*w*, 1301*w*, 1245*s*, 1172*m*, 1081*m*, 1034*s*, 970*m*, 819*m*, 757*w*, 720*w*, 637*w*. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.28–7.19 (*m*, 4 arom. H); 6.91–6.85 (*m*, 4 arom. H); 5.73 (*dtd*, *J* = 15.2, 7.2, 0.4, H–C(5)); 5.51 (*ddt*, *J* = 15.4, 8.2, 1.1, H–C(4)); 4.55, 4.29 (2*d*, *J* = 11.4, ArC*H*$_2$); 4.48, 4.44 (2*d*, *J* = 11.7, 2 ArC*H*$_2$); 3.92 (*ddd*, *J* = 8.2, 5.3, 0.4, H–C(3)); 3.81, 3.80 (2*s*, 2 MeO); 3.66–3.61 (*m*, H–C(2)); 3.59 (*dd*, *J* = 9.6, 4.4, H_a–C(1)); 3.54 (*dd*, *J* = 9.8, 6.9, H_b–C(1)); 3.23–3.11 (*m*, 2 H–C(6)); 2.48 (*t*, *J* = 7.4, 2 H–C(1′)); 1.59 (*q*, *J* = 7.3, 2 H–C(2′)); 1.35–1.22 (*m*, 16 H); 0.89 (*t*, *J* = 6.7, Me). ^{13}C-NMR ($CDCl_3$, 100 MHz): 159.34, 159.27 (2*s*); 132.84 (*d*, C(4)); 129.94, 129.90 (2*s*); 129.30 (4*d*); 128.72 (*d*, C(5)); 113.86 (2*d*); 113.84 (2*d*); 78.39 (*d*, C(3)); 73.07, 70.05 (2*t*, 2 Ar*C*H$_2$); 69.10 (*t*, C(1)); 64.32 (*d*, C(2)); 55.26 (*q*, 2 MeO); 33.41 (*t*, C(6)); 31.94, 30.10 (2*t*); 29.65–29.33 (several *t*); 28.99, 24.17, 22.71 (3*t*); 14.14 (*q*, Me). HR-MALDI-MS: 622.3075 (100, $[M+K]^+$, $C_{33}H_{49}KN_3O_4S^+$; calc. 622.3081). Anal. calc. for $C_{33}H_{49}N_3O_4S$ (583.3444): C 67.89, H 8.46, N 7.20; found: C 68.14, H 8.52, N 6.94.

*(*E*)-2-Azido-2,4,5,6-tetradeoxy-6-(undecylsulfanyl)-*D*-erythro-hex-4-enitol* (**19**). A soln. of **18** (58.5 mg, 0.1 mmol) in CH_2Cl_2 (4.5 ml) was cooled to 0° and treated with TFA (0.5 ml, 6.5 mmol). The mixture was stirred for 45 min, and diluted with CH_2Cl_2 (20 ml) and H_2O. After separation of the layers, the aq. phase was extracted with CH_2Cl_2 (3 × 30 ml). The combined org. phases were washed with brine, dried (Na_2SO_4), and evaporated to afford crude **19** (34.3 mg, >99%), which was pure enough to be used directly in the next step. IR (ATR): 3371*w* (br.), 2922*s*, 2852*s*, 2099*s*, 1464*m*, 1419*w*, 1265*m*, 1063*m*, 1008*m*, 969*m*, 721*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 5.84 (*dtd*, *J* = 15.3, 7.2, 1.2, H–C(5)); 5.65 (*ddt*, *J* = 15.0, 6.6, 0.9, H–C(4)); 4.32 (*t*, *J* = 6.0, H–C(3)); 3.80 (br. *d*, *J* = 4.9, 2 H–C(1)); 3.53 (*q*, *J* = 5.2, H–C(2)); 3.16 (*d*, *J* = 7.1, 2 H–C(6)); 2.46 (*t*, *J* = 7.5, 2 H–C(1′)); 2.21 (br. *s*, HO–C(3)); 2.09 (br. *s*, HO–C(1)); 1.61–1.52 (*m*, 2 H–C(2′)); 1.39–1.20 (*m*, 16 H); 0.88 (*t*, *J* = 6.7, Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 130.73 (*d*, C(4)); 130.45 (*d*, C(5)); 72.73 (*d*, C(3)); 66.47 (*d*, C(2)); 62.36 (*t*, C(1)); 32.20 (*t*, C(6)); 31.79, 31.08 (2*t*); 29.50 (2*t*); 29.22 (2*t*); 29.43, 29.18 (2*t*); 28.80, 22.58 (2*t*); 14.01 (*q*, Me). HR-MALDI-MS: 366.2198 (100, $[M+Na]^+$, $C_{17}H_{33}N_3NaO_2S^+$; calc. 366.2191).

*(*E*)-2-Amino-2,4,5,6-tetradeoxy-6-(undecylsulfanyl)-*D*-erythro-hex-4-enitol* (**5**). A soln. of crude **19** (34.3 mg, 0.1 mmol) in THF (4 ml) was cooled to 0° and treated dropwise with 1M PMe_3 in THF (0.15 ml, 0.15 mmol). The mixture was warmed to 25° and stirred for 1 h, when the TLC showed complete conversion of **19**, treated with H_2O (1 ml), and stirred for 15 h. The mixture was diluted with AcOEt (30 ml) and H_2O. After separation of the layers, the aq. phase was extracted with AcOEt (3 × 30 ml). The combined org. phases were washed with H_2O and brine, dried (Na_2SO_4), and evaporated. FC (CH_2Cl_2/MeOH 100:0 → 9:1) yielded **5** (26.5 mg, 83.5%). Colourless solid. M.p. 62°. R_f (CH_2Cl_2/MeOH 4:1) 0.12. $[\alpha]_D^{25} = +3.3$ ($c = 0.25$, $CHCl_3$). IR (ATR): 3358 (br.), 3326 (br.), 3286 (br.), 3261 (br.), 3176*w*, 2955*w*, 2917*s*, 2850*s*, 1615*w*, 1579*w*, 1467*w*, 1431*w*, 1337*w*, 1101*w*, 1052*m*, 1038*m*, 1021*m*, 994*m*, 967*m*, 957*m*, 889*w*, 812*w*, 720*w*, 646*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 5.77 (*dtd*, *J* = 15.3, 7.2, 0.9, H–C(5)); 5.58 (br. *dd*, *J* = 15.3, 6.6, H–C(4)); 4.11 (*t*, *J* ≈ 5.7, H–C(3)); 3.64 (*d*, *J* = 4.8, 2 H–C(1)); 3.14 (*d*, *J* = 7.2, 2 H–C(6)); 2.83 (*q*, *J* ≈ 5.1, H–C(2)); 2.52 (br. *s*, exchanged with D_2O, NH_2, 2 OH); 2.45 (*t*, *J* = 7.3, 2 H–C(1′)); 1.60–1.50 (*m*, 2 H–C(2′)); 1.38–1.25 (*m*, 16 H); 0.87 (*t*, *J* = 6.6, Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 132.04 (*d*, C(4)); 129.30 (*d*, C(5)); 74.60 (*d*, C(3)); 63.85 (*t*, C(1)); 56.05 (*d*, C(2)); 33.56 (*t*, C(6)); 32.00 (*t*, C(1′)); 31.43 (*t*); 29.73–29.41 (several *t*); 29.03, 22.81 (2*t*); 14.26 (*q*, Me). HR-MALDI-

MS: 318.2465 (100, $[M+H]^+$, $C_{17}H_{36}NO_2S^+$ calc. 318.2467). Anal. calc. for $C_{17}H_{35}NO_2S$ (317.53): C 64.30, H 11.11, N 4.41; found: C 64.21, H 11.11, N 4.41.

*(*E*)-2-Azido-2,4,5,6-tetradeoxy-1,3-bis*-O-*(methoxybenzyl)-6-(undecylsulfonyl)*-D-erythro-*hex-4-enitol* (**20**). A soln. of **18** (82 mg, 0.14 mmol) in CH_2Cl_2/MeOH 2:1 (6 ml) was treated with oxone (432 mg, 0.7 mmol), stirred at 25° for 24 h, and filtered through a short pad of silica gel (filter cake was washed with AcOEt/CH_2Cl_2 1:2). The combined filtrate and washings were evaporated. FC (AcOEt/hexane 1:4) gave **20** (67 mg, 78%). Colourless syrup. R_f (AcOEt/hexane 1:4) 0.15. $[\alpha]_D^{25} = -16.7$ ($c = 0.55$, $CHCl_3$). IR (ATR): 2923*m*, 2854*w*, 2096*m*, 1612*w*, 1586*w*, 1512*m*, 1464*w*, 1403*w*, 1361*w*, 1301*m*, 1245*s*, 1173*m*, 1127*m*, 1033*s*, 977*m*, 892*w*, 819*s*, 757*w*, 721*w*. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.26–7.17 (*m*, 4 arom. H); 6.90–6.83 (*m*, 4 arom. H); 5.89–5.78 (*m*, H–C(4), H–C(5)); 4.53, 4.32 (2*d*, $J = 11.4$, ArCH_2); 4.45 (*s*, ArCH_2); 3.99 (*dt*, $J = 6.0, 2.0$, H–C(3)); 3.80, 3.79 (2*s*, 2 MeO); 3.71 (*d*, $J = 5.8$, 2 H–C(6)); 3.65 (*td*, $J = 6.1, 4.5$, H–C(2)); 3.58 (*dd*, $J = 10.0, 4.8$, H_a–C(1)); 3.54 (*dd*, $J = 10.0, 6.4$, H_b–C(1)); 2.97–2.91 (*m*, 2 H–C(1′)); 1.86–1.78 (*m*, 2 H–C(2′)); 1.45–1.38 (*m*, 2 H–C(3′)); 1.32–1.25 (*m*, 14 H); 0.88 (*t*, $J = 6.9$, Me). ^{13}C-NMR ($CDCl_3$, 100 MHz): 159.38 (2*s*); 136.84 (*d*, C(4)); 129.69, 129.46 (2*s*); 129.40 (2*d*); 129.37 (2*d*); 122.05 (*d*, C(5)); 113.90 (2*d*); 113.88 (2*d*); 77.67 (*d*, C(3)); 73.10, 70.61 (2*t*, 2 ArCH_2); 68.79 (*t*, C(1)); 63.98 (*d*, C(2)); 56.26 (*t*, C(6)); 55.27 (*q*, 2 MeO); 51.74 (*t*, C(1′)); 31.88 (*t*); 29.54–29.08 (several *t*); 28.51, 22.66, 21.87 (3*t*); 14.09 (*q*, Me). HR-MALDI-MS: 638.3242 (100, $[M+Na]^+$, $C_{33}H_{49}N_3NaO_6S^+$; calc. 638.3240). Anal. calc. for $C_{33}H_{49}N_3O_6S$ (615.82): C 64.36, H 8.02, N 6.82; found: C 64.49, H 8.07, N 6.76.

*(*E*)-2-Azido-2,4,5,6-tetradeoxy-6-(undecylsulfonyl)*-D-erythro-*hex-4-enitol* (**21**). An ice-cold soln. of **20** (65 mg, 0.1 mmol) in CH_2Cl_2 (4.5 ml) was treated dropwise with TFA (0.5 ml), stirred for 1 h, diluted with CH_2Cl_2 (20 ml), and washed with H_2O (20 ml). The aq. phase was extracted with CH_2Cl_2 (3 × 50 ml). The combined org. phases were washed with brine, dried (Na_2SO_4), and evaporated to afford crude **21** (37 mg, >99%), which was anal. pure to be used directly in the next step. IR (ATR): 3433 (br.), 2922*s*, 2853*m*, 2098*s*, 1465*w*, 1405*w*, 1281*s*, 1162*w*, 1125*s*, 1064*m*, 1009*m*, 975*m*, 891*w*, 763*w*, 721*w*, 654*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 6.00 (*dd*, $J = 15.6, 5.4$, H–C(4)); 5.91 (*dt*, $J = 15.6, 6.9$, H–C(5)); 4.38 (*q*, $J \approx 4.7$, addn. of $CD_3OD \rightarrow t$, $J = 5.8$, H–C(3)); 3.82 (*t*, $J \approx 4.7$, addn. of $CD_3OD \rightarrow d$, $J = 5.2$, 2 H–C(1)); 3.73 (*d*, $J \approx 6.3$, 2 H–C(6)); 3.54 (*q*, $J = 4.8$, H–C(2)); 3.37 (*d*, $J \approx 4.5$, exchange with CD_3OD, HO–C(3)); 3.01–2.95 (*m*, 2 H–C(1′)); 2.77 (*t*, $J \approx 5.2$, exchange with CD_3OD, HO–C(1)); 1.87–1.77 (*m*, 2 H–C(2′)); 1.48–1.38 (*m*, 2 H–C(3′)); 1.34–1.26 (*m*, 14 H); 0.87 (*t*, $J = 6.5$, Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 139.02 (*d*, C(4)); 118.87 (*d*, C(5)); 72.00 (*d*, C(3)); 66.23 (*d*, C(2)); 62.20 (*t*, C(1)); 55.98 (*t*, C(6)); 52.28 (*t*, C(1′)); 31.99 (*t*); 29.66, 29.62 (2*t*); 29.41 (2*t*); 29.20, 28.56, 22.79, 21.99 (4*t*); 14.26 (*q*, Me). HR-MALDI-MS: 398.2086 (100, $[M+Na]^+$, $C_{17}H_{33}N_3NaO_4S^+$; calc. 398.2089).

*(*E*)-2-Amino-2,4,5,6-tetradeoxy-6-(undecylsulfonyl)*-D-erythro-*hex-4-enitol* (**6**). An ice-cold soln. of crude **21** in THF (4 ml) was treated dropwise with 1M PMe_3 in THF (0.16 ml, 0.16 mmol) and stirred at 25° for 1 h. When the TLC showed complete conversion of **21**, the mixture was treated with H_2O (1 ml), stirred for 15 h, and diluted with AcOEt (20 ml). After separation of the layers, the aq. phase was extracted with AcOEt (3 × 20 ml). The combined org. phases were washed with H_2O and brine, dried (Na_2SO_4), and evaporated. FC (CH_2Cl_2/MeOH 1:0 → 9:1) yielded **6** (31 mg, 84%). M.p. 98.6°. R_f (CH_2Cl_2/MeOH 4:1) 0.12. $[\alpha]_D^{25} = +9.1$ ($c = 0.15$, $CHCl_3$). IR (ATR): 3460 (br.), 3407 (br.), 3371 (br.), 3348 (br.), 3314*w*, 3290*w*, 3066*m* (sh), 2920*s*, 2848*s*, 2718*m* (sh), 1585*w*, 1467*w*, 1320*m*, 1287*m*, 1275*m*, 1255*w*, 1119*s*, 1048*m*, 992*m*, 980*m*, 947*w*, 764*w*, 725*w*. ^{1}H-NMR (CD_3OD, 300 MHz): 5.98 (br. *dd*, $J = 15.6, 6.3$, H–C(4)); 5.81 (*dtd*, $J = 15.3, 7.2, 0.9$, H–C(5)); 4.13 (br. *t*, $J \approx 5.1$, H–C(3)); 3.92–3.78 (AB, 2 H–C(6)); 3.66 (*dd*, $J = 11.1, 4.8$, H_a–C(1)); 3.52 (*dd*, $J = 10.8, 6.3$, H_b–C(1)); 3.10–3.05 (*m*, 2 H–C(1′)); 2.81 (*td*, $J = 6.6, 5.1$, H–C(2)); 1.84–1.74 (*m*, 2 H–C(2′)); 1.52–1.41 (*m*, 2 H–C(3′)); 1.41–1.29 (*m*, 14 H); 0.89 (*t*, $J = 6.7$, Me). ^{13}C-NMR (CD_3OD, 75 MHz): 141.14 (*d*, C(4)); 119.21 (*d*, C(5)); 73.66 (*d*, C(3)); 63.78 (*t*, C(1)); 57.64 (*t*, C(6)); 56.67 (*d*, C(2)); 52.26 (*t*, C(1′)); 32.79 (*t*); 30.45–29.25 (several *t*); 23.48, 22.42 (2*t*); 14.23 (*q*, Me). HR-MALDI-MS: 351.2360 (100, $[M+Na]^+$, $C_{17}H_{37}NO_4S^+$; calc. 351.2443).

X-Ray Analysis of **6**. Single crystal was obtained by isothermal crystallization from MeOH and H_2O. A soln. of **6** in MeOH (*ca.* 5 mg/300 µl), in a microtube (diameter *ca.* 5 mm), was immersed in a chamber containing H_2O and kept for 7 d undisturbed. The resulting plates of **6** were good enough for X-ray crystallographic analysis. Dimensions: 0.4 × 0.4 × 0.005 mm. Colourless crystals: $C_{17}H_{35}NO_4S$ (349.534),

triclinic P_1, $a = 4.7826(5)$, $b = 6.2512(8)$, $c = 16.624(2)$ Å, $\alpha = 90.343(9)$, $\beta = 96.817(10)$, $\gamma = 98.494(4)^\circ$, $V = 487.94(10)$ Å^3, $Z = 1$, $D_{calc} = 1.190$ Mg/m^3. All reflections were measured using a *Bruker Nonius-Kappa CCD* diffractometer (MoK_α radiation, $\lambda = 0.71073$) at 203 K. 2764 measured reflections, 2764 independent reflections, 2499 observed reflections. Refinement of F^2: full-matrix least-squares refinement, R(all) = 0.0596, R(gt) = 0.0522, wR(ref) = 0.1433, wR(gt) = 0.1353. All diagrams and calculations were performed using maXus (*Bruker Nonius*, *Delft & MacScience*, *Japan*). Programme used to solve structure: SIR97; programme used to refine structure: SHELXL-97.

*(*E*)-2-Azido-6-{[(*tert*-butoxy)carbonyl](undecyl)amino}-2,4,5,6-tetradeoxy-1,3-bis*-O-*(methoxybenzyl)*-D-erythro-*hex-4-enitol* (**22**). A soln. of **17** (100 mg, 0.2 mmol) in THF (2 ml) was treated with LiBr (dried in a *Kugelrohr* at 250° for 24 h and cooled in a vacuum desiccator over KOH; 177 mg, 2 mmol), and stirred at 25° for 2.5 h when TLC (AcOEt/pentane 1:4) showed complete conversion of **17**. The mixture was diluted with H_2O and extracted with Et_2O (3 × 50 ml). The combined org. phases were dried (Na_2SO_4) and evaporated to yield the crude bromide, which was used for the next step without purification. An ice-cold suspension of NaH (60% in oil, 24.4 mg, 0.609 mmol) in DMF (1 ml) was treated with $C_{11}H_{23}NHBoc$ (83 mg, 0.3 mmol) and stirred for 15 min. The mixture was treated with a soln. of the above crude bromide in DMF (1 ml) and stirred at 0° for 3 h. After the portionwise addition of crushed ice and sat. aq. NH_4Cl soln. and extraction with Et_2O (3 × 25 ml), the combined org. phases were dried (Na_2SO_4) and evaporated. FC (Et_2O/pentane 3:17) gave crude **22** (93 mg, 70%). Colourless syrup solidifying upon standing. R_f (AcOEt/hexane 1:4) 0.23. IR (ATR): 2924*m*, 2853*m*, 2097*m*, 1612*w*, 1586*w*, 1513*s*, 1464*m*, 1412*w*, 1365*w*, 1301*m*, 1246*s*, 1172*m*, 1090*m*, 1035*m*, 976*w*, 819*m*, 764*w*. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.32–7.16 (*m*, 4 arom. H); 6.90–6.83 (*m*, 4 arom. H); 5.70 (*dt*, *J* = 15.7, 5.6, H–C(5)); 5.52 (br. *dd*, *J* = 15.6, 8.2, H–C(4)); 4.52, 4.27 (2*d*, *J* = 11.5, ArCH_2); 4.45 (*s*, ArCH_2); 3.92 (*dd*, *J* = 8.0, 5.4, H–C(3)); 3.89–3.82 (*m*, 2 H–C(6)); 3.80, 3.79 (2*s*, 2 MeO); 3.62 (*m*, 2 H–C(1), H–C(2)); 3.20–3.11 (*m*, 2 H–C(1′)); 1.52–1.44 (*m*, 2 H–C(2′)); 1.46 (*s*, Me_3C); 1.33–1.19 (*m*, 16 H); 0.88 (*t*, *J* = 6.6, Me). HR-MALDI-MS: 689.4249 (100, $[M + Na]^+$, $C_{38}H_{58}N_4NaO_6S^+$; calc. 689.4254).

*(*E*)-2-Amino-6-{[(*tert*-butoxy)carbonyl](undecyl)amino}2,4,5,6-tetradeoxy-1,3-bis*-O-*(methoxybenzyl)*-D-erythro-*hex-4-enitol* (**23**). An ice-cold soln. of crude **22** (150 mg, 0.225 mmol) in THF (4 ml) was treated dropwise with 1M PMe_3 in THF (0.45 ml, 0.45 mmol) and stirred at 25° for 1 h when the TLC showed complete conversion of **22**. The mixture was treated with H_2O (1 ml), stirred for 10 h, and diluted with AcOEt. After the separation of the layers, the aq. phase was extracted with AcOEt (3 × 100 ml). The combined org. phases were washed with H_2O, and brine, dried (Na_2SO_4), and evaporated to afford crude **23** (114 mg, > 99%), which was used for the next step without further purification. R_f (CH_2Cl_2/MeOH 95:5) 0.25. IR (ATR): 3387*w*, 2924*m*, 2853*m*, 1690*s*, 1612*m*, 1586*w*, 1512*m*, 1463*m*, 1411*m*, 1389*w*, 1364*w*, 1301*m*, 1245*s*, 1170*s*, 1078*m*, 1035*s*, 975*w*, 875*w*, 844*m*, 819*s*, 772*w*, 721*w*. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.24–7.16 (*m*, 4 arom. H); 6.88–6.83 (*m*, 4 arom. H); 5.69 (*dt*, *J* = 15.4, 5.7, H–C(5)); 5.51 (br. *dd*, *J* = 15.5, 8.1, H–C(4)); 4.49, 4.40 (2*d*, *J* = 11.4, ArCH_2); 4.43, 4.24 (2*d*, *J* = 11.4, ArCH_2); 3.88 (br. *s*, 2 H–C(6)); 3.80, 3.79 (2*s*, 2 MeO); 3.81–3.75 (*m*, H–C(3)); 3.55 (*dd*, *J* = 9.1, 4.0, H–C(1)); 3.43 (*dd*, *J* = 9.1, 6.7, H′–C(1)); 3.16 (br. *s*, exchange with D_2O, NH_2); 3.05 (*td*, *J* = 6.5, 4.0, H–C(2)); 1.52–1.48 (*m*, 2 H–C(1′)); 1.46 (*s*, Me_3C); 1.33–1.19 (*m*, 18 H); 0.88 (*t*, *J* = 6.9, Me). ^{13}C-NMR ($CDCl_3$, 100 MHz): 159.21, 159.15 (2*s*); 155.41 (*s*, C=O); 131.94 (*d*, C(4)); 130.48 (2*s*); 129.31 (*d*, 4 arom. C and C(5)); 113.79 (2*d*); 113.78 (2*d*); 80.66 (*d*, C(3)); 79.39 (*s*, Me_3C); 72.97 (*t*, ArCH_2); 71.58 (*t*, C(1)); 70.03 (*t*, ArCH_2); 55.26 (*q*, 2 MeO); 54.37 (*d*, C(2); 46.89 (*t*, C(6)); 31.91 (*t*); 29.66–29.34 (several *t*); 28.50 (*q*, Me_3C); 26.91, 22.69 (2*t*); 14.11 (*q*, Me). HR-MALDI-MS: 641.4531 (100, $[M + H]^+$, $C_{38}H_{61}N_2O_6^+$; calc. 641.4530). Anal. calc. for $C_{38}H_{60}N_2O_6$ (640.8928): C 71.21, H 9.44, N 4.37; found: C 71.00, H 9.49, N 4.27.

*(*E*)-2-Amino-2,4,5,6-tetradeoxy-6-{[(trifluoromethyl)carbonyl](undecyl)amino}-*D-erythro-*hex-4-enitol* (**24**). A soln. of **23** (70 mg, 0.11 mmol) in CH_2Cl_2 (4.5 ml) was cooled to 0°, treated with CF_3COOH (0.5 ml), stirred for 45 min, diluted with CH_2Cl_2 (20 ml), and washed with H_2O (3 × 20 ml). The combined aq. phases were extracted with CH_2Cl_2 (3 × 100 ml). The combined org. phases were washed with brine, dried (Na_2SO_4), and evaporated. FC (silica gel (NH_2), CH_2Cl_2/MeOH 9:1) yielded **24** (21 mg, 65%). Colourless gum solidifying into a glassy substance upon cooling. R_f (CH_2Cl_2/MeOH 4:1) 0.12. $[\alpha]_D^{25} = -4.5$ ($c = 0.25$, $CHCl_3$). IR (ATR): 3505*w*, 3272*m*, 3107*w*, 2922*s*, 2853*m*, 2681*w*, 1696*s*, 1561*m*, 1468*w*, 1459*w*, 1366*w*, 1342*w*, 1295*w*, 1206*m*, 1183*s*, 1168*s*, 1123*w*, 1079*w*, 1055*w*, 1032*w*, 974*w*, 910*w*, 875*w*, 747*w*, 728*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 5.93 (*dt*, *J* = 15.6, 6.6, H–C(5)); 5.71 (br. *dd*, *J* = 15.6, 5.4,

H–C(4)); 4.38 (*t*, $J \approx 4.5$, H–C(3)); 4.04 (*dd*, $J = 11.4, 4.8$, H_a–C(1)); 3.92 (*q*, $J \approx 3.5$, H–C(2)); 3.67 (*dd*, $J = 11.7, 3.6$, H_b–C(1)); 3.25 (*d*, $J = 6.3$, 2 H–C(6)); 3.25 (br. *s*, exchange with D_2O, NH_2, 2 OH); 2.57 (*t*, $J = 7.3$, 2 H–C(1′)); 1.50–1.42 (*m*, 2 H–C(2′)); 1.32–1.22 (*m*, 16 H); 0.88 (*t*, $J = 6.5$, Me). ^{19}F-NMR ($CDCl_3$, 300 MHz): −75.5 (*s*, CF_3). ^{13}C-NMR ($CDCl_3$, 100 MHz): 157.58 (*q*, ^2J(C,F) = 36.7, C=O); 131.81 (*d*, C(4)); 130.73 (*d*, C(5)); 116.30 (*q*, ^1J(C,F) = 286.0, CF_3); 73.31 (*d*, C(3)); 61.18 (*t*, C(1)); 54.22 (*d*, C(2)); 51.12 (*t*, C(6)); 49.98 (*t*, C(1′)); 32.29 (*t*); 29.99–29.72 (several *t*); 27.64, 23.07 (2*t*); 14.49 (*q*, Me). HR-ESI-MS: 397.2650 (100, $[M + H]^+$, $C_{19}H_{36}F_3N_2O_3^+$; calc. 397.2678).

*(*E*)-2-Amino-2,4,5,6-tetradeoxy-6-(undecylamino)-*D-erythro-*hex-4-enitol* (**7**). A soln. of **24** (5 mg, 0.0126 mmol) in MeOH (1 ml) was treated with *Amberlyst A-26* (OH^- form) (300 mg) and stirred at 25° for 7 h when MALDI-MS showed the complete conversion of **24**. The mixture was filtered and the filtrate evaporated to dryness. FC (NH_2 silica gel, CH_2Cl_2/MeOH 1:0 → 85:15) gave **7** (3.0 mg, 79%). $[\alpha]_D^{25} = +3.6$ ($c = 0.135$, $CHCl_3$). IR (ATR): 3350*w*, 3279*w*, 3172*w*, 2953*m*, 2921*s*, 2852*s*, 1574*w*, 1464*m*, 1409*w*, 1377*w*, 1304*w*, 1260*w*, 1095*w*, 1027*m*, 974*m*, 867*w*, 802*m*, 720*w*. ^{1}H-NMR ($CDCl_3$, 400 MHz): 5.87 (*dtd*, $J =$ 15.6, 6.0, 1.0, H–C(5)); 5.68 (*ddt*, $J = 15.5, 6.7, 1.3$, H–C(4)); 4.11 (*t*, $J = 6.0$, H–C(3)); 3.70–3.61 (*m*, 2 H–C(1)); 3.27 (*dd*, $J = 6.0, 0.4$, 2 H–C(6)); 2.89 (*q*, $J = 5.3$, H–C(2)); 2.60 (*t*, $J = 7.3$, 2 H–C(1′)); 1.88 (br. *s*, NH_2, HO–C(1), HO–C(3), NH); 1.49 (*quint.*, $J = 7.1$, 2 H–C(2′)); 1.34–1.26 (*m*, 18 H); 0.88 (*t*, $J = 6.9$, Me). ^{13}C-NMR ($CDCl_3$, 100 MHz): 131.61 (*d*, C(4)); 131.20 (*d*, C(5)); 75.02 (*d*, C(3)); 64.20 (*t*, C(1)); 56.08 (*d*, C(2)); 51.07 (*t*, C(6)); 49.65 (*t*, C(1′)); 31.90 (*t*); 29.98–29.33 (several *t*); 27.37, 22.67 (2*t*); 14.09 (*q*, Me). HR-MALDI-MS: 301.2850 (100, $[M + H]^+$, $C_{17}H_{37}N_2O_2^+$; calc. 301.2855).

2. *Biological Tests.* – 2.1. *Phosphorylation by Sphingosine Kinase* (SPHK). The phosphorylation reactions were performed essentially as described in [28]. Briefly, the cytoplasmic fraction of recombinant HEK-293 cells overexpressing human SPHK1 or SPHK2 was incubated at 30° in total volumes of 100 µl with sphingosine derivatives (20 mM; added from stock solns. in DMSO), 1 mM of ATP, and 2 mCi [γ-^{32}P]ATP in 50 mM *Hepes* buffer (pH 7.4) containing 15 mM $MgCl_2$, 0.005% *Triton X-100*, 10 mM KCl, 10 mM NaF, and 1.5 mM semicarbazide. Following incubations for different time points up to 2 h, lipids were extracted and separated by TLC plates (Merck). Radiolabeled sphingosine phosphate derivatives were visualized and quantified using a *Molecular Dynamics Storm PhosphorImager* (Sunnyvale, CA). The rate of phosphorylation was calculated and is reported for the derivatives as value relative to the rate for sphingosine (for which the rate was 41 and 25 nmol/(min·mg) with SPHK1 and SPHK2, resp.).

2.2. *T-Cell Suppression: T-Cell Clones and Antigen-Presenting Cells* (*APC*). Human CD1d-restricted iNKT-cell clones were established and cultured as described in [29]. Human CD1d-transfected THP1 (THP1-CD1d) cells or monocyte-derived dendritic cells were used as antigen-presenting cells (APCs) in the experiments.

Cytotoxicity Assays. To test cell toxicity sonicated compounds were incubated overnight with different numbers of cells. Cells were labelled with 5 µg/ml propidium iodide (*Sigma-Aldrich*) or 7-aminoactinomycin D (*Invitrogen*, Carlsbad CA), and cell death was assessed by flow cytometry on a *CYAN*™ ADP cytometer (*Beckman Coulter*, Fullerton CA).

Antigen Presentation and Competition Assays with Living APC. THP1-CD1d Cells ($2.5 \cdot 10^4$/well) in RPMI-1640 medium containing 10% fetal calf serum (FCS) were incubated during the assays at 37° with sonicated compounds at the indicated concentrations in the presence of T cells ($7.5 \cdot 10^4$/well). For competition assays, a fixed dose of the compounds was given 4.5 h before addition of different doses of α-GalCer. Supernatants were harvested after 24 h, and released cytokines were measured by ELISA.

Plate-Bound Human CD1d Activation and Competition Assays. All incubations were performed overnight at r.t. with extensive washing between each step. Plates were coated with 10 µg/ml BIR1.4 monoclonal antibody (mAb). Soluble recombinant human CD1d purified by isoelectric focusing was incubated on BIR1.4-coated plates at twofold molar excess. Sonicated compounds were added and given 4.5 h in advance of α-GalCer to plate-bound human CD1d before competition. T Cells ($1.5 \cdot 10^5$/well) were plated in RPMI-1640 medium containing 5% HS and 100 U/ml human IL-2. After 24 h at 37°, supernatants were harvested, and released cytokines were measured by ELISA.

ELISA. Plates coated with 8D4-8 (anti-human IL-4 mAb, BD), MAb1 (anti-human TNF-α mAb, BD), 6804 (anti-human GM-CSF mAb, R&D), or HB-8700 (anti-human IFN-γ mAb, ATCC) were blocked, and then incubated with the freshly collected T-cell supernatants. Detection of cytokines was

performed using MP4-25D2 (anti-human IL-4 biotin labelled mAb, BD), MAb11 (anti-human TNF-α biotin-labelled mAb, BD), 3209 (anti-human GM-CSF biotin labelled mAb, R&D), or γ69 [30] (anti-human IFN-γ-biotin labelled mAb, provided by *G. Garotta*) with streptavidin-HRP (*Zymed*, *Invitrogen*) and *o*-phenylenediamine dihydrochloride (*Sigma*, according to the manufacturer's instructions) as substrate.

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