

Synthesis Of Labeled Glycosyl Phosphatidyl Inositol (GPI) Anchors

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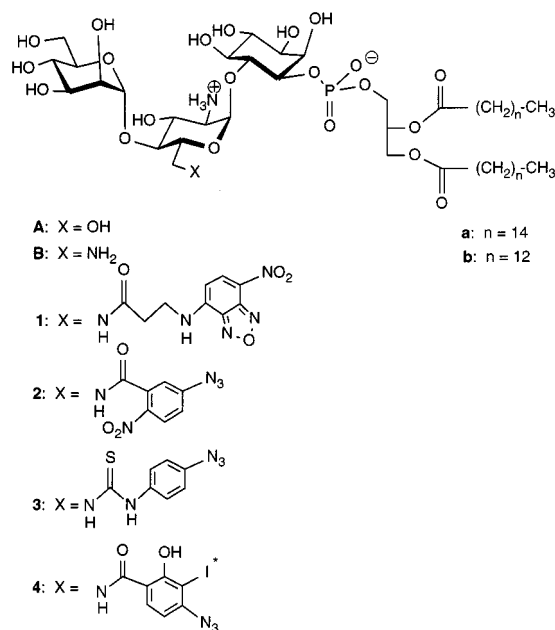
The exploration of the molecular and structural basis for the sorting of GPI-anchored proteins is based on labeled partial structures of GPI's which can be incorporated into the GPI anchor biosynthesis and cellular transport systems. To this end, from mannosyl donor **6** and the D-glucosaminyl-(1→6)-D-myoinositol derivative **7** as acceptor, the pseudotrisaccharide **8** was prepared. Compound **8** was transformed into the GPI partial structures **5a,b** which contain the pseudotrisaccharide ligated to two different phosphatidyl residues. Compounds **5a,b** have Boc protection at the 2-

amino group of the glucosamine residue (2b-position) and a free amino group at the 6b-position. The 6b-amino group was used for the ligation of the 3-(7-nitrobenzofurazan-4-yl)-aminopropanoyl group as a fluorescent label, the 5-azido-2-nitrobenzoyl and 4-azidophenylaminothiocarbonyl groups as photolabels, and the 4-azido-2-hydroxybenzoyl group as a radiolabel after the introduction of radioactive iodine by an electrophilic aromatic substitution. Thus, after acid-catalyzed removal of the protective groups, the unprotected target molecules **1–4** were obtained.

Many cell surface proteins are anchored by glycosyl phosphatidylinositols (GPI's) to the cell surface membrane.^{[1][2]} This protein modification is involved in intramolecular signaling and seems to act as an apical targeting signal for proteins in epithelial cells.^[3] However, the mechanism by which the GPI anchors confer the ability to sort membrane proteins is unknown, and the importance of specialized membrane domains in this process remains to be elucidated.^{[4][5]}

Labeled GPI partial structures should be important tools for the exploration of the cellular and molecular basis for the sorting of GPI-anchored proteins because their fate can be followed in the endocytic and exocytic pathways of both polarized and nonpolarized cells.^{[5][6]} Based on the biosynthesis of GPI anchors,^[1,2,7] compound **A** (Scheme 1), with one mannosyl residue at the glucosaminylinositol phosphatide moiety, seemed to be a particularly versatile intermediate for these studies as it is present in almost all species-specific variations of the GPI anchor biosynthesis. Thus, it should be incorporated into the GPI anchor biosynthesis and cellular transport systems. For the label attachment, the 6-position of the glucosamine residue (6b-position) was chosen because this position is at a reasonable distance from the membrane surface and from the site of further elongation of the GPI anchor. Therefore, modification at the 6b-position of **A** should be tolerated by the biological systems involved in GPI anchor metabolism and transport. In order to generate a firm connection between the 6b-position and the label, replacement of the 6b-hydroxy group by an amino group (→ **B**) was performed, thus providing a convenient means for attaching the labels by amide bonds. Fluorescent labels, photolabels and radio-

labels were desirable for the biological studies; therefore compounds **1–4** were chosen as target molecules.



Scheme 1. Target molecules

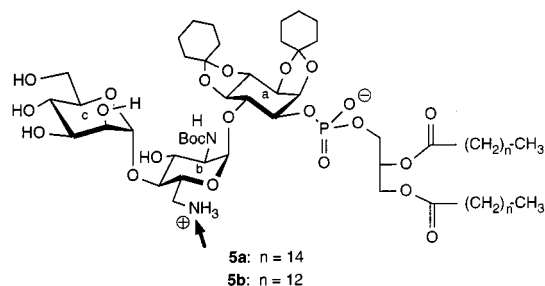
Synthesis of the 6b-Amino-6-deoxy GPI Anchor Intermediates **5a,b**

For the synthesis of compounds **1–4** intermediate **B** is not useful because the presence of two amino groups will prevent regioselective label attachment at the 6b-position. Additionally, the sensitivity of the envisaged labels to hydrogenolytic conditions requires that protective groups which are cleaved by hydrogenolysis are removed prior to label attachment. The known stability of the labels to mild acid treatment, led to the choice of compounds **5a,b**

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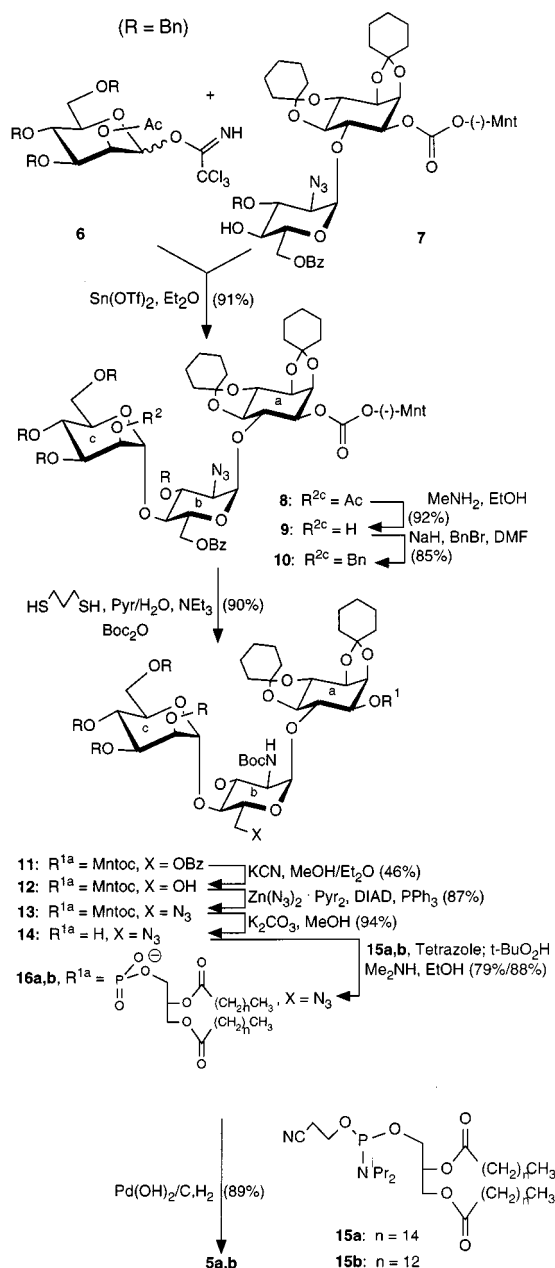
(Scheme 2) as decisive intermediates for the label attachment. Compounds **5a,b** were also selected as targets because readily accessible building blocks, employed in our previous successful GPI anchor syntheses,^[8–11] can be also utilized here. For the lipidic part two different chain lengths (C_{16} , C_{14}) were chosen because cellular uptake and transport are highly dependent on the lipid composition.^[12]



Scheme 2. Decisive intermediates

The synthesis of the required α -glucosaminyl(1 $\rightarrow$ 6)inositol building block **7** (Scheme 3), which permits regioselective access to the 4b- and also 6b- and 1a-*O*-positions, was performed as previously described.^[11] For the attachment of the mannosyl residue the known donor **6**^{[8][11]} was selected. The efficiency of the synthesis of **6** could be improved by the direct and almost quantitative transformation of 3,4,6-tri-*O*-benzyl-1,2-*O*-[1-(1*R*)-methoxyethylidene]- β -D-mannopyranose^[13] into 2-*O*-acetyl-3,4,6-tri-*O*-benzyl- α -D-mannopyranose,^[8,11,14] the precursor for the synthesis of **6**. Reaction of **6** with **7** as acceptor in diethyl ether as solvent, in the presence of tin(II)trifluoromethanesulfonate as catalyst, afforded the desired α -linked pseudotrisaccharide **8** in 91% yield [^1H NMR: δ = 5.47 (d, 1 H, $J_{1,2}$ = 1.7 Hz, 1c-H), 5.33 (d, 1 H, $J_{1,2}$ = 3.6 Hz, 1b-H)]. The 2-*O*-acetyl group could be selectively removed by treatment with methylamine in ethanol ($\rightarrow$ **9**). Subsequent 2*c*-*O*-benzylation with benzyl bromide in the presence of NaH as base afforded compound **10** in high yield. Next, the 2b-azido group was transformed into the amino function by treatment with propanedithiol in pyridine/water in the presence of catalytic amounts of triethylamine,^[15] and the ensuing reaction with di-*tert*-butyl-dicarbonate (Boc₂O)^[16] afforded the desired *N*-Boc protected derivative **11**.

The regioselective removal of the 6b-*O*-benzoyl group from **11** turned out to be a critical step. Treatment with potassium cyanide as base, in methanol, gave the desired 6b-*O*-deprotected compound **12**. However, the reaction had to be carefully monitored and stopped after 50% completion otherwise partial loss of the menthyloxycarbonyl (Mntoc) group also occurred. After chromatography, **12** could be isolated in 46% yield and 40% of the starting material **11** could be recovered. For the azide introduction at the 6b-position, a modified Mitsunobu reaction was employed:^[17] treatment of the diisopropylazodicarboxylate (DIAD)/triphenylphosphane-activated 6b-hydroxy group with the zinc azide pyridine complex^[17] as azide group donor afforded directly the 6b-azido compound **13**. The menthyloxy carbonyl group was then removed by addition of



Scheme 3. Synthesis of **5a, b**

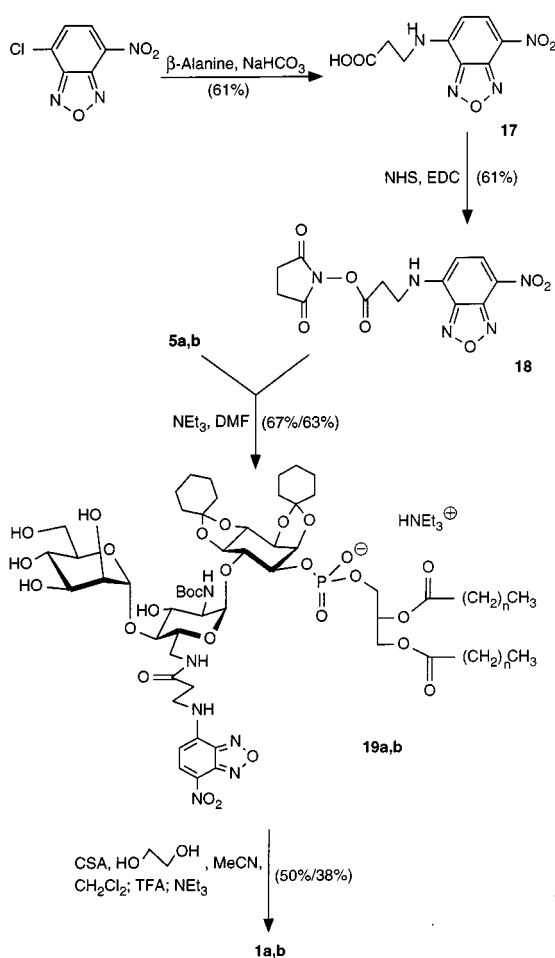
potassium carbonate as base, in methanol, thus providing the 1a-*O*-unprotected compound **14**.

Attachment of the phosphatidyl residues to **14** was performed with the phosphoramidites **15a,b**, which were readily obtained from bis(diisopropylamino)cyanoethoxyphosphane^[18] and 1,2-di-*O*-palmitoyl- or 1,2-di-*O*-myristoyl-sn-glycerol.^[19] Reaction of **14** with **15a,b** in the presence of tetrazole followed by oxidation with *tert*-butylhydroperoxide led to the corresponding phosphotriesters, which gave, on treatment with dimethylamine in ethanol, the diesters **16a** and **16b**. Hydrogenolysis with palladium hydroxide on carbon as catalyst (Pearman's catalyst^[20]), in a mixture of dichloromethane/methanol/water as solvent, led to clean *O*-debenzylation and to concomitant liberation of the am-

ino group in the 6b-position, thus providing target molecules **5a** and **5b** in high yields.

Attachment of the Labels to **5a,b**

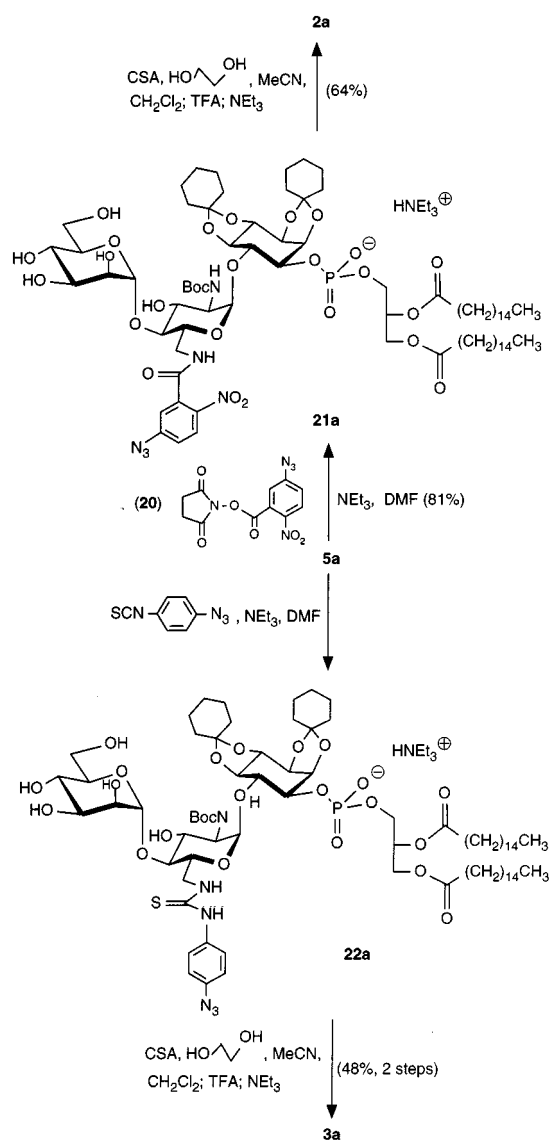
From the various fluorophores which have been employed for labeling experiments, the 7-nitrobenzofurazan (NBD) moiety seemed to be particularly interesting because it combines compactness with high sensitivity.^[21] In order to increase the distance to the GPI anchor structure a β -alanine spacer was inserted. Thus, commercially available 4-chloro-7-nitro-benzofurazan was treated with β -alanine in the presence of sodium hydrogencarbonate to give the substitution product **17** (Scheme 4). Activation of the acid moiety by treatment with *N*-hydroxysuccinimide (NHS) in the presence of the water-soluble carbodiimide EDC as condensing agent gave **18**. Reaction of **5a** or **5b** with **18** in DMF in the presence of triethylamine as base led to attachment of the NBD residue affording compounds **19a** and **19b**. Their treatment with camphorsulfonic acid (CSA) in the presence of ethylene glycol, in order to facilitate cyclohexylidene cleavage, and then with trifluoroacetic acid (TFA) and ensuing neutralization with triethylamine afforded the target molecules **1a** and **1b**. After flash chroma-



Scheme 4. Synthesis of **1a, b**

tography on silica gel with a mixture of butyl alcohol/ethanol/aqueous ammonia as eluent, **1a** and **1b** were obtained in satisfactory yields. The structures could be fully assigned based on DQF-COSY and HMBC NMR correlations (see Experimental Section).

The 5-azido-2-nitrobenzoyl (ANB) group is an excellent crosslinker because photoactivation is possible at 320–350 nm and the reactivity of the generated aryl nitrene intermediate is very high.^[22] Therefore the activated 5-azido-2-nitrobenzoate derivative **20** (Scheme 5) was prepared following literature procedures.^[22] Reaction of **20** with **5a** in DMF as solvent and in the presence of triethylamine led to amide bond formation affording **21a** in high yield. Ensuing de-*O*-cyclohexylidenation and removal of the *N*-Boc group was performed as described above furnishing the target molecule **2a** in 64% yield. The structural assignment was again based on DQF-COSY and HMBC correlations.



Scheme 5. Synthesis of **2a** and **3a**

Isothiocyanate addition to amino groups has been extensively employed for label attachment to peptides and pro-

teins. Therefore, direct reaction of **5a** with 4-azidophenyl isothiocyanate^[23] was investigated. In DMF as solvent and in the presence of triethylamine the 4-azidophenyl thiourea derivative **22a** was obtained, which exhibits a shoulder in the UV absorption spectrum at 320 nm, which can be used for the photoactivation. Compound **22a** was immediately deprotected as described above to afford the target molecule **3a** in 48% overall yield. Compound **3a** could also be fully structurally assigned.

Iodine isotopes are often chosen as radioactive labels for biological studies because they can be readily introduced into phenols by electrophilic aromatic substitution at the position *ortho* to the hydroxy group; an additional advantage of this choice is that γ -radiation of ^{125}I with a half life of 60 days makes this radiolabel readily detectable.^[24] A photoreactive compound which can be radiolabeled with ^{125}I is 4-azido salicylic acid. On activation with NHS this salicylic acid gave intermediate **23** (Scheme 6), which underwent reaction with **5a** in the presence of triethylamine and led to the amide derivative **24a**. The removal of all protective groups under the conditions described above furnished the desired 6b-(4-azido-2-hydroxybenzoylamido-6-deoxy) compound **25a**. Compounds **24a** and **25a** were fully characterized by NMR spectroscopy and mass spectrometry. Direct iodination of **25a** was performed on a small scale with

immobilized chloramine T (ODO BEADS)^[25]; the FAB-MS data of the product indicated that target molecule **4a** had been generated [$m/z = 1420$ (M. – H^+), 1407 (M. – $\text{H}^+ - \text{N}$), 1392 (M. – $\text{H}^+ - \text{N}_2$)].

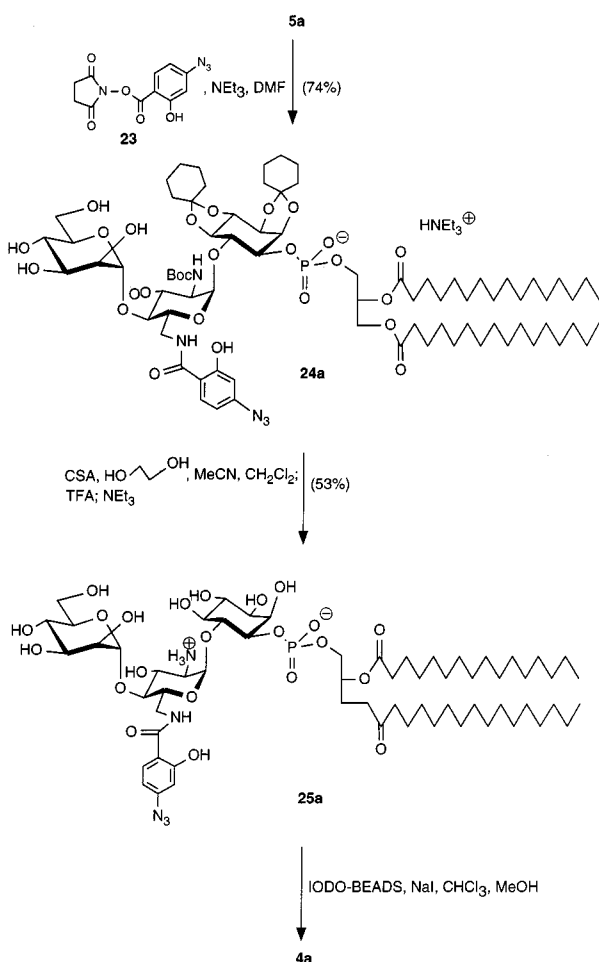
Successful biological studies have been most recently performed with target molecule **1a**,^[26] which confirmed the utility of the conceptual approach to cellular sorting studies with the help of the target molecules described in this paper.

Experimental Section

General: Solvents were purified in the usual way; boiling range of petroleum ether: 35–65°C. – Optical rotations: Perkin–Elmer polarimeter MC; 1 dm cell. – Thin-layer chromatography: Plastic sheets, silica gel 60 F₂₅₄ (Merck; layer thickness 0.2 mm). – Flash chromatography: Silica gel (J.T. Baker particle size 40 μm). – ^1H NMR: Bruker AC 250 (250 MHz) Cryospec, Bruker DRX 600 (600 MHz), internal standard tetramethylsilane (TMS). – ^{31}P NMR: Jeol JNM-GX 400; external standard 85% phosphoric acid. – Elemental analyses: Heraeus CHN-O-Rapid.

O-(2-O-Acetyl-3,4,6-tri-O-benzyl- α/β -D-mannopyranosyl)trichloroacetimidate (6): 3,4,6-Tri-*O*-benzyl-1,2-*O*-[1-(1*R*)-methoxyethylidene]- β -D-mannopyranose^[13] (21.4 g) was stirred for 2 h in 60% acetic acid (950 mL) and then the acetic acid was removed by evaporation. Flash chromatography of the residue yielded 2-*O*-acetyl-3,4,6-tri-*O*-benzyl- α/β -D-mannopyranose^[8,11,14] (20.8 g, 98%). An anomeric ratio of α/β (6.5:1) was determined by NMR spectroscopy. TLC (petroleum ether/ethyl acetate, 2:1): $R_f = 0.28$ (α -anomer), $R_f = 0.18$ (β -anomer). – ^1H NMR (250 MHz, CDCl_3): $\delta = 2.14$ (s, COCH_3), 2.19 (s, COCH_3), 3.42–3.78 (m), 3.99–4.09 (m), 4.45–4.86 (m, CH_2Ph , 1-H), 5.19 (br. s, 1-H), 5.35 (dd, $^3J_{1,2} = 1.9$ Hz, $^3J_{2,3} = 3.2$ Hz, 2-H), 5.44 (dd, $^3J_{1,2} = 1$ Hz, $^3J_{2,3} = 2$ Hz, 2-H), 7.13–7.35 (m, 15 H, Ph). – $\text{C}_{29}\text{H}_{32}\text{O}_7$ (492.6): calcd. C 70.71, H 6.55; found C 70.03, H 6.27. – This compound was transformed into **6** as previously described.^[11]

O-(2-O-Acetyl-3,4,6-tri-O-benzyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 4)-O-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4,5-di-*O*-cyclohexylidene-1-*O*-(1*R*)-menthyloxycarbonyl-D-myoinositol (8): To a solution of compounds **7**^[11] (3.4 g, 3.76 mmol) and **6** (3.4 g, 5.34 mmol) in dry diethyl ether were added powdered molecular sieves and the mixture stirred for 15 min under argon. Tin(II) trifluoromethanesulfonate (63 mg, 0.15 mmol) was then added and the reaction was stopped after 20 min by neutralization with solid NaHCO_3 . After evaporation of the solvent, the residue was purified by flash chromatography (petroleum ether/ethyl acetate, 6:1) to yield **8** (4.7 g, 91%). – TLC (petroleum ether/ethyl acetate, 4:1): $R_f = 0.50$. – $[\alpha]_D^{25} = +52$ ($c = 1$, CHCl_3). – ^1H NMR (250 MHz, CDCl_3): $\delta = 0.74$ –0.77 (d, $^3J = 6.9$ Hz, 3 H, CH_3), 0.81–1.08 (2 d, m, 9 H, 2 CH_3 , 3 H_{Mnt}), 1.30–1.78 (m, 24 H, 20 $\text{H}_{\text{Cyclohex}}$, 4 H_{Mnt}), 1.88–2.09 (m, 2 H, 2 H_{Mnt}), 1.98 (s, 3 H, COCH_3), 3.37–3.43 (m, 2 H, 2b-, 6c-H), 3.60 (dd, $^3J_{4a,5a} = 10.8$ Hz, $^3J_{5a,6a} = 8.7$ Hz, 1 H, 5a-H), 3.68 (dd, $^3J_{5c,6c'} = 2.9$ Hz, $J_{\text{gem}} = 10.6$ Hz, 1 H, 6c'-H), 3.75 (m 1 H, 5c-H), 3.89–3.93 (m 3 H, 3c-, 4c-H), 3.96–4.04 (m 3 H, 4a-, 3b-, 4b-H), 4.09 (dd, $^3J_{1a,6a} = 2.9$ Hz, $^3J_{5a,6a} = 8.6$ Hz, 1 H, 6a-H), 4.15–4.20 (m, 2 H, 0.5 CH_2Ph , 5b-H), 4.37–4.61 (m, 7 H, 1.5 CH_2Ph , 2a-, 3a-, 6b-H, H_{Mnt}), 4.67–4.77 (m, 3 H, CH_2Ph , 6b'-H), 4.82 (d, $J_{\text{gem}} = 10.7$ Hz, 1 H, 0.5 CH_2Ph), 4.93 (d, $J_{\text{gem}} = 10.7$ Hz, 1 H, 0.5 CH_2Ph), 4.96 (dd, $^3J_{1a,2a} = ^3J_{1a,6a} = 3.8$ Hz, 1 H, 1a-H), 5.33 (d, $^3J_{1b,2b} = 3.6$ Hz, 1 H, 1b-H), 5.47 (d, $^3J_{1c,2c} = 1.7$ Hz, 1 H, 1c-H), 5.54 (br. s, 1 H, 2c-H), 7.06–7.48 (m, 22 H, 4 Ph, *m*-COPh), 7.55–7.59 (m, 1 H,



Scheme 6. Synthesis of **4a**

p-COPh), 8.03–8.06 (m, 2 H, *o*-COPh). – $C_{78}H_{95}N_3O_{19}$ (1378.6): calcd. C 67.96, H 6.95, N 3.05; found C 67.91, H 6.96, N 3.33.

***O*-(3,4,6-Tri-*O*-benzyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 4)-*O*-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4,5-di-*O*-cyclohexylidene-1-*O*-(1*R*)-menthyloxycarbonyl-D-*myo*-inositol (9):** Compound **8** (3.1 g, 2.25 mmol) was dissolved in a solution of methylamine (33%) in dry ethanol (15 mL) and then stirred until the reaction was complete (2 h, monitored by TLC). After evaporation and flash chromatography (petroleum ether/ethyl acetate, 4:1) compound **9** (2.77 g, 92%) was obtained as a colorless foam. – TLC (petroleum ether/ethyl acetate, 7:3): R_f = 0.65. – $[\alpha]_D^{25} = +61$ (c = 1, $CHCl_3$). – 1H NMR (250 MHz, $CDCl_3$): δ = 0.74–0.77 (d, 3J = 6.9 Hz, 3 H, CH_3), 0.80–1.08 (2 d, m, 9 H, 2 CH_3 , 3 H_{Mnt}), 1.26–1.79 (m, 24 H, 20 $H_{Cyclohex.}$, 4 H_{Mnt}), 1.90–2.07 (m, 2 H, 2 H_{Mnt}), 2.19 (d, $^3J_{2c,OH}$ = 2.9 Hz, 1 H, OH), 3.37–3.48 (m, 2 H, 2b-, 6c-H), 3.56–3.84 (m, 4 H), 3.92–4.23 (m, 8 H), 4.38–4.77 (m, 11 H, 3 CH_2Ph , 2a-, 3a-, 6b-, 6b'-H, H_{Mnt}), 4.92–4.99 (m, 2 H, 0.5 CH_2Ph , 1a-H), 5.34 (d, $^3J_{1b,2b}$ = 3.5 Hz, 1 H, 1b-H), 5.39 (d, $^3J_{1c,2c}$ = 1.4 Hz, 1 H, 1c-H), 7.09–7.44 (m, 22 H, 4 Ph, *m*-COPh), 7.51–7.57 (m, 1 H, *p*-COPh), 8.03–8.07 (m, 2 H, *o*-COPh). – $C_{76}H_{93}N_3O_{18}$ (1336.6): calcd. C 68.29, H 7.01, N 3.14; found C 67.96, H 7.04, N 3.29.

***O*-(2,3,4,6-Tetra-*O*-benzyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 4)-*O*-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4,5-di-*O*-cyclohexylidene-1-*O*-(1*R*)-menthyloxycarbonyl-D-*myo*-inositol (10):** To a solution of compound **9** (2.2 g, 1.65 mmol) in dry dimethylformamide (5 mL) was added in portions sodium hydride (55 mg, 2.4 mmol) at 0°C. After hydrogen evolution stopped benzyl bromide (0.59 mL, 4.95 mmol) was added and the mixture warmed to room temperature. If some starting material remained after 24 h the solution was stirred at 40°C for a few more hours. The excess of sodium hydride was destroyed with small amounts of ethyl acetate and the reaction mixture was concentrated at 35°C under high vacuum. Flash chromatography of the residue (petroleum ether/ethyl acetate, 9:1) gave compound **10** (2.0 g, 85%). – TLC (petroleum ether/ethyl acetate, 4:1): R_f = 0.50. – $[\alpha]_D^{25} = +39$ (c = 0.86, $CHCl_3$). – 1H NMR (250 MHz, $CDCl_3$): δ = 0.74–0.77 (d, 3J = 6.9 Hz, 3 H, CH_3), 0.82–1.08 (2 d, m, 9 H, 2 CH_3 , 3 H_{Mnt}), 1.34–1.78 (m, 24 H, 20 $H_{Cyclohex.}$, 4 H_{Mnt}), 1.88–2.08 (m, 2 H, 2 H_{Mnt}), 3.41 (dd, $^3J_{1b,2b}$ = 3.7 Hz, $^3J_{2b,3b}$ = 9.5 Hz, 1 H, 2b-H), 3.54–4.78 (m, 26 H), 4.93–5.00 (m, 2 H, 0.5 CH_2Ph , 1a-H), 5.36–5.37 (m, 2 H, 1b-, 1c-H), 7.11–7.49 (m, 27 H, 5 Ph, *m*-COPh), 7.50–7.56 (m, 1 H, *p*-COPh), 8.04–8.07 (m, 2 H, *o*-COPh). – $C_{83}H_{99}N_3O_{18}$ (1426.7): calcd. C 69.88, H 6.99, N 2.95; found C 69.79, H 7.02, N 3.54.

***O*-(2,3,4,6-Tetra-*O*-benzyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 4)-*O*-(6-*O*-benzoyl-3-*O*-benzyl-2-*N*-(*tert*-butoxycarbonyl)amino-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4,5-di-*O*-cyclohexylidene-1-*O*-(1*R*)-menthyloxycarbonyl-D-*myo*-inositol (11):** Compound **10** (1 g, 0.701 mmol) was dissolved in pyridine (50 mL), water (7 mL) and propanedithiol (1.5 mL). An alkaline pH was established by addition of triethylamine and the mixture was stirred until the free amine had formed (16 h). TLC (petroleum ether/ethyl acetate, 7:3): R_f = 0.19. The solution was diluted with 150 mL of toluene/ethanol (5:1) and concentrated to 25% of its volume at 30°C. This procedure was repeated three times in order to coevaporate water. Di-*tert*-butyldicarbonate (0.76 g, 3.50 mmol) was then added and the mixture stirred for a few hours at room temperature. The solution was concentrated and coevaporated with toluene. Flash chromatography (petroleum ether $\rightarrow$ petroleum ether/ethyl acetate, 7:1) yielded compound **11** (0.95 g, 90%) as colorless foam. TLC (petroleum ether/ethyl acetate, 5:1): R_f = 0.32. – $[\alpha]_D^{25} = +27$ (c = 1,

$CHCl_3$). – 1H NMR (250 MHz, $CDCl_3$): δ = 0.72–0.75 (d, 3J = 6.9 Hz, 3 H, CH_3), 0.78–1.07 (2 d, m, 9 H, 2 CH_3 , 3 H_{Mnt}), 1.26–1.77 [s, m, 33 H, $C(CH_3)_3$, 20 $H_{Cyclohex.}$, 4 H_{Mnt}], 1.89–2.05 (m, 2 H, 2 H_{Mnt}), 3.52–4.82 (m, 29 H), 4.93 (bt, 1 H, 1a-H), 5.17 (d, $^3J_{1b,2b}$ = 3.0 Hz, 1 H, 1b-H), 5.29 (s, 1 H, 1c-H), 7.08–7.34 (m, 25 H, 5 Ph), 7.49–7.58 (m, 3 H, *m*-, *p*-COPh), 8.03–8.06 (m, 2 H, *o*-COPh). – $C_{88}H_{109}NO_{20}$ (1500.8): calcd. C 70.43, H 7.32, N 0.9; found C 70.42, H 7.53, N 1.0.

***O*-(2,3,4,6-Tetra-*O*-benzyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 4)-*O*-(3-*O*-benzyl-2-*N*-(*tert*-butoxycarbonyl)amino-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4,5-di-*O*-cyclohexylidene-1-*O*-(1*R*)-menthyloxycarbonyl-D-*myo*-inositol (12):** Compound **11** (0.9 g, 0.60 mmol) was dissolved in a mixture of dry diethyl ether and methanol (30 mL 1:1); then potassium cyanide (0.45 g) was added and the mixture was heated to 40°C. After 50% conversion, the reaction was stopped (7 h, monitored by TLC) by careful separation of the potassium cyanide by filtration through Celite. The solvents were evaporated and flash chromatography [petroleum ether/ethyl acetate, (5:1) $\rightarrow$ (4:1)] yielded compound **12** (384 mg, 46%). Starting material (360 mg, 40%) was also recovered. TLC (petroleum ether/ethyl acetate, 4:1): R_f = 0.23. – $[\alpha]_D^{25} = +17$ (c = 1, $CHCl_3$). – 1H NMR (250 MHz, $CDCl_3$): δ = 0.73–0.76 (d, 3J = 6.9 Hz, 3 H, CH_3), 0.79–1.13 (2 d, m, 9 H, 2 CH_3 , 3 H_{Mnt}), 1.24–1.77 [s, m, 33 H, $C(CH_3)_3$, 20 $H_{Cyclohex.}$, 4 H_{Mnt}], 1.89–2.00 (m, 1 H, H_{Mnt}), 2.10–2.19 (m, 1 H, H_{Mnt}), 3.12 (bt, 1 H, OH), 3.50–4.13 (m, 17 H), 4.40–4.81 (m, 12 H, 4 CH_2Ph , $NHCOO$, 2a-, 3a-, H_{Mnt}), 4.94 (bt, 1 H, 1a-H), 5.11 (d, $^3J_{1b,2b}$ = 3.3 Hz, 1 H, 1b-H), 5.29 (br. s, 1 H, 1c-H), 7.09–7.37 (m, 25 H, 5 Ph). – FAB-MS (positive-mode, matrix: 3-nitrobenzyl alcohol with NaI): m/z = 1419 [$M + Na^+$]. – $C_{81}H_{105}NO_{19}$ (1396.72).

***O*-(2,3,4,6-Tetra-*O*-benzyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 4)-*O*-(6-azido-3-*O*-benzyl-2-*N*-(*tert*-butoxy-carbonyl)amino-2,6-di-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4,5-di-*O*-cyclohexylidene-1-*O*-(1*R*)-menthyloxycarbonyl-D-*myo*-inositol (13):** Zinc azide dipyrindine complex^[17] (0.39 g, 2.55 mmol) was suspended in a solution of compound **12** (0.47 g, 0.34 mmol) and triphenylphosphane (0.89 g, 3.4 mmol) in dry toluene (5 mL). Diisopropyl azodicarboxylate (DIAD, 0.68 mL, 3.4 mmol) was added dropwise while stirring. The mixture was heated to 50°C for 30 min to accelerate the reaction. The heterogeneous mixture was filtered through Celite and the Celite was washed several times with toluene. The solvent was removed under reduced pressure and flash chromatography (petroleum ether/ethyl acetate, 4:1) yielded compound **13** (0.42 g, 87%) as a colorless foam. TLC (petroleum ether/ethyl acetate, 4:1): R_f = 0.40. – $[\alpha]_D^{25} = +35$ (c = 0.33, $CHCl_3$). – 1H NMR (250 MHz, $CDCl_3$): δ = 0.73–0.76 (d, 3J = 6.9 Hz, 3 H, CH_3), 0.86–1.13 (2 d, m, 9 H, 2 CH_3 , 3 H_{Mnt}), 1.25–1.75 [s, m, 33 H, $C(CH_3)_3$, 20 $H_{Cyclohex.}$, 4 H_{Mnt}], 1.88–1.98 (m, 1 H, H_{Mnt}), 2.06–2.15 (m, 1 H, H_{Mnt}), 3.45–4.22 (m, 17 H), 4.40–4.84 (m, 12 H, 4 CH_2Ph , $NHCOO$, 2a-, 3a-, H_{Mnt}), 4.91 (bt, 1 H, 1a-H), 5.18 (m, 2 H, 1b-H, 1c-H), 7.09–7.32 (m, 25 H, 5 Ph). – FAB-MS (positive-mode, matrix: 3-nitrobenzyl alcohol with NaI): m/z = 1445 [$M + Na^+$]. – $C_{81}H_{104}N_4O_{18}$ (1421.73).

***O*-(2,3,4,6-Tetra-*O*-benzyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 4)-*O*-(6-azido-3-*O*-benzyl-2-*N*-(*tert*-butoxycarbonyl)-amino-2,6-di-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4,5-di-*O*-cyclohexylidene-D-*myo*-inositol (14):** Potassium carbonate (1.0 g) was suspended in a solution of compound **13** (0.42 g, 0.30 mmol) in dry methanol (25 mL) and the mixture was heated to 60°C under vigorous stirring. After the reaction was complete (1–2 h, TLC monitoring) the solvent was removed, the residue was mixed with water and ethyl acetate and the organic layer separated. The aqueous layer was then

washed again with ethyl acetate. The combined organic layers were dried with Na_2SO_4 and the solvent evaporated. Flash chromatography (petroleum ether/ethyl acetate, 4:1) yielded compound **14** (0.35 g, 94%). TLC (petroleum ether/ethyl acetate, 4:1): $R_f = 0.13$. – $[\alpha]_D = +67$ ($c = 0.5$, CHCl_3). – ^1H NMR (250 MHz, CDCl_3): $\delta = 1.25\text{--}1.78$ [s, m, 29 H, $\text{C}(\text{CH}_3)_3$, 20 $\text{H}_{\text{Cyclohex}}$], 2.61 (br. s, 1 H, OH), 3.40–3.60 (m, 4 H), 3.70–4.12 (m, 12 H), 4.25–4.84 (m, 13 H, 5 CH_2Ph , NHCOO , 2a-, 3a-H), 5.11 (br. s, 1 H, 1b-H), 5.22 (br. s, 1 H, 1c-H), 7.15–7.34 (m, 25 H, 5 Ph). – $\text{C}_{70}\text{H}_{86}\text{N}_4\text{O}_{16}$ (1239.5): calcd. C 67.83, H 6.99, N 4.52; found C 67.51, H 7.05, N 4.65.

General Procedure for the Synthesis of Compounds 15a,b: (S)-1,2-Di-O-acylglycerols (0.70 mmol) and (2-cyanoethoxy)bis(diisopropylamino)phosphane^[18] were dissolved in dry acetonitrile/dichloromethane (15 mL, 2:3). After addition of tetrazole (30 mg, 0.42 mmol) the mixture was stirred for 4 h. The solution was diluted with dichloromethane and washed with saturated sodium hydrogencarbonate solution, and water. The organic layer was dried (Na_2SO_4), the solvents were removed and then fast flash chromatography (petroleum ether/ethyl acetate, 4:1) was performed in order to purify compounds **15a,b**.

[(R)-2,3-Bis{(1-oxohexadecyl)oxy}propyloxy](2-cyanoethoxy)-(diisopropylamino)phosphane (15a): Treatment of (S)-glycerine-1,2-dipalmitate^[19] (400 mg) according to the general procedure afforded **15a** (470 mg, 87%) as a waxy solid. TLC (petroleum ether/ethyl acetate, 4:1): $R_f = 0.67$. – $[\alpha]_D = -5$ ($c = 4$, CHCl_3). – ^1H NMR (250 MHz, CDCl_3): $\delta = 0.81\text{--}0.86$ (t, $^3J = 6.2$ Hz, 6 H, 2 CH_3), 1.11–1.15 [2 d, m, 12 H, 2 $\text{CH}(\text{CH}_3)_2$], 1.17–1.29 (m, 48 H, 24 CH_2), 1.51–1.61 (m, 4 H, 2 COCH_2CH_2), 2.22–2.31 (m, 4 H, 2 COCH_2), 2.56–2.61 (t, $^3J = 6.4$ Hz, 2 H, CH_2CN), 3.48–3.85 [m, 6 H, 2 $\text{CH}(\text{CH}_3)_2$, OCH_2 , 1-, 1'-H], 4.07–4.17 (m, 1 H, 3 -H), 4.25–4.34 (m, 1 H, 1'-H), 5.11–5.20 (m, 1 H, 2-H). – ^{31}P NMR [161.7 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$ (1:1)]: $\delta = 150.05$, 150.19. – FAB-MS (positive-mode, matrix: 3-nitrobenzyl alcohol): $m/z = 808$ [$\text{M} + \text{K}^+$]. – $\text{C}_{44}\text{H}_{85}\text{N}_2\text{O}_6\text{P}$ (769.14).

[(R)-2,3-Bis{(1-oxotetradecyl)oxy}propyloxy](2-cyanoethoxy)-(diisopropylamino)phosphane (15b): Treatment of (S)-glycerine-1,2-dimyristate^[19] (359 mg) according to the general procedure afforded **15b** (261 mg, 52%) as a waxy solid. TLC (petroleum ether/ethyl acetate, 4:1): $R_f = 0.67$. – ^1H NMR (250 MHz, CDCl_3): $\delta = 0.85\text{--}0.90$ (t, $^3J = 6.2$ Hz, 6 H, 2 CH_3), 1.15–1.20 [2 d, m, 12 H, 2 $\text{CH}(\text{CH}_3)_2$], 1.22–1.35 (m, 40 H, 20 CH_2), 1.55–1.68 (m, 4 H, 2 COCH_2CH_2), 2.27–2.36 (m, 4 H, 2 COCH_2), 2.60–2.67 (t, $^3J = 6.4$ Hz, 2 H, CH_2CN), 3.53–3.90 [m, 6 H, 2 $\text{CH}(\text{CH}_3)_2$, OCH_2 , 1-, 1'-H], 4.11–4.22 (m, 1 H, 3 -H), 4.29–4.40 (m, 1 H, 1'-H), 5.13–5.23 (m, 1 H, 2-H). – ^{31}P NMR (161.7 MHz, CDCl_3): $\delta = 149.90$, 150.13. – FAB-MS (positive-mode, matrix: 3-nitrobenzyl alcohol with NaI): $m/z = 735$ [$\text{M} + \text{Na}^+$]. – $\text{C}_{40}\text{H}_{77}\text{N}_2\text{O}_6\text{P}$ (713.03).

General Procedure for the Synthesis of Compounds 16a,b: Compound **14** (340 mg, 0.274 mmol), phosphitamides **15a,b** (0.520 mmol) and tetrazole (50 mg, 0.71 mmol) were dried in the reaction flask under high vacuum for 1 h, then dry dichloromethane (8 mL) was added and the mixture was stirred for 2 h to obtain the phosphite [TLC (petroleum ether/ethyl acetate, 4:1): $R_f = 0.38$]. After the reaction was complete, *tert*-butylhydroperoxide (2 mL, 3 M in toluene) was added and oxidation to the phosphate occurred within 1.5 h [TLC (petroleum ether/ethyl acetate, 4:1): $R_f = 0.06$]. Subsequently the mixture was concentrated to a volume of 2 mL. A solution of dimethylamine (33% in dry ethanol, 5 mL) was added and stirred for 30 min to cleave the cyanoethoxy group [TLC (toluene/acetone, 1:1): $R_f = 0.45$]. The concentrated mixture (1 mL) was

diluted with chloroform and washed with half-saturated sodium hydrogencarbonate solution. The aqueous layer was extracted once more with chloroform, and the organic layer was dried (Na_2SO_4) and concentrated. Purification by flash chromatography yielded compounds **16a,b**.

O-(2,3,4,6-Tetra-O-benzyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 4)-O-[6-azido-3-O-benzyl-2-N-(*tert*-butoxycarbonyl)amino-2,6-dideoxy- α -D-glucopyranosyl]-(1 $\rightarrow$ 6)-2,3,4,5-di-O-cyclohexylidene-1-[(R)-2,3-bis{(1-oxohexadecyl)oxy}propyldimethylammonium phosphate]-D-myo-inositol (16a): Treatment of **15a** (400 mg) according to the general procedure afforded **16a** (495 mg, 79%) after flash chromatography (toluene $\rightarrow$ toluene/acetone, 9:1 $\rightarrow$ toluene/acetone, 5:3 $\rightarrow$ toluene/acetone, 1:2) [TLC (toluene/acetone, 1:1): $R_f = 0.10\text{--}0.20$]. – $[\alpha]_D = +25$ ($c = 0.5$, CHCl_3). – ^1H NMR [250 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$ (1:1)]: $\delta = 0.83\text{--}0.90$ (t, $^3J = 6.4$ Hz, 6 H, 2 CH_3), 1.22–1.75 [m, 84 H, $\text{C}(\text{CH}_3)_3$, 20 $\text{H}_{\text{Cyclohex}}$, 26 CH_2 , CH_3NH_3^+], 2.17–2.40 (m, 4 H, 2 COCH_2), 3.25–4.92 (m, 32 H), 5.21–5.36 (m, 3 H, 1b-, 1c-, 2 g-H), 7.09–7.35 (m, 25 H, 5 Ph). – ^{31}P NMR [161.7 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$ (1:1)]: $\delta = -0.90$. – FAB-MS (positive-mode, matrix: 3-nitrobenzyl alcohol with NaI): $m/z = 1915$ [$\text{M} - \text{Na}^+ \text{Na}^+$]. – $[\text{C}_{105}\text{H}_{152}\text{N}_4\text{O}_{23}\text{P}]^-$ (1869.34).

O-(2,3,4,6-Tetra-O-benzyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 4)-O-[6-azido-3-O-benzyl-2-N-(*tert*-butoxycarbonyl)amino-2,6-di-deoxy- α -D-glucopyranosyl]-(1 $\rightarrow$ 6)-2,3,4,5-di-O-cyclohexylidene-1-[(R)-2,3-bis{(1-oxotetradecyl)oxy}propyldimethylammonium phosphate]-D-myo-inositol (16b): Treatment of **15b** (371 mg) according to the general procedure afforded **16b** (437 mg, 88%) after flash chromatography (toluene/acetone, 9:1 $\rightarrow$ toluene/acetone, 1:1 $\rightarrow$ toluene/acetone, 1:2) [TLC (toluene/acetone, 1:1): $R_f = 0.10\text{--}0.20$]. – ^1H NMR [250 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$ (1:1)]: $\delta = 0.82\text{--}0.90$ (t, $^3J = 6.4$ Hz, 6 H, 2 CH_3), 1.15–1.75 [m, 76 H, $\text{C}(\text{CH}_3)_3$, 20 $\text{H}_{\text{Cyclohex}}$, 22 CH_2 , CH_3NH_3^+], 2.18–2.30 (m, 4 H, 2 COCH_2), 3.25–4.92 (m, 32 H), 5.18–5.37 (m, 3 H, 1b-, 1c-, 2 g-H), 7.08–7.35 (m, 25 H, 5 Ph). – FAB-MS (positive-mode, matrix: 3-nitrobenzyl alcohol with NaI): $m/z = 1859$ [$\text{M} - \text{Na}^+ \text{Na}^+$]. – $[\text{C}_{101}\text{H}_{144}\text{N}_4\text{O}_{23}\text{P}]^-$ (1813.23).

General Procedure for the Synthesis of Compounds 5a,b: Pearlman catalyst [$\text{Pd}(\text{OH})_2$ on charcoal, 20 mg] was added to a solution of compound **16a,b** (80 μm) in methanol (4 mL), dichloromethane (4 mL), and water (0.1 mL), and the mixture was stirred under a hydrogen atmosphere. The reaction was monitored by TLC [**5a,b**: (chloroform/methanol, 4:1), $R_f = 0.28$] at 5 min intervals. If the reaction did not start within 20 min more Pearlman catalyst (20 mg) was added. At the end of the reaction (0.5–2 h) triethylamine was added until pH = 7 was reached. The mixture was then filtered through Celite. The Celite was washed thoroughly with chloroform/methanol (3:2). Clean **5a,b** was obtained directly after evaporation of the solvents. For the following reaction the compound was dissolved in dimethylformamide (4 mL) and triethylamine (0.1 mL).

O-(α -D-Mannopyranosyl)-(1 $\rightarrow$ 4)-O-[6-amino-2-N-(*tert*-butoxycarbonyl)amino-2,6-di-deoxy- α -D-glucopyranosyl]-(1 $\rightarrow$ 6)-2,3,4,5-di-O-cyclohexylidene-1-[(R)-2,3-bis{(1-oxohexadecyl)oxy}propyldimethylammonium phosphate]-D-myo-inositol (5a): Treatment of **16a** (150 mg) according to the general procedure afforded **5a** (81 mg, 89%). TLC (chloroform/methanol, 4:1), $R_f = 0.28$. – ^1H NMR [250 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$ (1:1)]: $\delta = 0.83\text{--}0.92$ (t, $^3J = 6.4$ Hz, 6 H, 2 CH_3), 1.21–1.81 [m, 81 H, $\text{C}(\text{CH}_3)_3$, 20 $\text{H}_{\text{Cyclohex}}$, 26 CH_2], 2.27–2.38 (m, 4 H, 2 COCH_2), 3.07 (dd, $^3J_{1b,2b} = 3.5$ Hz, $^3J_{2b,3b} = 8.0$ Hz, 1 H, 2b-H), 3.40–4.53 (m, 21 H), 5.19–5.25 (m, 2 H, 1c-, 2 g-H), 5.35 (d, $^3J_{1b,2b} = 3.6$ Hz, 1 H, 1b-H). – ^{31}P NMR [161.7 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$ (1:1)]: $\delta = -1.24$. – FAB-MS [nega-

tive-mode, matrix: 3-nitrobenzyl alcohol/glycerol (1:1); m/z = 1392 [M^-]. – $C_{70}H_{125}N_2O_{23}P$ (1393.73).

***O*-(α -D-Mannopyranosyl)-(1 $\rightarrow$ 4)-*O*-[6-amino-2-*N*-(*tert*-butoxycarbonyl)amino-2,6-di-deoxy- α -D-glucopyranosyl]-(1 $\rightarrow$ 6)-2,3,4,5-di-*O*-cyclohexylidene-1-[(*R*)-2,3-bis{(1-oxotetradecyl)oxy}propyl]dimethylammonium phosphate]-D-*myo*-inositol (**5b**): Treatment of **16b** (145 mg) according to the general procedure afforded **5b** (96 mg, 89%). TLC (chloroform/methanol, 4:1), R_f = 0.28. – 1H NMR [250 MHz, $CDCl_3/CD_3OD$ (1:1)]: δ = 0.84–0.92 (t, 3J = 6.4 Hz, 6 H, 2 CH_3), 1.21–1.81 [m, 73 H, $C(CH_3)_3$, 20 $H_{Cyclohex.}$, 22 CH_2], 2.28–2.36 (m, 4 H, 2 $COCH_2$), 3.07 (dd, $^3J_{1b,2b}$ = 3.5 Hz, $^3J_{2b,3b}$ = 8.0 Hz, 1 H, 2b-H), 3.40–4.53 (m, 21 H), 5.17–5.30 (m, 2 H, 1c-, 2g-H), 5.37 (d, $^3J_{1b,2b}$ = 3.6 Hz, 1 H, 1b-H). – FAB-MS [positive-mode, matrix: 3-nitrobenzyl alcohol/glycerol (1:1)]; m/z = 1338 [M^+]. – $C_{66}H_{117}N_2O_{23}P$ (1337.62).**

3-[*N*-(7'-Nitrobenz-2'-oxa-1',3'-diazol-4'-yl)amino]propanoic Acid (17**):** β -Alanine (0.23 g, 2.5 mmol) and sodium hydrogencarbonate (0.63 g, 7.5 mmol) were dissolved in water (5 mL). After addition of a solution of 4-chloro-7-nitro-benzofurazane (0.5 g, 2.5 mmol) in methanol (20 mL) the mixture was stirred for 1 h at 50°C. The mixture was then cooled to room temperature and the pH was adjusted to 1 by the addition of 2 M hydrochloric acid (2–3 mL). After evaporation of the solvents, the product was crystallized by addition of water. The crystals were dissolved in sodium hydrogencarbonate solution (0.25 g in 5 mL water) and crystallized once more by addition of 2 M hydrochloric acid (2–3 mL) to yield pure compound **17** (0.39 g, 61%) as a brown solid. m.p.: > 199°C, decomposition. – TLC (chloroform/methanol, 4:1): R_f = 0.32. – 1H NMR [250 MHz, $CDCl_3/CD_3OD$ (1:1)]: δ = 2.80 (t, 3J = 6.7 Hz, 2 H, $COCH_2$), 3.85 (bt, 2 H, CH_2NH), 6.33 (d, $^3J_{5',6'}$ = 8.7 Hz, 1 H, 5'-H), 8.54 (d, $^3J_{5',6'}$ = 8.7 Hz, 1 H, 6'-H). – EI-MS (positive-mode, 70 eV, 195°C): m/z = 252 [M^+]. – radical]. – $C_9H_8N_4O_5$ (252.19).

***N*-[2-{*N*-(7'-Nitrobenz-2'-oxa-1',3'-diazol-4'-yl)amino}ethyl-carbonyloxy]succinimide (**18**):** Compound **17** (250 mg, 1.0 mmol) and *N*-hydroxysuccinimide (138 mg, 1.2 mmol) were dissolved in a mixture of dry dioxane (5 mL) and dry dichloromethane (5 mL). After addition of *N*-(3-dimethylaminopropyl)-*N'*-ethyl-carbodiimide hydrochloride (230 mg, 1.2 mmol) the mixture was stirred for 2 h. The solvents were then removed and the residue was poured into a half-saturated sodium chloride solution and ethyl acetate. After separation of the organic layer the aqueous layer was extracted once more with ethyl acetate. The dried organic layers (Na_2SO_4) were concentrated and purified by flash chromatography (ethyl acetate) to yield **18** (210 mg, 61%) as a red orange powder. m.p.: > 188°C, decomposition. – TLC (ethyl acetate): R_f = 0.68. – 1H NMR [250 MHz, $CDCl_3/CD_3OD$ (1:1)]: δ = 2.79 (s, 4 H, 4 $H_{Succ.}$), 3.07 (t, 3J = 6.6 Hz, 2 H, $COCH_2$), 3.88 (bt, 2 H, CH_2NH), 6.29 (d, $^3J_{5',6'}$ = 8.7 Hz, 1 H, 5'-H), 8.45 (d, $^3J_{5',6'}$ = 8.7 Hz, 1 H, 6'-H). – EI-MS (positive-mode, 70 eV, 220°C): m/z = 349 [M^+]. – radical]. – $C_{13}H_{11}N_5O_7$ (349.26).

General Procedure for the Synthesis of Compounds 19a,b: To a solution of compound **5a,b** (58 μ mol) in dimethylformamide (4 mL) and triethylamine (0.1 mL) was added compound **18** (81 mg, 234 μ mol). The solution was stirred for 1 h and then the solvents were evaporated under high vacuum. The residue was purified by flash chromatography (ethyl acetate/methanol, 4:1) to yield compound **19a,b**.

***O*-(α -D-Mannopyranosyl)-(1 $\rightarrow$ 4)-*O*-[6-{3-[*N*-(7'-nitrobenz-2'-oxa-1',3'-diazol-4'-yl)amino]-1-oxopropyl}amino-2-*N*-(*tert*-butoxycarbonyl)amino-2,6-di-deoxy- α -D-glucopyranosyl]-(1 $\rightarrow$ 6)-2,3,4,5-di-*O*-cyclohexylidene-1-[(*R*)-2,3-bis{(1-oxohexadecyl)oxy}propyl]tri-**

ethylammonium phosphate]-D-*myo*-inositol (19a**):** Treatment of **5a** (81 mg) according to the general procedure afforded **19a** (72 mg, 67%) as an amorphous red solid. TLC (ethyl acetate/methanol, 4:1): R_f = 0.08. – $[\alpha]_D$ = +86 (c = 1, $CHCl_3$). – 1H NMR [600 MHz, $CDCl_3/CD_3OD$ (1:1)]: δ = 0.87–0.90 (t, 3J = 7.1 Hz, 6 H, 2 CH_3), 1.24–1.77 [m, 90 H, $C(CH_3)_3$, 20 $H_{Cyclohex.}$, 26 CH_2 , $(CH_3CH_2)_3NH^+$], 2.29–2.33 (m, 4 H, 2 $COCH_2$), 2.70–2.85 (m, 2 H, CH_2NH), 3.14–3.18 [m, 6 H, $(CH_3CH_2)_3NH^+$], 3.48–4.58 (m, 24 H), 5.24–5.31 (m, 3 H, 1b-, 1c-, 2g-H), 6.40 (br. s, 1 H, 5'-H), 8.52 (d, $^3J_{5',6'}$ = 8.8 Hz, 1 H, 6'-H). – ^{31}P NMR [$CDCl_3/CD_3OD$ (1:1)]: δ = 0.008. – FAB-MS [negative-mode, matrix: 3-nitrobenzyl alcohol/glycerol (1:1)]; m/z = 1627 [M^+]. – $[C_{79}H_{130}N_6O_{27}P]^-$ (1626.89).

***O*-(α -D-Mannopyranosyl)-(1 $\rightarrow$ 4)-*O*-[6-{3-[*N*-(7'-nitrobenz-2'-oxa-1',3'-diazol-4'-yl)amino]-1-oxopropyl}amino-2-*N*-(*tert*-butoxycarbonyl)amino-2,6-di-deoxy- α -D-glucopyranosyl]-(1 $\rightarrow$ 6)-2,3,4,5-di-*O*-cyclohexylidene-1-[(*R*)-2,3-bis{(1-oxotetradecyl)oxy}propyl]triethylammonium phosphate]-D-*myo*-inositol (**19b**):** Treatment of **5b** (78 mg) according to the general procedure afforded **19b** (58 mg, 63%) as an amorphous red solid. TLC (ethyl acetate/methanol, 4:1): R_f = 0.08. – 1H NMR [250 MHz, $CDCl_3/CD_3OD$ (1:1)]: δ = 0.87–0.90 (t, 3J = 6.6 Hz, 6 H, 2 CH_3), 1.23–1.75 [m, 82 H, $C(CH_3)_3$, 20 $H_{Cyclohex.}$, 22 CH_2 , $(CH_3CH_2)_3NH^+$], 2.29–2.35 (m, 4 H, 2 $COCH_2$), 2.75–2.85 (m, 2 H, CH_2NH), 3.14–3.23 [m, 6 H, $(CH_3CH_2)_3NH^+$], 3.48–4.46 (m, 24 H), 5.26–5.31 (m, 3 H, 1b-, 1c-, 2g-H), 6.38–6.42 (d, $^3J_{5',6'}$ = 8.8 Hz, 1 H, 5'-H), 8.53–8.56 (d, $^3J_{5',6'}$ = 8.8 Hz, 1 H, 6'-H). – FAB-MS (positive-mode, matrix: 3-nitrobenzyl alcohol with NaI): m/z = 1594 [M^+ + Na^+]. – $C_{75}H_{123}N_6O_{27}P$ (1570.78).

General Procedure for the Synthesis of Compounds 1a,b: Compound **19a,b** (26.0 μ mol) was dissolved in a mixture of dichloromethane/acetonitrile, (4 mL, 1:1) and ethanediol (135 μ L). The solution was acidified to pH = 1 with camphor-10-sulfonic acid and stirred for 4 h under argon. The *tert*-butoxycarbonyl group was cleaved on addition of trifluoroacetic acid (1 mL). After 2 h, triethylamine was added until the pH was alkaline. The mixture was then concentrated under high vacuum. Ethanediol was removed by flash chromatography (*n*-butyl alcohol/ethanol/conc. ammonia solution/water, 30:30:2:2) and pure compound **1a,b** was obtained after elution with *n*-butyl alcohol/ethanol/conc. ammonia solution/water = 30:30:5:5.

***O*-(α -D-Mannopyranosyl)-(1 $\rightarrow$ 4)-*O*-[2-amino-2,6-di-deoxy-6-{3-[*N*-(7'-nitrobenz-2'-oxa-1',3'-diazol-4'-yl)amino]-1-oxopropyl}amino- α -D-glucopyranosyl]-(1 $\rightarrow$ 6)-2,3,4,5-di-*O*-cyclohexylidene-1-[(*R*)-2,3-bis{(1-oxohexadecyl)oxy}propyl] hydrogen phosphate]-D-*myo*-inositol (**1a**):** Treatment of **19a** (45 mg) according to the general procedure afforded **1a** (19 mg, 50%) as an orange solid. TLC (*n*-butyl alcohol/ethanol/conc. ammonia/water, 4:4:1:1): R_f = 0.24. – 1H NMR [600 MHz, $CDCl_3/CD_3OD/D_2O$ (1:1:0.15)]: δ = 0.87–0.90 (2 t, 3J = 7.1 Hz, 6 H, 2 CH_3), 1.25–1.35 (m, 48 H, 24 CH_2), 1.57–1.65 (m, 4 H, 2 $COCH_2CH_2$), 2.31–2.37 (2 t, 4 H, 2 $COCH_2$), 2.74 (t, 2 H, 3J = 6.8 Hz, CH_2NH), 3.20 (dd, $^3J_{1b,2b}$ = 3.8 Hz, $^3J_{2b,3b}$ = 10.7 Hz, 1 H, 2b-H), 3.24 (dd, J_{gem} = 13 Hz, $^3J_{5b,6b}$ = 8.0 Hz, 1 H, 6b-H), 3.36 (dd, $^3J_{4a,5a}$ = $^3J_{5a,6a}$ = 9.3 Hz, 1 H, 5a-H), 3.46 (dd, $^3J_{3a,4a}$ = 9.2 Hz, 1 H, 3a-H), 3.48 (dd, $^3J_{3b,4b}$ = $^3J_{4b,5b}$ = 9.5 Hz, 1 H, 4b-H), 3.64 (dd, $^3J_{3c,4c}$ = $^3J_{4c,5c}$ = 7.8 Hz, 1 H, 4c-H), 3.66 ($^3J_{3a,4a}$ = 9.2 Hz, $^3J_{4a,5a}$ = 9.3 Hz, 1 H, 4a-H), 3.67 ($^3J_{4c,5c}$ = 7.8 Hz, 1 H, 5c-H), 3.72 ($^3J_{3c,4c}$ = 7.8 Hz, 1 H, 3c-H), 3.74 (J_{gem} = 11 Hz, 1 H, 6c-H), 3.76 (bd, J_{gem} = 13 Hz, 1 H, 6b'-H), 3.87 (J_{gem} = 11 Hz, 1 H, 6c'-H), 3.96 (dd, $^3J_{1a,6a}$ = 9.5 Hz, $^3J_{5a,6a}$ = 9.3 Hz, 1 H, 6a-H), 3.98 (1 H, 2c-H), 3.99 ($^3J_{2b,3b}$ = 10.7 Hz, $^3J_{2b,3b}$ = 9.5 Hz, 1 H, 3b-H), 4.03 (1 H, 1g'-H), 4.10 (bt, 1 H,

2a-H), 4.19 (1 H, 1a-H), 4.21 (1 H, 3 g-H), 4.29 (m, $^3J_{4b,5b} = 9.5$ Hz, $^3J_{5b,6b} = 8.0$ Hz, 1 H, 5b-H), 4.42 (1 H, 3g'-H), 5.25 (s, 1 H, 1c-H), 5.28 (1 H, 2 g-H), 5.49 (d, $^3J_{1b,2b} = 3.8$ Hz, 1 H, 1b-H), 6.42 (d, $^3J_{5',6'} = 8.8$ Hz, 1 H, 5'-H), 8.56 (d, $^3J_{5',6'} = 8.8$ Hz, 1 H, 6'-H). – ^{31}P NMR [161.7 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}/\text{D}_2\text{O}$ (1:1:0.15)]: $\delta = -0.95$. – FAB-MS [negative-mode, matrix: 3-nitrobenzyl alcohol/glycerol (1:1)]: $m/z = 1365$ [M^-]. – $[\text{C}_{62}\text{H}_{107}\text{N}_6\text{O}_{25}\text{P}]^-$ (1367.53).

O-(α -D-Mannopyranosyl)-(1 $\rightarrow$ 4)-O-[2-amino-2,6-di-deoxy-6-{3-[N-(7'-nitrobenz-2'-oxa-1',3'-diazol-4'-yl)amino]-1-oxopropyl}amino- α -D-glucopyranosyl]-(1 $\rightarrow$ 6)-2,3:4,5-di-O-cyclohexylidene-1-[(R)-2,3-bis{(1-oxotetradecyl)oxy}propyl hydrogen phosphate]-D-myo-inositol (1b): Treatment of 19b (41 mg) according to the general procedure afforded 1b (13 mg, 38%) as an orange solid. TLC (*n*-butyl alcohol/ethanol/conc. ammonia solution/water, 4:4:1:1): $R_f = 0.24$. – ^1H NMR [250 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}/\text{D}_2\text{O}$ (1:1:0.15)]: $\delta = 0.87\text{--}0.90$ (2 t, $^3J = 7.1$ Hz, 6 H, 2 CH_3), 1.25–1.35 (m, 40 H, 20 CH_2), 1.55–1.70 (m, 4 H, 2 COCH_2CH_2), 2.30–2.40 (2 t, 4 H, 2 COCH_2), 2.72–2.77 (t, 2 H, $^3J = 6.8$ Hz, CH_2NH), 3.15–4.35 (m, 24 H), 5.25–5.30 (m, 2 H, 1c-, 2-H), 5.48 (d, 1 H 1b-H), 6.42–6.46 (d, $^3J_{5',6'} = 8.8$ Hz, 1 H, 5'-H), 8.56–8.60 (d, $^3J_{5',6'} = 8.8$ Hz, 1 H, 6'-H). – FAB-MS [negative-mode, matrix: 3-nitrobenzyl alcohol/glycerol (1:1)]: $m/z = 1310$ [M^-]. – $[\text{C}_{58}\text{H}_{99}\text{N}_6\text{O}_{25}\text{P}]^-$ (1311.42).

O-(α -D-Mannopyranosyl)-(1 $\rightarrow$ 4)-O-[6-N-(5'-azido-2'-nitrobenzoyl)amino-2-N-(*tert*-butoxycarbonyl)amino-2,6-di-deoxy- α -D-glucopyranosyl]-(1 $\rightarrow$ 6)-2,3:4,5-di-O-cyclohexylidene-1-[(R)-2,3-bis{(1-oxohexadecyl)oxy}propyltriethylammonium phosphate]-D-myo-inositol (21a): To a solution of compound 5a in dimethylformamide/triethylamine (1 mL, 19.8 mg, 14.2 μmol) was added *N*-(5-azido-2-nitrobenzoyloxy)succinimide^[22] (13 mg, 42.6 μmol) and the mixture stirred for 2 h at room temperature. The solution was then concentrated under high vacuum. Flash chromatography (loroform $\rightarrow$ chloroform/methanol (9:1), 1% triethylamine) yielded the triethylammonium salt of compound 21a (16 mg, 81%) as an amorphous solid. TLC (chloroform/methanol, 4:1): $R_f = 0.59$. – $[\alpha]_D = +22$ ($c = 1$, CHCl_3). – ^1H NMR [600 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$ (1:1)]: $\delta = 0.87\text{--}0.90$ (t, $^3J = 7.1$ Hz, 6 H, 2 CH_3), 1.25–1.85 [m, 90 H, $\text{C}(\text{CH}_3)_3$, 20 $\text{H}_{\text{Cyclohex.}}$, 26 CH_2 , $(\text{CH}_3\text{CH}_2)_3\text{NH}^+$], 2.30 (t, 4 H, 2 COCH_2), 3.13–3.17 (q, $\text{CH}_3\text{CH}_2)_3\text{NH}^+$], 3.49 (dd, $^3J_{4a,5a} = 10.3$ Hz, $^3J_{5a,6a} = 8.6$ Hz, 1 H, 5a-H), 3.54 (dd, $^3J_{3b,4b} = ^3J_{4b,5b} = 9.9$ Hz 1 H, 4b-H), 3.59 ($^3J_{\text{gem}} = 12.7$ Hz, 1 H, 6b-H), 3.60 ($^3J_{1b,2b} = 3.2$ Hz, 1 H, 2b-H), 3.62 (1 H, 5c-H), 3.73 (1 H, 3c-H), 3.75 (1 H, 4c-H), 3.75 (1 H, 6c-H), 3.76 (1 H, 3b-H), 3.89 (1 H, 6b'-H), 3.94 (1 H, 6c'-H), 3.97 (1 H, 5b-H), 3.99 (1 H, 1 g-H), 3.99 (1 H, 2c-H), 4.07 (1 H, 1g'-H), 4.10 ($^3J_{3a,4a} = 7.3$ Hz, $^3J_{4a,5a} = 10.3$ Hz 1 H, 4a-H), 4.13 (1 H, 3 g-H), 4.24 (m, $^3J_{1a,2a} = 6.3$ Hz, $^3J_{1a,6a} = 3.2$ Hz, 1 H, 1a-H), 4.28 (dd, $^3J_{1a,6a} = 3.2$ Hz, $^3J_{5a,6a} = 8.6$ Hz 1 H, 6a-H), 4.35 (dd, $^3J_{2a,3a} = ^3J_{3a,4a} = 7.3$ Hz 1 H, 3a-H), 4.47 (1 H, 3g'-H), 4.47 ($^3J_{1a,2a} = 6.3$ Hz, $^3J_{2a,3a} = 3.2$ Hz, 1 H, 2a-H), 5.22 (1 H, 2 g-H), 5.26 (d, $^3J_{1b,2b} = 3.2$ Hz, 1 H, 1b-H), 5.32 (s, 1 H, 1c-H), 7.20 (d, $^4J_{4',6'} = 2.6$ Hz, 1 H, 6'-H), 7.20 (dd, $^4J_{4',6'} = 2.6$ Hz, $^3J_{3',4'} = 8.9$ Hz, 1 H, 4'-H), 8.17 (d, $^3J_{3',4'} = 8.9$ Hz, 1 H, 3'-H). – ^{31}P NMR [161.7 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$ (1:1)]: $\delta = -1.11$. – FAB-MS (negative-mode, matrix: 3-nitrobenzyl alcohol): $m/z = 1582$ [M^-]. – $[\text{C}_{77}\text{H}_{126}\text{N}_6\text{O}_{26}\text{P}]^-$ (1582.84).

O-(α -D-Mannopyranosyl)-(1 $\rightarrow$ 4)-O-[2-amino-6-N-(5'-azido-2'-nitrobenzoyl)amino-2,6-di-deoxy- α -D-glucopyranosyl]-(1 $\rightarrow$ 6)-1-[(R)-2,3-bis{(1-oxohexadecyl)oxy}propyl hydrogen phosphate]-D-myo-inositol (2a): Compound 21a (14 mg, 8.3 μmol) was dissolved in a mixture of dichloromethane/acetonitrile (2 mL, 1:1) and ethanediol (40 μL). The solution was acidified to pH = 1 with camphor-10-

sulfonic acid and stirred for 4 h under argon. The *tert*-butoxycarbonyl group was cleaved on addition of trifluoroacetic acid (0.5 mL). After 2 h, triethylamine was added until the pH was alkaline and the mixture was concentrated under high vacuum. Purification by flash chromatography [*n*-butyl alcohol/ethanol/conc. ammonia solution/water (30:30:5:5) $\rightarrow$ (30:30:8:8)] yielded compound 2a (7.0 mg, 64%) as an amorphous solid. TLC (*n*-butyl alcohol/ethanol/conc. ammonia solution/water, 2:2:1:0.3): $R_f = 0.45$. – ^1H NMR [600 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$ (1:1)]: $\delta = 0.88\text{--}0.90$ (t, $^3J = 6.9$ Hz, 6 H, 2 CH_3), 1.23–1.34 (m, 48 H, 24 CH_2), 1.57–1.64 (m, 4 H, 2 COCH_2CH_2), 2.30–2.37 (m, 4 H, 2 COCH_2), 3.24 (dd, $^3J_{1b,2b} = 3.8$ Hz, $^3J_{2b,3b} = 9.9$ Hz, 1 H, 2b-H), 3.34 (dd, $^3J_{4a,5a} = ^3J_{5a,6a} = 9.2$ Hz, 1 H, 5a-H), 3.44 (dd, $^3J_{2a,3a} = 2.8$ Hz, $^3J_{3a,4a} = 9.2$ Hz 1 H, 3a-H), 3.58 (1 H, 6b-H), 3.59 (1 H, 4b-H), 3.62 ($^3J_{3a,4a} = ^3J_{4a,5a} = 9.2$ Hz 1 H, 4a-H), 3.66 (dd, $^3J_{3c,4c} = ^3J_{4c,5c} = 7.8$ Hz, 1 H, 4c-H), 3.74 ($^3J_{4c,5c} = 7.8$ Hz, 1 H, 5c-H), 3.74 (1 H, 6c-H), 3.77 ($^3J_{3c,4c} = 7.8$ Hz, 1 H, 3c-H), 3.89 (1 H, 6b'-H), 3.89 (1 H, 6c'-H), 3.96 (dd, $^3J_{1a,6a} = 9.4$ Hz, $^3J_{5a,6a} = 9.2$ Hz 1 H, 6a-H), 4.02 (1 H, 1 g-, 1g'-H), 4.03 (1 H, 2c-H), 4.04 ($^3J_{2b,3b} = 9.9$ Hz, 1 H, 3b-H), 4.09 (dd, $^3J_{1a,2a} = 2.8$ Hz, $^3J_{2a,3a} = 2.8$ Hz, 1 H, 2a-H), 4.18 ($^3J_{1a,2a} = 2.8$ Hz, $^3J_{1a,6a} = 9.4$ Hz, 1 H, 1a-H), 4.20 (1 H, 3 g-H), 4.40 (1 H, 3g'-H), 4.41 (1 H, 5b-H), 5.26 (1 H, 2 g-H), 5.27 (s, 1 H, 1c-H), 5.55 (d, $^3J_{1b,2b} = 3.8$ Hz, 1 H, 1b-H), 7.28–7.31 (d, dd, $^4J_{4',6'} = 2.5$ Hz, $^3J_{3',4'} = 8.6$ Hz, 2 H, 4'-, 6'-H), 8.18 (d, $^3J_{3',4'} = 8.6$ Hz, 1 H, 3'-H). – ^{31}P NMR (161.7 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}/\text{D}_2\text{O}$, 1:1:0.15): $\delta = 0.02$. – FAB-MS [negative-mode, matrix: 3-nitrobenzyl alcohol/glycerol (1:1)]: $m/z = 1322$ [M^-]. – $[\text{C}_{60}\text{H}_{103}\text{N}_6\text{O}_{24}\text{P}]^-$ (1323.47).

O-(α -D-Mannopyranosyl)-(1 $\rightarrow$ 4)-O-[6-{[N-(4'-azidophenyl)amino]thiocarbonyl}amino-2-N-(*tert*-butoxycarbonyl)amino-2,6-di-deoxy- α -D-glucopyranosyl]-(1 $\rightarrow$ 6)-2,3:4,5-di-O-cyclohexylidene-1-[(R)-2,3-bis{(1-oxohexadecyl)oxy}propyltriethylammonium phosphate]-D-myo-inositol (22a): To a solution of 5a in dimethylformamide/triethylamine (0.73 mL, 14.4 mg, 10.3 μmol) was added 4-azidophenyl isothiocyanate^[23] (4.5 mg, 26 μmol) and the mixture was stirred for 30 minutes at room temperature. The reaction was quenched by methylamine and the solvents removed under high vacuum. Flash chromatography (loroform $\rightarrow$ chloroform/methanol (9:1), 1% triethylamine) yielded crude compound 22a as its triethylammonium salt which was pure enough for the following reaction. TLC (chloroform/methanol, 4:1): $R_f = 0.57$. – ^{31}P NMR [161.7 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$ (1:1:0.15)]: $\delta = -1.20$.

O-(α -D-Mannopyranosyl)-(1 $\rightarrow$ 4)-O-[2-amino-6-{[N-(4'-azidophenyl)amino]thiocarbonyl}amino-2,6-di-deoxy- α -D-glucopyranosyl]-(1 $\rightarrow$ 6)-1-[(R)-2,3-bis{(1-oxohexadecyl)oxy}propyl hydrogen phosphate]-D-myo-inositol (3a): Compound 22a (14 mg, 8.3 μmol) was dissolved in a mixture of dichloromethane/acetonitrile, (2 mL, 1:1) and ethanediol (40 μL). The solution was acidified to pH = 1 with camphor-10-sulfonic acid and stirred for 4 h under argon. The *tert*-butoxycarbonyl group was cleaved on addition of trifluoroacetic acid (0.5 mL). After 2 h, triethylamine was added until the pH was alkaline. The mixture was then concentrated under high vacuum. Purification by flash chromatography [*n*-butyl alcohol/ethanol/conc. ammonia solution/water (30:30:2:2) $\rightarrow$ (30:30:7:7)] yielded compound 3a (6.5 mg, 48% for two steps) as an amorphous solid. The compound is thermolabile and was stored at 4°C. TLC (*n*-butyl alcohol/ethanol/conc. ammonia/water, 2:2:1:0.3): $R_f = 0.46$. – ^1H NMR [250 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}/\text{D}_2\text{O}$ (1:1:0.15)]: $\delta = 0.81\text{--}0.88$ (t, $^3J = 7.4$ Hz, 6 H, 2 CH_3), 1.18–1.31 (m, 48 H, 24 CH_2), 1.50–1.62 (m, 4 H, 2 COCH_2CH_2), 2.24–2.34 (2 t, 4 H, 2 COCH_2), 3.16–4.5 (m, 22 H), 5.19–5.27 (m, 2 H, 1c-, 2 g-H), 5.49 (d, $^3J_{1b,2b} = 4.0$ Hz, 1 H 1b-H), 7.04 (d, $^3J = 9.5$ Hz, 2 H, 2 H_{Ar}), 7.49 (d, $^3J = 9.5$ Hz, 2 H, 2 H_{Ar}). – ^{31}P NMR

[161.7 MHz, CDCl₃/CD₃OD/D₂O (1:1:0.1)]: δ = 0.41. – FAB-MS [positive-mode, matrix: 3-nitrobenzyl alcohol/glycerol (1:1) mit NaI]: m/z = 1310 [M + H⁺], 1332 [M + Na⁺], 1354 [MNa + Na⁺]. – C₆₀H₁₀₅N₆O₂₁PS (1309.55).

O-(α -D-Mannopyranosyl)-(1 $\rightarrow$ 4)-O-[6-(N-(4'-azido-2'-hydroxybenzoyl)amino)-2-N-(tert-butoxycarbonyl)amino-2,6-dideoxy- α -D-glucopyranosyl]-(1 $\rightarrow$ 6)-2,3,4,5-di-O-cyclohexylidene-1-[(R)-2,3-bis{(1-oxohexadecyl)-oxy}propyltriethylammonium phosphate]-D-myo-inositol (24a): To a solution of compound 5a in dimethylformamide/triethylamine (1 mL, 19.8 mg, 14.2 μ mol) was added N-(4-azido-2-hydroxybenzoyloxy)succinimide (23)^[24] (9.8 mg, 36 μ mol) and the mixture stirred for 1.5 h at room temperature. The solution was then concentrated under high vacuum. Flash chromatography (ethyl acetate/methanol, 5:1, 1% triethylamine) yielded the triethylammonium salt of compound 24a (17 mg, 74%) as an amorphous solid. TLC (chloroform/methanol, 4:1): R_f = 0.19. – [α]_D = +26 (c = 1, CHCl₃). – ¹H NMR [600 MHz, CDCl₃/CD₃OD (1:1)]: δ = 0.87–0.90 (2 t, 6 H, 2 CH₃), 1.20–1.85 [m, 90 H, C(CH₃)₃, 20 H_{Cyclohex.}, 26 CH₂, (CH₃CH₂)₃NH⁺], 2.29–2.33 (2 t, 4 H, 2 COCH₂), 3.12–3.16 [q, ³J = 7.3 Hz, 6 H, CH₃CH₂)₃NH⁺], 3.17–3.22 (m, 1 H, 6b-H), 3.46–3.55 (m, 2 H, 4b-, 5a-H), 3.60–3.81 (m, 6 H, 2b-, 3b-, 3c-, 4c-, 5c-, 6c-H), 3.93–4.04 (1 H, 5b-, 2c-, 6c'-H), 4.05–4.09 (m, 2 H, 1g-, 1g'-H), 4.13–4.24 (1 H, 4a-, 6b'-, 3g-H), 4.32–4.45 (m, 5 H, 1a-, 2a-, 3a-, 6a-, 3g'-H), 5.24 (1 H, 2g-H), 5.35 (s, 1 H, 1c-H), 5.44 (s 1 H, 1b-H), 6.58 (d, ³J_{5',6'} = 8.6 Hz, 1 H, 5'-H), 6.62 (s, 1 H, 3'-H), 7.94 (d, ³J_{5',6'} = 8.5 Hz, 1 H, 6'-H). – ³¹P NMR [161.7 MHz, CDCl₃/CD₃OD (1:1)]: δ = –1.47. – FAB-MS [negative-mode, matrix: 3-nitrobenzyl alcohol/glycerol (1:1)]: m/z = 1553 [M[–]]. – [C₇₇H₁₂₇N₅O₂₅P][–] (1553.84).

O-(α -D-Mannopyranosyl)-(1 $\rightarrow$ 4)-O-[2-amino-6-N-(4'-azido-2'-hydroxybenzoyl)amino-2,6-di-deoxy- α -D-glucopyranosyl]-(1 $\rightarrow$ 6)-1-[(R)-2,3-bis{(1-oxohexadecyl)-oxy}propyl hydrogen phosphate]-D-myo-inositol (25a): Compound 24a (10 mg, 6.0 μ mol) was dissolved in a mixture of dichloromethane/acetonitrile, (2 mL, 1:1) and ethanediol (40 μ l). The solution was acidified to pH = 1 with camphor-10-sulfonic acid and stirred for 4 h under argon. The tert-butoxycarbonyl group was cleaved on addition of trifluoroacetic acid (0.5 mL). After 2 h, triethylamine was added until the pH was alkaline. The mixture was then concentrated under high vacuum. Purification by flash chromatography [*n*-butyl alcohol/ethanol/conc. ammonia solution/water (30:30:3:3)] yielded compound 25a (4.1 mg, 53%) as a white solid. TLC (*n*-butyl alcohol/ethanol/conc. ammonia/water, 30:30:7:7): R_f = 0.18. – ¹H NMR [250 MHz, CDCl₃/CD₃OD/D₂O (1:1:0.15)]: δ = 0.86–0.91 (t, ³J = 7.2 Hz, 6 H, 2 CH₃), 1.22–1.36 (m, 48 H, 24 CH₂), 1.52–1.66 (m, 4 H, 2 COCH₂CH₂), 2.29–2.38 (2 t, 4 H, 2 COCH₂), 3.24 (dd, ³J_{1b,2b} = 4.0 Hz, ³J_{2b,3b} = 10.2 Hz, 1 H, 2b-H), 3.28–4.5 (m, 21 H), 5.20–5.29 (m, 1 H, 2g-H), 5.29 (d, ³J_{1c,2c} = 1.5 Hz, 1 H, 1c-H), 5.55 (d, ³J_{1b,2b} = 4.0 Hz 1 H, 1b-H),), 6.58 (d, ⁴J_{3',5'} = 2.2 Hz, 1 H, 3'-H), 6.66 (dd, ⁴J_{3',5'} = 2.2 Hz, ³J_{5',6'} = 8.6 Hz, 1 H, 5'-H), 7.87 (d, ³J_{5',6'} = 8.6 Hz, 1 H, 6'-H). – ³¹P NMR [161.7 MHz, CDCl₃/CD₃OD/D₂O (1:1:0.15)]: δ = 0.37. – FAB-MS [negative-mode, matrix: 3-nitrobenzyl alcohol/glycerol (1:1)]: m/z = 1292 [M. – H⁺]. – C₆₀H₁₀₄N₅O₂₃P (1294.48)

O-(α -D-Mannopyranosyl)-(1 $\rightarrow$ 4)-O-[2-amino-6-N-(4'-azido-2'-hydroxy-3'-iodobenzoyl)amino-2,6-di-deoxy- α -D-glucopyranosyl]-(1 $\rightarrow$ 6)-2,3,4,5-di-O-cyclohexylidene-1-[(R)-2,3-bis{(1-oxohexadecyl)-oxy}propyl hydrogen phosphate]-D-myo-inositol (4a): IODO-BEAD^[25] was added to a solution of compound 25a (0.2 mg) in chloroform/methanol/0.1 M sodium iodide in H₂PO₄^{2–}/HPO₄^{2–} buffer in an Eppendorf cup and the mixture was allowed to stand for

20 min. After preparative TLC [*n*-butyl alcohol/ethanol/conc. ammonia solution/water (2:2:0.5:1.5)] the silica gel containing the product was scratched off and suspended in chloroform/methanol/water (1:1:0.15). After the silica gel was removed by centrifugation, the presence of compound 4a in the solution was determined by FAB-MS. TLC (*n*-butyl alcohol/ethanol/conc. ammonia/water, 2:2:0.5:1.5): R_f = 0.58. – FAB-MS [negative-mode, matrix: 3-nitrobenzyl alcohol/glycerol (1:1)]: m/z = 1392 [M. – H⁺. – N₂], 1407 [M. – H⁺. – N], 1420 [M. – H⁺]. – C₆₀H₁₀₃IN₅O₂₃P (1420.37).

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