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Synthesis of 7-Oxasphingosine and -ceramide Analogues and Their Evaluation in a Model for Apoptosis

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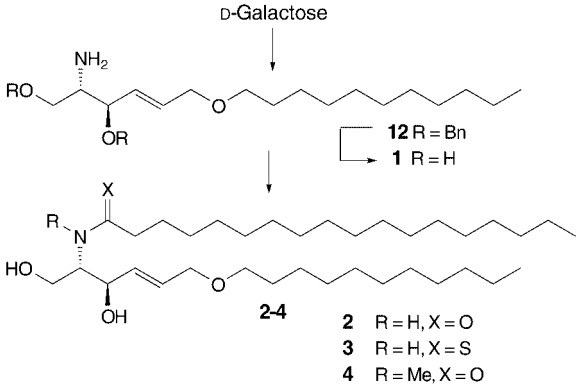
The 7-oxasphingosine (**1**), 7-oxaceramide (**2**), the thio-oxaceramide **3**, and *N*-methyloxaceramide **4** were synthesised from D-galactose *via* the building block **9**. The apoptosis-inducing properties of **1**–**4** were compared to those of sphingosine (Sph) and ceramide (Cer) using a human neuroblastoma (SK-N-BE) and a murine-promyelocyte-derived (32d) cell line. There were no differences between **2**–**4** and Cer in terms of their effects on the viability of cells and their ability to trigger cell proliferation. However, in the presence of *N*,*N*-dimethylsphingosine, an inhibitor of sphingosine kinase (SPHK), Cer was more potent than thio-ceramide **3** in 32d cells, while thio-ceramide **3** was more potent and efficacious in SK-N-BE cells, where it showed an IC_{50} value of 3 nM compared to 100 nM for Cer. In both SK-N-BE and 32d cells, 7-oxasphingosine (**1**) and Sph were equally toxic, even in the presence of *N*,*N*-dimethylsphingosine.

Introduction. – The growing interest in sphingolipids and glycosphingolipids [1–4] based on their ability to modulate apoptotic [5–12] and immune responses [13–16] prompts us to disclose a synthesis of 7-oxasphingosine (**1**) and the corresponding ceramide **2** that we developed in 1985 [17]. We wished to synthesise sphingolipid analogues in a modular way to independently vary the structure of the polar headgroup (including the (*E*)-alkenyl group) and the lipid moiety. Building block **9**, possessing a primary allylic OH group, appeared appropriate; a variety of lipid moieties should be readily attached. We recently turned back to this synthesis with the goal of determining, in a model for apoptosis, first the effect of 7-oxasphingosine (**1**) and the corresponding ceramide **2** as compared to sphingosine (Sph) and ceramide (Cer), and then the effect of two analogues **3** and **4**. These two analogues should allow to evaluate the effect on apoptosis of the H-bond donating and accepting properties of the amide group. *N*-Methylation of ceramide should also lower the energy difference between the conformers, resulting from rotation about the C(2)–N bond and between the (*E*)- and (*Z*)-amide conformers; this is expected to result in a different population of conformers in addition to removing any H-bond from the original amide group. In view of recent interest in 7-oxasphingosine [18], we wish to describe in detail the original synthesis and first biological results.

Synthesis. – The synthesis of 7-oxasphingosine is based on the known $HgSO_4$-promoted transformation of tri-*O*-benzyl-D-galactal **5** (available in an overall yield of



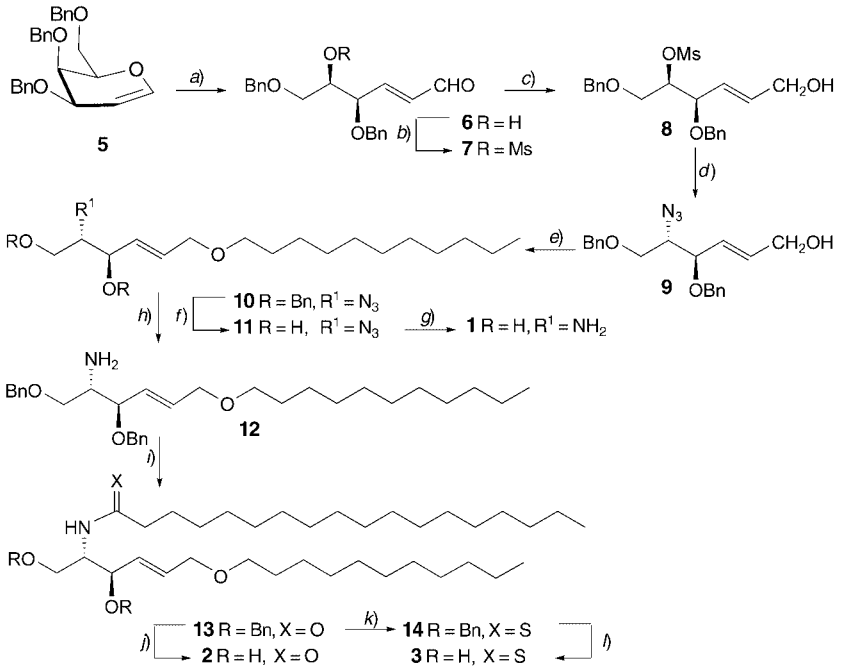
71% from galactose) [19][20] to the α,β-unsaturated aldehyde **6** [21][22] (*Scheme 1*). *O*-Mesylation of **6** provided the mesyloxy aldehyde **7** that was reduced to the alcohol **8**. Replacement of the MsO group of **8** by treatment with NaN_3 in DMF at 140° provided the azido alcohol **9** in an overall yield of 43% from **5** (30% from galactose). *O*-Alkylation of **9** with undecyl bromide gave the ether **10** that was reduced with $LiAlH_4$ to the amine **12**, which was *N*-acylated with stearoyl chloride to the protected oxaceramide **13** (72 % from **9**). Finally, debenzylation of **12** with $AlCl_3$ in the presence of anisole [23] provided 7-oxaceramide (**2**). To prepare 7-oxasphingosine (**1**), we similarly debenzylated **10** to the azido-7-oxasphingosine **11** that was reduced with Me_3P/NaOH to **1** (48% from **10**). Treatment of **13** with *Lawesson*'s reagent afforded 84% of the thioamide **14** that was deprotected in a yield of 70% to the thioceramide **3**. Attempts to thiocarbonylate the 7-oxaceramide **2** failed. To prepare the *N*-methylated analogue **4**, we initially treated the protected oxaceramide **13** with MeI in the presence of bases such as K_2CO_3, NaH, or KH; but these conditions did not affect **13**. Hence, the amine **12** was nosylated with *o*-nitrobenzenesulfonyl (Ns) chloride to **15**, which was readily *N*-methylated to **16** with MeI and K_2CO_3 (*Scheme 2*). The Ns group was removed by treatment with PhSH and KOH in MeCN at 60° [24][25] to give **17**, which was *N*-acylated with stearoyl chloride to provide **18** (76% from **12**). Deprotection of **18** afforded the *N*-methyl-oxaceramide **4** in 74% yield. According to the ^{1}H-NMR spectrum ($CDCl_3$), **18** is a 2:1 and **4** a 7:1 mixture of rotamers.

The D-*erythro*-configuration of 7-oxasphingosine (**1**) is evidenced by the similarity of the coupling constants for **1** (and for the azide **11**) and for D-*erythro*-sphingosine [26] (*Table 1*). It is confirmed by the ^{13}C(3) signal of the ceramide **2**, resonating at 74.19 ppm, downfield from the one of *threo*-configured ceramides (71–72 ppm) [27], and by the similarity of the other chemical shifts and coupling constants to those of ceramide [28] (*Table 2*).

The chemical shift and coupling constant of the N*H d* (6.33 ppm, 7.5 Hz) of **2** evidences the (*Z*)-*anti*-conformation that was also found in the solid state [29]. The C=S group of **3** is evidenced by the ^{13}C(1′) *s* at 206.23 ppm (δ(C(1′)) for **2** 174.17 ppm) and the downfield shift for all ^{1}H in its vicinity.

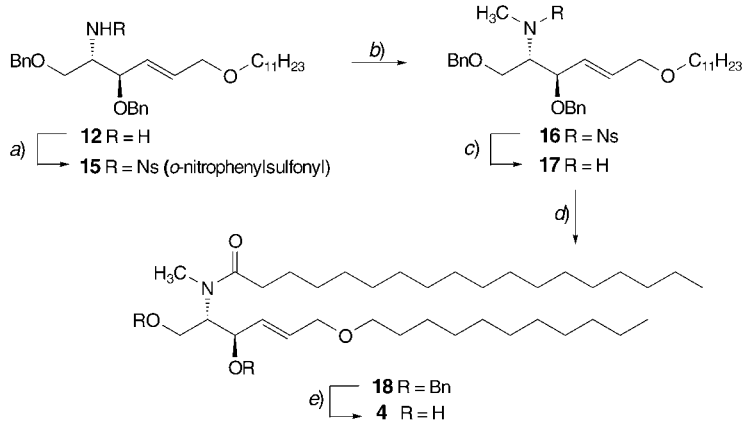
The ^{1}H-NMR spectrum of **4** shows MeN signals at 3.02 and 2.82 ppm, indicating a 7:1 mixture of rotamers (*cf.* [30][31]). The slight upfield shift for H–C(2), as

Scheme 1



a) $HgSO_4$, 0.02N H_2SO_4, dioxane; 84%. *b*) MsCl, Et_3N, CH_2Cl_2; 74%. *c*) $NaBH_4$, MeOH; 97%. *d*) NaN_3, DMF; 71%. *e*) NaH, $C_{11}H_{23}Br$, DMF; 87%. *f*) $AlCl_3$, anisole, CH_2Cl_2; 65%. *g*) Me_3P, THF, then NaOH; 74%. *h*) $LiAlH_4$, THF. *i*) $C_{17}H_{35}COCl$, pyridine, CH_2Cl_2; 83% from **10**. *j*) $AlCl_3$, anisole, $ClCH_2CH_2Cl$; 73%. *k*) *Lawesson*'s reagent, toluene; 84%. *l*) $AlCl_3$, anisole, CH_2Cl_2; 70%.

Scheme 2



a) *o*-Nitrobenzenesulfonyl chloride, Et_3N, CH_2Cl_2; 87%. *b*) MeI, K_2CO_3, DMF; 100%. *c*) PhSH, KOH, CH_3CN; 95%. *d*) Stearoyl chloride, pyridine, CH_2Cl_2; 92%. *e*) $AlCl_3$, anisole, $ClCH_2CH_2Cl$; 74%.

Table 1. *Selected ^{1}H-NMR Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *of Azido-7-oxasphingosine* **11**, *7-Oxasphingosine* (**1**), *and* D-erythro-*Sphingosine in* $CDCl_3$

	11	**1**	D-erythro-*Sphingosine*[a])
H–C(1)	3.82	3.71–3.61	3.67
H–C(1′)	–	3.71–3.61	3.60
H–C(2)	3.52	2.90	2.87
H–C(3)	4.34	4.14	4.04
H–C(4)	5.81	5.76	5.46
H–C(5)	5.94	5.89	5.75
2 H–C(6)	3.99	3.98	2.04
HO–C(3)	2.34	2.25–1.82	1.95
HO–C(1)	2.19	2.25–1.82	1.95
NH_2	–	2.25–1.82	1.95
J(1,2)	5.4	5.1	5.9
J(1′,2)	–	–	4.5
J(2,3)	4.5	5.1	5.1
J(3,4)	6.6	6.3	6.9
J(4,5)	15.6	15.6	15.5
J(4,6)	1.3	–	–
J(5,6)	5.1	5.1	7.0

[a]) [26].

Table 2. *Selected ^{1}H-NMR Chemical Shifts* [ppm], *and Coupling Constants* [Hz] *of Ceramide (Cer), the 7-Oxaceramides* **2**–**4**, *and the Protected 7-Oxaceramides* **13**, **14**, *and* **18** *in* $CDCl_3$

	Cer[a])	**2**	**3**	$\mathbf{4}_{major}$	$\mathbf{4}_{minor}$	**13**	**14**	$\mathbf{18}_{major}$	$\mathbf{18}_{minor}$
H–C(1)	3.93	3.96–3.88	4.13	4.07–3.83	[b])	3.80	3.86	3.88	3.64
H′–C(1)	3.68	3.74–3.65	3.83	4.07–3.83	[b])	3.53	3.67	3.73	3.78
H–C(2)	3.88	3.96–3.88	4.64–4.56	3.66	[b])	4.25	4.96	4.70–4.60	3.90–3.87
H–C(3)	4.31	4.38	4.74–4.66	4.59	4.36	3.96	4.19	4.07	4.06
H–C(4)	5.51	5.79	5.87	5.76	[b])	5.65	5.73	5.61	5.57
H–C(5)	5.76	5.91	5.99	5.85	[b])	5.79	5.85	5.79	5.82
2 H–C(6)	2.03	3.98	4.01	3.95	[b])	3.97	3.97	4.00–3.93	4.00–3.93
NH	6.21	6.33	8.07	–	[b])	5.72	7.39		
HO–C(3)	2.70	3.26	2.56	3.57	[b])				
HO–C(1)	2.70	2.97	1.66–1.52	3.57–3.50	[b])				
J(1′,2)	11.2	[c])	10.5	[c])		9.9	10.0	9.6	10.2
J(1,1′)	3.4	[c])	2.0	6.4		4.5	4.4	8.4	8.4
J(1,2)	3.4	[c])	< 1.5	3.8		3.9	4.4	3.6	3.3
J(2,3)	3.6	4.0	[c])	6.5		7.2	6.2	8.1	8.1
J(3,4)	6.5	5.3	5.0	6.3		7.5	7.5	8.3	8.3
J(4,5)	15.4	15.6	15.6	15.6		15.9	15.9	15.6	15.6
J(5,6)	6.8	5.1	5.4	5.0		5.4	5.2	5.7	5.3
J(NH,2)	7.3	7.5	7.2	–		9.3	9.0		
J(OH,3)	[c])	5.4	[c])	3.0					
J(OH,1)	3.6	7.2	[c])	[c])					
J(OH,1′)	7.5	3.6	[c])	[c])					

[a]) [28]. [b]) Not observed as a separate signal. [c]) Not assigned.

compared to ceramide and to **2**, may reflect a rotation about the C(2)–N bond, and a (partial) C(1)– or C(3)–OH···O=C H-bond, in keeping with a downfield shift for C(1)–O*H* and to a smaller extent for C(3)–O*H*.

Biological Studies. – The behaviour of 7-oxasphingosine (**1**) and 7-oxaceramide (**2**) was compared to that of Sph and Cer using a human neuroblastoma (SK-N-BE) cell line and a murine-promyelocyte-derived (32d) cell line; the thioceramide **3** and the *N*-methylceramide **4** were tested in the same models. These cell lines were used because of their different ability to respond to Cer, as observed in a preliminary screening (see *Fig. 1*). Thus, Cer is not toxic in SK-N-BE cells, while it induces cell death in 32d cells (*Fig. 1*,*a* and *b*). To quantitatively analyse the differences between the synthesised compounds in the two cell lines, we investigated the viability and proliferation triggered by Cer, Sph, and the analogues **1**–**4** in concentration/response curves by means of the MTT (tetrazolium salt conversion) metabolic assay [32].

A 72-h treatment with Cer induced a decrease in viability in 32d cells, with the maximal effect observed at 10 μM (37 ± 3% of control; *Fig. 1*,*a*). In contrast, the same concentration of Cer reduced the viability in SK-N-BE cells by only 6 ± 2% (*Fig. 1*,*b*). We speculated that the relative Cer resistance might depend on differential expression or differential activity of the ceramide kinase (CERK) and/or sphingosine kinase (SPHK) in the two cell lines. To show that, in principle, this hypothesis is well-founded, we performed exploratory RT-PCR experiments. These experiments suggest that different CERK splice variants are present in the two cell lines (*Fig. 2*). To test the hypothesis functionally, both cell lines were treated with Cer in the presence of *N*,*N*-dimethylsphingosine (*N*,*N*-DMS), a synthetic inhibitor of SPHK that blocks the conversion of Sph to the anti-apoptotic sphingosine-1-phosphate (Sph-1-P) [33]. Inhibition of CERK could not be performed, since no valid inhibitors were reported in the literature. In agreement with our hypothesis, *N*,*N*-DMS (5 μM) potentiated the cytotoxicity of Cer, although to different extents in the two cell lines. *N*,*N*-DMS, at a concentration that *per se* is ineffective, brought the maximal toxicity of Cer from 6 ± 2% to 45 ± 2.8% (*Fig. 1*,*b*). Such treatment also increased the potency of Cer. In the 32d cells, co-treatment with *N*,*N*-DMS did not alter the efficacy as incisively (from 37 ± 3% to 55 ± 2%), but greatly increased its potency (IC_{50} 3 μM *vs.* 100 nM) (*Fig. 1*,*a*). The effect of *N*,*N*-DMS on both cell lines suggests that conversion of Cer to Sph-1-P plays an important part in rendering cells less sensitive to Cer-induced toxicity. When Sph was tested (*Fig. 1*,*c* and *d*), a higher degree of toxicity as compared to Cer (measured both in potency and efficacy) was evident in both cell lines. 7-Oxasphingosine (**1**) behaved similarly to Sph (*Fig. 1*,*c* and *d*). This suggests that, at high Sph concentrations, SPHK activity is limiting, and the Sph/Sph-1-P ratio favours toxicity rather than protection. This hypothesis also assumes that ceramidase does not convert all exogenous Cer to Sph, and that other pathways (including CERK) are active. 32d Cells behaved differently in response to Sph. As with SK-N-BE cells, Sph proved toxic, but its potency was increased by the addition of *N*,*N*-DMS (IC_{50} 3 μM in the absence; 100 nM in the presence of *N*,*N*-DMS). These data suggest that the pathways utilising and metabolising sphingolipids in 32d and SK-N-BE are different.

In this case, it is possible that the synthetic analogues in this study elicit different effects in the two cell lines. All of the Cer analogues grossly recapitulated the effects

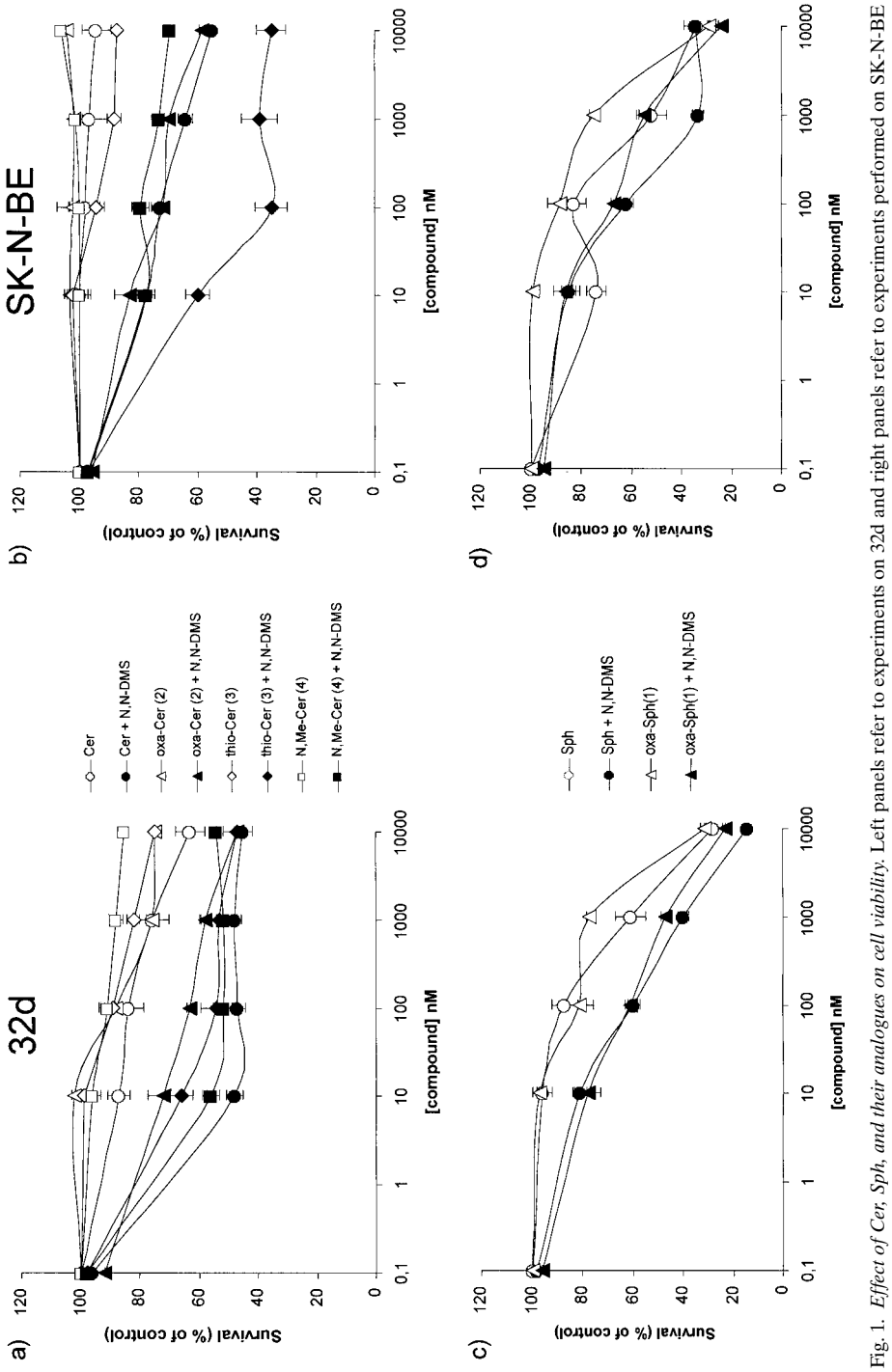


Fig. 1. *Effect of Cer, Sph, and their analogues on cell viability.* Left panels refer to experiments on 32d and right panels refer to experiments performed on SK-N-BE cells. Values are mean ± S.E.M. of at least 32 replicates in four experiments.

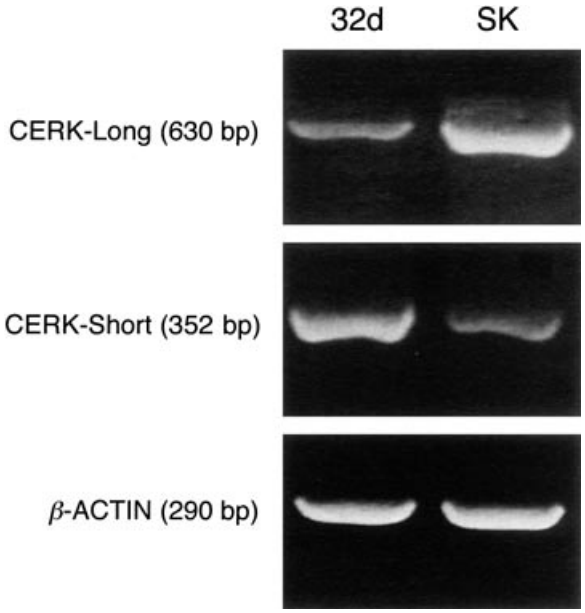


Fig. 2. *Presence of different isoforms of CERK in 32d and SK-N-BE cells.* The figure is representative of three separate experiments (SK = SK-N-BE).

induced by commercial Cer (*C2-Ceramide*, *Sigma*) in both cell lines, with 32d cells more susceptible to the treatment, as expected (*Fig. 1*, *a* and *b*). The difference between 32d and SK-N-BE cells was more evident when the relative rank order of potency of these analogues was evaluated. Testing compounds on their own showed no statistically significant difference as compared to Cer. However, in the presence of *N*,*N*-DMS, where effects were more pronounced, the rank order of potency was significantly different. In particular, Cer was more potent than thio-ceramide **3** in 32d cells, while thio-ceramide **3** was more potent and efficacious in SK-N-BE cells. Indeed, in SK-N-BE cells **3** was by far the most lethal compound according to the MTT assay. In this system, the apparent IC_{50} value of **3** was 3 nM and that for Cer 100 nM (*Fig. 1*, *b*). Furthermore, evaluating the maximal effects induced, **3** killed $65 \pm 6.0\%$ of cells compared to the $45 \pm 2.8\%$ killed by Cer.

One of the major concerns about the role of Cer and Sph as second messengers in cell-death programs derives from the consideration that such lipophilic molecules may physically impair cell-membrane function, rather than activating a well-defined apoptotic pathway [5]. In view of this consideration, we studied apoptotic death by testing for nuclear chromatin condensation and fragmentation, two well-known markers of programmed cell death [34]. After treating the cell lines as described above, nuclear chromatin was labelled with a fluorescent DNA dye (*Hoechst 33258*) and visualised with a fluorescent microscope under UV light. As shown in *Fig. 3*, all of the compounds found to be cytotoxic in the presence of *N*,*N*-DMS (Cer, thio-ceramide, Sph, 7-oxasphingosine) induced nuclear condensation and fragmentation at concen-

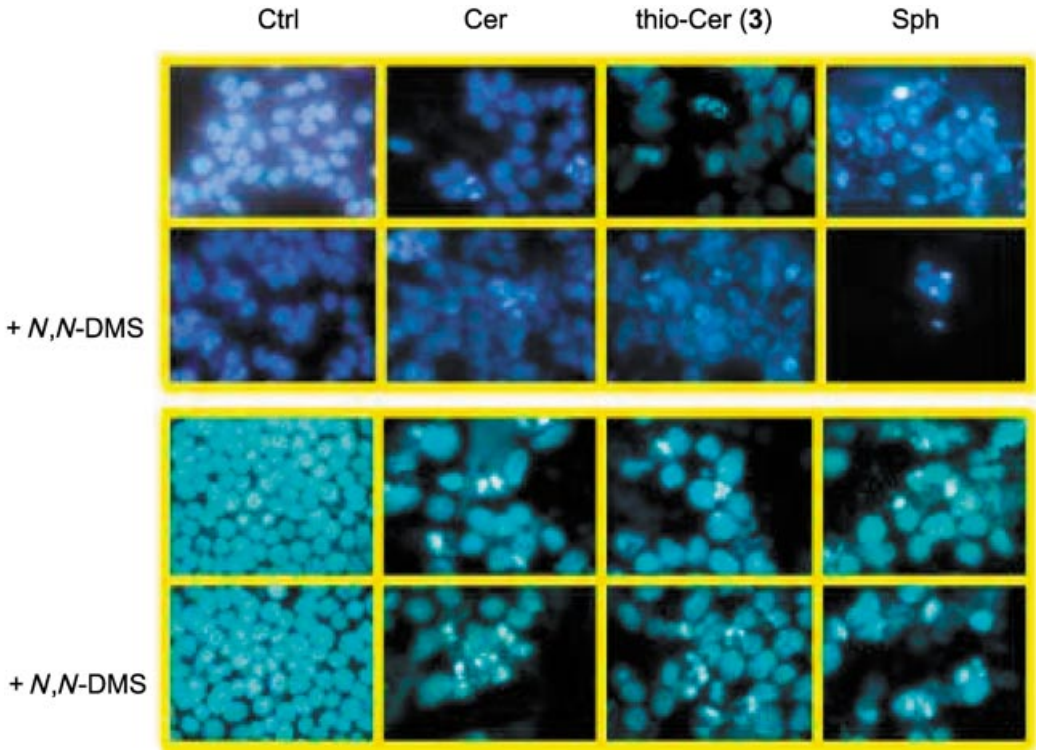


Fig. 3. Hoechst *staining of cells treated for 72 h with Cer, Sph, thioceramide, and 7-oxasphingosine in 32d and SK-N-BE cells.* Images are representative of at least ten fields in three separate experiments.

trations consistent with those suggested by MTT assays, further evidencing the specificity of Cer and its analogues in triggering apoptotic death.

These data sustain the relevance of SPHK in disabling, in part, the cytotoxic effect of Cer by phosphorylation and highlight the importance in this respect of either the absolute levels of Sph, or of the sphingosine/sphingosine 1-phosphate ratio. Our data also suggest that there are no major differences in the way in which Cer and its oxa and *N*-Me analogues are metabolised to the Sph derivative, and in the way that the resulting Sph and oxasphingosine are recognised by SPHK. On the other hand, the greater potency showed by thio-ceramide **3** in SK-N-BE cells devoid of SPHK activity (as the result of *N,N*-DMS treatment) strongly supports the idea that the thio group changes the biological characteristics of this compound. Although, at present, it is difficult to speculate on the reasons of such a difference, thio-ceramide **3** might have a higher affinity than Cer to death effectors (ion channels, protein kinases, phosphatases, *etc.*) or a decreased capacity to be phosphorylated by either CERK or SPHK.

Experimental Part

1. Synthesis. – *General*. Solvents were distilled: THF from Na and benzophenone, CH_2Cl_2 from P_2O_5, MeOH from CaH_2. Reactions were carried out under Ar, 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). Optical rotations: 1-dm cell at 25°, 589 nm. FT-IR Spectra: KBr or *ca.* 2% soln. in $CHCl_3$, absorption 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.

*(*E*)-4,6-Di*-O-*benzyl-2,3-dideoxy*-D-threo-*hex-2-enose* (**6**) [22]. Prepared from tri-*O*-benzyl-D-galactal **5** according to [22]. R_f (AcOEt/hexane 2:3) 0.22. IR ($CHCl_3$): 3574*w*, 3089*w*, 3067*w*, 3027*w*, 3015*w*, 2904*w*, 2869*w*, 2824*w*, 2737*w*, 1692*s*, 1603*w*, 1496*w*, 1454*w*, 1388*w*, 1364*w*, 1259*w*, 1164*w*, 1102*s*, 1028*m*, 1007*m*, 981*m*, 911*w*, 877*w*. ^1H-NMR ($CDCl_3$, 300 MHz): 9.56 (*d*, $J=7.8$, H–C(1)); 7.39–7.26 (*m*, 10 arom. H); 6.78 (*dd*, $J=15.9$, 6.0, H–C(3)); 6.34 (*ddd*, $J=15.9$, 7.8, 1.2, H–C(2)); 4.63 (*d*, $J=11.7$, PhC*H*); 4.54 (*d*, $J=11.7$, PhC*H*); 4.48 (*d*, $J=11.7$, PhC*H*); 4.43 (*d*, $J=11.4$, PhC*H*); 4.29 (*t*, $J\approx 5.6$, H–C(4)); 3.91–3.82 (*m*, H–C(5)); 3.59 (*dd*, $J=9.6$, 4.8, H–C(6)); 3.51 (*dd*, $J=9.9$, 5.4, H′–C(6)); 2.62 (br. *s*, OH). ^{13}C-NMR ($CDCl_3$, 75 MHz): 192.95 (*d*, C(1)); 153.03 (*d*, C(3)); 137.48, 137.02 (2*s*); 133.66 (*d*, C(2)); 128.51 (2*d*); 128.43 (2*d*); 128.12 (*d*); 127.96 (2*d*); 127.89 (*d*); 127.85 (2*d*); 78.51 (*d*, C(4)); 73.58 (*t*, PhCH$_2$); 72.30 (*d*, C(5)); 72.24 (*t*, PhCH$_2$); 70.02 (*t*, C(6)). HR-MALDI-MS: 349.1407 (100, $[M+Na]^+$, $C_{20}H_{22}NaO_4^+$; calc. 349.1410).

*(*E*)-4,6-Di*-O-*benzyl-2,3-dideoxy-5*-O-*(methylsulfonyl)*-D-threo-*hex-2-enose* (**7**). An ice-cold soln. of **6** (13.16 g, 40.32 mmol) in dry CH_2Cl_2 was treated with Et_3N (8.14 g, 80.64 mmol) and MsCl (13.86 g, 120.96 mmol), stirred for 4 h at r.t., and then treated with H_2O. After extraction with CH_2Cl_2 (4×200 ml), the combined org. layers were washed with brine, dried (Na_2SO_4), and evaporated. FC (silica gel; AcOEt/hexane 2:8) gave solid **7** (12.06 g, 74%), which was recrystallised in Et_2O-hexane. White crystals. R_f (AcOEt/hexane 2:3) 0.38. M.p. 71–73°. $[\alpha]_D^{25}=+21.9$ ($c=2.00$, $CHCl_3$). IR (KBr): 3361*w*, 3086*w*, 3062*w*, 3028*m*, 2934*w*, 2905*m*, 2875*m*, 2774*w*, 1689*s*, 1603*w*, 1494*m*, 1469*w*, 1453*m*, 1396*m*, 1354*s*, 1328*s*, 1275*w*, 1245*w*, 1204*m*, 1176*s*, 1156*m*, 1113*s*, 1090*s*, 1076*s*, 1062*s*, 1043*s*, 1025*s*, 977*s*, 969*s*, 931*s*. ^1H-NMR ($CDCl_3$, 300 MHz): 9.57 (*d*, $J=7.8$, H–C(1)); 7.39–7.26 (*m*, 10 arom. H); 6.74 (*dd*, $J=15.9$, 5.1, H–C(3)); 6.40 (*ddd*, $J=15.9$, 7.8, 1.2, H–C(2)); 4.84 (*ddd*, $J=6.5$, 5.6, 3.1, H–C(5)); 4.64 (*d*, $J=11.5$, PhC*H*); 4.56 (*d*, $J=11.8$, PhC*H*); 4.49 (*d*, $J=11.5$, PhC*H*); 4.47 (*d*, $J=11.8$, PhC*H*); 4.47–4.44 (*m*, H–C(4)); 3.74 (*dd*, $J=10.9$, 3.1, H–C(6)); 3.63 (*dd*, $J=11.2$, 6.5, H′–C(6)); 2.99 (*s*, MsO). ^{13}C-NMR ($CDCl_3$, 75 MHz): 192.53 (*d*, C(1)); 150.13 (*d*, C(3)); 136.94, 136.55 (2*s*); 134.30 (*d*, C(2)); 128.55 (2*d*); 128.51 (2*d*); 128.26 (*d*); 128.06 (*d*); 127.95 (2*d*); 127.84 (2*d*); 81.05 (*d*, C(5)); 76.94 (*d*, C(4)); 73.66, 72.54 (2*t*, 2 PhCH$_2$); 68.57 (*t*, C(6)); 38.57 (*q*, MsO). HR-ESI-MS: 427.1181 (40, $[M+Na]^+$, $C_{21}H_{24}NaO_6S^+$; calc. 427.1186). Anal. calc. for $C_{21}H_{24}O_6S$ (404.48): C 62.36, H 5.98, S 7.93; found: C 62.20, H 5.73, S 7.84.

*(*E*)-4,6-Di*-O-*benzyl-2,3-dideoxy-5*-O-*(methylsulfonyl)*-D-threo-*hex-2-enitol* (**8**). An ice-cold soln. of **7** (12.06 g, 29.82 mmol) in CH_2Cl_2 (75 ml) and MeOH (300 ml) was treated with $NaBH_4$ (1.13 g, 29.82 mmol). The mixture was stirred for 5 min, diluted with H_2O (2 ml), and evaporated. A soln. of the residue in $CHCl_3$ was washed with H_2O. The combined org. layers were dried (Na_2SO_4) and evaporated. Crystallisation from Et_2O gave **8** (11.76 g, 97%). Colourless crystals. R_f (AcOEt/hexane 3:2) 0.24. M.p. 88–89.5°. $[\alpha]_D^{25}=-7.7$ ($c=2.00$, $CHCl_3$). IR (KBr): 3434*m* (br.), 3088*w*, 3060*w*, 3037*w*, 3006*w*, 2941*w*, 2924*m*, 2881*m*, 2861*m*, 1666*w*, 1498*w*, 1454*m*, 1397*w*, 1363*s*, 1349*s*, 1277*w*, 1260*w*, 1252*w*, 1238*w*, 1209*w*, 1174*s*, 1143*w*, 1096*s*, 1068*s*, 1038*m*, 1030*m*, 1021*m*, 981*s*, 968*s*, 931*s*. ^1H-NMR ($CDCl_3$, 300 MHz): 7.38–7.26 (*m*, 10 arom. H); 5.99 (*dtd*, $J=15.6$, 5.3, 0.9, H–C(2)); 5.65 (*ddt*, $J=15.5$, 7.4, 1.8, H–C(3)); 4.76 (*td*, $J=5.6$, 4.0, H–C(5)); 4.63 (*d*, $J=12.0$, PhC*H*); 4.57 (*d*, $J=11.7$, PhC*H*); 4.47 (*d*, $J=12.0$, PhC*H*); 4.38 (*d*, $J=11.7$, PhC*H*); 4.18–4.14 (*m*, H–C(4), 2 H–C(1)); 3.73 (*dd*, $J=11.2$, 4.2, H–C(6)); 3.69 (*dd*, $J=11.2$, 4.1, H′–C(6)); 2.97(*s*, MsO); 1.80 (br. *s*, OH). ^{13}C-NMR ($CDCl_3$, 75 MHz): 137.52, 137.32 (2*s*); 135.29 (*d*, C(3)); 128.40 (2*d*); 128.36 (2*d*); 127.85 (3*d*); 127.79 (3*d*); 125.58 (*d*, C(2)); 83.06 (*d*, C(5)); 77.89 (*d*, C(4)); 73.50, 70.97 (2*t*, 2 PhCH$_2$); 69.22 (*t*, C(6)); 62.57 (*t*, C(1)); 38.67 (*q*, MsO). HR-ESI-MS: 429.1336 (58, $[M+Na]^+$, $C_{21}H_{26}NaO_6S^+$; calc. 429.1342). Anal. calc. for $C_{21}H_{24}O_6S$ (406.49): C 62.05, H 6.45, S 7.89; found: C 61.86, H 6.37, S 7.75.

*(*E*)-2-Azido-1,3-di*-O-*benzyl-2,4,5-trideoxy*-D-erythro-*hex-4-enitol* (**9**). A soln. of NaN_3 (11.28 g, 173.58 mmol) and **8** (11.76 g, 28.93 mmol) in dry DMF (300 ml) was heated to 140° for 6 h, cooled to r.t., diluted with H_2O (100 ml), and extracted with Et_2O (2×200 ml). The combined org. layers were dried (Na_2SO_4) and evaporated. FC (silica gel; AcOEt/hexane 3:7) gave **9** (7.26 g, 71%). Colourless oil. R_f (AcOEt/hexane 2:3) 0.22. $[\alpha]_D^{25}=-32.4$ ($c=2.00$, $CHCl_3$). IR ($CHCl_3$): 3446*w* (br.), 3091*w*, 3066*w*, 3014*m*, 2920*w*, 2867*w*, 2102*s*, 1598*w*, 1496*w*, 1454*w*, 1384*w*, 1363*w*, 1311*w*, 1269*w*, 1168*w*, 1092*s*, 1028*w*, 978*m*, 910*w*. ^1H-NMR ($CDCl_3$, 300 MHz): 7.39–7.25 (*m*, 10 arom. H); 5.92 (*dtd*, $J=15.6$, 4.8, 0.6, H–C(5)); 5.70 (*ddt*, $J=15.6$, 8.1, 1.5, H–C(4)); 4.61 (*d*, $J=12.0$, PhC*H*); 4.57 (*d*, $J=12.0$, PhC*H*); 4.51 (*d*, $J=12.0$, PhC*H*); 4.39 (*d*, $J=12.0$, PhC*H*); 4.20 (br. *t*, $J\approx 5.0$, 2 H–C(6)); 4.01 (*ddd*, $J=8.4$, 5.1, 0.6, H–C(3)); 3.69 (*ddd*, $J=6.8$, 5.2, 4.1, H–C(2)); 3.65 (*dd*, $J=9.9$, 4.2, H–C(1)); 3.59 (*dd*, $J=9.9$, 6.8, H′–C(1)); 1.59 (*t*, $J=5.7$, OH). ^{13}C-NMR ($CDCl_3$, 75 MHz): 137.83, 137.66 (2*s*); 135.28 (*d*, C(4)); 128.39 (2*d*); 128.35 (2*d*); 127.74 (*d*); 127.64 (5*d*); 127.00 (*d*, C(5)); 78.83 (*d*,

C(3)); 73.44, 70.61 (2*t*, 2 Ph*C*H$_2$); 69.28 (*t*, C(1)); 64.29 (*d*, C(2)); 62.68 (*t*, C(6)). HR-ESI-MS: 376.1626 (100, $[M+Na]^+$, $C_{20}H_{23}N_3NaO_3^+$; calc. 376.1632). Anal. calc. for $C_{20}H_{23}N_3O_3$ (353.41): C 67.97, H 6.56, N 11.89; found: C 67.74, H 6.76, N 11.89.

*(*E*)-2-Azido-1,3-di*-O-*benzyl-2,4,5-trideoxy-6*-O-*undecyl*-D-erythro-*hex-4-enitol* (**10**). A suspension of **9** (500 mg, 1.41 mmol) and 60% NaH in oil (101 mg, 3.00 mmol) in dry DMF (10 ml) was stirred for 15 min, treated dropwise with 1-bromoundecane (499 mg, 2.12 mmol), stirred for 12 h, and treated dropwise with MeOH until complete consumption of NaH. After dilution with H_2O, the mixture was extracted with Et_2O (3 × 100 ml). The combined org. layers were dried (Na_2SO_4) and evaporated. FC (silica gel; AcOEt/hexane 1:19) gave **10** (630 mg, 88%). Colourless oil. R_f (AcOEt/hexane 1:9) 0.25. $[\alpha]_D^{25} = -26.7$ ($c = 2.00$, $CHCl_3$). IR ($CHCl_3$): 3089*w*, 3066*w*, 3032*w*, 3011*w*, 2928*s*, 2856*s*, 2102*s*, 1602*w*, 1496*w*, 1454*w*, 1365*w*, 1310*w*, 1265*w*, 1098*s*, 1072*m*, 1028*w*, 976*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.38–7.22 (*m*, 10 arom. H); 5.85 (*dt*, $J = 15.6, 5.4$, H–C(5)); 5.68 (*dd*, $J \approx 15.6, 7.8$, H–C(4)); 4.62 (*d*, $J = 11.7$, PhC*H*); 4.56 (*d*, $J = 12.0$, PhC*H*); 4.51 (*d*, $J = 12.3$, PhC*H*); 4.36 (*d*, $J = 11.7$, PhC*H*); 4.02 (*d*, $J = 4.8$, 2 H–C(6)); 3.99 (*dd*, $J = 7.8, 5.4$, H–C(3)); 3.69 (*ddd*, $J = 6.8, 5.1, 3.9$, H–C(2)); 3.64 (*dd*, $J = 9.6, 4.2$, H–C(1)); 3.59 (*dd*, $J = 9.6, 6.9$, H′–C(1)); 3.43 (*t*, $J \approx 6.8$, 2 H–C(1′)); 1.64–1.53 (*m*, 2 H–C(2′)); 1.38–1.22 (*m*, 16 H); 0.88 (*t*, $J \approx 6.6$, Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 137.75, 137.61 (2*s*); 133.18 (*d*, C(4)); 128.28 (2*d*); 128.24 (2*d*); 128.06 (*d*, C(5)); 127.61 (*d*); 127.49 (5*d*); 78.86 (*d*, C(3)); 73.35, 70.61 (2*t*, 2 Ph*C*H$_2$); 70.46, 70.38 (2*t*, C(6), C(1′)); 69.29 (*t*, C(1)); 64.27 (*d*, C(2)); 31.97, 29.81 (2*t*); 29.69 (3*t*); 29.59, 29.40, 26.28, 22.76 (4*t*); 14.22 (*q*, Me). HR-ESI-MS: 530.3344 (100, $[M+Na]^+$, $C_{31}H_{45}N_3NaO_3^+$; calc. 530.3353). Anal. calc. for $C_{31}H_{45}N_3O_3$ (507.71): C 73.34, H 8.93, N 8.28; found: C 73.46, H 8.73, N 8.16.

*(*E*)-2-Amino-1,3-di*-O-*benzyl-2,4,5-trideoxy-6*-O-*undecyl*-D-erythro-*hex-4-enitol* (**12**). A suspension of $LiAlH_4$ (94 mg, 2.48 mmol) in dry Et_2O (20 ml) was cooled to 0°, treated dropwise with a soln. of **10** (630 mg, 1.24 mmol) in dry Et_2O (10 ml), stirred for 1 h, warmed to r.t., stirred for 6 h, cooled to 0°, and treated dropwise with H_2O (1 ml), 1N NaOH (2 ml), and H_2O (3 ml). The suspension was filtered through *Celite*, and the filtrate was extracted with AcOEt (3 × 100 ml). The combined org. layers were dried (Na_2SO_4) and evaporated affording crude **12** (667 mg), which was used for the next step without further purification. IR ($CHCl_3$): 3379*w*, 3089*w*, 3067*w*, 2951*m*, 2928*s*, 2856*s*, 1602*w*, 1586*w*, 1496*w*, 1465*w*, 1454*w*, 1364*w*, 1264*m*, 1097*s*, 1026*m*, 1014*m*, 911*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.37–7.23 (*m*, 10 arom. H); 5.85 (*dt*, $J = 15.6, 5.3$, H–C(5)); 5.67 (*ddt*, $J = 15.6, 8.1, 1.2$, H–C(4)); 4.59 (*d*, $J = 12.0$, PhC*H*); 4.49 (*s*, PhC*H*$_2$); 4.34 (*d*, $J = 12.0$, PhC*H*); 4.02 (*dd*, $J = 5.3, 1.2$, 2 H–C(6)); 3.84 (*dd*, $J = 7.8, 6.3$, H–C(3)); 3.61 (*dd*, $J = 9.2, 4.1$, H–C(1)); 3.49 (*dd*, $J = 9.3, 6.6$, H′–C(1)); 3.42 (*t*, $J \approx 6.7$, 2 H–C(1′)); 3.13 (*td*, $J = 6.3, 3.9$, H–C(2)); 1.89 (br. *s*, NH_2); 1.65–1.52 (*m*, 2 H–C(2′)); 1.38–1.22 (*m*, 16 H); 0.88 (*t*, $J = 6.9$, Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 138.27, 138.15 (2*s*); 132.62 (*d*, C(4)); 129.46 (*d*, C(5)); 128.23 (2*d*); 128.20 (2*d*); 127.59 (4*d*); 127.48, 127.40 (2*d*); 80.93 (*d*, C(3)); 73.29, 71.69 (2*t*, 2 Ph*C*H$_2$); 70.63, 70.56 (2*t*, C(6), C(1′)); 70.46 (*t*, C(1)); 54.44 (*d*, C(2)); 31.97, 29.82 (2*t*); 29.69 (3*t*); 29.60, 29.41, 26.30, 22.76 (4*t*); 14.21 (*q*, Me). HR-ESI-MS: 482.3623 (100, $[M+H]^+$, $C_{31}H_{48}NO_3^+$; calc. 482.3629).

*(*E*)-1,3-Di*-O-*benzyl-2,4,5-trideoxy-2-[(octadecanoyl)amino]-6*-O-*undecyl*-D-erythro-*hex-4-enitol* (**13**). A soln. of crude **12** (667 mg, *ca.* 1.38 mmol) in dry CH_2Cl_2 (20 ml) was treated with pyridine (0.22 ml, 2.76 mmol) and stearoyl chloride (630 mg, 2.08 mmol), stirred at r.t. for 3 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 (silica gel; AcOEt/hexane 2:8) gave **13** (744 mg, 80%). White crystalline solid. R_f (AcOEt/hexane 1:4) 0.20. M.p. 59.5–61.5°. $[\alpha]_D^{25} = -20.2$ ($c = 2.00$, $CHCl_3$). IR (KBr): 3450*w* (br.), 3306*m*, 3087*w*, 3062*w*, 3033*w*, 2954*s*, 2918*s*, 2850*s*, 1704*w*, 1645*s*, 1541*m*, 1495*w*, 1470*m*, 1453*m*, 1417w, 1386*w*, 1374*w*, 1359*w*, 1297*w*, 1255*w*, 1239*w*, 1221*w*, 1205*w*, 1098*m*, 1056*m*, 1026*m*, 987*w*, 929*w*, 901*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.35–7.24 (*m*, 10 arom. H); 5.79 (*dt*, $J = 15.9, 5.4$, H–C(5)); 5.72 (*d*, $J = 9.3$, NH); 5.65 (br. *dd*, $J = 15.6, 7.9$, H–C(4)); 4.60 (*d*, $J = 12.0$, PhC*H*); 4.49 (*d*, $J = 11.7$, PhC*H*); 4.43 (*d*, $J = 11.7$, PhC*H*); 4.30 (*d*, $J = 12.0$, PhC*H*); 4.25 (*ddt*, $J = 9.0, 7.2, 4.2$, H–C(2)); 3.97 (br. *d*, $J = 5.4$, 2 H–C(6)); 3.96 (*t*, $J \approx 7.5$, H–C(3)); 3.80 (*dd*, $J = 9.9, 4.5$, H–C(1)); 3.53 (*dd*, $J = 9.6, 3.9$, H′–C(1)); 3.45–3.33 (*AB*, 2 H–C(1′)); 2.15–1.99 (*AB*, 2 H–C(2″)); 1.64–1.48 (*m*, 2 H–C(2′), 2 H–C(3″)); 1.38–1.22 (*m*, 44 H); 0.88 (*t*, $J \approx 7.0$, 2 Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 172.38 (*s*, C(1″)); 138.15, 137.97 (2*s*); 132.26 (*d*, C(4)); 129.95 (*d*, C(5)); 128.29 (2*d*); 128.27 (2*d*); 127.72 (2*d*); 127.65 (3*d*); 127.52 (*d*); 78.95 (*d*, C(3)); 73.13, 70.66 (2*t*, 2 Ph*C*H$_2$); 70.61, 70.55 (2*t*, C(6), C(1′)); 68.41 (*t*, C(1)); 51.51 (*d*, C(2)); 37.05 (*t*); 32.04 (2*t*); 29.90 (*t*); 29.83–29.76 (several *t*); 29.68, 29.65, 29.52 (3*t*); 29.48 (2*t*); 29.43, 26.36, 25.85 (3*t*); 22.83 (2*t*); 14.28 (*q*, 2 Me). HR-MALDI-MS: 770.6056 (100, $[M+Na]^+$, $C_{49}H_{81}NNaO_4^+$; calc. 770.6058). Anal. calc. for $C_{49}H_{81}NO_4$ (748.17): C 78.66, H 10.91, N 1.87; found: C 78.81, H 11.07, N 1.75.

*(*E*)-2,4,5-Trideoxy-2-[(octadecanoyl)amino]-6*-O-*undecyl*-D-erythro-*hex-4-enitol* (**2**). A soln. of **13** (300 mg, 0.40 mmol) in dry $ClCH_2CH_2Cl$ (20 ml), was treated with anisole (518 mg, 4.80 mmol) and $AlCl_3$ (480 mg, 3.60 mmol), stirred at 60° for 18 h, cooled to 0°, treated dropwise with 10% aq. HCl (3 ml), and

extracted with AcOEt (5 × 100 ml). The combined org. layers were dried (Na_2SO_4), evaporated, and purified by FC (silica gel; MeOH/CH_2Cl_2 2:8) to give **2** (166 mg, 73%). White crystals. R_f (AcOEt) 0.26. M.p. 84–87°. $[\alpha]_D^{25} = -2.3$ ($c = 2.00$, $CHCl_3$). IR (KBr): 3367*m*, 3284*s*, 3081*w*, 2954*s*, 2919*s*, 2849*s*, 2797*m*, 1639*s*, 1551*s*, 1466*m*, 1416*w*, 1370*m*, 1340*w*, 1276*w*, 1204*w*, 1136*m*, 1118*m*, 1094*m*, 1057*m*, 1039*m*, 985*m*, 961*w*, 894*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 6.33 (*d*, $J = 7.5$, NH); 5.91 (br. *dt*, $J = 15.6$, 5.1, H–C(5)); 5.79 (br. *dd*, $J = 15.6$, 5.3, H–C(4)); 4.38 (br. *q*, $J \approx 4.0$, addn. of $D_2O \rightarrow$ br. *t*, $J \approx 4.0$, H–C(3)); 3.98 (*d*, $J = 5.4$, 2 H–C(6)); 3.96–3.88 (*m*, addn. of $D_2O \rightarrow$ change, H–C(1), H–C(2)); 3.74–3.65 (*m*, addn. of $D_2O \rightarrow$ change, H′–C(1)); 3.41 (*t*, $J = 6.6$, 2 H–C(1′)); 3.26 (*d*, $J = 5.4$, exchange with D_2O, HO–C(3)); 2.97 (*dd*, $J = 7.2$, 3.6, exchange with D_2O, HO–C(1)); 2.22 (*t*, $J = 7.6$, 2 H–C(2″)); 1.68–1.50 (*m*, 2 H–C(2′), 2 H–C(3″)); 1.38–1.28 (*m*, 44 H); 0.87 (*t*, $J \approx 6.8$, 2 Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 174.17 (*s*, C(1″)); 131.55 (*d*, C(4)); 129.58 (*d*, C(5)); 74.19 (*d*, C(3)); 71.08 (*t*, C(6)); 70.67 (*t*, C(1′)); 62.45 (*t*, C(1)); 54.28 (*d*, C(2)); 37.00 (*t*); 32.10 (2*t*); 29.96–29.68 (several *t*); 29.54 (2*t*); 29.48, 26.35, 25.93 (3*t*); 22.87 (2*t*); 14.30 (*q*, 2 Me). HR-MALDI-MS: 590.5113 (100, $[M + Na]^+$, $C_{35}H_{69}NNaO_4^+$; calc. 590.5119). Anal. calc. for $C_{35}H_{69}NO_4$ (567.93): C 74.02, H 12.25, N 2.47; found: C 73.99, H 11.98, N 2.45.

*(*E*)-2-Azido-2,4,5-trideoxy-6-*O*-undecyl-*D*-erythro-hex-4-enitol* (**11**). A soln. of **10** (475 mg, 0.937 mmol) in CH_2Cl_2 (20 ml) was treated with anisole (810 mg, 7.49 mmol) and $AlCl_3$ (749 mg, 5.62 mmol), stirred for 18 h, cooled to 0°, treated dropwise with 10% aq. HCl (3 ml), and extracted with AcOEt (5 × 100 ml). The combined org. layers were dried (Na_2SO_4) and evaporated. FC (silica gel; AcOEt/hexane 1:1) gave **11** (200 mg, 65%). Colourless oil. R_f (AcOEt/hexane 3:2) 0.33. $[\alpha]_D^{25} = -17.2$ ($c = 0.84$, $CHCl_3$). IR ($CHCl_3$): 3336*w* (br.), 2923*s*, 2853*s*, 2096*s*, 1465*w*, 1365*w*, 1266*m*, 1110*m*, 1064*m*, 1012*m*, 972*m*, 852*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 5.94 (br. *dt*, $J = 16.2$, 5.4, H–C(5)); 5.81 (*ddt*, $J = 15.6$, 6.6, 1.3, H–C(4)); 4.34 (br. *q*, $J = 4.5$, H–C(3)); 3.99 (*d*, $J = 5.1$, 2 H–C(6)); 3.82 (*t*, $J \approx 5.4$, 2 H–C(1)); 3.52 (*q*, $J = 5.1$, H–C(2)); 3.43 (*t*, $J = 6.8$, 2 H–C(1′)); 2.34 (*d*, $J = 4.2$, exchange with D_2O, HO–C(3)); 2.19 (*t*, $J \approx 5.8$, exchange with D_2O, HO–C(1)); 1.66–1.50 (*m*, 2 H–C(2′)); 1.38–1.20 (*m*, 16 H); 0.88 (*t*, $J \approx 6.8$, Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 130.79 (*d*, C(4)); 130.27 (*d*, C(5)); 72.98 (*d*, C(3)); 71.00 (*t*, C(6)); 70.41 (*t*, C(1′)); 66.40 (*d*, C(2)); 62.47 (*t*, C(1)); 32.02, 29.79 (2*t*); 29.74 (3*t*); 29.62, 29.45, 26.27, 22.82 (4*t*); 14.27 (*q*, Me). HR-ESI-MS: 350.2405 (67, $[M + Na]^+$, $C_{17}H_{33}N_3NaO_3^+$; calc. 350.2414). Anal. calc. for $C_{17}H_{33}N_3O_3$ (327.46): C 62.35, H 10.16, N 12.83; found: C 62.60, H 10.24, N 12.55.

*(*E*)-2-Amino-2,4,5-trideoxy-6-*O*-undecyl-*D*-erythro-hex-4-enitol* (**1**). A soln. of **11** (190 mg, 0.58 mmol) in dry THF (15 ml) was treated with 1M Me_3P (2.9 ml, 2.9 mmol), stirred for 16 h, treated with aq. 1M NaOH (5.8 ml, 5.8 mmol), stirred for 2 h, and evaporated. A soln. of the residue in H_2O was extracted with AcOEt (5 × 100 ml). The combined org. layers were dried (Na_2SO_4) and evaporated. FC (silica gel; AcOEt/hexane 1:1) gave **1** (130 mg, 74%). White crystals. R_f (MeOH/CH_2Cl_2 1:4) 0.12. M.p. 52–57°. $[\alpha]_D^{25} = +3.5$ ($c = 1.00$, $CHCl_3$). IR ($CHCl_3$): 3352*w* (br.), 3301*w* (br.), 2955*m*, 2922*s*, 2852*s*, 1587*w*, 1466*w*, 1364*w*, 1261*w*, 1105*m*, 1042*m*, 972*m*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 5.89 (br. *dt*, $J = 15.9$, 5.7, H–C(5)); 5.76 (br. *dd*, $J = 15.6$, 6.3, H–C(4)); 4.14 (br. *t*, $J \approx 5.7$, H–C(3)); 3.98 (br. *d*, $J = 5.1$, 2 H–C(6)); 3.71–3.61 (*AB*, 2 H–C(1)); 3.42 (*td*, $J = 6.8$, 0.9, 2 H–C(1′)); 2.90 (*quint.*, $J \approx 5.1$, H–C(2)); 2.25–1.82 (2 br. *s*, exchange with D_2O, NH_2, 2 OH); 1.63–1.50 (*m*, 2 H–C(2′)); 1.38–1.16 (*m*, 16 H); 0.88 (*t*, $J \approx 6.8$, Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 132.24 (*d*, C(4)); 129.70 (*d*, C(5)); 74.59 (*d*, C(3)); 71.09 (*t*, C(6)); 70.79 (*t*, C(1′)); 63.84 (*t*, C(1)); 56.16 (*d*, C(2)); 32.08, 29.87 (2*t*); 29.80 (3*t*); 29.69, 29.52, 26.34, 22.86 (4*t*); 14.27 (*q*, Me). HR-ESI-MS: 302.2685 (100, $[M + H]^+$, $C_{17}H_{36}NO_3^+$; calc. 302.2690).

*(*E*)-1,3-Di-*O*-benzyl-2,4,5-trideoxy-2-[(octadecanethioyl)amino]-6-*O*-undecyl-*D*-erythro-hex-4-enitol* (**14**). A mixture of **13** (100 mg, 0.13 mmol) and *Lawesson*'s reagent (38 mg, 0.09 mmol) in toluene was stirred for 90 min at 75° and then evaporated. The residue was diluted with H_2O (20 ml) and extracted with CH_2Cl_2 (2 × 50 ml). The combined org. layers were washed with sat. $NaHCO_3$ soln. and brine, dried (Na_2SO_4), and evaporated. FC (silica gel; AcOEt/hexane 1:9) gave **14** (86 mg, 84%). Colourless oil. R_f (AcOEt/hexane 1:9) 0.15. $[\alpha]_D^{25} = -26.6$ ($c = 2.00$, $CHCl_3$). IR ($CHCl_3$): 3387*w*, 3089*w*, 3066*w*, 3017*w*, 2927*s*, 2855*s*, 1601*w*, 1513*w*, 1465*w*, 1455*w*, 1405w, 1364*w*, 1263*w*, 1217*m*, 1097*m*, 1028*w*, 974*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.39 (*d*, $J = 9.0$, NH); 7.35–7.24 (*m*, 10 arom. H); 5.85 (br. *dt*, $J = 15.9$, 5.2, H–C(5)); 5.73 (br. *dd*, $J = 15.9$, 7.5, H–C(4)); 4.96 (*ddt*, $J = 9.0$, 6.2, 4.4, H–C(2)); 4.62 (*d*, $J = 11.8$, PhC*H*); 4.50 (*d*, $J = 11.8$, PhC*H*); 4.45 (*d*, $J = 11.8$, PhC*H*); 4.30 (*d*, $J = 11.8$, PhC*H*); 4.19 (br. *t*, $J \approx 6.9$, H–C(3)); 3.97 (br. *d*, $J = 5.0$, 2 H–C(6)); 3.86 (*dd*, $J = 10.0$, 4.4, H–C(1)); 3.67 (*dd*, $J = 10.0$, 4.4, H′–C(1)); 3.45–3.35 (*AB*, $J = 6.6$, 1.7, 2 H–C(1′)); 2.60–2.46 (*AB*, 2 H–C(2″)); 1.73–1.61 (*m*, 2 H–C(3″)); 1.60–1.51 (*m*, 2 H–C(2′)); 1.35–1.21 (*m*, 44 H); 0.88 (*t*, $J = 6.6$, 2 Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 205.79 (*s*, C(1″)); 138.27, 138.07 (2*s*); 132.64 (*d*, C(4)); 129.29 (*d*, C(5)); 128.60 (4*d*); 128.01 (2*d*); 127.97 (3*d*); 127.90 (*d*); 78.32 (*d*, C(3)); 73.33, 70.94 (2*t*, 2 PhCH_2); 70.84, 70.71 (2*t*, C(6), C(1′)); 67.18 (*t*, C(1)); 57.55 (*d*, C(2)); 47.82 (*t*, C(2″)); 32.12 (2*t*); 29.98–29.55 (several *t*); 29.10, 26.43 (2*t*); 22.89

(2*t*); 14.33 (*q*, 2 Me). HR-MALDI-MS: 786.5840 (100, $[M+Na]^+$, $C_{49}H_{81}NNaO_3S^+$; calc. 786.5829). Anal. calc. for $C_{49}H_{81}NO_3S$ (764.24): C 77.01, H 10.68, N 1.83; found: C 77.01, H 10.85, N 1.99.

*(*E*)-2,4,5-Trideoxy-2-[(octadecanethioyl)amino]-6-*O*-undecyl-*D*-erythro-hex-4-enitol* (**3**). A soln. of **14** (86 mg, 0.11 mmol) in CH_2Cl_2 (10 ml) was treated with anisole (90 mg, 0.68 mmol) and $AlCl_3$ (97 mg, 0.90 mmol), stirred at r.t. for 16 h, cooled to 0°, treated with 10% aq. HCl (1 ml), diluted with H_2O and extracted with CH_2Cl_2 (3 × 100 ml). The combined org. layers were dried (Na_2SO_4) and evaporated. FC (silica gel; AcOEt/hexane 1:1) gave **3** (46 mg, 70%). White solid. M.p. 73–75°. R_f (AcOEt) 0.63. $[\alpha]_D^{25} = -4.4$ ($c = 0.63$, $CHCl_3$). IR (KBr): 3214*m*, 3054*m*, 2955*m*, 2918*s*, 2850*s*, 1545*m*, 1467*m*, 1409*m*, 1366*w*, 1244*w*, 1172*w*, 1114*m*, 1096*w*, 1073*w*, 1037*w*, 1024*w*, 1004*w*, 974*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 8.07 (*d*, $J = 7.2$, NH); 5.99 (br. *dt*, $J =$ 15.6, 5.4, H–C(5)); 5.87 (br. *dd*, $J = 15.6$, 5.0, H–C(4)); 4.74–4.66 (*m*, H–C(3)); 4.64–4.56 (*m*, H–C(2)); 4.13 (*dd*, $J \approx 11.6$, 2.0, H–C(1)); 4.01 (*d*, $J = 5.4$, 2 H–C(6)); 3.83 (br. *d*, $J = 10.5$, H′–C(1)); 3.43 (*t*, $J \approx 6.8$, 2 H–C(1′)); 2.71 (*t*, $J \approx 7.7$, 2 H–C(2″)); 2.56 (br. *s*, exchange with D_2O, OH); 1.86–1.72 (*m*, 2 H–C(3″)); 1.66–1.52 (*m*, 2 H–C(2′), OH); 1.38–1.22 (*m*, 44 H); 0.88 (*t*, $J \approx 6.5$, 2 Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 206.23 (*s*, C(1′)); 131.01 (*d*, C(4)); 129.77 (*d*, C(5)); 73.90 (*d*, C(3)); 71.16 (*t*, C(6)); 70.55 (*t*, C(1′)); 61.13 (*t*, C(1)); 58.29 (*d*, C(2)); 47.65 (*t*, C(2″)); 32.10 (2*t*); 29.88–29.54 (several *t*); 29.10, 26.35 (2*t*); 22.87 (2*t*); 14.30 (*q*, 2 Me). HR-MALDI-MS: 606.4883 (100, $[M+Na]^+$, $C_{35}H_{69}NNaO_3S^+$; calc. 606.4890). Anal. calc. for $C_{35}H_{69}NO_3S$ (583.49): C 71.98, H 11.91, N 2.40, S 5.49; found: C 71.90, H 11.93, N 2.45, S 5.31.

*(*E*)-1,3-Di-*O*-benzyl-2,4,5-trideoxy-2-[[(2-nitrophenyl)sulfonyl]amino]-6-*O*-undecyl-*D*-erythro-hex-4-enitol* (**15**). A soln. of **12** (250 mg, 0.52 mmol) in dry CH_2Cl_2 (20 ml) was treated with NsCl (126 mg, 0.57 mmol) and Et_3N (0.09 ml, 0.62 mmol), stirred for 16 h at r.t., diluted with H_2O, and extracted with CH_2Cl_2 (3 × 100 ml). The combined org. layers were dried (Na_2SO_4) and evaporated. FC (silica gel; AcOEt/hexane 2:8) gave **15** (300 mg, 87%). Yellow oil. R_f (AcOEt/hexane 1:4) 0.18. $[\alpha]_D^{25} = -4.7$ ($c = 2.00$, $CHCl_3$). IR ($CHCl_3$): 3382*w*, 3089*w*, 3066*w*, 3029*w*, 3018*w* 2928*s*, 2856*s*, 1596*w*, 1541*s*, 1496*w*, 1454*w*, 1442*w*, 1418*m*, 1357*s*, 1263*m*, 1217*m*, 1168*m*, 1098*s*, 1027*m*, 1017*m*, 855*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 8.03 (*dd*, $J = 7.3$, 1.8, H–C(2″)); 7.63 (*dd*, $J =$ 7.2, 2.1, H–C(6″)); 7.51 (*td*, $J = 7.3$, 1.8, H–C(4″)); 7.46 (*td*, $J = 7.2$, 1.8, H–C(5″)); 7.32–7.25 (*m*, 6 arom. H); 7.17–7.10 (*m*, 4 arom. H); 5.83 (br. *dt*, $J = 15.0$, 6.3, H–C(5)); 5.82 (*d*, $J = 8.7$, NH); 5.59 (*ddt*, $J = 15.6$, 7.2, 1.1, H–C(4)); 4.43 (*d*, $J = 11.1$, PhC*H*); 4.30 (br. *s*, PhCH_2); 4.19 (*d*, $J = 11.7$, PhC*H*); 4.08 (br. *t*, $J \approx 6.1$, H–C(3)); 3.95 (*dd*, $J = 4.8$, 0.8, 2 H–C(6)); 3.75 (*dq*, $J = 8.0$, 5.6, H–C(2)); 3.64 (*dd*, $J = 9.9$, 5.7, H–C(1)); 3.41 (*dd*, $J =$ 9.8, 5.7, H′–C(1)); 3.40 (*t*, $J = 6.8$, 2 H–C(1′)); 1.63–1.53 (*m*, 2 H–C(2′)); 1.37–1.22 (*m*, 16 H); 0.88 (*t*, $J = 6.9$, Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 147.56 (*s*, C(2″)); 137.64, 137.41 (2*s*); 134.79 (*s*, C(1″)); 132.98, 132.74, 132.42 (3*d*, C(4″), C(5″), C(4)); 130.56 (*d*, C(5)); 128.25 (4*d*); 127.73 (4*d*); 127.67, 127.58 (2*d*); 127.42 (*d*, C(6″)); 125.16 (*d*, C(3″)); 78.84 (*d*, C(3)); 73.32, 70.94 (2*t*, 2 PhCH_2); 70.75 (*t*, C(6)); 70.37 (*t*, C(1′)); 69.06 (*t*, C(1)); 57.89 (*d*, C(2)); 32.03, 29.87 (2*t*); 29.75 (3*t*); 29.66, 29.46, 26.33, 22.83 (4*t*); 14.27 (*q*, Me). HR-ESI-MS: 689.3240 (100, $[M+Na]^+$, $C_{37}H_{50}N_2NaO_7S^+$; calc. 689.3231). Anal. calc. for $C_{37}H_{50}N_2O_7S$ (666.87): C 66.64, H 7.56, N 4.20; found: C 66.83, H 7.66, N 4.18.

*(*E*)-1,3-Di-*O*-benzyl-2,4,5-trideoxy-2-[[(2-nitrophenyl)sulfonyl](methyl)amino]-6-*O*-undecyl-*D*-erythro-hex-4-enitol* (**16**). A soln. of **15** (300 mg, 0.45 mmol) in dry DMF (10 ml) was treated with K_2CO_3 (124 mg, 0.90 mmol), heated to 60°, stirred for 5 min, treated with MeI (0.05 ml, 0.90 mmol), stirred for 1 h, cooled to r.t., diluted with H_2O, and extracted with Et_2O (4 × 50 ml). The combined org. layers were dried (Na_2SO_4) and evaporated. FC (silica gel; AcOEt/hexane 2:8) gave **16** (306 mg, 100%). Yellow oil. R_f (AcOEt/hexane 1:4) 0.18. $[\alpha]_D^{25} = -9.7$ ($c = 2.00$, $CHCl_3$). IR ($CHCl_3$): 3089*w*, 3066*w*, 3031*w*, 3011*w*, 2928*s*, 2856*s*, 1594*w*, 1541*s*, 1496*w*, 1465*w*, 1455*w*, 1441*w*, 1371*m*, 1350*m*, 1265*w*, 1217*w*, 1157*m*, 1102*m*, 1059*m*, 1027*w*, 984*w*, 911*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.98 (*d*, $J = 8.1$, H–C(2″)); 7.43 (*d*, $J = 3.9$, 2 arom. H); 7.35–7.23 (*m*, 9 arom. H); 7.14–7.09 (*m*, 2 arom. H); 5.85 (br. *td*, $J = 15.6$, 5.4, H–C(5)); 5.65 (br. *dd*, $J = 15.6$, 7.5, H–C(4)); 4.57 (*d*, $J = 11.4$, PhC*H*); 4.34 (*d*, $J = 11.4$, PhC*H*); 4.30 (*d*, $J = 11.7$, PhC*H*); 4.26 (*d*, $J = 11.7$, PhC*H*); 4.19 (*ddd*, $J = 7.8$, 6.0, 4.2, H–C(2)); 4.13 (*t*, $J \approx 6.8$, H–C(3)); 4.03–3.91 (*AB*, 2 H–C(6)); 3.76 (*dd*, $J = 10.5$, 7.5, H–C(1)); 3.74 (*dd*, $J =$ 10.8, 4.5, H′–C(1)); 3.41 (*t*, $J = 6.6$, 2 H–C(1′)); 2.97 (*s*, MeN); 1.64–1.53 (*m*, 2 H–C(2′)); 1.37–1.22 (*m*, 16 H); 0.88 (*t*, $J = 6.9$, Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 147.97 (*s*, C(2″)); 137.78, 137.57 (2*s*); 133.27 (*s*, C(1″)); 132.64, 132.51, 130.98, 130.81 (4*d*, C(4″), C(5″), C(4), C(5)); 128.95 (*d*, C(6″)); 128.32 (2*d*); 128.23 (2*d*); 127.83 (2*d*); 127.68 (2*d*); 127.62 (*d*); 127.59 (*d*); 123.54 (*d*, C(3″)); 80.65 (*d*, C(3)); 73.27, 71.06 (2*t*, 2 PhCH_2); 70.69 (*t*, C(6)); 70.43 (*t*, C(1′)); 66.78 (*t*, C(1)); 60.41 (*d*, C(2)); 32.03, 31.71 (2*t*); 31.17 (*q*, MeN); 29.87 (*t*); 29.75 (2*t*); 29.66, 29.46, 26.33, 22.82 (4*t*); 14.27 (*q*, Me). HR-ESI-MS: 703.3390 (100, $[M+Na]^+$, $C_{38}H_{52}N_2NaO_7S^+$; calc. 703.3387). Anal. calc. for $C_{38}H_{52}N_2O_7S$ (680.89): C 67.03, H 7.70, N 4.11; found: C 67.23, H 7.73, N 4.10.

*(*E*)-1,3-Di-*O*-benzyl-2,4,5-trideoxy-2-(methylamino)-6-*O*-undecyl-*D*-erythro-hex-4-enitol* (**17**). A mixture of **16** (306 mg, 0.45 mmol) and 5.0M aq. KOH soln. (0.45 ml, 2.25 mmol) in dry MeCN was heated to 60°, treated with PhSH (0.23 ml, 2.25 mmol), stirred for 48 h, cooled to r.t., and evaporated. An emulsion of the residue in

H_2O was extracted with AcOEt (4 × 50 ml). The combined org. layers were dried (Na_2SO_4) and evaporated. FC (silica gel; MeOH/CH_2Cl_2 1:49) gave **17** (212 mg, 95%). Colourless oil. R_f (MeOH/CH_2Cl_2 1:19) 0.20. $[\alpha]_D^{25} = -5.6$ ($c = 2.00$, $CHCl_3$). IR ($CHCl_3$): 3390*w* (br.), 3089*w*, 3066*w*, 3031*w*, 3009*m*, 2928*s*, 2856*s*, 2802*w*, 1599*w*, 1541*s*, 1496*w*, 1463*w*, 1454*m*, 1365*w*, 1263*m*, 1097*s*, 1027*m*, 978*w*, 911*w*. ^{1}H-NMR($CDCl_3$, 300 MHz): 7.36–7.24 (*m*, 10 arom. H); 5.83 (br. *dt*, $J = 15.6$, 5.3, H–C(5)); 5.70 (br. *dd*, $J = 15.6$, 7.6, H–C(4)); 4.60 (*d*, $J = 11.7$, PhC*H*); 4.53 (*d*, $J = 12.0$, PhC*H*); 4.47 (*d*, $J = 12.0$, PhC*H*); 4.37 (*d*, $J = 11.7$, PhC*H*); 4.06 (br. *dd*, $J = 7.8$, 5.0, H–C(3)); 4.00 (br. *d*, $J = 5.3$, 2 H–C(6)); 3.61 (*dd*, $J = 9.7$, 5.6, H–C(1)); 3.57 (*dd*, $J = 9.7$, 5.3, H′–C(1)); 3.42 (*t*, $J \approx 6.7$, 2 H–C(1′)); 2.88 (*q*, $J \approx 5.3$, H–C(2)); 2.76 (br. *s*, NH), 2.45 (*s*, MeN); 1.63–1.54 (*m*, 2 H–C(2′)); 1.38–1.22 (*m*, 16 H); 0.88 (*t*, $J = 6.9$, Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 138.25, 138.06 (2*s*); 132.14 (*d*, C(4)); 129.42 (*d*, C(5)); 128.22 (2*d*); 128.19 (2*d*); 127.64 (2*d*); 127.59 (2*d*); 127.50, 127.38 (2*d*); 79.05 (*d*, C(3)); 73.28, 70.63 (2*t*, 2 Ph*C*H_2); 70.57, 70.51 (2*t*, C(6), C(1′)); 68.48 (*t*, C(1)); 62.91 (*d*, C(2)); 34.70 (*q*, MeN); 31.99, 29.84 (2*t*); 29.71 (3*t*); 29.62, 29.42, 26.31, 22.78 (4*t*); 14.24 (*q*, Me). HR-ESI-MS: 496.3779 (100, $[M+H]^+$, $C_{32}H_{50}NO_3^+$; calc. 496.3785). Anal. calc. for $C_{32}H_{49}NO_3$ (495.74): C 77.53, H 9.96, N 2.83; found: C 77.49, H 9.95, N 2.96.

*(*E*)-1,3-Di-*O*-benzyl-2,4,5-trideoxy-2-[(octadecanoyl)(methyl)amino]-6-*O*-undecyl-*D*-erythro-hex-4-enitol* (**18**). A soln. of **17** (212 mg, 0.43 mmol) in dry CH_2Cl_2 (10 ml) was treated with pyridine (0.05 ml, 0.65 mmol) and stearoyl chloride (0.19 ml, 0.56 mmol) at r.t. for 3 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 (silica gel; AcOEt/hexane 2:8) gave **18** (302 mg, 92%). Colourless oil. R_f (AcOEt/hexane 1:4) 0.20. $[\alpha]_D^{25} = -25.0$ ($c = 2.00$, $CHCl_3$). IR ($CHCl_3$): 3443*w*, 3087*w*, 3066*w*, 3015*w*, 2927*s*, 2855*s*, 1630*m*, 1494*w*, 1465*w*, 1455*w*, 1405*w*, 1364*w*, 1308*w*, 1218*w*, 1100*m*, 1071*w*, 1028*w*, 975*w*, 907*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz, 2:1 mixture of diastereoisomers): 7.36–7.22 (*m*, 10 arom. H); 5.82 (br. *dt*, $J = 15.6$, 5.3, 0.37 H), 5.79 (*dt*, $J = 15.6$, 5.7, 0.63 H) (H–C(5)); 5.61 (br. *dd*, $J = 15.6$, 8.3, 0.63 H), 5.57 (*dd*, $J = 15.6$, 8.3, 0.37 H) (H–C(4)); 4.70–4.60 (br. *s*, 0.63 H–C(2)); 4.59 (*d*, $J = 11.7$, 0.37 H), 4.57 (*d*, $J = 11.7$, 0.63 H) (PhC*H*); 4.52 (*d*, $J = 12.3$, PhC*H*); 4.45 (*d*, $J = 11.4$, 0.37 H), 4.41 (*d*, $J = 12.0$, 0.63 H) (PhC*H*); 4.28 (*d*, $J = 12.0$, PhC*H*); 4.07 (*t*, $J = 8.1$, 0.63 H), 4.06 (*t*, $J \approx 8.1$, 0.37 H) (H–C(3)); 4.00–3.93 (*AB*, 2 H–C(6)); 3.88 (*dd*, $J = 9.6$, 8.4, 0.63, H′–C(1)); 3.90–3.87 (*m*, 0.37 H–C(2)); 3.78 (*dd*, $J \approx 10.2$, 3.3, 0.37 H), 3.73 (*dd*, $J \approx 10.8$, 3.6, 0.63 H) (H–C(1)); 3.64 (*dd*, $J = 10.2$, 8.4, 0.37 H′–C(1)); 3.43–3.32 (*AB*, 2 H–C(1′)); 2.91 (*s*, 1.89 H), 2.75 (*s*, 1.11 H) (MeN); 2.46–2.32 (*m*, 0.63 H), 2.32–2.15 (*m*, 1.37 H) (2 H–C(2″)); 1.74–1.50 (*m*, 2 H–C(2′) 2 H–C(3″)); 1.36–1.22 (*m*, 44 H); 0.88 (*t*, $J \approx 6.5$, 2 Me). ^{13}C-NMR ($CDCl_3$, 75 MHz, 2:1 mixture of diastereoisomers): signals of major isomer: 173.74 (*s*, C(1′)); 138.22, 138.12 (2*s*); 131.79 (*d*, C(4)); 130.21 (*d*, C(5)); 128.48–127.31 (several *d*); 78.77 (*d*, C(3)); 72.74 (*t*, Ph*C*H_2); 70.67, 70.53, 70.41 (3*t*, Ph*C*H_2, C(6), C(1′)); 67.66 (*t*, C(1)); 58.20 (br. *d*, C(2)); 34.10 (*t*); 31.99 (2*t*); 29.90–29.10 (several *t*); 28.62 (*q*, MeN); 26.32, 25.15 (2*t*); 22.77 (2*t*); 14.21 (*q*, 2 Me); signals of minor isomer: 173.91 (*s*, C(1″)); 137.87, 137.57 (2*s*); 132.75 (*d*, C(4)); 130.21 (*d*, C(5)); 128.48–127.31 (several *d*); 78.67 (*d*, C(3)); 73.21, 70.75 (2*t*, 2 Ph*C*H_2); 70.67 (*t*, C(6)); 70.22 (*t*, C(1′)); 67.72 (*t*, C(1)); 60.39 (*d*, C(2)); 33.71 (*t*); 31.97 (2*t*); 29.90–29.10 (several *t*); 29.21 (*q*, MeN); 26.32, 25.48 (2*t*); 22.77 (2*t*); 14.21 (*q*, 2 Me). HR-MALDI-MS: 784.6205 (100, $[M+Na]^+$, $C_{50}H_{83}NNaO_4^+$; calc. 784.6214). Anal. calc. for $C_{50}H_{83}NO_4$ (762.20): C 78.79, H 10.98, N 1.84; found: C 78.88, H 11.06, N 2.07.

*(*E*)-2,4,5-Trideoxy-2-[(octadecanoyl)(methyl)amino]-6-*O*-undecyl-*D*-erythro-hex-4-enitol* (**4**). A soln. of **18** (302 mg, 0.40 mmol) in $ClCH_2CH_2Cl$ (20 ml) was treated with anisole (0.52 ml, 4.80 mmol) and $AlCl_3$ (480 mg, 3.6 mmol), stirred at 60° for 18 h, cooled to 0°, treated dropwise with 10% aq. HCl (3 ml), diluted with H_2O, and extracted with AcOEt (3 × 100 ml). The combined org. layers were dried (Na_2SO_4) and evaporated. FC (silica gel; MeOH/CH_2Cl_2 2:8) gave **4** (172 mg, 74%). White solid. R_f (AcOEt) 0.24. $[\alpha]_D^{25} = +3.0$ ($c = 1.00$, $CHCl_3$). IR (KBr): 3391*m*, 2956*s*, 2918*s*, 2850*s*, 1712*w*, 1607*s*, 1468*m*, 1408*m*, 1374*m*, 1268*w*, 1115*m*, 1088*m*, 1025*m*, 1000*m*, 970*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz 7:1 mixture of diastereoisomers): 5.85 (br. *dt*, $J = 15.6$, 5.0, H–C(5)); 5.76 (br. *dd*, $J = 15.6$, 6.0, H–C(4)); 4.59 (br. *t*, $J \approx 6.3$, 0.87 H), 4.40–4.32 (*m*, 0.13 H) (H–C(3)); 4.07–3.83 (*m*, 2 H–C(1)); 3.95 (*d*, $J = 4.5$, 2 H–C(6)); 3.66 (br. *td*, $J \approx 6.5$, 3.8, H–C(2)); 3.57 (*d*, $J = 3.0$, exchange with D_2O, HO–C(3)); 3.57–3.50 (br. *s*, exchange with D_2O, HO–C(1)); 3.40 (*t*, $J \approx 6.8$, 2 H–C(1′)); 3.02 (*s*, 2.61 H), 2.82 (*s*, 0.39 H) (MeN); 2.30 (*t*, $J \approx 7.8$, 2 H–C(2″;)); 1.65–1.50 (*m*, 2 H–C(2′), 2 H–C(3″)); 1.36–1.22 (*m*, 44 H); 0.88 (*t*, $J \approx 6.8$, 2 Me). ^{13}C-NMR ($CDCl_3$, 75 MHz 7:1 mixture of diastereoisomers): signals of major isomer: 175.10 (*s*, C(1″)); 132.10 (*d*, C(4)); 129.33 (*d*, C(5)); 71.38 (*d*, C(3)); 70.70 (*t*, C(6)); 70.53 (*t*, C(1′)); 64.91 (*t*, C(1)); 61.51 (*d*, C(2)); 36.30 (*q*, MeN); 34.52 (*t*); 31.98 (2*t*); 29.80–29.35 (several *t*); 26.27, 25.05 (2*t*); 22.75 (2*t*); 14.20 (*q*, 2 Me); signals of minor isomer: 174.02 (*s*, C(1″)); 131.34 (*d*, C(4)); 130.09 (*d*, C(5)); 72.23 (*d*, C(3)); 70.92 (*t*, C(6)); 70.26 (*t*, C(1′)); 62.21 (*t*, C(1)); 62.02 (*d*, C(2)); 33.79 (*t*); 31.98 (2*t*); 29.80–29.35 (several *t*); 28.58 (*q*, MeN); 26.27, 25.46 (2*t*); 22.75 (2*t*); 14.20 (*q*, 2 Me). HR-MALDI-MS: 604.5267 (100, $[M+Na]^+$, $C_{36}H_{71}NNaO_4^+$; calc. 604.5275). Anal. calc. for $C_{36}H_{71}NO_4$ (581.95): C 74.30, H 12.30, N 2.41; found: C 74.03, H 12.24, N 2.42.

2. Biological Tests. – *Materials and Methods. Cell Lines.* The SK-N-BE human neuroblastoma cell lines were obtained from the *European Collection of Cell Cultures* (*ECACC*; England) and cultured in 50% *Dulbecco's* Modified Eagle's Medium (DMEM) and 50% F-12 supplemented with 10% foetal bovine serum, 2 mmol/l L-glutamine, penicillin (100 μg/ml), and streptomycin (100 μg/ml). The 32d mouse promielocytic cell line was obtained from *ECACC* and cultured in *Iscove's* modified *Dulbecco's* medium supplemented with 10% foetal bovine serum, 2 mmol/l L-glutamine, penicillin (100 μg/ml), and streptomycin (100 μg/ml).

Thiazolyl Blue Tetrazolium Bromide (*MTT*) *Assay.* Experiments were perfomed in *Locke's* soln. (134 mmol/l NaCl, 5 mmol/l KCl, 4 mmol/l $NaHCO_3$, 10 mmol/l *HEPES* (pH 7.6), 2.3 mmol/l $CaCl_2$, 1 mmol/l $MgCl_2$, 5 mmol/l sucrose) with a final MTT (1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan) concentration of 250 μg/ml. Cells were washed twice with *Locke's* soln. and then grown for 1 h with MTT at 37. Reactions were then stopped, and the crystals were solubilised in i-PrOH/HCl before being read at 570 nm in a spectrophotometer [32].

Nuclear DNA Staining. For fluorescence-microscopy analysis of DNA integrity, SK-N-BE or 32d cells (cytospun with a *Shannon* cytocentrifuge, Shannon, USA) were plated on 12-mm glass cover slips and maintained for 24 h with different sphingosine or ceramide analogues at different concentrations. Bisbenzimide (*Hoechst 33258*; *Roche Diagnostic SpA*, Milan, Italy) DNA staining was used to detect nuclear condensation as apoptotic marker. Briefly, cells were washed once in PBS and fixed with a soln. of paraformaldehyde 4% for 30 min at 4°. Cells were permeabilised with PBS/*TRITON X*-100 0.1% for 5 min. Following PBS washing, cells were stained with a soln. (0.8 mg/ml) of bisbenzimide in PBS (2 mg/ml in water) for 30 min at 37°. Cells were washed twice with H_2O, and mounted onto a cover slip to be visualised with a UV filter (360/395 nm) [35].

Reverse Transcriptase Polymerase Chain Reaction (*RT-PCR*). Total RNA was isolated from cells using *TRI REAGENT* (*Sigma*), and cDNA was made using the *StrataScript* amplification system (*Stratagene*) according to the manufacturer's protocol. PCR Using specific primers for CERK was carried out in a volume of 25 μl of the reaction mixture supplemented with 10 × reaction buffer, 0.5 mM of each dNTP, 1.5 mM $MgCl_2$, and 0.5 μl of Taq polymerase. Three μg of template and 0.5 μM of each primer were used. Samples were amplified with an initial denaturation at 94° for 2 min, followed by 30 cycles of denaturation at 94° for 30 s, annealing at 56° for 30 s, and extension at 72° for 1 min.

Primer sequences (all 5′ → 3′) were: CERKshort forward GTTTCTGGCCATCAATGCCAC, CERKshort reverse CTGGCAGTGGACTCTGACCTC; CERKlong forward TGTCACTTGTGGCTGCAGACC, CERK-long reverse GTTTGTGGCATTGATGGCCAG. The amplification of a single-size product was further verified by gel electrophoresis. The expression levels of CERK were compared to actin as an internal standard.

Data Analysis. Results are expressed as mean ± SE. Statistical analysis was performed by analysis of variance (*ANOVA*). A *p*-value of ≤ 0.05 was considered to be statistically significant.

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