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Synthesis and Evaluation of Morpholino- and Pyrrolidinosphingolipids as Inhibitors of Glucosylceramide Synthase

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Abstract—This paper describes the new synthesis and evaluation of some morpholino- and pyrrolidinosphingolipids and mimics as inhibitors of glucosylceramide synthase. It was found that the pyrrolidino derivatives are generally more active than the morpholino derivatives and the best one was shown to be a nanomolar inhibitor.

Introduction

UDP-Glucose: *N*-acylsphingosine glucosyltransferase (glucosylceramide synthesase, EC 2, 4.1. 80) catalyses the glucosyl transfer from UDP-glucose to the primary hydroxyl group of ceramide to form glucosylceramide (GlcCer)[1] (**1**), which serves as a key precursor to numerous glycosphingolipids (GSLs) for important biological functions and to many unique structures involved in cancer cell growth and metabolism.[2] In addition, the slow catabolism of GSL could cause lysosomal storage diseases, such as Gaucher and Tay-Sachs diseases, which are known to result from the accumulation of GlcCer[3]. Inhibition of the biosynthesis of GlcCer is therefore considered to be a useful strategy for the treatment of cancers and these two types of diseases. In fact, 1*R*-phenyl-2*R*-decanoylamino-3-morpholino-l-propanol D-*threo* (PDMP), a mimic of the transition state of the enzymatic synthesis of glucosylceramide and a competitive inhibitor of glucosylceramide synthase ($K_i = 0.7\,\mu M$) has been shown to be active as an anticancer agent.[4] *N*-Butyldeoxynojirimycin is also an inhibitor of the enzyme and has been shown to be active against Tay-Sachs disease in a mouse model.[5]

Recent studies have also shown that the other three stereoisomers of PDMP are inactive as inhibitors of glycosylceramide synthase.[4a] Both morpholino- and pyrrolidino-sphingolipids with the same stereochemistry as PDMP are more active than the ones with the natural ceramide stereochemistry[4d,g] and pyrrolidinosphingolipids are slightly more potent than the corresponding morpholinosphingolipids. The synthesis of these inhibitors was usually carried out using a lengthy procedure and required separation of diastereomers, with the exception that Evans' enantioselective aldol reaction was used in the synthesis of morpholino-ceramide.[4d] Here we report the new synthesis of morpholino- and pyrrolidino-ceramide derivatives (**1**–**4**) and the pyrrolidino version of PDMP (D-*threo*-PDPP and the L-enantiomer) analogues, and evaluation of their inhibition activities against glucosylceramide synthase (Fig. 1).

Results and Discussion

Synthesis of L-*threo*-PDMP (9)

First, we have established a new synthetic route to L-*threo*-PDMP (**9**) from compound **5** in a model study (Scheme 1). Treatment of compound **5** with decanoyl

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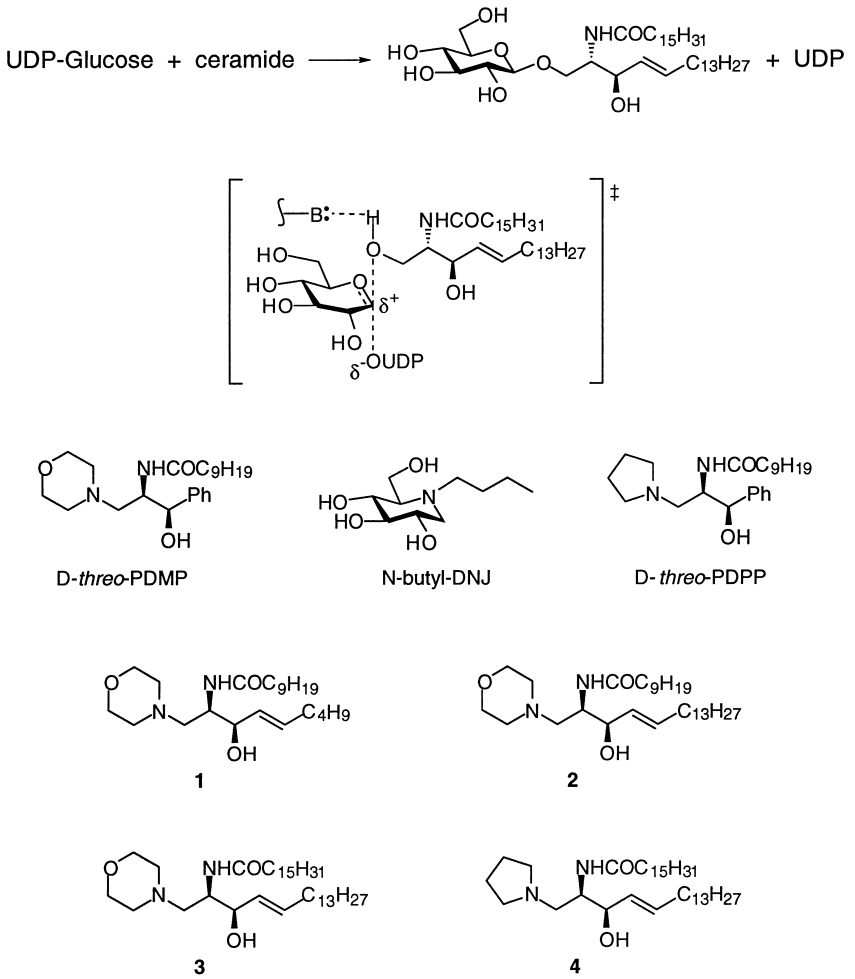
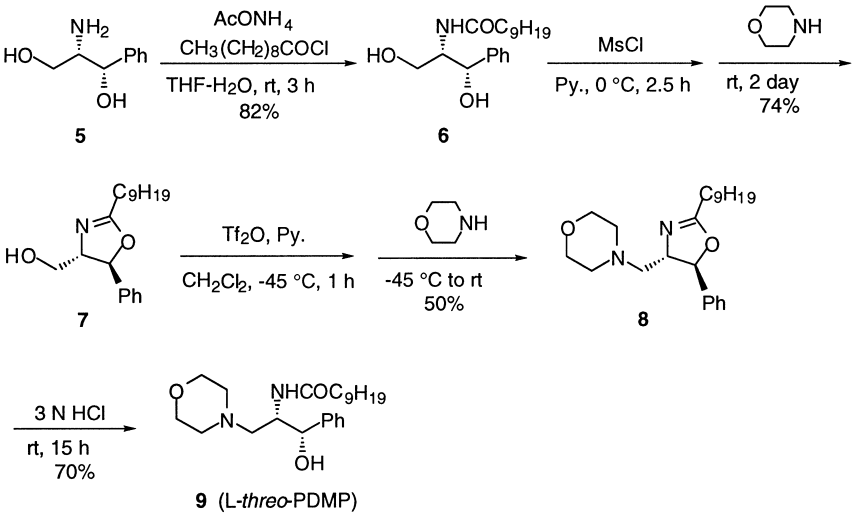


Figure 1. Glycosylceramide synthase-catalyzed reaction and the proposed transition-state structure.



Scheme 1.

chloride in THF and H_2O gave compound **6** in 82% yield. Compound **6** was then converted to the oxazoline derivative (**7**) (using MsCl/Py/morpholine) in 74% yield. Compound **7** was then reacted with Tf_2O and morpholine in CH_2Cl_2 to give compound **8** in 50% yield. Treatment of compound **8** with 3 N HCl at room temperature for 15 h gave L-*threo*-PDMP (**9**) in 70% yield. Using the enantiomer of **5**, D-*threo*-PDMP has been prepared.

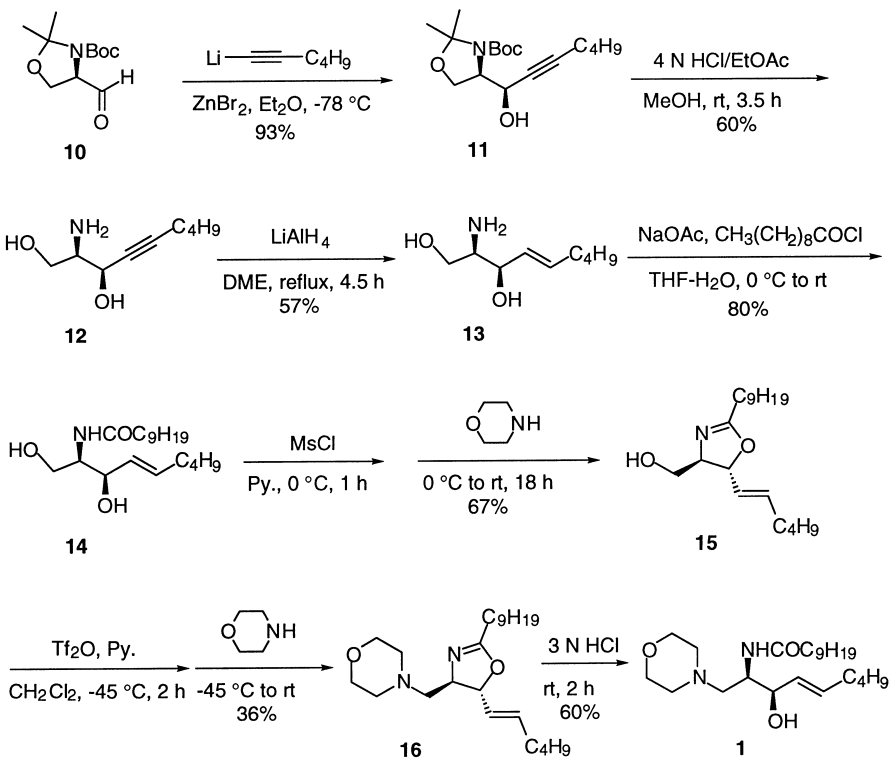
Synthesis of 1–4

As shown in Scheme 2, D-*threo*- **13** and the *N*-acyl derivative **17** were prepared from the Gamer aldehyde[6] according to Herold's procedure.[7] Using the same strategy described for the synthesis of L-*threo*-PDMP (**9**), compound **1** was prepared as shown in Scheme 2. Compounds **2**, **3**, and **4** have been synthesized in a similar manner as above (Schemes 3–5).

It is worth noting that the conversion of **6** to oxazoline **7** under the condition described in Scheme 1 is unusual and deserves further study. We thought the reaction might proceed through the oxazoline intermediate (**26**) (Scheme 6). The primary hydroxy group of **6** may react with MsCl to afford compound **25**, followed by the formation of oxazoline (**26**). Ring closure of **26** would afford compound **7** (Scheme 6). The formation of compounds **25** and **26** were indeed observed by ^1H NMR measurement in pyridine-d_5. It is noted that addition of base (such as morpholine or piperzine) is very important, and the amount of morpholine added determines the extent to which the reaction goes to completion. The less morpholine was added, the less compound **7** was formed and the ring-opening product **27** was produced (Scheme 7). Addition of morpholine ($pK_a = 8.42$) or piperizine ($pK_a = 11.11$), however, accelerates the formation of compound **7**. These results support the mechanism described in Scheme 6.

Inhibition Analysis

Compounds **1**–**4** were tested as inhibitors of glucosylceramide synthase and the relative inhibition activities were shown in Table 1. It is clear that D-*threo*-PDMP is slightly better than the corresponding ceramide derivatives including the ones with different chain length. The pyrrolidino derivatives are, however, more potent than the morpholino derivatives and the one corresponding to the PDMP side chain is the best. The enantiomer had no inhibition activity at 0.1 M. This study suggests that the pyrrolidino group is perhaps a better mimic of the cationic character of the glycosyl moiety in the transition state of

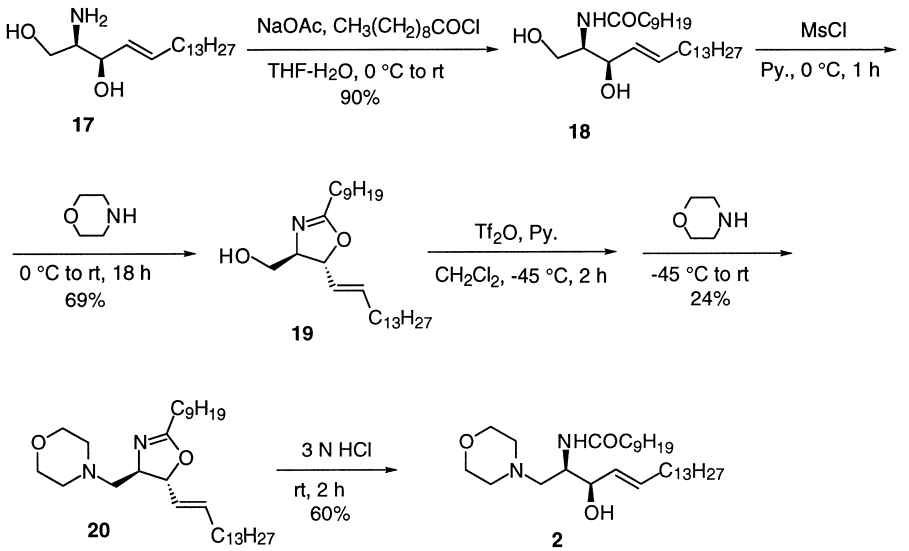


Scheme 2.

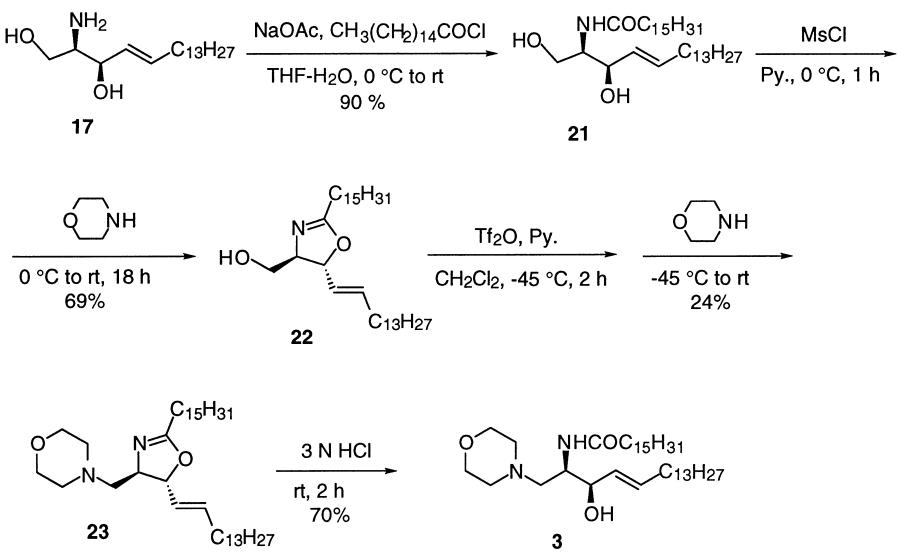
the enzymatic reaction, and the two stereogenic centers both with the *R* configuration exhibit the best activity. Work is in progress to incorporate a better glucosyl cation mimic into the designed inhibitor to further enhance the inhibition potency.

Experimental

Compound 7. MsCl (0.53 mL, 6.84 mmol) was added dropwise to a solution of **6** (2.00 g, 6.22 mmol) in pyridine (5 mL) at 0 °C under nitrogen atmosphere. After 2.5 h of stirring at 0 °C, morpholine (5.4 mL, 62.2 mmol) was added. After 2 days of stirring at room temperature, the reaction mixture was extracted three times with EtOAc. The combined EtOAc extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The crude product thus obtained was purified by column chromatography on silica gel (hexane:AcOEt, 2.5:1) to give **7** (1.40 g, 74%) as colorless prisms; mp 62 °C; $[\alpha]_D^{23}$ −60.0° (ca. 2.09, $CHCl_3$); ^1H NMR ($CDCl_3$; D_2O exchange) δ 0.88 (3H, t, J=6.9 Hz, Me), 1.27 (12H, m), 1.71 (2H, m, C=N-CH_2-CH_2), 2.39 (2H, t, J=7.6 Hz, C-N-CH_2), 3.66 (1H,



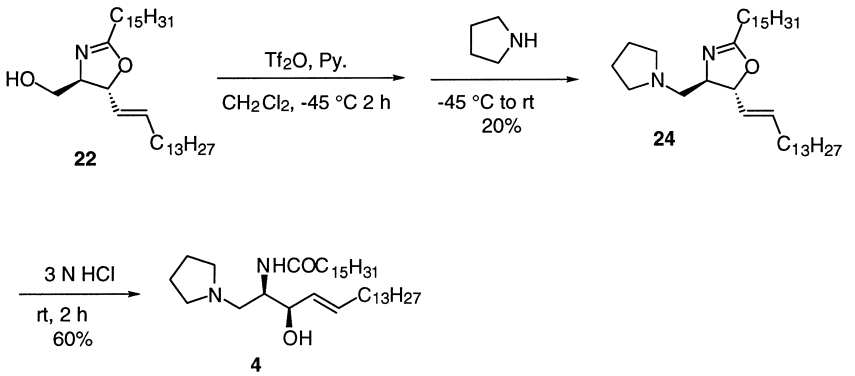
Scheme 3.



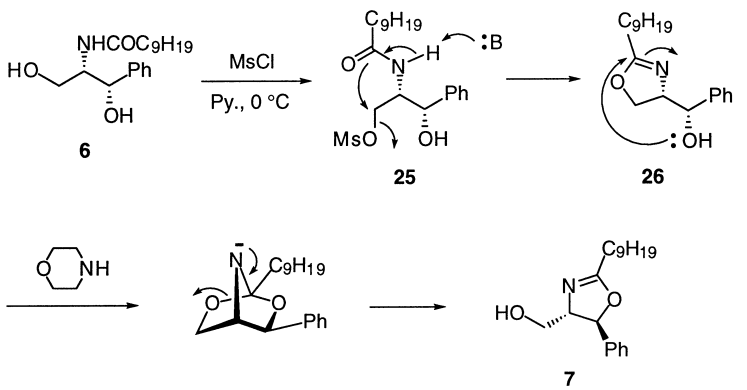
Scheme 4.

dd, $J=11.7$, 4.3 Hz, O-CH_2), 3.91 (1H, dd, $J=11.7$, 3.8 Hz, O-CH_2), 4.05 (1H, m, N-CH), 5.29 (1H, d, $J=7.6$ Hz, O-CH), 7.33 (5H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 14.1 (q), 22.6 (t), 26.1 (t), 28.3 (t), 29.2 (t), 29.4 (t), 31.8 (t), 63.5 (d), 76.2 (d), 82.7 (d), 125.6 (d), 128.3 (d), 128.8 (d), 140.5 (s), 169.5 (s). TOF Mass: 304 ($M+H^+$), ($C_{19}H_{29}NO_2$ 303). Anal. calcd for $C_{19}H_{29}NO_2$: C 75.21; H, 9.63; N, 4.62. Found: C, 74.82; H, 9.63; N, 4.57.

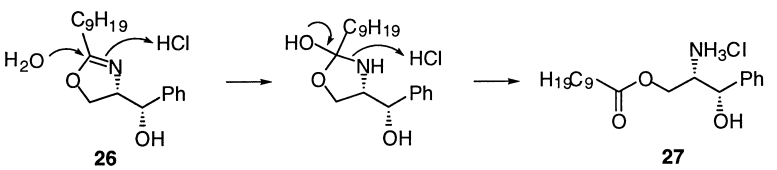
Compound 8. Tf_2O (72 mL, 0.43 mmol) was added dropwise to a solution of **7** (100 mg, 0.33 mmol) in CH_2Cl_2 (2 mL) and pyridine (53 mL, 0.66 mmol) at 0 °C under nitrogen atomosphere. After 30 min of stirring at 0 °C, morpholine (288 mL, 3.30 mmol) was added at the same temperature. After 1 h (0 °C) and 1.5 h (room temperature) the reaction mixture was extraced three times with EtOAc. The combined EtOAc extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated. The crude product thus obtained was purified by column chromatography on silica gel (hexane:AcOEt, 4:1) to give **8** (29.3 mg, 24%) as colorless oil. $[\alpha]^{23}_D$ 7.4° (ca. 3.04, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, $J=6.9$ Hz, CH_3), 1.27 (12H, m), 1.70 (2H, m, N=C-CH_2-CH_2), 2.22 (5H, m, N-$CH_2\times5/2$), 2.71 (1H, dd, $J=12.5$, 5.3 Hz, N-$CH_2\times1/2$), 3.67 (4H, m, O-$CH_2\times2$), 4.08 (1H, m, N-CH), 5.23 (1H, d, $J=6.9$ Hz, O-CH), 7.32 (5H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 14.1



Scheme 5.



Scheme 6.

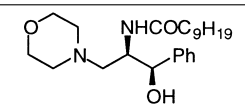
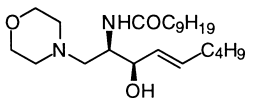
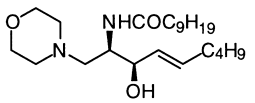
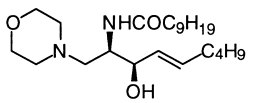
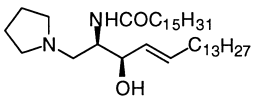
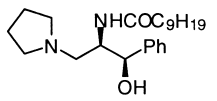
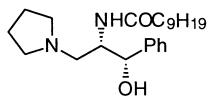


Scheme 7.

(Q), 22.6 (t), 26.0 (t), 28.3 (t), 29.2 (t), 29.4 (t), 31.8 (t), 54.1 (t), 63.5 (t), 66.8 (t), 72.5 (d), 85.0 (d), 125.5 (d), 127.9 (d), 128.6 (d), 141.2 (s), 168.0 (s). TOF Mass: 373 ($M+H^+$), ($C_{23}H_{36}N_2O_2$ 372). HRMS (FAB) calcd for $C_{23}H_{37}N_2O_2$ ($M+H^+$): 373.2855. Found: 373.2853.

L-*threo*-PDMP (9). One millilitre of 3 N HCl was added to **8** (9.3 mg, 0.025 mmol) and the mixture was stirred at room temperature for 15 h. The aqueous layer was adjusted to pH 9–10 with 1 N NaOH. The reaction mixture was extracted with EtOAc×3. The combined EtOAc extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo to give enantiomericaly pure **9**.

Table 1. Inhibitory activities of PDMP analogs against glycosylceramide synthase

Compd	Relative IC_{50} value[a]
 D-*threo*-PDMP	1[b]
 **1**	2.3
 **2**	2.3
 **3**	2.5
 **4**	1.6
 D-*threo*-PDPP	0.2
 L-*threo*-PDPP	>5.0

[a]Expressed as the ratio of the IC_{50} value of the compound to that of D-*threo*-PDMP.

[b]IC_{50} = 20 μM, K_i = 0.7 μM.

***tert*-Butyl(4*R*,1′*R*)-2,2-dimethyl-4-(1′-hydroxyhept-2′-ynyl)-oxazolidine-3-carboxylate (11).** A solution of 1.7 M *n*-BuLi (43 mL, 73.3 mmol) was added dropwise to a solution of 1-hexyne (10.5 mL, 91.6 mmol) in dry ether (200 mL) at −20 °C. The white suspension was stirred at −20 °C for 1 h, then anhydrous $ZnBr_2$ (23.4 g, 104 mmol) was added at 0 °C. After 1 h at 0 °C and 1 h at room temperature, a solution of 1,1-dimethylethyl 2,2-dimethyl-4(*R*)-formyloxazolidine-3-carboxylate (**10**) (14.0 g, 61.1 mmol) in dry ether (35 mL) was added dropwise at −78 °C. The mixture was allowed to warm to room temperature overnight and then quenched by the addition of saturated NH_4Cl (280 mL) at −20 °C. After dilution with H_2O (350 mL), the aqueous layer was separated and extracted two times with EtOAc. The combined EtOAc extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated. The crude product thus obtained was purified by column chromatography on silica gel (hexane:AcOEt, 6:1) to give **11** (17.8 g, 93%) as a colorless oil. $[\alpha]_D^{23}$ 41.4° (ca. 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$; D_2O exchange) δ 0.90 (3H, t, J = 7.3 Hz, CH_2-CH_3), 1.50 (20H, m), 2.22 (2H, dt, J = 2.0, 6.9 Hz, ≡C-CH_2), 4.06 (3H, m), 4.48 (1H, m, CH-C≡), ^{13}C NMR ($CDCl_3$) δ 13.3 (q), 18.3 (t), 21.7 (t), 24.2 (q), 26.9 (q), 28.1 (q), 30.3 (t), 62.3 (d), 65.1 (t), 65.6 (d), 78.7 (s), 81.2 (s), 86.1 (s), 94.4 (s), 154.6 (s). HRMS (FAB) calcd for $C_{17}H_{30}NO_4$ ($M+H^+$): 312.2175. Found: 312.2176.

(2*R*,3*R*)-2-Amino-4-nonyn-1,3-diol (12). A solution of 4 N HCl/ROAc (20 mL) was added to a solution of **11** (17.7 g, 56.8 mmol) in methanol (20 mL) at 0 °C. After stirring for 3 h at room temperature, a solution of 1 N NaOH was added, and the aqueous layer was adjusted to pH 9. Water was evaporated in vacuo, and the residue was extracted with Extrelute® (eluent: $CHCl_3$). The solvent was evaporated. The crude product thus obtained was purified by column chromatography on silica gel ($CHCl_3$:MeOH:H_2O, 8:2:0.1) to give **12** (5.81 g, 60%) as yellow oil. ^1H NMR ($CDCl_3$) δ 0.91 (3H, t, J = 7.3 Hz, Me), 1.44 (4H, m), 2.23 (2H, t, J = 6.9 Hz, ≡C-CH_2), 2.52 (4H, brs, NH_2 and 0H×2), 2.96 (1H, m, N-CH), 3.66 (1 H, dd, J = 10.9, 5.6 Hz, HO-CH_2), 3.78 (1H, dd, J = 10.9, 4.6 Hz, HO-CH_2), 4.35 (1H, d, J = 5.3 Hz, CH-C≡). TOF Mass: 172 ($M+H^+$), 194 ($M+Na^+$), ($C_9H_{17}NO_2$ 171).

(2*R*,3*R*)-2-Amino-4-(*E*)-nonyn-1,3-diol (13). A solution of **12** (5.71 g, 33.4 mmol) in 1,2-dimethoxyethane (60 mL) was added to a suspension of $LiAlH_4$ (3.80 g, 100 mmol) in 1,2-dimethoxyethane (40 mL) under nitrogen, and the mixture was stirred at reflux temperature for 15 h. After cooling, H_2O (3.8 mL), 15% NaOH (3.8 mL), and H_2O (11.4 mL) were added at 0 °C, and the mixture was stirred at room temperature for 30 min. The mixture was filtered through a Celite pad, and

washed with hot $CHCl_3$, then the solvent of filtrate was removed under reduced pressure. Hexane was added to the residue, the crystal formed was filtered to give **13** (3.29 g, 57%) as a colorless prism; mp 68 °C; $[\alpha]_D^{23}$ 12.8° (ca. 0.50, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, J = 7.3 Hz, Me), 1.34 (4H, m), 2.06 (2H, q, J = 6.6 Hz, =CH-CH_2), 2.77 (1H, q, J = 5.6 Hz, N-CH), 3.53 (1H, dd, J = 10.9, 6.3 Hz, HO-CH_2), 3.67 (1 H, dd, J = 10.9, 4.0 Hz, HO-CH_2), 4.01 (1H, t, J = 5.9 Hz, CH-CH=), 5.45 (1H, dd, J = 15.5, 6.9 Hz, CH-CH=), 5.74 (1H, dt, J = 15.5, 6.6 Hz, =CH-CH$_2$); ^{13}C NMR ($CDCl_3$) δ 13.9 (q), 22.3 (t), 31.3 (t), 32.0(t), 56.5 (d), 64.0 (t), 73.4 (d), 129.8 (d), 133.8 (d). TOF Mass: 174 ($M+H^+$), 197 ($M+Na+H^+$) ($C_9H_{19}NO_2$ 173).

(2*R*,3*R*)-2-Decanoylamino-4-(*E*)-nonen-1,3-diol (14). $AcONa \cdot H_2O$ (4.62 g, 33.9 mmol) was added to a solution of **13** (2.94 g, 17.0 mmol) in THF (30 mL) and water (30 mL). Decanoyl chloride (4.2 mL, 20.4 mmol) was added dropwise at 0 °C. After stirring for 0.5 h at 0 °C and 1.5 h at room temperature, the solvent was evaporated under reduced pressure. The reaction mixture was extracted three times with EtOAc. The combined EtOAc extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated The crude product thus obtained was purified by column chromatography on silica gel to give **14** (4.43 g, 80%) as a colorless prism; mp 74 °C; $[\alpha]_D^{23}$ 63° (ca. 1.48, $CHCl_3$); ^{1}H NMR ($CDCl_3$: D_2O exchange) δ 0.88 (6H, m, CH_3×2), 1.27 (16H, m) 1.62 (2H, m), 2.04 (2H, q, J = 6.9 Hz, =CH-CH_2), 2.22 (2H, t, J = 7.9 Hz, CO-CH$_2$), 3.76 (2H, d, J = 4.6 Hz, HO-CH_2), 3.90 (1H, m, N-CH), 4.39 (1H, m, HO-CH), 5.46 (1 H, dd, J = 15.5, 6.3 Hz, CH-CH=), 5.74 (1H, dt, J = 15.5, 6.9 Hz, =CH-CH$_2$), 6.23 (1H, d, J = 7.9 Hz, NH); ^{13}C NMR ($CDCl_3$) δ 13.8 (q), 14.0 (q), 22.1 (t), 22.6 (t), 25.8 (t), 29.2 (t), 29.3 (t), 29.4 (t), 31.2 (t), 31.8 (t), 31.9 (t), 36.7 (t), 54.8 (d), 63.1 (t), 7116 (d), 129.0 (d), 133.2 (d), 174.6 (s). Anal. calcd for $C_9H_{19}NO_2$: C, 69.68; H, 11.39; N, 4.28. Found: C, 69.67; H, 11.44; N, 4.25.

Compound 15. MsCl (0.65 mL, 8.43 mmol) was added dropwise to a solution of **14** (2.3 g, 7.02 mmol) in pyridine (10 mL) at 0 °C under nitrogen atmosphere. After 1 h of stirring at 0 °C, morpholine (6.1 mL, 70.2 mmol) was added. After another 44 h of stirring at room temperature, the reaction mixture was extracted three times with EtOAc. The combined EtOAc extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The crude product thus obtained was purified by column chromatography on silica gel (CH_3Cl only) to give **15** (1.46 g, 67%) as a colorless prism. $[\alpha]_D^{23}$ 75.9° (ca. 1.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$; D_2O exchange) δ 0.89 (6H, m, CH_3×2), 1.26 (16H, m), 1.62 (2H, m), 2.07 (2H, q, J = 6.6 Hz, =CH-CH_2), 2.27 (2H, t, J = 7.6 Hz, N=C-CH$_2$), 3.50 (1H, m, HO-CH_2), 3.80 (2H, m), 4.69 (1H, t, J = 8.3 Hz, O-CH), 5.49 (1H, dd, J = 15.5, 8.3 Hz, CH-CH=), 5.77 (1H, dt, J = 15.5, 6.6 Hz, =CH-CH$_2$). TOF Mass: 310 ($M+H^+$), 333 ($M+Na+H^+$). ($C_{19}H_{35}NO_2$ 309). HRMS (FAB) calcd for $C_{19}H_{36}NO_2$ ($M+H^+$): 310.2746. Found: 310.2750.

Compound 16. Tf_2O (0.46 mL, 2.71 mmol) was added dropwise to a solution of **15** (700 mg, 2.26 mmol) in CH_2Cl_2 (15 mL) and pyridine (0.55 mL, 6.79 mmol) at −45 °C under nitrogen atmosphere. After 1.5 h of stirring at −45 °C, morpholine (1.98 mL, 22.6 mmol) was added at the same temperature. After further stirring for 1 h at −45 °C and 2 h at room temperature the reaction mixture was extracted three times with EtOAc. The combined EtOAc extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The crude product thus obtained was purified by column chromatography on silica gel (hexane:AcOEt, 2:1) to give **16** (141 mg, 39%) as colorless oil; $[\alpha]_D^{23}$ 32.5° (*c* 0.43, $CHCl_3$); ^{1}H NMR ($CDC1_3$) δ 0.89 (6H, m, CH_3×2), 1.26 (16H, m), 1.61 (2H, m), 2.06 (2H, m, =EH-CH_2), 2.26 (2H, t, J = 8.0 Hz, N=C-CH$_2$), 2.32–2.63 (6H, m, N-CH$_2$×3), 3.68 (4H, m, O-CJ$_2$×2), 3.87 (1H, q, J = 6.9 Hz, N-CH), 4.62 (1H, t, J = 7.6 Hz, O-CH), 5.46 (1H, dd, J = 15.2, 7.6 Hz, CH-CH=), 5.76 (1H, dt, J = 15.2, 6.9 Hz, =CH-CH$_2$); ^{13}C NMR ($CDCl_3$) δ 13.7 (q), 14.0 (q), (22.0 (t), 22.5 (t), 25.9 (t), 28.2 (t), 29.0 (t), 29.1 (t), 29.3 (t), 31.0 (t), 31.7 (t), 54.0 (t), 62.9 (t), 66.7 (t), 69.7 (d), 84.5 (d), 128.1 (d), 134.7 (d), 167.7 (s). TOF Mass: 379 ($M+H^+$), ($C_{23}H_{42}N_2O_2$ 378). HRMs (FAB) calcd for $C_{23}H_{43}N_2O_2$ ($M+H^+$): 379.3325. Found: 379.3322.

(2*R*,3*R*)-2-Decanoylamino-1-morpholino-4-(*E*)-nonen-3-ol (1). A solution of 3 N HCl (3 mL) was added to **16** (39 mg, 0.103 mmol). After 13 h of stirring at room temperature, 1 N NaOH was added, and the aqueous layer was adjusted to pH 9. The reaction mixture was extracted three times with EtOAc. The combined EtOAc extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The crude product thus obtained was purified by column chromatography on silica gel (hexane:AcOEt, 1:3) to give **1** (24.5 mg, 60%) as colorless oil; $[\alpha]_D^{23}$ 23.0° (ca. 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$: D_2O exchange) δ 0.89 (6H, m, CH_3×2), 1.26 (16H, m), 1.60 (2H, m), 2.06 (2H, m, =CH-CH_2), 2.17 (2H, t, J = 7.9 Hz, CO-CH$_2$), 2.51 (1H, dd, J = 13.2, 5.9 Hz, H-1a), 2.56 (4H, t, J = 4.6 Hz, H-2′) 2.69 (1H, dd, J = 13.2, 6.6 Hz, H-1b), 3.69 (4H, t, J = 4.6 Hz, H-1′), 4.05 (1H, m, N-CH), 4.28 (1H, dd, J = 5.9, 3.3 Hz, HO-CH) 5.42 (1H, dd, J = 15.2, 6.3 Hz, CHCH=), 5.73 (1H, dt, J = 15.5, 6.6 Hz, =CH-CH$_2$); ^{13}C NMR ($CDCl_3$) δ 13.8 (q), 14.0 (q), 22.1 (t), 22.5 (t), 25.7 (t), 29.1 (t), 29.26 (t), 29.33 (t), 31.2 (t), 31.9 (t), 36.7 (t), 49.8 (d), 54.1 (t), 66.7 (t), 73.5 (d), 128.8 (d),

133.5 (d), 173.5 (s). TOF Mass: 397 ($M+H^+$), 420 ($M+Na^+$), ($C_{23}H_{44}N_2O_3$ 396), HRMS (FAB) calcd for $C_{23}H_{45}N_2O_3$ ($M+H^+$) 397.3430; Found: 397.3430

Compound 18. AcON·H_2O (1.68 g, 12.4 mmol) was added to a solution of D-*threo*-sphingosine (**17**) (1.85 g, 6.18 mmol) in THF (30 mL) and water (30 mL). Decanoyl chloride (1.54 mL, 7.41 mmol) was added dropwise at 0 °C. After stirring for 0.5 h at 0 °C and 2 h at room temperature, the solvent was evaporated. The reaction mixture was extracted three times with $CHCl_3$. The combined EtOAc extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated under a reduced pressure. Hexane was added to the residue, the deposited crystal was filtered to give **18** (2.53 g, 90%) as a colorless prism; mp 91 °C; $[\alpha]_D^{23}$ 5.7° (ca. 0.96, $CHCl_3$); ^{1}H NMR ($CDCl_3$; D_2O exchange) δ 0.88 (6H, t, J=6.9 Hz, $CH_3\times2$), 1.26 (34H, m), 1.62 (2H, m), 2.03 (2H, q, J=6.9 Hz, =CH-CH_2), 2.23 (2H, t, J=7.9 Hz, CO-CH_2), 3.80 (2H, m, HO-CH_2), 3.91 (1H, m, N-CH), 4.38 (1H, dd, J=6.3, 3.3 Hz, HO-CH), 5.47 (1H, dd, J=15.2, 6.3 Hz, CH-CH=), 5.75 (1H, dt, J=15.2, 6.9 Hz, =CH-CH_2), 6.13 (1H, d, J=7.9 Hz, NH). TOF Mass: 477 ($M+Na^+$), 493 ($M+K^+$) ($C_{28}H_{55}NO_3$ 453).

Compound 19. MsCl (0.55 mL, 7.11 mmol) was added dropwise to a solution of **18** (2.48 g, 5.47 mmol) in pyridine (20 mL) at 0 °C under nitrogen atmosphere. After 1 h of stirring at 0 °C, morpholine (4.8 mL, 54.7 mmol) was added. After 15 h of stirring at room temperature, the reaction mixture was extracted three times with $CHCl_3$. The combined $CHCl_3$ extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated. Hexane was added to the residue, the crystal formed was filtered off. The filtrate was purified by column chromatography on silica gel ($CHCl_3$ only) to give **19** (1.64 g, 69%) as a colorless prism; mp 38 °C; $[\alpha]_D^{23}$ 54.7° (ca. 2.94, $CHDl_3$); ^{1}H NMR ($CDCl_3$; D_2O exchange) δ 0.88 (6H, t, J=6.9 Hz, $CH_3\times2$), 1.26 (34H, m), 1.61 (2H, m, N=C-CH_2-CH_2), 2.05 (2H, q, J=6.6 Hz, =CH-CH_2), 2.26 (2H, t, J=7.9 Hz, N=C-CH_2), 3.49 (1H, dd, J=11.6, 4.3 Hz, HO-CH_2), 3.80 (2H, m, HO-CH_2 and N-CH), 4.70 (1H, t, J=8.1 Hz, O-CH), 5.48 (1H, dd, J=15.3, 8.1 Hz, CH-CH=), 5.77 (1H, dt, J=15.3, 6.6 Hz, =CH-CH_2;. ^{13}C NMR ($CDCl_3$) δ 14.0 (q), 22.6(t), 25.9 (t), 28.2 (t), 28.7 (t), 29.0 (t), 29.1 (t), 29.2 (t), 29.3 (t), 29.47 (t), 29.54 (t), 31.75 (t), 31.81 (t), 32.1 (t), 62.6 (t), 73.4 (d), 82.4 (d), 127.5 (d), 135.6 (d, 169.2(s). TOF Mass: 436 ($M+H^+$), 459 ($M+Na+H^+$) ($C_{28}H_{53}NO_2$, 435). HRMS (FAB) calcd for $C_{28}H_{54}NO_2$ ($M+H^+$): 436.4155. Found: 436.4147.

Compound 20. A solution of Tf_2O (0.73 mL, 4.35 mmol) was added dropwise to a solution of **19** (1.58 mg, 3.63 mmol) in CH_2Cl_2 (200 mL) and pyridine (0.88 mL, 10.9 mmol) at −45 °C under nitrogen. After 2 h of stirring at −45 °C, morpholine (3.2 mL, 36.3 mmol) was added at the same temperature. After further stirring for 2 h at −45 °C and 8 h at room temperature, the reaction mixture was extracted three times with $CHCl_3$. The combined $CHCl_3$ extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The crude product thus obtained was purified by column chromatography on silica gel (hexane: E_tOA_c, 3:1) to give **20** (432 mg, 24%) as colorless oil; $[\alpha]_D^{23}$ 19.4° (ca. 0.32, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.9 Hz, $CH_3\times2$), 1.26 (34H, m), 1.62 (2H, m, N=C-CH_2-CH_2), 2.05 (2H, q, J=6.9 Hz, =CH-CH_2), 2.26 (2H, t, J=8.0 Hz, N=C-CH_2), 2.32–2.63 (6H, m, N-$CH_2\times3$), 3.68 (4H, m, O-$CH_2\times2$), 3.87 (1H, q, J=6.9 Hz, N-CH), 4.62 (1H, t, J=7.6 Hz, O-CH), 5.46 (1H, dd, J=15.2, 7.6 Hz, CH-CH=), 5.75 (1H, dt, J=15.2, 6.9 Hz, =CH-CH_2); ^{13}C NMR ($CDCl_3$) δ 14.0 (q), 22.6 (t), 26.0 (t), 28.3 (t), 28.9 (t), 29.08 (t), 29.15 (t), 29.26 (t), 29.33 (t), 29.4(t), 29.5 (t), 29.6 (t), 31.77 (t), 31.83 (t), 31.83 (t), 32.1 (t), 54.1 (t), 62.9 (t), 66.8 (t), 69.7 (d), 84.5 (d), 128.1(d), 134.8 (d), 167.8 (s). TOF Mass: 505 ($M+H^+$), ($C_{32}H_{60}N_2O_2$, 504). HRMS (FAB) calcd for $C_{32}H_{61}N_2O_2$ ($M+H^+$): 505.4733. Found: 505.4736.

(2*R*,3*R*)-2-Decanoylamino-1-morpholino-4-(*E*)-octadecen-3-ol (2). A solution of 3 N HCl (3 mL) was added to **20** (314 mg, 0.62 mmol). After stirring for 2 h at room temperature, the aqueous layer was adjusted to pH 9 with NaOH. The reaction mixture was extracted three times with EtOAc. The combined EtOAc extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated. The crude product thus obtained was purified by column chromatography on silica gel (hexane:AcOEt, 1:4) to give **2** (187 mg, 58%) as a colorless oil; mp 41 °C; $[\alpha]_D^{23}$ 16.9° (ca. 0.95, CHC_3; ^{1}H NMR ($CDCl_3$; D_2O exchange) δ 0.88 (6H, t, J=6.9 Hz, $CH_3\times2$), 1.26 (34H, m), 1.60 (2H, m, CO-CH_2CH_2), 2.04 (2H, q, J=6.9 Hz, =CH-CH_2). 2.17 (2H, t, J=7.6 Hz, CO-CH_2), 2.49 (1H, dd, J=12.9, 5.6 Hz, H-1a), 2.55 (4H, t, J=4.6 Hz, H-2′), 2.69 (1H, dd, J=12.9, 6.6 Hz, H-1b), 3.69 (4H, t, J=4.6 Hz, H-1′), 4.04 (1H, m, H-2), 4.27 (1H, dd, J=6.3, 3.6 Hz, H-3), 5.42 (1H, dd, J=15.2, 6.3 Hz, H-4), 5.73 (1H, dt, J=15.2, 6.9 Hz H-5); ^{13}C NMR ($CDCl_3$) δ 14.0 (q), 22.5 (t), 25.7 (t), 29.15 (t), 29.24 (t), 29.27 (t), 29.36 (t), 29.40 (t), 29.56 (t), 31.74 (t), 31.79 (t), 32.24 (t), 36.7 (t), 49.8 (d), 54.2 (t), 59.7 (t), 66,8 (t), 73.6 (d), 128.8 (d), 133.5 (d), 173.4 (s). TOF Mass: 524 ($M+H^+$), 546 ($M+Na^+$), ($C_{32}H_{62}N_2O_3$, 522). HRMS (FAB) calcd for $C_{32}H_{63}N_2O_3$ ($M+H^+$): 523.4838. Found: 523.4837.

Compound 22. Melting point 56 °C; $[\alpha]_D^{23}$ 53.80 (ca. 0.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$; D_2O exchange) δ 0.88 (6H, t, J=6.9 Hz, $CH_3\times2$), 1.26 (46 H, m), 1.62 (2H, m, Co-CH_2-CH_2), 2.06 (2H, q, J=7.3 Hz, =CH-CH_2), 2.28 (2H, t, J=7.6 Hz, N=C-CH_2), 3.52 (1H, dd,

$J = 12.2$, 5.3 Hz, O-C*H*H), 3.83 (2H, m, O-CH*H* and N-CH), 4.69 (1H, t, $J = 8.3$ Hz, O-CH), 5.49 (1H, dd, $J = 15.5$, 8.3 Hz, CH-C*H*=), 5.78 (1H, dt, $J = 15.5$, 6.6 Hz, =C*H*-CH_2); ^{13}C NMR ($CDCl_3$) δ 14.1 (q), 22.7 (t), 26.1 (t), 28.4 (t), 28.9 (t), 29.2 (t), 29.3 (t), 29.4 (t), 29.5 (t), 29.6 (t), 29.7 31.9 (t), 32.2 (t), 63.0 (t), 73.6 (d), 82.5 (d), 127.7 (d), 135.9 (d), 169.4 (s). HRMS (FAB) calcd for $C_{34}H_{66}NO_3$ ($M + H^+$): 520.5094. Found: 520.5054.

(2*R*,3*R*)-2-Palmitoylamino-1-morpholino-4-(*E*)-octadecen-3-o1 (3). Melting point 43 °C; $[\alpha]_D^{23}$ 10.8° (ca. 0.45, $CHCl_3$); ^{1}H NMR ($CDCl_3$; D_2O exchange) δ 0.88 (6H, t, $J = 6.6$ Hz, $CH_3 \times 2$), 1.25 (46H, m), 1.60 (2H, m, CO-CH_2C*H*$_2$), 2.05 (2H, q, $J = 6.6$ Hz, H-6), 2.17 (2H, t, $J = 7.9$ Hz, CO-CH_2), 2.50 (1H, dd, $J = 12.9$, 5.9 Hz, H-1a), 2.56 (4H, t, $J = 4.3$ Hz, H-2′) 2.69 (1H, dd, $J = 12.9$, 6.6 Hz, H-1b), 3.70 (4H, t, $J = 4.3$ Hz, H-1′), 4.05 (1H, m, H-2), 4.28 (1H, rn, H-3), 5.42 (1H, dd, $J = 15.2$, 6.3 Hz, H-4), 5.73 (1H, dt, $J = 15.2$, 7.6 Hz, H-5); ^{13}C NMR ($CDCl_3$) δ 14.1 (q), 22.7 (t), 25.8 (t), 29.3 (t), 29.4 (t), 29.5 (t), 29.7 (t), 49.8 (d). 54.3 (t), 59.9 (t), 66.8 (t), 74.2 (d), 128.6 (d), 143.0 (d), 173.6 (s). HRMS (FAB) calcd for $C_{38}H_{75}N_2O_3$ ($M + H^+$): 607.5778. Found: 607.5773

(2*R*,3*R*)-2-Palmitoylamino-1-pyrrolidino-4-(*E*)-octadecen-3-o1 (4). $[\alpha]_d^{23}$ 15.1° (*c* 1.08, $CHDl_3$); ^{1}H NMR ($CDC1_3$; D_2O exchange) δ 0.88 (6H, t, $J = 6.6$ Hz, $CH_3 \times 2$), 1.25 (46H, m), 1.60 (2H, m, CO-CH_2C*H*$_2$), 1.80 (4H, m, N-CH_2C*H*$_2 \times$z), 2.03 (2H, q, $J = 6.7$ Hz, H-6), 2.18 (2H, t, $J = 7.9$ Hz, CO-CH_2), 2.71 (4H, m, N-$CH_2 \times 2$), 2.84 (1H, dd, $J = 12.9$, 4.9 Hz, H-1a), 2.92 (1H, dd, $J = 12.9$, 5.6 Hz, H-1b), 4.00 (1H, m, H-2), 4.37 (1H, m, H-3), 5.41 (1H, dd, $J = 15.5$ Hz, H-4), 5.73 (1H, dt, $J = 15.5$, 6.7 Hz, H-5;. ^{13}C NMR ($CDCl_3$) δ 14.1 (q), 22.7 (t), 23.5 (t), 25.8 (t), 29.22 (t), 29.27 (t), 29.35 (t), 29.5 (t), 29.6 (t), 29.7 (t), 31,9 (t), 32.3 (t), 36.8 (t), 51.2 (d), 55.1 (t), 57.6 (t), 74.0 (d), 128.7 (d), 133.3 (d), 173.5 (s). TOF Mass: 592 ($M + H^+$), ($C_{38}H_{74}N_2O_2$ 591). HRMS (FAB) calcd for $C_{38}H_{75}N_2O$ ($M + H^+$): 591.5829. Found: 591.5844.

Compound 26. Melting point 47 °C; $[\alpha]^{23}{}_D$ 59.4° (ca. 1.58, $CHDl_3$); ^{1}H NMR ($CDCl_3$; D_2O exchange) δ 0.88 (3H, t, $J = 6.9$ Hz, CH_3), 1.27 (12H, m), 1.61 (2H, m, N=C-CH_2-C*H*$_2$), 2.27 (2H, t, $J = 7.9$ Hz, N=C-CH_2), 3.94 (1H, t, $J = 8.6$ Hz, O-C*H*H), 4.05 (1H, t, $J = 9.2$ Hz, O-CH*H*), 4.33 (1H, q, $J = 8.3$ Hz, N-CH), 4.46 (1H, d, $J = 7.6$ Hz, O-CH), 7.38 (5H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 14.0 (q), 22.6 (t), 25.8 (t), 27.9 (t), 29.08 (t), 29.11 (t), 29.15 (t), 29.3 (d), 31.7 (t), 68.9 (t), 72.1 (d), 76.7 (d), 126.9 (d), 127.9 (d), 128.3 (d), 140.2 (s), 169.8 (s).

Compound 27. Melting point 75 °C; $[\alpha]^{23}{}_D$ 19.7° (ca. 1.79, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, t, $J = 6.9$ Hz, CH_3), 1.22 (12H, m), 1.45 (2H, m, CO-CH_2-C*H*$_2$), 2.26 (2H, m, $J = 7.6$ Hz, CO-CH_2), 4.00 (2H, m, N-CH and O-C*H*H), 4.24 (1H, m, O-CH*H*), 5.06 (1H, d, $J = 8.3$ Hz, O-CH), 5.86 (1H, brs, OH; exchanged with D_2O), 7.32 (3H, m, ArH), 7.43 (2H, m, ArH), 8.51 (3H, brs NH_3; exchanged with D_2O); ^{13}C NMR ($CDCl_3$) δ 14.1 (q), 22.6 (t), 24.5 (t), 29.1 (t), 29.26 (t), 29.34 (t), 29.44 (t), 31.8 (t), 33.9 (t), 56.9 (d), 60.7 (t), 71.9 (d), 127.1 (d), 129.0) (d), 138.9 (s), 173.8 (s).

Inhibition analysis

Glucosylceramide synthase was assayed according to the procedure described previously,[4a] with liver microsomes as enzyme source. Liposomes were prepared from *N*-octanoylsphingosine, dioleoyl phosphotidylcholine and brain sulfatide, and the mixture was incubated for 1 h with UDP-[^{3}H]·glucose, NAD, DTT, EDTA, Mgck and Tris-HCl (pH 7.4). The labeled GlcCer formed was isolated by partitioning between *t*-butyl methyl ether and 2-propanol-aqueous Na_2SO_4 and counted without removing the precipitated proteins.

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