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Ceramide-1-phosphate sugars: new types of glycophospholipids

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Ceramide-1-phosphate sugars were synthesized by direct glycosyl phosphite/phosphate and *O*-glycosyl trichloroacetimidate/phosphate exchange reactions, respectively. Thus, ceramide-1-*O*-phosphoric acid **5** gave with sialyl phosphite **1** as sialyl donor directly β-linked sialyl phosphate **6**; deprotection afforded the corresponding glycophospholipid ceramide-1-phosphate *N*-acetylneuraminate **7**. Similarly, from *O*-glucosyl- and *O*-galactosyltrichloroacetimidate **10** and **13** with phosphoric acid derivative **5** glycosyl ceramide-1-phosphate sugars **12** and **15**, respectively, were obtained.

Keywords: glycophospholipids, ceramide-1-phosphate sugars, azidosphingosine-1-phosphate sugars, glycosyl phosphite, glycosyl phosphate, *O*-glycosyl trichloroacetimidate, *N*-acetylneuraminic acid (Neu5Ac).

Introduction

In addition to nucleosidemono- and -diphosphate sugars and glycosyl pyrophosphates, nature has also generated glycophospholipids which serve as glycosyl donors in glycosyltransferase catalysed reactions [1, 2]. Thus, the leaving group character of the phosphate or pyrophosphate group linked to a nucleoside or to a lipid moiety is utilized to generate glycosyl donor properties. Therefore, these compounds are typical metabolic intermediates which, due to their hydrolytic lability, are not accumulated in larger quantities. Even the glycosyl phosphatidates, which possess typical amphiphilic properties, are not employed as stable membrane constitutents. For this purpose, nature has instead utilized the corresponding much more stable pseudo sugar analogues which are based on the inositol moiety, i.e. the glycosylphosphatidyl inositols (GPIs); they serve as stable membrane anchors for various proteins and glycoproteins [3, 4].

Therefore, it is tempting to generate glycophospholipids of typical sugars because they may exhibit interesting biological properties in between the two above described roles for such compounds, namely glycosyl donor or stable membrane constituent. This view is supported by the finding that the antibiotic moenomycin, a transglycosidation inhibitor, possesses such a structure, and derives some stabilization from a uronate moiety [5, 6]. We present here two convenient procedures for preparing such ceramide-1-phosphate sugars [7, 8]. α- and β-D-glycopyranosyl phosphatidates having different fatty acyl residues have been already prepared [9–11].

Results and discussion

The first target molecule was Neu5Ac-1-phospho-ceramide **7** (Scheme 1) because it contains a stabilizing carboxylate and a destabilizing deoxy moiety. The synthesis was based on the previously introduced phosphite/phosphate exchange reaction, as exhibited in our CMP-Neu5Ac synthesis [12, 13]. Firstly, as a model compound 3-*O*-acetyl protected azidosphingosine-1-phosphoric acid **2** was prepared from the corresponding azidosphingosine-1-phosphate [14]. Reaction with known sialyl phosphite **1** [15, 16] in DMF:CH_3CN (2:1) as solvents at −20 °C afforded directly and in high yield the desired β-phosphate **3**; the configurational assignment is based on comparison of the NMR data of **3** with those of previously obtained β-phosphate derivatives of neuraminic acid [15, 16]. Treatment with sodium methanolate in methanol and then the addition of water gave the *O*-deprotected azidosphingosine-1-phosphate *N*-acetylneuraminic acid which was isolated as stable disodium salt **4**;

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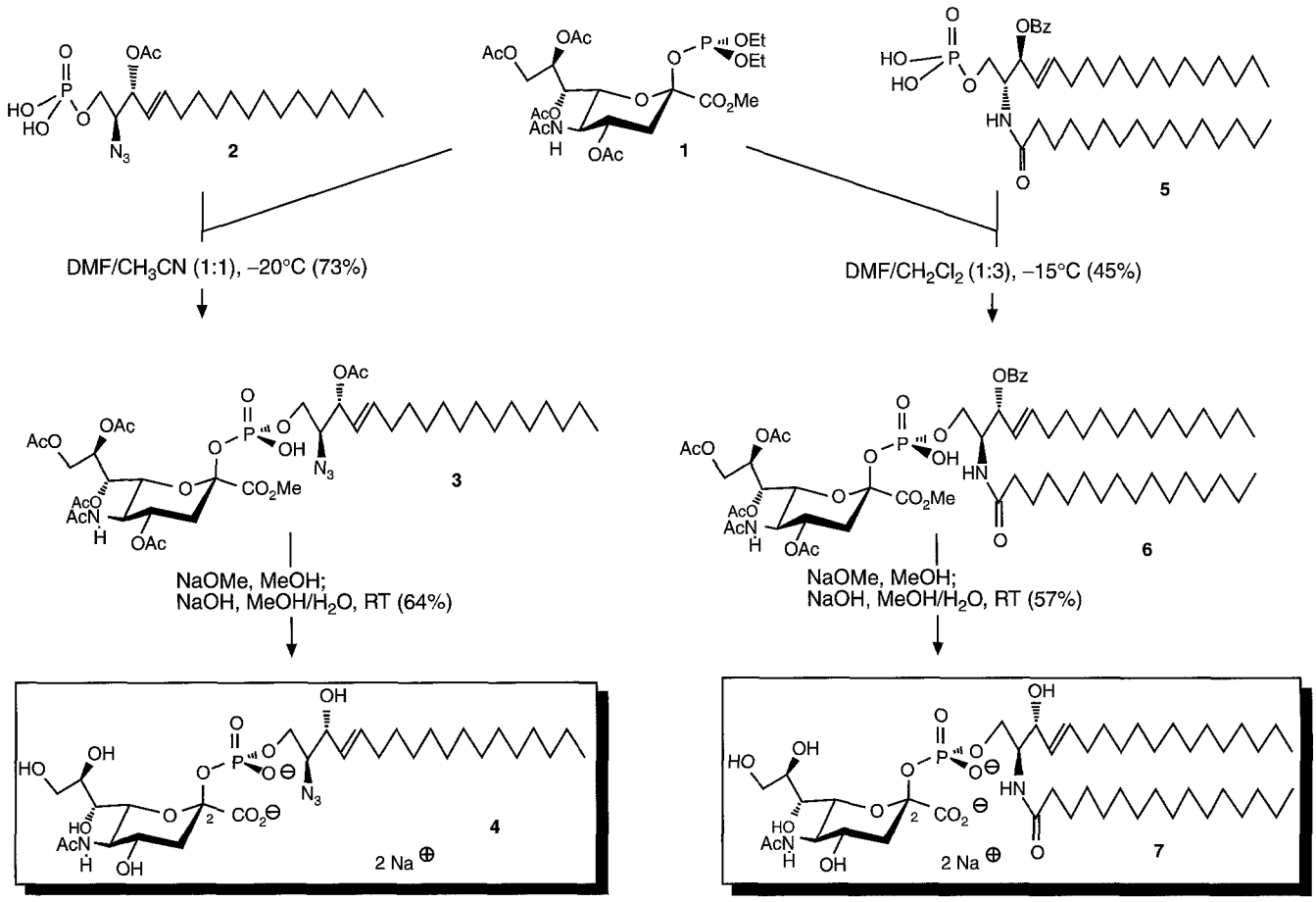


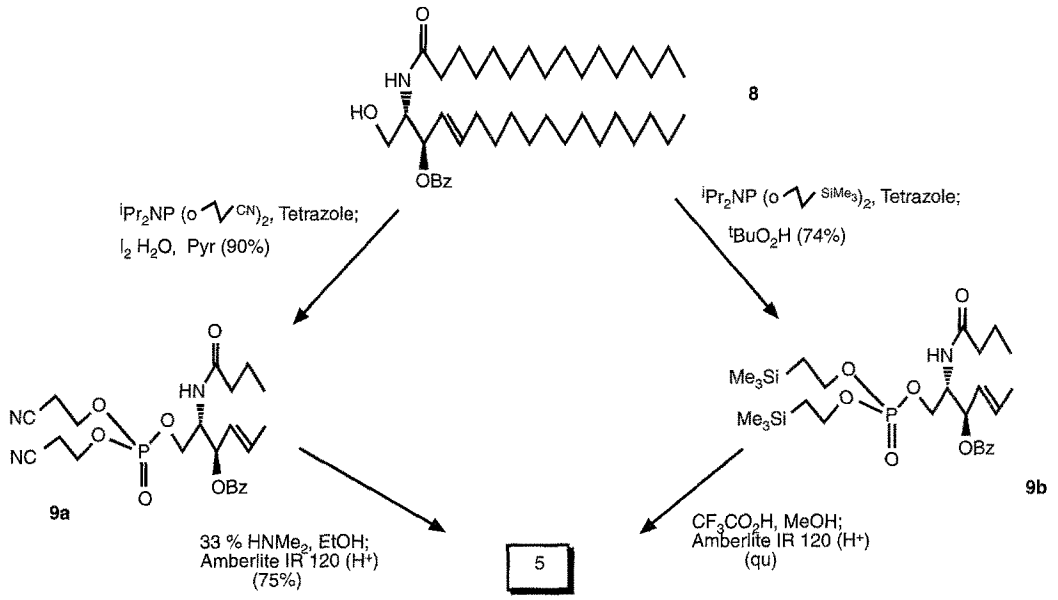
Scheme 1.

this compound exhibited NMR- and MS-data in accordance with the proposed structure.

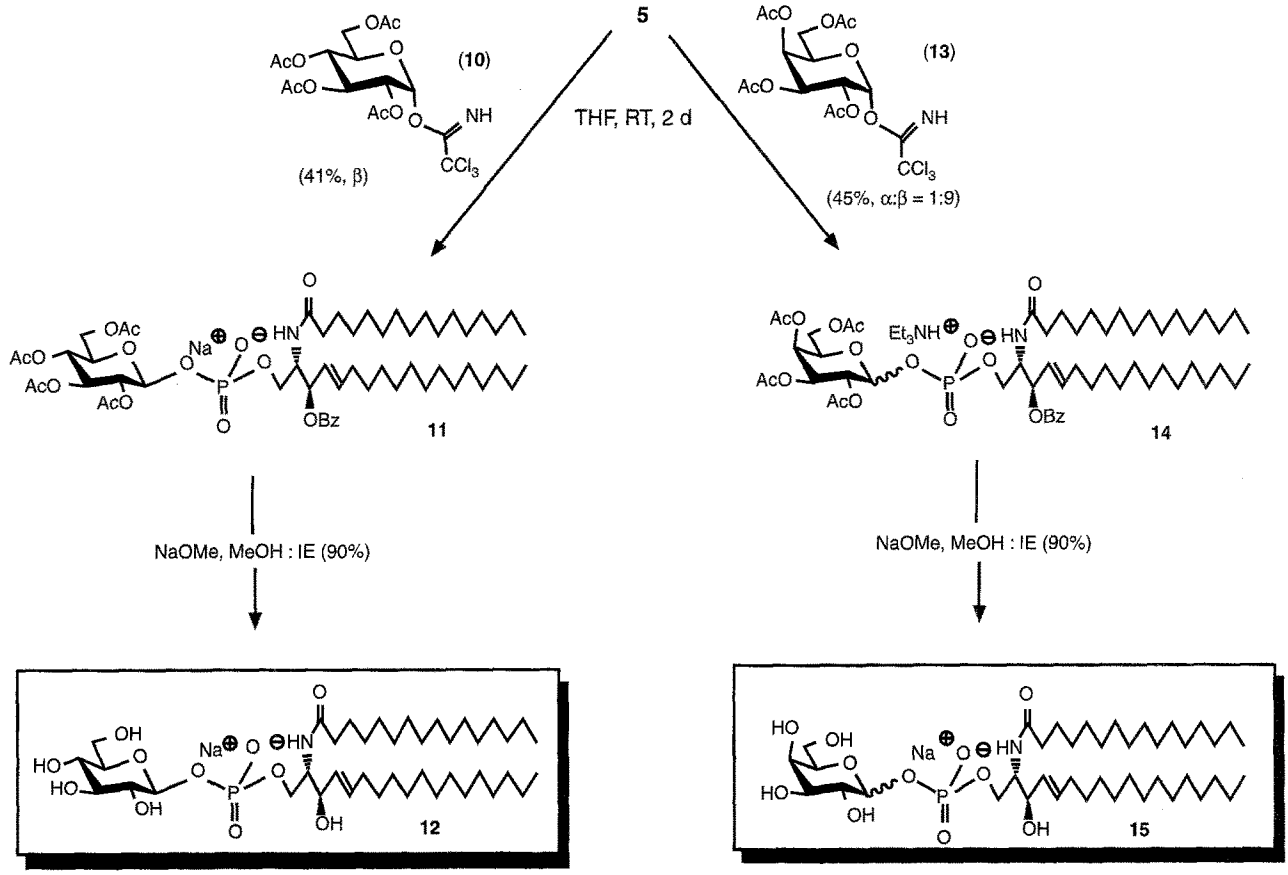
Then, for the synthesis of target molecule **7** (Scheme 1), known 3-*O*-benzoyl ceramide **8** [17] was transformed into the corresponding 1-phosphate derivative **5** (Scheme 2). To this aim, **8** was converted with the monofunctional phosphitylating agent bis(2-cyanoethoxy)(diisopropylamino)phosphane [18, 19] under nitrogen in the presence of freshly sublimated tetrazole into the corresponding trialkyl phosphite which upon oxidation with iodine in water/pyridine afforded trialkyl phosphate **9a**. Removal of the cyanoethyl protective groups with dimethylamine in ethanol and then acidification with ion exchange resin afforded the desired 3-*O*-benzoylceramide-1-phosphoric acid **5**. This compound could be also obtained from **8** with the monofunctional phosphitylating agent bis(2-trimethylsilylethoxy)(diisopropylamino)phosphane [20] and tetrazole and then oxidation with *tert*-butylhydroperoxide furnishing trialkyl phosphite **9b**. Removal of the trimethylsilylethyl groups could be readily accomplished by acid hydrolysis yielding **5** quantitatively. Reaction of **5** with sialyl phosphite **1** in DMF:CH_2Cl_2 (1:3) as solvents at −15 °C furnished directly the desired β-phosphate **6** in 45% yield. Treatment of **6** as described for **3** (see above) furnished target molecule **7** as stable disodium salt in 57% isolated yield. The structural assignment of compounds **6** and **7** could be based on NMR (^{1}H, ^{31}P) and MS data.

Amongst hexoaldoses, glucopyranosides for instance exhibit higher hydrolytic stability than the corresponding galactopyranosides. Therefore, ceramide-1-phosphate glucopyranose **12** (Scheme 3) was the next target molecule. For glycosyl phosphate formation the previously reported direct trichloroacetimidate/phosphate exchange reaction [21–23] was envisaged. To this aim 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate **10** [24] was reacted with **5** in THF as solvent for 2 days, affording, due to the participating 2-*O*-acetyl group, exclusively β-product **11** which was isolated as sodium salt in 41% yield. Treatment of **11** with sodium methoxide in methanol and then neutralizing the reaction mixture with ion exchange resin (H^+-form) furnished target molecule **12** in very high yield. Compounds **11** and **12** were structurally assigned by NMR (^{1}H, ^{31}P) and MS data (**12**: $J_{1,2} = 7.6$ Hz). Surprisingly, for the synthesized β-D-glycopyranosyl phosphatidates the ^{1}H-NMR data of the anomeric protons could not be assigned [10, 11].

Similarly, from *O*-galactosyl trichloroacetimidate **13**



Scheme 2.



Scheme 3.

[24] and ceramide-1-phosphoric acid **5** a 1:9 mixture of α/β-anomers **14** was obtained, thus exhibiting a higher tendency to anomerization of galactopyranose compared with glucopyranose under the same reaction conditions. De-*O*-acylation of **14** as described for **11** led to the sodium salt of target molecule **15**, which, due to hydrolytic lability, was much more difficult to purify than glucose derivative **12**. Therefore, not surprisingly,

extension of this reaction to pentoaldoses and to deoxysugars led to very labile ceramide-1-phosphate sugars, which were not obtained in an entirely pure form [8].

Materials and methods

General methods

Solvents were purified and dried in the usual way; the boiling range of the petroleum ether used was 35–65 °C. ^{1}H-NMR spectra: Bruker WM 250 cryospec and Jeol JNM-GX 400, internal standard tetramethylsilane (TMS). Flash chromatography: J.T. Baker silica gel 60 (30–60 μm) at a pressure of 0.3 bar. Medium pressure chromatography: Merck silica gel LiChroprep Si 60 (15–25 μm) at a pressure of up to 1 mPa (10 bar). Thin-layer chromatography (TLC): Merck plastic plates silica gel 60 F_{254}, layer thickness 0.2 mm, detection by treatment with a solution of 15% H_2SO_4, followed by heating at 120 °C. Optical rotations: Perkin-Elmer polarimeter 241/MS, 1 dm cell.

[(2S, 3R, 4E)-3-Acetyloxy-2-azido-4-octadecen-1-yl]dihydrogenphosphate (**2**)

[(2S, 3R, 4E)-2-Azido-3-hydroxy-4-octadecen-1-yl]dihydrogenphosphate [14] (0.22 g, 0.54 mmol) was dissolved in pyridine:acetic anhydride (3:1, 16 ml) and stirred for 12 h. After removal of the solvent in vacuo and codistillation with toluene (2 × 15 ml) the residue was treated with ion exchange resin (Amberlite IR 120, H^+) to yield **5** (0.15 g, 97%) as a slightly yellow amorphous solid, which is homogeneous on the basis of TLC and ^{1}H-NMR data. TLC (*n*-butanol:acetic acid:water, 3:1:1): R_F = 0.64.- ^{1}H-NMR (250 MHz, MeOD): R_F = 0.86–0.89 (t, J = 6.3 Hz, 3 H, CH_3), 1.28–1.38 (m, 22 H, 11 CH_2), 2.01–2.14 (m, 5 H, 6a-H, 6b-H, COCH_3), 3.84–4.05 (m, 3 H, 1a-H, 1b-H, 2-H), 5.31–5.36 (dd, $J_{2,3}$ = 4.5 Hz, $J_{3,4}$ = 7.7 Hz, 1 H, 3-H), 5.42–5.51 (dd, $J_{3,4}$ = 7.7 Hz, $J_{4,5}$ = 15.2 Hz, 1 H, 4-H), 5.79–5.91 (dt, $J_{4,5}$ = 15.2 Hz, $J_{5,6}$ = 6.8 Hz, 1 H, 5-H).

*Methyl (5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-glycero-D-galacto-2-nonulopyranosyl)onate [(2S, 3R, 4E)-3-acetyloxy-2-azido-4-octadecen-1-yl]hydrogenphosphate* (**3**)

To a stirred solution of **2** (150 mg, 0.335 mmol) in DMF:CH_3CN (2:1, 3.5 ml) was added a solution of **1** [15, 16] (328 mg, 0.536 mmol) in dry CH_3CN (1.5 ml) at −20 °C. After 4 h the solution was allowed to warm up to room temperature. Then the reaction mixture was evaporated in vacuo, finally at 13 Pa (10^{-2} torr). The residue was purified by silica gel, flash chromatography with chloroform:methanol (9:1). Compound **3** (225 mg, 73%) was obtained as a colourless oil. **3**: R_F [silica gel plates, $CHCl_3$:MeOH (9:1)] 0.24.- ^{1}H-NMR (250 MHz, MeOD): δ 0.90(t, J = 6.5 Hz, 3 H, CH_3), 1.29–1.43 (m, 24 H, 12 CH_2), 1.84–2.10 (6 s, 21 H, 3a-H, C=C—CH—CH_2, 6 $COCH_3$), 2.71 (dd, $J_{3e,4}$ = 4.8 Hz, $J_{3a,3e}$ = 13.2 Hz, 1 H, 3e-H), 3.81 (s, 3 H, $COOCH_3$), 3.82–4.02 (m, 4 H, CH-N_3, CH_2-O, 5-H), 4.21 (dd, $J_{8,9'}$ = 7.1 Hz, $J_{9,9'}$ = 12.3 Hz, 1 H, 9′-H), 4.47 (dd, $J_{6,7}$ = 2.1 Hz, $J_{5,6}$ = 10.7 Hz, 1 H, 6-H), 4.61 (dd, $J_{8,9}$ = 2.6 Hz, $J_{9,9'}$ = 12.3 Hz, 1 H, 9′-H), 5.30 (m, 1 H, 8-H), 5.36–5.46 (m, 3 H, 4-H, 7-H, CH-OAc), 5.51 (dd, J = 7.8 Hz, J_{trans} = 15.0 Hz, 1 H, CH=CH—CH_2), 5.88 (dt, J = 6.7 Hz, J_{trans} = 15.0 Hz, 1 H, CH=CH—CH_2). ^{31}P-NMR (161.7 MHz, MeOD): δ–6.94 (only β). MS (FAB, negative Mode): $(M-H)^-$ 919.

Disodium (5-Acetamido-3,5-dideoxy-β-D-glycero-D-galacto-2-nonulopyranosyl)onate [(2S, 3R, 4E)-3-Hydroxy-2-azido-4-octadecen-1-yl]phosphate (**4**)

To a solution of **3** (110 mg, 0.119 mmol) in dry methanol (5 ml) was added under stirring sodium methanolate (1 ml of a 0.1 M solution in methanol) at room temperature. After 3–4 h (TLC monitoring) water (0.5 ml) was added to the reaction mixture. After stirring again for 18 h (TLC monitoring) at room temperature, the mixture was neutralized (pH ~ 7–8) by adding a cation exchange resin (Amberlite IR, H^+). After filtration, the solvents were evaporated in vacuo. The residue was purified by flash chromatography with chloroform:methanol:2 N ammonia (65:35:8).

Compound **4** (53 mg, 64%) was obtained as an amorphous mass. **4**: R_F [silica gel plates, $CHCl_3$:MeOH:2 N NH_3 (65:35:8)] 0.26.- ^{1}H-NMR (250 MHz, MeOD): δ 0.94 (t, J = 6.6 Hz, 3 H, CH_3), 1.33–1.45 (m, 24 H, 12 CH_2), 1.65 (dd, $J_{3a,4}$ = 10.7 Hz, $J_{3a,3e}$ = 13.0 Hz, 1 H, 3a-H), 2.05 (s, 3 H, $NCOCH_3$), 2.07–2.19 (m, 2 H, C=CH—CH_2), 2.54 (dd, $J_{3e,4}$ = 4.4 Hz, $J_{3a,3e}$ = 13.0 Hz, 1 H, 3e-H), 3.46 (d, J = 9.4 Hz, 1 H, 7-H), 3.58–3.74 (m, $J_{3e,4}$ = 4.4 Hz, 2 H, 4-H, 6-H), 3.84–4.10 (m, 5 H, 5-H, 9′-H, CH-N_3, CH_2—O), 4.16–4.27 (m, 3 H, CH—OH, 8-H, 9″-H), 5.56 (dd, J = 7.5 Hz, J_{trans} = 15.3 Hz, 1 H, CH=CH—CH_2), 5.83 (dt, J = 6.7 Hz, J_{trans} = 15.3 Hz, 1 H, CH=CH—CH_2). ^{31}P-NMR (161.7 MHz, MeOD): δ–3.34 (only β). MS (FAB, negative mode): $(M—H)^-$ 695, $(MNa-H)^-$ 717.

[(2S, 3R, 4E)-3(Benzoyloxy)-2-(hexadecanoylamino)-4-octadecen-1-yl]dihydrogen-phosphate (**5**)

(a) *From* **9a**: A solution of **9a**, see below, (100 mg, 0.12 mmol) in ethanolic dimethylamine (33% in dry ethanol, 6 ml) was stirred at 40 °C for 3 d. The reaction mixture was concentrated in vacuo and purified by silica gel flash chromatography with chloroform:methanol:water (8:2:0.2). The residue was then dissolved in chloroform:methanol (1:2, 8 ml) and the solution was stirred with ion exchange resin (Amberlite IR 120, H^+ form). After 3 h,

the reaction mixture was filtered and the filtrate concentrated under reduced pressure. Compound **5** (65 mg, 75%) was obtained as colourless crystals, m.p. 75 °C. **5**: R_F [silica gel plates, $CHCl_3$:MeOH:H_2O (8:2:0.2)] 0.2 $[\alpha]_D^{20}$-5.02 (*c* 1.0, $CHCl_3$).- ^{1}H-NMR (250 MHz, $CDCl_3$:MeOD, 1:1), δ 0.79–0.98 (m, 6 H, 2 CH_3), 1.26–1.42 (m, 46 H, CH_2), 1.58–1.69 (m, 2 H, $COCH_2CH_2$), 2.02–2.12 (m, 2 H, C=CHCH_2), 2.21–2.29 (m, 2 H, COCH_2), 4.12–4.19 (m, 2 H, CH_2O), 4.48–4.53 (m, 1 H, CHN), 5.48–5.52 (m, 2 H, CHOBz, C*H*=CHCH$_2$), 5.95 (dt, $J_{6,5}$ = 7.0 Hz, $J_{4,5}$ = 14.6 Hz, 1 H, CH=C*H*—CH_2), 7.39–7.52 (m, 2 H, BB′, Ph), 7.52–7.67 (m, 1 H, C, Ph), 7.99–8.1 (m, 2 H, AA′, Ph). ^{31}P-NMR ($CDCl_3$:MeOD, 1:1) δ 0.603. MS (EI) [M 98]$^+$ 623.

(b) *From* **9b**: **9b**, see below, (7.15 mg, 0.79 mmol) was dissolved in dry methanol under a nitrogen atmosphere. A solution of CF_3COOH:MeOH (1:2, 3 ml) was added dropwise and the reaction mixture was stirred at 40 °C for 1 d. The solution was then neutralized with solid sodium hydrogen carbonate and concentrated under reduced pressure. The obtained residue was purified by silica gel flash chromatography with chloroform:methanol:water (8:2:0.2) and treated with an ion exchange resin (Amberlite IR 120, H^+ form) as described above to give compound **5** (540 mg, 93%).

*Methyl (5-Acetamido-4,7,8,9-tetra-*O*-acetyl–3,5-dideoxy-β-*D*-glycero-*D*-galacto-2-nonulopyranosyl)onate [(2*S*, 3*R*, 4*E*)-3-Benzoyloxy-2-hexadecanoylamino-4-actadecen-1-yl] hydrogenphosphate* (**6**)

To a stirred mixture **5** (80 mg, 0.111 mmol) in DMF:CH_2Cl_2 (1:2, 3 ml) was added a solution of **1** [12, 13] (135 mg, 0.222 mmol) in dry CH_2Cl_2 (1 ml) at −15 °C. After 5 h the solution was allowed to warm up to room temperature. Then the reaction mixture was evaporated in vacuo, finally at 13 Pa (10^{-2} torr). The residue was purified by silica gel flash chromatography with chloroform:methanol (8.5:1.5). Compound **6** (83 mg, 63%) was obtained as colourless oil. **6**: R_F [silica gel plates, $CHCl_3$:MeOH (8.5:1.5)] 0.33.- ^{1}H-NMR (250 MHz, MeOD): δ 0.89 (t, *J* = 6.5 Hz, 6 H, 2 CH_3), 1.14–1.35 (m, 46 H, 23 CH_2), 1.61–1.71 (m, 3 H, 3a-H, CH_2), 1.83–2.15 (m, 17 H, CH=CH—CH_2, 5 $COCH_3$), 2.20–2.32 (m, 2 H, NCO—CH_2), 2.74 (dd, $J_{3e,4}$ = 5.0 Hz, $J_{3e,3a}$ = 13.3 Hz, 1 H, 3e-H), 3.76 (s, 3 H, $COOCH_3$), 3.99 (dd, $J_{4,5}$ = $J_{5,6}$ = 10.7 Hz, 1 H, 5-Hz), 4.10 (m, 2 H, O—CH_2), 4.15 (dd, $J_{8,9''}$ = 6.6 Hz, $J_{9',9''}$ = 12.3 Hz, 1 H, 9″-H), 4.42 (dd, $J_{6,7}$ = 2.2 Hz, $J_{5,6}$ = 10.7 Hz, 1 H, 6-H), 4.52 (dd, $J_{8,9'}$ = 2.5 Hz, $J_{9',9''}$ = 12.3 Hz, 1 H, 9′-H), 4.52–4.55 (m, 1 H, CH—N), 5.23 (ddd, $J_{8,9'}$ = 2.5 Hz, $J_{8,9''}$ = 6.6 Hz, 1 H, 8-H), 5.37 (ddd, $J_{3e,4}$ = 5.0 Hz, $J_{4,5}$ = 10.7 Hz, $J_{3a,4}$ = 11.0 Hz, 1 H, 4-H), 5.44 (dd, $J_{6,7}$ = 2.3 Hz, 1 H, 7-H), 5.68–5.79 (m, *J* = 7.6 Hz, 2 H, C*H*=CH—CH_2, CHOBz), 5.92 (dt, *J* = 6.6 Hz, J_{trans} = 13.7 Hz, 1 H, CH=C*H*—CH_2), 7.44–7.50 (m, 2 H, BB′, Ph), 7.58–7.64 (m, 1 H, C, Ph), 8.04–9.07 (m, 2 H, AA′, Ph). ^{31}P-NMR (161.7 MHz, MeOD): δ −6.37 (only β). MS (FAB, negative mode): $(M—H)^-$ 1195.

*Disodium (5-Acetamido-3,5-dideoxy-β-*D*-glycero-*D*-galacto-2-nonulopyranosyl)onate [(2*S*, 3*R*, 4*E*)-3-hydroxy-2-hexadecanoylamino-4-octadecen-1-yl] phosphate* (**7**)

Deprotection of compound **6** (50 mg, 42 μmol) in dry methanol (3 ml) with sodium methanolate (0.5 ml of a 0.1 M solution in methanol), then water (0.5 ml), as described for compound **4**, afforded **7** (27 mg, 69%) as an amorphous mass. **7**: R_F [silica gel plates, $CHCl_3$:MeOH:2 N NH_3 (65:35:8)] 0.35.- ^{1}H-NMR (250 MHz, MeOD): δ 0.90 (t, 6 H, 2 CH_3), 1.20–1.29 (m, 46 H, 23 CH_2), 1.55–1.66 (m, 3 H, 3a-H, CH_2), 2.01 (s, 3 H, $NCOCH_3$), 2.10–2.27 (m, 2 H, CH=CH—CH_2), 2.50 (dd, $J_{3e,4}$ = 4.6 Hz, $J_{3a,3e}$ = 13.1 Hz, 1 H, 3e-H), 3.42 (d, *J* = 9.3 Hz, 1 H, 7-H), 3.59–3.67 (m, $J_{8,9'}$ = 5.9 Hz, $J_{9',9''}$ = 11.4 Hz, 2 H, 5-H, 9′-H), 3.80 (dd, $J_{8,9''}$ = 3.0 Hz, $J_{9',9''}$ = 11.4 Hz, 1 H, 9″-H), 3.85–4.01 (m, $J_{6,7}$ = 2.7 Hz, $J_{5,6}$ = 10.2 Hz, 6 H, 4-H, 6-H, 8-H, CH-N_3, CH_2—O), 4.09–4.19 (m, 1 H, C*H*—OH), 5.44 (dd, *J* = 7.5 Hz, J_{trans} = 15.2 Hz, 1 H, C*H*=CH—CH_2), 5.71 (dt, *J* = 6.4 Hz, J_{trans} = 15.1 Hz, 1 H, CH=C*H*—CH_2).- ^{31}P-NMR (161 MHz, MeOD:$CDCl_3$ = 3:1): δ −3.83 (only β).

*[(2*S*, 3*R*, 4*E*)-3-Benzoyloxy-2-hexadecanoylamino-4-octadecen-1-yl]-bis(2-cyanoethyl)phosphate* (**9a**)

To a stirred solution of **8** (100 mg, 0.16 mmol) and freshly sublimated tetrazole (22 mg, 31 mmol) in dry CH_2Cl_2:CH_3CN (1:1, 3 ml) was added a solution of bis(2-cyanoethoxy)(diisopropylamino)phosphane [15, 16] (69 mg, 0.25 mmol) in dry CH_2Cl_2 (1 ml) at room temperature. After 20 min (monitoring by TLC) a solution of iodine in pyridine:dichloromethane:water (3:1:1, 0.4 M) was added dropwise until iodine decolorisation ceased. After 10 min the reaction mixture was treated with a saturated disodiumthiosulfate solution (10 ml) and then extracted with dichloromethane (10 ml). The extract was dried ($MgSO_4$), concentrated in vacuo and the residue was purified by silica gel flash chromatography (toluene:ethyl acetate, 1:1) to yield **9a** (115 mg, 90%) as colourless crystals, m.p. 75 °C.- $[\alpha]_D^{20}$ 10.3 (*c* 1.0, $CHCl_3$).- R_F (silica gel plates, toluene:ethyl acetate, 1:1) 0.28.- ^{1}H-NMR (250 MHz, $CDCl_3$) δ 0.76–0.93 (m, 6 H, 2 CH_3), 1.19–1.34 (m, 46 H, 23 CH_2), 1.51–1.63 (m, 2 H, $COCH_2CH_2$), 1.96–2.06 (m, 2 H, C=CH—CH_2), 2.12–2.26 (m, 2 H, $COCH_2$), 2.59–2.68 (m, 2 H, 2 NCCH_aH_b), 2.70–2.77 (m, 2 H, 2NCH_aH_b), 4.12–4.33 (m, 6 H, $NCCH_2CH_2O$, OCH_2), 4.53–4.64 (m, 1 H, CHN), 5.42 (dd, $J_{5,4}$ = 14.9 Hz, $J_{3,4}$ = 7.8 Hz, 1 H, C*H*=CH—CH_2), 5.58 (dd, $J_{3,2}$ = 7.8 Hz, 1 H, CHOBZ), 5.91 (dt, $J_{6,5}$ = 7.6 Hz, $J_{4,5}$ = 14.6 Hz, 1 H, CH=C*H*—CH_2), 6.15

(d, 1 H, $J = 9.4$ Hz, NH), 7.38–7.49 (m, 2 H, BB′, Ph), 7.51–7.60 (m, 1 H, C, Ph), 7.95–8.08 (m, 2 H, AA′, Ph), $C_{47}H_{78}N_3O_7P$ (828.1)

Ber.:	C 68.17	H 9.49	N 5.08
Gef.:	C 68.13	H.9.40	N 5.18

[(2S, 3R, 4E)-3-Benzoyloxy-2-hexadecanoylamino-4-octadecen-1-yl]-bis(trimethylsilylethyl) phosphate (**9b**)

To a stirred solution of **5** (660 mg, 1.03 mmol) and freshly sublimated tetrazole (114 mg, 2.06 mmol) in dry CH_2Cl_2:CH_3CN (1:1, 8 ml) was added a solution of bis[2-(trimethylsilylethoxy)-(diisopropylamino)phosphane [20] (600 mg, 1.65 mmol) in dry CH_2Cl_2 (2 ml) at room temperature. After 60 min (monitoring by TLC) a solution of *tert*-butylhydroperoxide (3 M in abs. toluene 0.55 ml, 1.6 mmol) was added dropwise and the mixture was stirred for another 2 h. The solution was then diluted with dichloromethane (20 ml) and washed with satd. sodium hydrogen carbonate solution (15 ml). The organic layer was dried ($MgSO_4$) and concentrated to yield **9b** (715 mg, 75%) which was not further purified. -R_F (silica gel plates, toluene:acetone, 5:1) 0.51.- ^{1}H-NMR (250 MHz, $CDCl_3$) δ 0.08 [s, 18 H, 2 $Si(CH_3)_3$], 0.79–1.11 (m, 6 H, 2 CH_3), 1.19–1.40 (m, 48 H, CH_2), 1.51–1.68 (m, 2 H, $COCH_2CH_2$), 1.93–2.08 (m, 2 H, C=CH—CH_2), 2.12–2.25 (m, 2 H, $COCH_2$), 4.02–4.3 (m, 6 H, 3 CH_2O), 4.47–4.61 (m, 1 H, CHN), 5.4–5.6 (m, 2 H, CH=CH—CH_2, CHOBz), 5.81–5.98 (dt, 1 H, $J_{6,5} = 7.6$ Hz, $J_{4,5} = 14.6$ Hz, 1 H, CH=CH—CH_2), 6.24 (d, 1 H, $J = 9.4$ Hz, NH), 7.38–7.49 (m, 2 H, BB′, Ph), 7.5–7.6 (m, 1 H, C, Ph), 7.94–8.08 (m, 2 H, AA′, Ph).

Sodium [(2R, 3R, 4E)-3-benzoyloxy-2-hexadecanoylamino-4-octadecen-1-yl]-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl) phosphate (**11**)

A solution of **5** (100 mg, 0.14 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate **10** [24] (100 mg, 0.21 mmol) in dry THF (3 ml) was stirred at room temperature under a nitrogen atmosphere for 2 d. One drop of triethylamine was added and the reaction mixture was concentrated under reduced pressure. The residue was purified by silica flash chromatography with chloroform:methanol:water (12:1:0.1 → 4:1:0.1) and then dissolved in $CHCl_3$:MeOH (1:2, 3 ml). The solution was stirred with ion exchange resin (Amberlite IR 120, Na^+ form) for 3 h. After filtration the solvent was removed to yield **11** (60 mg, 41%) as an amorphous mass. **11**: R_F (silica gel plates, $CHCl_3$:MeOH:H_2O, 6:1:0.05) 0.33.- $[\alpha]_D^{20}$ 5.76 (*c* 1, $CHCl_3$).- ^{1}H-NMR (250 MHz, $CDCl_3$/MeOD, 1:1) δ 0.81–0.97 (m, 6 H, CH_3), 1.19–1.42 (m, 46 H, 23 CH_2), 1.62–1.68 (m, 2 H, $COCH_2CH_2$), 2.0–2.19 (m, 14 H, 4 $COCH_3$, CH=CH—CH_2), 2.2–2.27 (m, 2 H, $COCH_2$), 3.88 (ddd, 1 H, 5-H), 3.97–4.1 (m, 2 H, CH_2-O), 4.18 (dd, $J_{5,6} = 2.3$ Hz, $^2J_{6,6'} = 12.4$ Hz, 1 H, 6-H), 4.32 (dd, $J_{5,6'} = 3.9$ Hz, 1 H, 6′—H), 4.43–4.57 (m, 1 H, CHN), 4.95 (dd, $J = 8.1$ Hz, $J = 8.8$ Hz, 1 H, 2-H), 5.3 (dd, $J_{5,4} = J_{3,4} = 9.7$ Hz, 1 H, 4-H), 5.15–5.31 (m, 2 H, 1-Hβ, 3-H), 5.47–5.62 (m, 2 H, CHOBz, CH=CH—CH_2), 5.92 (dt, $J_{4,5} = 14.6$ Hz, $J_{6,5} = 7.6$ Hz, HC=CH—CH_2), 7.4–7.52 (m, 2 H, BB′, Ph), 7.52–7.66 (m, 1 H, C, Ph), 7.99–8.1 (m, 2 H, AA′, Ph).- ^{31}P-NMR (161.7 MHz, $CDCl_3$:MeOD, 1:1) δ–1.636 MS (FAB, negative mode): $[M-H]^-$ 1051.

Sodium [(2S, 3R, 4E)-2-hexadecanoylamino-3-hydroxy-4-octadecen-1-yl]-O-(β-D-glucopyranosyl)phosphate (**12**)

To a solution of **11** (20 mg, 0.02 mmol) in dry methanol (2 ml) was added under stirring sodium methanolate (0.3 ml of a 0.1 M solution in methanol) at room temperature. After 2 d (TLC monitoring) the mixture was neutralized (pH ~ 7–8) by adding a cation exchange resin (Amberlite IR 120, H^+). After filtration, the solvents were evaporated in vacuo. Compound **12** (12 mg, 90%) was obtained as an amorphous mass. **12**: R_F (silica gel plates, $CHCl_3$:MeOH:H_2O, 65:35:5) 0.3.- ^{1}H-NMR (250 MHz, $CDCl_3$:MeOD, 1:1) δ 0.8–0.95 (m, 6 H, CH_3) 1.12–1.40 (m, 46 H, CH_2), 1.52–1.62 (m, 2 H, $COCH_2CH_2$), 1.92–2.05 (m, 2 H, C=CH—CH_2), 2.11–2.25 (m, 2 H, $COCH_2$), 3.22 (ddd, $J_{2,P} = 3.2$ Hz, $J_{3,2} = 8.9$ Hz, $J_{4,3} = 9.2$ Hz, 1 H, 2-H), 3.42 (dd, $J_{2,3} = 8.9$ Hz, $J_{4,3} = 9.2$ Hz, 1 H, 3-H), 3.55–3.78 (m, 2 H, 4-, 5-H), 3.79–4.06 (m, OCH_aH_b-CHN, CHN, 6-, 6′-H), 4.11 (dd, 1 H, CHOH), 4.19–4.31 (m, 1 H, OCH_aH_b-CN), 4.87 (dd, $J_{P,1} = 7.6$ Hz, 1 H, 1-Hβ), 5.45 (dd, $J_{2,3} = 7.4$ Hz, $J_{6,5} = 7.2$ Hz, 1 H, CH=CH—CH_2), 5.71 (dt, $J_{4,5} = 14.9$ Hz, $J_{6,5} = 7.2$ Hz, 1 H, CH=CH—CH_2).- ^{31}P-NMR ($CDCl_3$:MeOD, 1:1): 4.235 MS (FAB, negative mode) $[M-H]^-$ 778.

Triethylammonium [(2S, 3R, 4E)-3-benzoyloxy-2-hexadecanoylamino-4-octadecen-1-yl]-O-(2,3,4,6-tetra-O-acetyl-α/β-D-galactopyranosyl)phosphate (**14**)

A solution of **5** (100 mg, 0.14 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (**13**) [24] (100 mg, 0.21 mmol) in dry THF (3 ml) was stirred at room temperature under a nitrogen atmosphere for 2 d. One drop of triethylamine was added and the reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel flash chromatography with chloroform:methanol:1 N NH_3 (10:1:0.1) to give **14** (62 mg, 45%) as an amorphous mass α:β = 1:9. **14**: R_F (silica gel plates, $CHCl_3$:MeOH:1 N NH_3 (65:35:5) 0.79.- ^{1}H-NMR (250 MHz, $CDCl_3$:MeOD, 1:1) δ 0.81–0.98 (m, 6 H, CH_3), 1.21–1.40 (m, 55 H, CH_2CH_3), 1.52–1.72 (m, $COCH_2CH_2$), 1.97–2.19 (m, 14 H, 4 $COCH_3$, CH=CH—CH_2), 2.19–2.32 (m, 2 H, $COCH_2$), 3.10–3.20 (q, 6 H, CH_2), 4.0–4.3 (m, 5 H, CH_2O, 5-, 6-, 6′-H), 4.39–4.53 (m, 1 H, CHN), 5.1–5.21 (m, 3 H, 1-Hβ, 2-H, 3-H), 5.42 (dd, 1 H, 4-H), 5.5–5.68 (m, 2 H, CHOBz, CH=CH—CH_2), 5.70–5.78 (dd, $J_{1,P} = 7.4$ Hz, $J_{1,2} = 3.5$ Hz, 0.1 H, 1-Hα), 5.95 (dt, $J_{6,5} = 7$ Hz, $J_{4,5} = 14.6$ Hz, 1 H, CH=CH—CH_2),

7.41–7.52 (m, 2 H, BB′, Ph), 7.53–7.67 (m, 1 H, C, Ph), 8.01–8.13 (m, 2 H, AA′, Ph).- ^{31}P-NMR ($CDCl_3$:MeOD, 1:1) δ -3.493 MS (FAB, negative mode) $[M-H]^-$ 1051.

Sodium [(2S, 3R, 4E)-2-hexadecanoylamino-3-hydroxy-4-octadecen-1-yl]-O-(α,β-D-galactopyranosyl)phosphate (**15**)

Deprotection of compound **14** (30 mg, 0.03 mmol) in dry methanol (2 ml) with sodium methanolate (0.1 ml of a 0.1 M solution in methanol) as described for compound **12**, afforded **15** (20 mg, 90%) as an amorphous mass. **15**: R_F (silica gel plates, $CHCl_3$:MeOH:H_2O, 65:35:5) 0.3 $\alpha:\beta = 1.9$.- ^{1}H-NMR (250 MHz, $CDCl_3$:MeOD, 1:1) δ 0.82–0.91 (m, 6 H, CH_3), 1.20–1.41 (m, 46 H, CH_2), 1.51–1.67 (m, 2 H, $COCH_2CH_2$), 1.98–2.08 (m, 2 H, CH=CH—CH_2), 2.12–2.22 (m, 2 H, $COCH_2$), 3.51 (dd, $J_{2,3} = 9.8$ Hz, $J_{4,3} = 3.0$ Hz, 1 H, 3-H), 3.59 (dd, $J_{1,2} = 7.4$ Hz, $J_{3,2} = 9.8$ Hz, 1 H, 2-H), 3.66–3.89 (m, 4 H, 4-, 5-, 6-, 6′-H), 3.89–4.0 (m, 2 H, OCH_aH_b, CHN), 4.06–4.13 (m, 1 H, CHOH), 4.18–4.20 (m, 1 H, OCH_aH_b), 4.81 (dd, $J_{P,1} = 7.4$ Hz, 1 H, 1-Hβ), 5.39–5.58 (dd, $J_{2,3} = 7.4$ Hz, $J_{4,3} = 14.9$ Hz, 1 H, CH=CH—CH_2, 0.1 H, 1-Hα), 5.62–5.76 (dt, $J_{4,5} = 14.9$ Hz, $J_{6,5} = 7.2$ Hz, 1 H, CH=CH—CH).- ^{31}P-NMR ($CDCl_3$:MeOD, 1:1) δ 0.185 MS (FAB, negative mode) $[M-H]^-$ 778.

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