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Enzymic Glycosphingolipid Synthesis on Polymer Supports.

I. Preparation of Modified Native Acceptors

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Two synthetic approaches leading to *N*-4-carboxymethyl-2-nitrobenzyloxycarbonyl derivatives of model compounds and of glycosylsphingosines are described. The carboxymethyl group can be converted into a hydrazido derivative and the resulting products react with an active ester polymer yielding light-sensitive polymeric products that may subsequently serve as acceptors in glycosyltransferase reactions.

Glycosphingolipid composition and metabolism is defined by genetic background, stage of differentiation and oncogenic transformation, the precise composition being important *inter alia* to cell-cell recognition and communication [1-7]. As a consequence, and due to necessity for biological and medical studies, intense efforts were recently channeled into the chemical synthesis of these compounds [8-16].

An obvious alternative may, however, be enzymic synthesis [17, 18], where the nature of newly-formed glycosidic bonds is determined by enzymic specificity circumventing blocking group chemistry. In addition to the possible preparative value of this approach, the structure of the products may reflect on enzymic specificity.

In view of the advantageous application of acceptor saccharides attached to polymer supports in the enzymic synthesis of oligosaccharides [19, 20], the preparation of modified native acceptors suitable for polymer-supported enzymic glycosphingolipid synthesis was explored.

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Table 1. Release (%) of free amino acids (determined by amino acid analysis). Prior to analysis, samples were dried *in vacuo*.

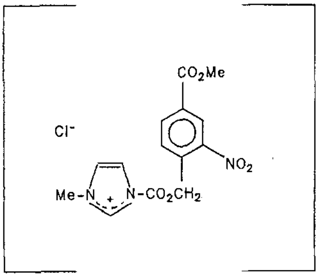
Compound	Irradiation 19 h	HBr/HOAC 6.5 h
3	74[a]	27[c]
4	82[a]	28[c]
7	78[b]	
8	94[b]	

[a] Samples of 160 nmol dissolved in a mixture of methanol (0.1 ml) and 0.5 M hydrochloric acid (1 ml).
[b] Samples of 17-20 mg dissolved in 0.5 M hydrochloric acid (1 ml).
[c] Samples of 160 nmol dissolved in 45% hydrobromic acid in acetic acid (0.2 ml) at room temperature.

Results and Discussion

Assuming that glycosylsphingosine derivatives, attached through a temporary protecting group of the 2-amino function to a carrier polymer, may serve as acceptors for glycosyltransferases involved in the biosynthesis of glycosphingolipids, 4-carboxymethyl-2-nitrobenzyloxycarbonyl was studied as a bifunctional protecting group meant to serve this purpose.

1-Methyl-3-(4-carboxymethyl-2-nitrobenzyloxycarboxyl)imidazolium chloride (**1**) was conveniently prepared [21] and served initially for *N*-acylation of model compounds: 2-amino-2-deoxy-D-glucose, glycine and L-phenylalanine yielding compounds **2** (after subsequent acetylation), **3** and **4**, respectively. As expected, benzyl urethans carrying electron withdrawing substituents were cleaved in low yields when treated with hydrobromic acid in acetic acid and, being 2-nitrobenzyl derivatives, photolysed efficiently (at 350 nm) [22] to yield glycine and phenylalanine (Table 1).



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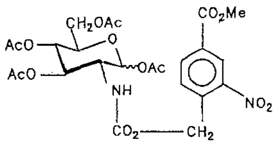

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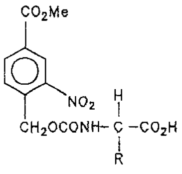
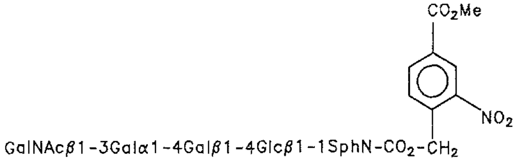


3 R = H
4 R = Ph



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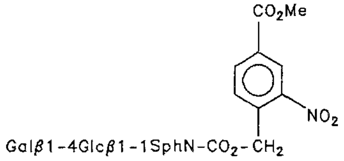

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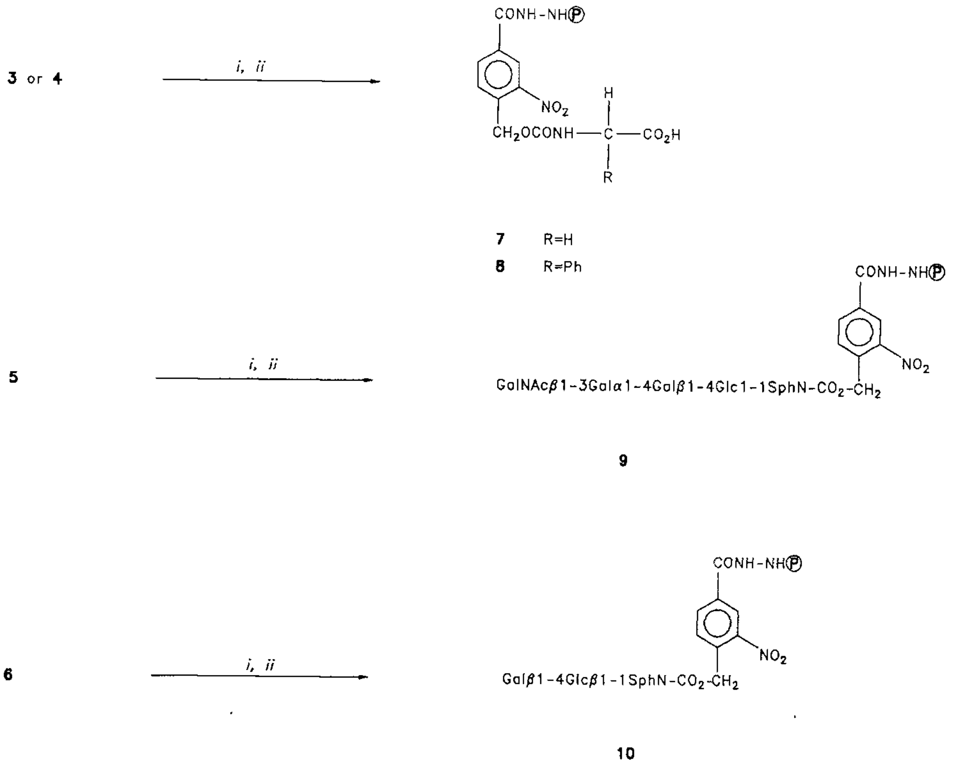
SphN = *N*-substituted sphingosine

The following step was to react compound **1** with suitable glycosylsphingosine derivatives. The preparation of two of these compounds from globoside (Gb_4) and from lactosylceramide was hampered, however, by the low yielding *N*-deacylation methodology available at the time [23]; this, however, might be improved now by using the method of Neuenhofer *et al.* [24]. *N*-Acylation provided, although in low yields, compounds **5** and **6**, respectively. In the first case, acylation of the deacylated globoside produced an isomer additional to compound **5** where the aromatic acyl substitution was on the amino function of the 2-amino-2-deoxy-D-galactopyranosyl residue (NMR, not shown). Compound **5** had, apparently, the proposed structure also being shown to possess antigenic properties similar to those of globoside (Gb_4) (E. Holmes and S. Hakomori, unpublished results).

In view of past experiences [19, 20], plans were drawn to saponify the methyl ester function present in compounds **2-6** and attach the carboxylic acid products to amino-substituted polymers. Base hydrolysis (under various conditions) resulted in extensive degradation of the urethan functions. Consequently, employing once again compounds **3** and **4** as models, the hydrazine/hydrazine sulfate treatment was optimized leading to the corresponding hydrazides. These were found to be suitable for attachment to poly(acrylamide)-poly(*N*-acryloxysuccinimide) [25] (PAN), an active ester-type, water soluble polymer, giving rise to polymers **7** and **8** [**P** , water soluble poly(acrylamide)]. As predicted, the two polymers were light-sensitive and photolysed to yield glycine and phenylalanine (Table 1). The same procedure was repeated in the attachment of compounds **5** and **6** to PAN giving polymers **9** and **10**.

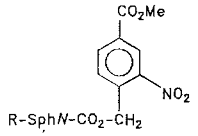
Based on the aforementioned experiments, an alternative approach was to react sphingosine (isolated following hydrolysis from bovine brain as the crystalline sulfate [26-28]) with compound **1**. The resulting urethan (**11**) was reacted with 2,3,4,6-tetra-*O*-acetyl-β-D-



Reagents: *i*, hydrazine, hydrazine sulphate, *ii*, PAN
Ⓟ= substituted poly(acrylamide)

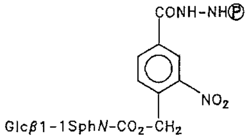
glucopyranosyl bromide, yielding the 1-*O*-β-*gluco* derivative **12**. The 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl moiety in compound **12** was unequivocally shown to be attached to C-1 of the substituted sphingosine by the ^{1}H-NMR spectra and n.O.e. experiments. Compound **12** reacted with hydrazine/hydrazine sulfate and then with PAN leading, following ultrafiltration, to the glucosylsphingosine polymer **13**.

In conclusion, the 4-carboxymethyl-2-nitrobenzyloxycarbonyl functionality has been demonstrated to be a useful bifunctional protecting group for attaching diverse amino derivatives *via* a temporary light-sensitive bond to a polymer or other carrier. More specifically, this protecting group has been utilized in two related synthetic approaches leading to potential acceptors for glycosyltransferases. In the first, a glycosphingolipid is deacylated, reacted with compound **1** and the product is attached to a polymer, while in the second, sphingosine is *N*-acylated with compound **1**, the product is glycosylated and subsequently attached to a carrier polymer. Further development of this synthetic methodology should lead, more efficiently [24], to additional, native glycosphingolipids of biological interest.



11 R=H

12 R=1-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)



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Experimental Procedures

General

Column chromatography was performed on silica gel (Baker, USA., 60-200 mesh), TLC was performed on Si-HPF TLC plates (Baker), and materials were viewed by u.v. or located by orcinol spray. HPLC was conducted on Iatrobeads (6RS-8010). Melting points were recorded with a Büchi 510 apparatus. Optical rotations were determined with a Bendix polarimeter; i.r. spectra, for potassium bromide discs, with a Perkin-Elmer Model 237; u.v. spectra with a Bausch and Lomb Spectronic 2000 instrument; ^{1}H-NMR spectra were recorded with a Varian Model FT80a (80 MHz) with a Bruker Model AH 300 (300 MHz) or with a Bruker Model AM-500 (500 MHz) instrument. The n.O.e. experiment was performed in difference mode on a 300 MHz instrument at 300 K; irradiation time 0.85 s at decoupler power of 40 L. Amino acid analysis was conducted on a Biotronik LC7000 instrument. Microanalyses were performed by Mrs. S. Ehrlich-Rogozinki and by Canadian Microanalytical Service, Vancouver, B.C., Canada. Photolysis was carried out in a Rayonet RPR-100 apparatus, with RPR 3500 Å lamps in Pyrex glassware.

*1-Methyl-3-(4-carboxymethyl-2-nitrobenzyloxycarbonyl)-imidazolium Chloride (**1**)*

Methyl 4-hydroxymethyl-3-nitrobenzoate [29] (139 mg, 0.66 mmol) was suspended, using efficient stirring, in toluene (6 ml). 1-Methylimidazole (81 μl, 1.0 mmol) was added, stirring was continued in an ice bath and phosgene (0.49 ml, 13.4% in toluene) was added. A heavy yellowish precipitate formed instantaneously and the stirring was continued for 1 h at 0°C and 1 h at room temperature. The product (**1**) was isolated by filtration as a crude yellowish solid; m.p. 81-83°C, dec.; 157 mg, 67% yield; i.r. data (KBr disc): 1785 (CO), 1735 (CO), 1630, 1600 and 1535 cm^{-1} (NO_2). The material could be used for further acylation without isolation.

*2-(4-Carboxymethyl-2-nitrobenzyloxycarbonylamino)-2-deoxy-1,3,4,6-tetra-O-acetyl-D-glucopyranose (**2**)*

2-Amino-2-deoxy-D-glucose hydrochloride (143 mg, 0.66 mmol) and sodium hydrogen carbonate (300 mg) were dissolved in water (50 ml) and stirred at 0°C. Compound **1** (starting from 139 mg of methyl 4-hydroxymethyl-3-nitrobenzoate) was transferred into the reaction mixture aided by 1,4-dioxane (10 ml) and water (5 ml), and the stirring was continued overnight. The reaction mixture was evaporated *in vacuo*, and pyridine (30 ml) and acetic anhydride (15 ml) were added. The acetylation was followed (after 10 h) by the addition of water, extraction with chloroform, and washing with water, dilute hydrochloric acid, water, saturated sodium hydrogen carbonate and water. The chloroform solution was dried over sodium sulfate, evaporated, and applied to a column of silica gel (20 g, 1 cm in diameter). The column was eluted with ethylacetate/hexane, 1/1 by vol, and 3 ml fractions were collected. Fractions 8-13 contained methyl 4-acetoxymethyl-3-nitrobenzoate (72 mg) while TLC pure (ethylacetate/hexane, 1/1 by vol) compound **2**, α- and β-anomers, eluted in fractions 38-48 (yellowish oil, 66 mg, 17% yield); $[\alpha]_D^{30}$ +42.9±4° (c 0.3, chloroform); u.v. data (chloroform): 257 sh (ε 5603), and 297 nm sh (1436); i.r. data (KBr disc): 3360 (NH), 2970, 1750 (CO), 1730 (CO) 1620 and 1540 cm^{-1} (NO_2); ^1H-NMR data (500 MHz, ^2H-chloroform) included: δ 8.71 (s, 1H, aromatic), 8.27 (d, 1H, *J* 7.7 Hz, aromatic), 7.60 (d, 1H, *J* 7.7 Hz, aromatic), 6.23 (d, 0.7H, $J_{1,2}$ 2.6 Hz, α-H-1), 5.75 (d, 0.3H, $J_{1,2}$ 8.6 Hz, β-H-1), 2.21 (s, 2.1 H, α-$OCOCH_3$) 2.10 (s, 0.9H, β-$OCOCH_3$), 2.08 (s, 3H, $OCOCH_3$), 2.05 (s, 3H, $OCOCH_3$), 2.04 (s, 3H, $OCOCH_3$), 3.98 (s, 3H, OCH_3). Analysis: Calculated. for $C_{24}H_{28}N_2O_{15}$: C, 49.32; H, 4.83; N, 4.79. Found: C, 49.15; H, 4.87; N, 4.67.

*4-Carboxymethyl-2-nitrobenzyloxycarbonyl Glycine (**3**)*

Glycine (25 mg, 0.33 mmol) and sodium hydrogen carbonate (50 mg) were dissolved in water (5 ml) and stirred at 0°C. Compound **1** (107 mg, 0.3 mmol) was added and the stirring was continued for 1 h at 0°C and 4 h at room temperature. The reaction mixture was diluted with water (50 ml), washed with 1,2-dichloromethane (10 ml), acidified with dilute hydrochloric acid and extracted with ethylacetate (60 ml). The ethylacetate solution was dried over sodium sulfate and evaporated to yield the crude oily product (**3**, 90 mg, 96% yield that was almost pure by TLC (chloroform/methanol, 14/9 by vol). A sample was further purified by TLC extracted first with chloroform/methanol,1/1 by vol, the extract was evaporated, extracted again with ethylacetate, washed with dilute hydrochloric acid, dried over sodium sulfate and evaporated. U.v. data (methanol): 256 sh (ε 5000), and 296 nm (1324); ^1H-NMR data (80 MHz, ^2H-chloroform/^2H-dimethylsulfoxide): δ 8.60 (s, 1H, aromatic), 8.25 (d, 1H, *J* 7.6 Hz, aromatic) 7.81 (d, *J* 7.6 Hz, 1H, aromatic) 5.48 (s, 2H, benzylic) and 3.94 (s, 3H, OCH_3). Analysis: Calculated for $C_{12}H_{12}N_2O_8$: N, 8.97. Found: N, 9.10.

*4-Carboxymethyl-2-nitrobenzyloxycarbonyl L-Phenylalanine (**4**)*

Compound **4** was prepared as described for compound **3**. Following the ethylacetate extraction, the residue was crystallized from ethylacetate/petroleum ether (52.6 mg, 43%); m.p. 119-121°, $[\alpha]_D^{30}$ -26.6±4° (c 0.25, chloroform); u.v. data (chloroform): 257 (ε 5167) and 295 nm sh (1162); i.r. data (chloroform): 3320 (NH), 1730 (CO), 1700 (CO), 1625 and 1535 (NO_2) cm^{-1}; ^1H-NMR data (80 MHz, ^2H-chloroform): δ 8.70 (s, 1H, aromatic), 8.23 (d, 1H, *J* 7.8 Hz, aromatic), 7.53 (d, 1H, aromatic, *J* 7.8 Hz), 7.26 (aromatic), 5.60 (m, 2H benzylic), 5.51 (s, 2H, benzylic), 3.97 (s, 3H, OCH_3), and 3.18 (m, 1H, after exchange with ^2H_2O). Analysis: Calculated for $C_{19}H_{19}N_2O_8$: N, 6.95. Found N, 6.60.

*1-O-[2-Acetamido-2-deoxy-β-D-galactopyranosyl-(1-3)-O-α-D-galactopyranosyl-(1-4)-β-D-galactopyranosyl-(1-4)-O-β-D-glucopyranosyl]-2-N-(4-carboxymethyl-2-nitrobenzyloxycarbonyl)-sphingosine (**5**)*

Globoside (Gb_4, 480 mg) was refluxed with stirring for 2 h in a mixture of 1-butanol (16.2 ml) and 10 M aqueous potassium hydroxide (1.8 ml). Water was added (10 ml) and the mixture was neutralized with hydrochloric acid. Sodium hydrogen carbonate (600 mg) and compound **1** (starting from 139 mg of methyl 4-hydroxymethyl-3-nitrobenzoate) were added as described in the preparation of compound **2**. Following overnight stirring, acetic anhydride (1.5 ml) was added and the stirring was continued for an additional 4 h. The reaction mixture was deionized with mixed bed resin (M-614, Baker), eluted with chloroform/methanol, 2/1 by vol, and the residue, following evaporation, was separated on a preparative (17", 0.5" in diameter) HPLC column eluted [1 ml/min, 4 ml fractions with linear gradients with the following proportions by vol of 2-propanol/hexane/water: 55/44/1 to 55/39/6 (80 min) to 55/37/8 (40 min) to 55/30/15 (80 min)]. Compound **5** eluted in fractions 68-71 was u.v. absorbing and orcinol positive, and was further purified by TLC (chloroform/methanol/water, 56/38/10 by vol). Assuming A_{256} nm, ε 5167, the yield was determined as 4.23 mg (3.45 μmol).

^{1}H-NMR data (500 MHz, ^{2}H-dimethylsulfoxide) included: δ 8.56 (s, 1H, aromatic), 8.30 (d, 1H, *J* 7.7 Hz, aromatic), 7.82 (d, 1H, *J* 7.7 Hz, aromatic), 5.57 (m, 1H, sphingosine vinyl H-5'), 5.34 (m, 1H, sphingosine vinyl H-4'), 4.79 (d, 1H, $J_{1,2}$ 3.0 Hz, α-Gal H-1), 4.55 (d, 1H, $J_{1,2}$ 8.5 Hz, β-GalNAc H-1), 4.28 (d, 1H, $J_{1,2}$ 7.3 Hz, β-Gal H-1), 4.20 (d, 1H, $J_{1,2}$ 7.7 Hz, β-Glc H-1), 4.16 (m, 1H, α-Gal H-5), 3.98 (d, 1H, $J_{3,4}$ 3.0 Hz, $J_{4,5}$ <1.5 Hz, α-Gal H-4), 3.07(dd, 1H, $J_{2,3}$ 7.7 Hz, β-Glc H-2), 1.63 (s, 3H, β-GalNAc $NCOCH_3$), 1.24 (mc, 22H, sphingosine $[CH_2]_{11}$), 0.85 (t, 3H, *J* 7 Hz, sphingosine CH_3), 3.92 (s, 3H, OCH_3). Compound **5** gave a positive radioimmunoassay with anti-globoside.

*2-N-(4-Carboxymethyl-2-nitrobenzyloxycarbonyl)-1-O-[β-D-galactopyranosyl-(1-4)-O-β-D-glucopyranosyl]-sphingosine (**6**)*

Lactosylceramide (61 mg) was deacylated and acylated with compound **1** as described for compound **5**. Following deionization, it was purified by HPLC (12", 0.25" diameter column, 0.5 ml/min, 1 ml fractions) using the same elution pattern. Compound **6** was isolated from fractions 8-38, and further purified by TLC (chloroform/methanol/water, 56/38/10 by vol). Assuming A_{256}, ε 5167, the yield was determined as 1.98 mg (2.31 μmol).

^{1}H-NMR data (500 MHz, ^{2}H-dimethylsulfoxide) included: δ 8.56 (s, 1H, aromatic), 8.30 (d, 1H, *J* 7.7 Hz, aromatic), 7.83 (d, 1H, *J* 7.7 Hz, aromatic), 5.57 (m, 1H, sphingosine H-5' vinyl), 5.34 (m, 1H, sphingosine vinyl H-4'), 4.23 (d, 1H, $J_{1,2}$ 7.5 Hz, β-Gal H-1), 4.19 (d, 1H, $J_{1,2}$ 7.8 Hz, β-Glc H-1), 3.07 (dd, 1H, $J_{2,3}$ 7.8 Hz, β-Glc H-2), 1.24 (mc, 22H, sphingosine $[CH_2]_{11}$), 0.85 (t, 3H, *J* 7 Hz, sphingosine CH_3), 3.92 (s, 3H, OCH_3).

*Attachment of Compounds **3**, **4**, **5**, **6** and **12** to a Soluble Poly(acrylamide)*

Compounds **3**, **4**, **5**, **6**, or **12** (1-3 µmol each) and hydrazine sulfate (1 mg) were dissolved in hydrazine (100 µl). The reaction mixture was mixed occasionally in a Vortex mixer and kept at room temperature for 30 min. The reaction mixture was frozen and freeze-dried. The residue always contained a major u.v. absorbing, light sensitive product, slower in TLC than the starting material. The last residue was dissolved in water (0.5 ml; in the case of compounds **5** and **6**, 0.5 ml dimethylsulfoxide also was added), triethylamine (7.5 µl) and PAN (100 mg, 0.54 mmol active ester/g) were added and stirring was continued at room temperature for 6 h. Following ultrafiltration and dialysis (Diaflo YM-2, Amicon, Lexington, MA, USA), compounds **3** and **4** yielded polymers **7** and **8**, respectively.

Starting from compound **5**, polymer **9** was isolated after dilution with 2 mM Triton X-100 followed by ultrafiltration and dialysis (Diaflo YM-2). The product still contained a proportion of Triton X-100. Starting from compounds **6** or **12**, polymer **10** or **13** was diluted to 5 ml and dialyzed extensively (spectra/Por3, Spectra Medical Industries, Los Angeles, CA, USA). The resulting solution was filtered, lyophilized (yielding 88 mg) and further purified by filtration (in three portions) through Sephadex G-25 (20 ml, 1 cm in diameter, 3 ml/fraction); compound **10** or **13** was collected in fractions 3-4.

The five polymeric products were isolated following lyophilization. Acid hydrolysis (6 M hydrochloric acid, 16 h, 120°C in a closed ampule) followed by amino acid analysis demonstrated 14.3 and 14.0% attachment in polymers **7** and **8**, respectively. Phenol sulfuric acid test [30] demonstrated 8.4% attachment in polymer **10** and 5.8% attachment in polymer **13**.

*2-N-(4-Carboxymethyl-2-nitrobenzyloxycarbonyl)-sphingosine (**11**)*

Sphingosine sulfate (3.45 g) was vigorously stirred at 0°C in a mixture of tetrahydrofuran (5 ml) and 10% sodium hydrogen carbonate (15 ml). Compound **1** (prepared from 2.18 g of methyl 4-hydroxymethyl-3-nitrobenzoate) and ether (150 ml) were added in small portions during 1 h and stirring was continued at room temperature overnight. The etheric phase was dried over sodium sulfate, evaporated and applied to a silica gel column (120 g, 2 cm in diameter) that was washed first with petroleum ether/ethylacetate, 2/1 by vol (600 ml), and eluted with petroleum ether/ ethylacetate, 1/1 by vol (5 ml/fraction). Fractions 156-163 contained pure (TLC, same solvent) compound **11** (yellowish solid, 1.6 g, 26%). Analytical samples were prepared by preparative HPLC (Merck RSIL-C 18-HL, 50 x 1 cm column eluted with methanol/water, 8/2 by vol).

^{1}H-NMR data (300 MHz, ^{2}H-chloroform): δ 8.73 (d, 1H, $J_{3,5}$ 1.5 Hz, H-3 aromatic), 8.30 (dd, 1H, $J_{5,6}$ 8.2 Hz, H-5 aromatic), 7.69 (d, 1H, H-6 aromatic), 5.82 (ddt, 1H, $J_{3,5}$ 1.0 Hz, $J_{4,5}$ 16.5 Hz, $J_{5,6}$ 6.5 Hz, H-5), 5.75 (d, 1H, $J_{NH,2}$ 8 Hz, NH), 5.58 (s, 2H, benzylic), 5.55 (ddt, 1H, $J_{3,4}$ 6.2 Hz, $J_{4,6}$ 1.4 Hz, H-4), 4.38 (ddd, 1H, $J_{2,3}$ 4.0 Hz, H-3), 4.02 (ddd, 1H, $J_{1,1'}$ 11.4 Hz, $J_{1,2} \approx 1.0$ Hz, $J_{1,OH}$ 4.0 Hz, H-1), 3.98 (s, 3H, OCH_3), 3.86 (d, 1H, OH-1), 3.67 (dddd, 1H $J_{1',2}$ 3.5 Hz, H-2), 3.75 (dd, 1H, H-1'), 2.07 (q, 2H, $J_{17,18}$ 6.8, CH_2-17), 1.26 (mc, 22H, ($CH_{2\text{-}11}$)), 0.88 (t, 3H, CH_3-18), assignment was supported by decoupling and deuterium exchange experiments.

*2-N-(4-Carboxymethyl-2-nitrobenzyloxycarbonyl)-1-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-sphingosine (**12**)*

Compound **11** (0.3 g) in a mixture of benzene (28 ml) and nitromethane (8 ml) was refluxed for 2 h and most of the solvent (26 ml) was removed by azeotropic distillation. 2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide (acetobromoglucose, 0.25 g) and mercuric cyanide (0.13 g), both dried over phosphorus pentoxide, were added and the reaction mixture was stirred overnight at 60°C under a calcium chloride seal. The reaction mixture was then filtered with some chloroform through a celite filter, the filtrate was diluted with chloroform (300 ml), washed with saturated sodium chloride, dried over calcium chloride and evaporated on silica gel (0.5 g) that was placed on top of a silica gel column (25 g, 0.9 cm in diameter) eluted with petroleum ether/ethylacetate, 1/1 by vol (3 ml/fraction). The impure product (**12**) eluted in fractions 28-33 (39 mg, 7%) was further purified by TLC (Merck, Silica gel 60 F_{254} plates) in the same solvent. $[\alpha]_D^{29}$ -9.2±3° (c 0.5, chloroform); ^{1}H-NMR data (300 MHz, ^{2}H-benzene): δ 8.54 (d, 1H, $J_{3,5}$ 1.6 Hz, H-3 aromatic), 7.34 (d, 1H, $J_{5,6}$ 8.0 Hz, H-6 aromatic), 7.16 (dd, 1H, H-5 aromatic), 5.70 (ddt, 1H, $J_{4a,5a}$ 15.2 Hz, $J_{3a,5a}$ 1.0 Hz, $J_{5a,6a}$ 6.2 Hz, H-5a), 5.49 (ddt, 1H, $J_{3a,4a}$ 6.2 Hz, $J_{4a,6a}$ ≈0.8 Hz), 5.46 (m, 2H, benzylic), 5.41 (d, 1H, $J_{NH,2a}$ 8.2 Hz), 5.36 (dd, 1H $J_{2,3}$ 9.4 Hz, $J_{3,4}$ 9.5 Hz, H-3), 5.20 (dd, 1H, $J_{4,5}$ 9.6 Hz, H-4), 5.21 (dd, 1H, $J_{1,2}$ 8.0 Hz, H-2), 4.19 (dd, 1H, $J_{5,6'}$ 4.8 Hz, $J_{6,6'}$ 12.4 Hz, H-6'), 4.18 (m, 1H, H-3a), 4.14 (d, 1H, H-1), 4.03 (dd, 1H, $J_{5,6}$ 3.9 Hz, H-6), 4.03 (dd, 1H, $J_{1,1a'}$ 10.2 Hz, $J_{1a',2}$ 4.0 Hz, H-1a'), 3.92 (m, 1H, $J_{1a,2}$ 3.6 Hz, H-2a), 3.64 (dd, 1H, H-1a), 3.40 (s, 3H, OCH_3), 3.21 (ddd, 1H, H-5), 1.97 (dq, 2H, $J_{6a,7a}$ 6.6 Hz,6a-CH_2), 1.84 (s, 3H, $OCOCH_3$), 1.75 (s, 3H, $OCOCH_3$), 1.69 (s, 3H, $OCOCH_3$), 1.67 (s, 3H, $OCOCH_3$), 1.31 (mc, 22H, $[CH_2]_{12}$), 0.91 (t, 3H, J 6.5 Hz, 18a-CH_3); n.O.e.: irradiation of H-1a (60% saturation) enhances H-1a' (75%) and H-1 (8.6%); the letter "a" after the number indicates sphingosine protons.

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