Pergamon

Tetrahedron 57 (2001) 4147–4160

TETRAHEDRON

Moenomycin analogues with long-chain amine lipid parts from reductive aminations

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Received 19 February 2001; accepted 13 March 2001

Abstract—From a moenomycin A glycolic aldehyde degradation product lacking the chromophore unit and most of the lipid part, a number of amines were prepared by reductive amination. Their interaction with artificial membranes as well as their transglycosylase inhibiting and antibiotic properties were studied.

1. Introduction

The transglycosylation reaction in peptidoglycan biosynthesis is a highly promising target for new antibiotics. The moenomycins,[1] [see moenomycin A (**1**) (Scheme 1)] have been shown to interfere with this biosynthetic step interacting with the enzyme(s).[2] A mechanism for their mode of action has been proposed.[3–5] It is assumed that they are anchored to the cytoplasmic membrane via the lipid part and then bind highly selectively to the active site of the enzyme via the C–E–F trisaccharide. Units A, B, and D have been shown to be of minor importance for the antibiotic activity.[6] Whereas the structural requirements for antibiotic activity in the carbohydrate part have been investigated in detail,[6,7] much less is known in which way the membrane anchoring locates moenomycin in the correct way for the interaction with the enzyme. Hydrogenation of the lipid part gives a decahydro derivative that is antibiotically fully active.[8] However, it has been shown previously that converting the glyceric acid part into its methyl ester or introducing a single OH group to C-17 or C-18 of the lipid part abolishes the antibiotic activity completely. Similarly, cleavage of the bond between the glyceric acid unit and the lipid part leads to a compound devoid of antibiotic activity.[9,10] In addition, membrane anchoring is of concern in the context of pharmacokinetics. First results on membrane binding of the moenomycins have been obtained making use of fluorescence methods.[11]

We have shown that moenomycin A can be degraded very efficiently by ozonolysis into a pentasaccharide derivative in which the chromophore part is removed and the lipid chain shortened to a glycolaldehyde unit (**1**→**2a**, Scheme 1). **2a** appears to be a very promising starting material for the synthesis of libraries of analogues with modified lipid chains provided reactions can be found that can be performed in water or mixtures of polar solvents with water. Indium-mediated Barbier reactions are of this type. Recently, we have reported on a whole series of analogues with new lipid chains which were constructued by Barbier reactions of **2a** with allylic and benzylic halides. We also studied the antibiotic properties of these compounds.[12]

Reductive amination is another reaction that can be employed for the conversion of carbonyl compounds into amines in aqueous solution. Of the common reducing agents such as $NaBH_4$, $NaB(OAc)_3H$,[13] and $NaBH_3CN$, only the latter seemed suitable for our purposes since it is stable in slightly acidic aqueous solution.[14]

1.1. Model aminations

It is assumed that a careful selection of the pH value minimizes the direct reduction of the aldehyde to give the corresponding primary alcohol **2b**. We were not able to suppress this side reaction completely. It was complicated to detect the primary alcohol by TLC since it had almost the same R_f value as the aldehyde (hydrate). However, in some mass spectra of crude reaction mixtures the presence of the primary alcohol was apparent. For the different series of amines the optimum pH value was about 7. In the model series (see Table 1) the yields were very satisfactory although the formation of primary amine **3a** was capricious and difficult to reproduce. **3a** and **3b** are, of course, very interesting compounds for the attachment of reporter groups

Keywords: antibiotics; reductive amination; phospholipids; carbohydrates.
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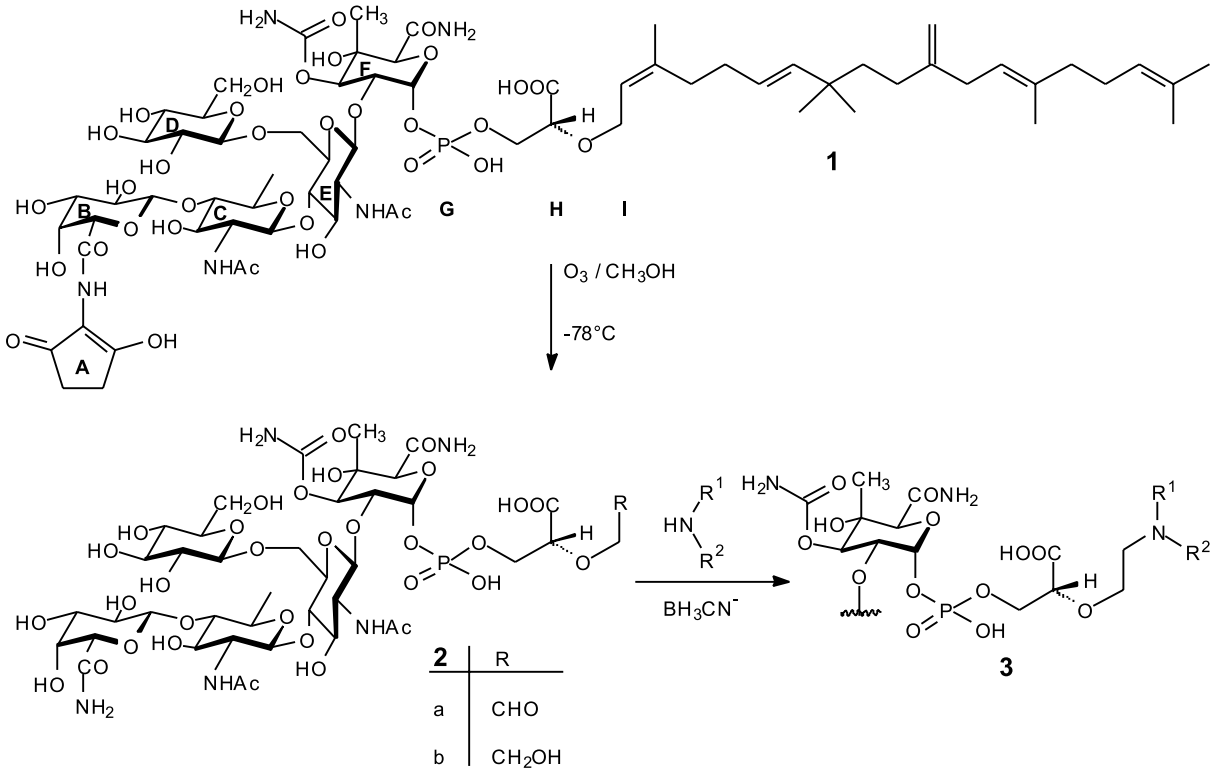
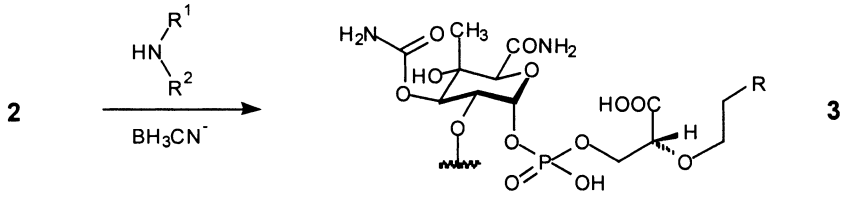
0040–4020/01/$ - see front matter
PII: S0040-4020(01)00306-4



Scheme 1.

to the B–C–E(D)–F–G–H core of the moenomycins. Use of such compounds will be reported in due course. In all reductive amination experiments an excess of the amine was used and great care was taken to obtain the reductive amination products in a highly pure form. Thus, after work-up the reaction products were purified by gel filtration followed by ion-exchange chromatography to liberate the acid form of the phosphoric acid diesters from the corresponding ammonium salts. Then side products were removed by flash chromatography and finally the amines were again purified by gel filtration to remove inorganic impurities.

Table 1. Model amines **3**



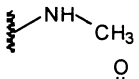
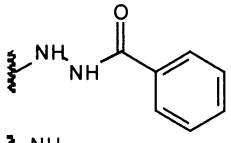
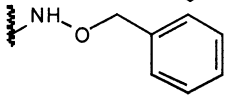
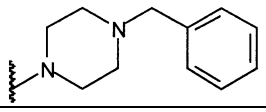
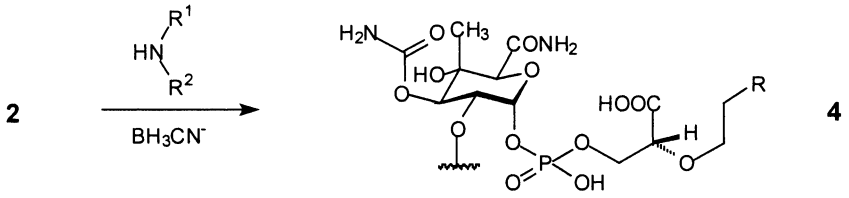
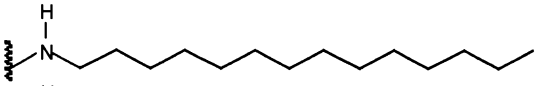
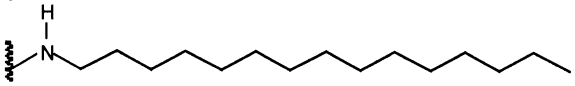
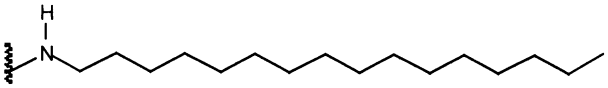
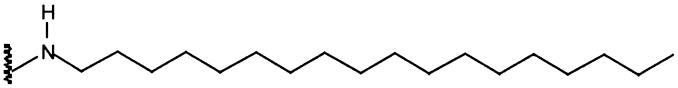
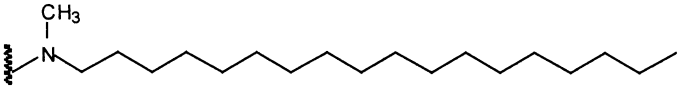
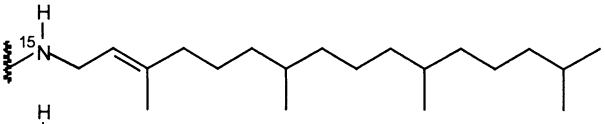
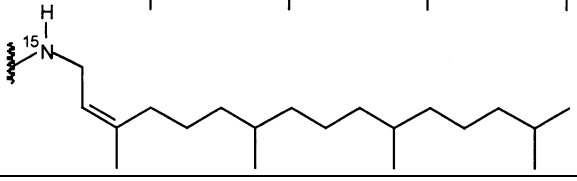
Product	R	Yield (%)
3a		78
3b		66
3c		59
3d		55
3e		54

Table 2. Secondary amines **4**

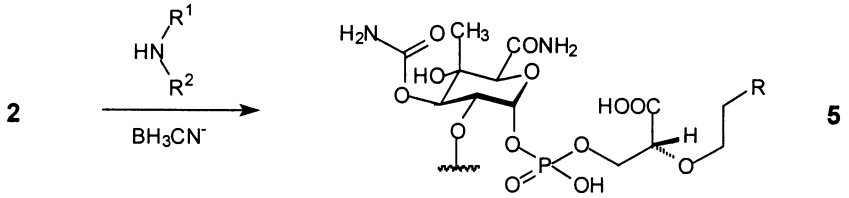


Product	R	Yield (%)
4a		68
4b		54
4c		63
4d		25
4e		9
4f		19
4g		8

1.2. Synthesis of long-chain secondary amines

In the synthesis of long-chain amines the solubility of the starting amines became a problem and the yields dropped with increasing chain length (see Table 2). If necessary, the reaction mixtures were sonicated. For the phytylamine-derived compounds a mixture of racemic phytols (60:40 *E*/*Z*, *R*/*S* at both stereogenic centres) was converted to the corresponding amines by a Gabriel synthesis. The *E*/*Z* isomers were then separated by flash chromatography and

Table 3. Long-chain tertiary amines **5**



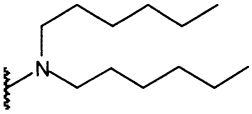
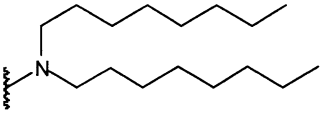
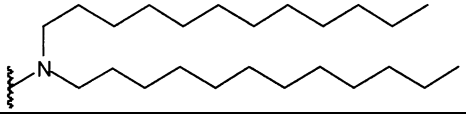
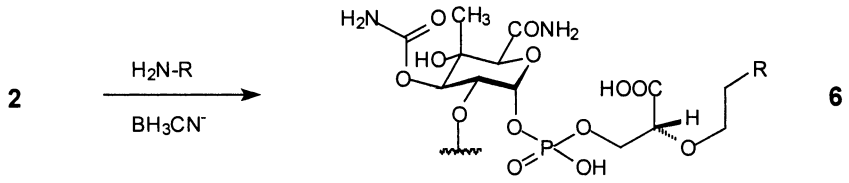
Product	R	Yield (%)
5a		38
5b		31
5c		9

Table 4. Amines **6**



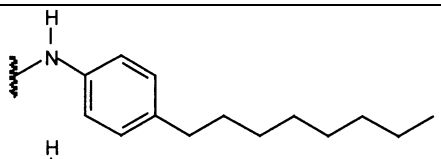
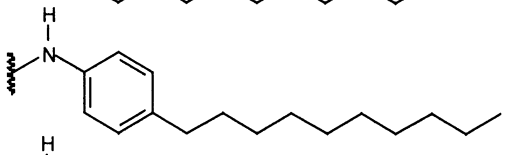
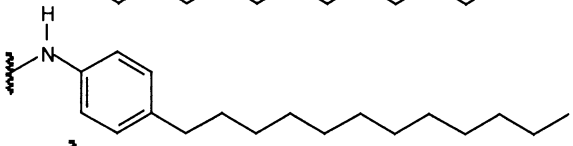
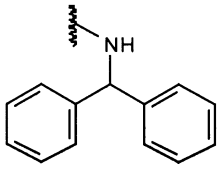
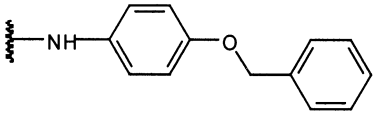
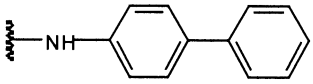
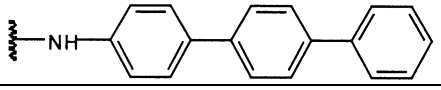
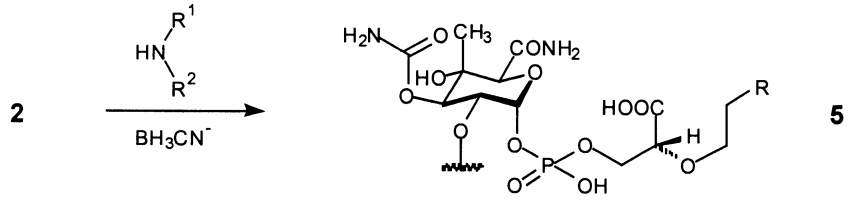
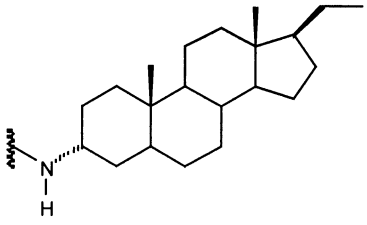
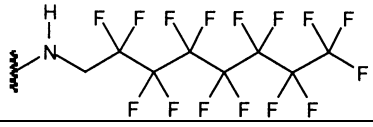
Product	R	Yield (%)
6a		71
6b		32
6c		31
6d		62
6e		51
6f		58
6g		10

Table 5. Amines **7**



Product	R	Yield (%)
7a		25
7b		10

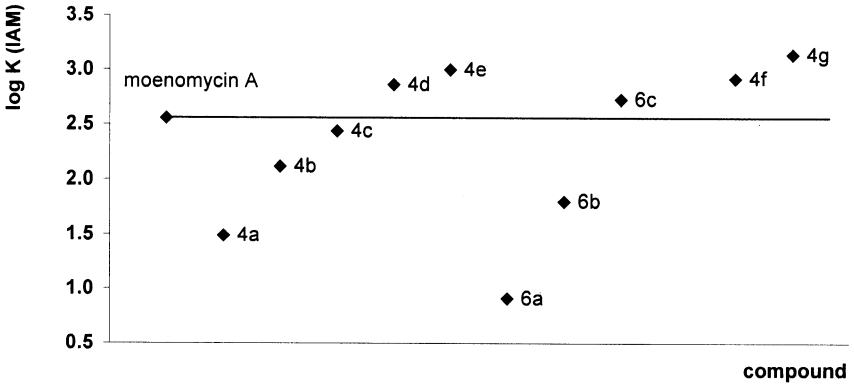


Figure 1. IAM chromatography data of amines **4a**–**4g** and **6a**–**6c**.

the configuration around the double bond was determined in both cases by $NOE_{difference}$ measurements. These two amines were separately used for the reductive amination of **2a** to give **4f** and **4g**, respectively. The phthalimide used for the preparation of the phytylamines was ^{15}N-labelled. Thus, the two phytyl analogues **4f** and **4g** might be interesting tools for NMR experiments.

1.3. Synthesis of aliphatic tertiary amines with two long chains

The compounds of this series are collected in Table 3. Again, the yields decreased with increasing chain length of the aliphatic residues, most probably due to solubility reasons.

1.4. Amines with aromatic partial structures

The choice of some aromatic amines (Table 4) was based on the structures of hydrophobic chains of antibiotically active semisynthetic glycopeptide antibiotics.[15] After reductive amination the ion-exchange step could be omitted since no ammonium salts were formed.

1.5. Miscellaneous compounds

Table 5 shows a number of special cases where the amines were either derived from steroids or fluorinated alkanes. These compounds may be interesting for their interaction with the cytoplasmic membrane.

Table 6. Minimum inhibitory concentration (MIC) against various test organisms

Strain	MIC (μg mL^{-1})							
	Moenomycin A	**4d**	**4e**	**4g**	**4f**	**5c**	**6c**	**7a**
ATCC 25923	0.125	>32	16	16	32	>32	>32	>32
ATCC 29213	0.06	>32	16	16	32	>32	>32	>32
PEG 18	0.03	>32	16	16	32	>32	>32	>32
PEG 5	0.06	>32	16	16	32	>32	>32	>32
MRSA 1309	0.125	>32	16	16	32	>32	>32	>32
ATCC 6538P	0.038	>32	8	16	32	>32	>32	>32
SG 511	0.125	>32	32	32	32	>32	>32	>32

1.6. Interaction of selected moenomycin analogues with immobilized artificial membranes

As summarized in the main introduction, the interaction of the lipid chain of moenomycin with the cytoplasmic membrane is of paramount importance for the antibiotic activity of the moenomycins. In addition, membrane binding is probably also of high importance in the context of pharmacokinetics. Recently, for the first time, quantitation of the interaction of moenomycin with artificial membranes was achieved by means of fluorescence methods. For the interaction of a coumarin-labelled moenomycin analogue with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) vesicles a partion coefficient $K_p = (2.2 \pm 0.7) \times 10^6$ was determined.[11] The availability of the series of compounds obtained by reductive amination of **2a** suggested studying their interactions with artificial membranes. In these experiments use was made of immobilized artificial membrane (IAM) chromatography. The stationary phase was IAM.PC.DD (an immobilized phosphatidylcholine ligand linked to silica propylamine via an ω-hydroxydodecanoyl chain). The retention times (t_r) of the moenomycin analogues on the IAM column were used to calculate the solute capacity factors (k'_{IAM}) using equation (1)

$$k'_{IAM} = \frac{(t_r - t_0)}{t_0} \quad (1)$$

where t_r is the retention time in minutes of the test compound and t_0 corresponds to the column dead time or void volume. The capacity factor k'_{IAM} is linearly related to the equilibrium partition coefficient K_{IAM} by

$$k'_{IAM} = \left(\frac{V_s}{V_m}\right) K_{IAM} \quad (2)$$

where V_m is the total volume of solvent within the HPLC column and V_s is the volume of the IAM interphase created by the immobilized phospholipids. For a given IAM column V_s/V_m is a constant. The results are collected in Fig. 1.

The results show that the K_{IAM} values increase with increasing chain length in two series of compounds. For comparison, the K_{IAM} of moenomycin A was also determined. The difference in the partition coefficient when compared to

Table 7. In vitro inhibition of the transglycosylase by amines **3c**, **3d**, **4a**, **4b**, and of moenomycin A

Product	IC_{50} (nM)	Product	IC_{50} (nM)	Poduct	IC_{50} (nM)	Product	IC_{50} (nM)
3c	3116	**4c**	152	**4g**	42	**6d**	1031
3d	1270	**4d**	89	**6a**	31	**6e**	2492
4a	247	**4e**	29	**6b**	12	**6f**	2541
4b	146	**4f**	41	**6c**	5	**6g**	277
Moenomycin A	1.5						

the value obtained for POPC vesicles reflects the different phospholipids used in the two series of experiments.[11]

1.7. Antibiotic activities

MIC values against a series of *Gram-positive* strains were determined by a serial two-fold micro-dilution method as described previously.[11] The results are collected in Table 6. All moenomycin-derived amines turned out to be antibiotically inactive.

1.8. In vitro activities of selected moenomycin analogues

The compounds were tested in an assay measuring the inhibition of incorporation of ^{3}H-labelled 2,6-diaminopimelic acid into peptidoglycan synthesized by toluene-permeabilized *E. coli K12* cells.[16] Most of the compounds are inactive in this test system (see Table 7), but there are some interesting observations. In the series of compounds **6** an increasing chain length leads to measurable activity. Compound **6c** is almost as active as moenomycin A.

2. Discussion

Starting from aldehyde **2a** a large number of moenomycin analogues with modified lipid chains have been obtained by reductive amination. Membrane binding of these compounds has been tested and antibiotic properties and in vitro inhibition of the transglycosylation step were studied. Wheras all compounds were antibiotically inactive (*S. aureus*) some of them with an alkylsubstituted aniline side chain were highly active inhibitors of the transglycosylation reaction in an *E. coli* in vitro system. The most active compound showed a membrane binding similar to that of moenomycin A. The results strengthen the view that proper orientation of the moenomycin analogues at the membrane by means of the phospholipid moiety is essential for correct recognition of the carbohydrate part at the transglycosylase domain of the *penicillin binding protein* and, thus, for antibiotic activity. Converting the glyceric acid part into its methyl ester, introducing a single OH group to the distal part (C-17 or C-18) of the lipid[9] or into the proximity of the phosphoglycerate unit (C-2 of the lipid chain),[10,12] or inserting an amino nitrogen between C-2 and C-3 of the lipid abolishes the antibiotic activity completely. Similarly, cleavage of the bond between the glyceric acid unit and the lipid part leads to a compound devoid of antibiotic activity.[10] Until now, a single analogue has been found in which the chain length of the lipid overrides the effect of a polar group at C-2, the Barbier addition product of phytyl bromide to aldehyde **2a**.[12]

3. Experimental

3.1. General

NMR: GEMINI 200 (Varian), GEMINI 2000 (Varian), GEMINI 300 (Varian), DRX 400 (Bruker), DRX 600 (Bruker); chemical shifts are given in δ values, CH_3, CH_2, CH groups and quaternary carbons when identified by APT are indicated by (−) (CH_3, CH) and (+) (CH_2, C_q), respectively. ^{31}P shifts are referenced to H_3PO_4 as external standard, ^{19}F shifts to external trifluoracetic acid. Mass spectrometry: FAB MS: VG Autospec (Fisons, 3-nitrobenzylalcohol matrix), ESI MS: FT-ICR-MS Apex II (Bruker Daltonics, water–methanol, negative ion mode). MIC values were determined by a serial two-fold microdilution method (Iso-Sensitest medium, Oxoid). A series of decreasing concentrations of the compound under investigation was prepared in the medium. For inoculations 1×10^5 cfu mL^{-1} were used. After 24 h at 37°C the MICs were determined (absence of visible turbidity). For the in vitro testing the method of McMurry et al. was used.[16]

3.1.1. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-(2-aminoethoxy)-propionic acid (3a). To a solution of **2a** (99.7 mg, 83 μmol) in a mixture of phosphate buffer (400 μl, 50 mM) and methanol (1.6 ml) a solution of ammonium acetate (64.7 mg, 830 μmol) in a mixture of water (400 μl) and methanol (400 μl) was added. The pH was corrected until pH 7.2 was reached. A solution of $NaBH_3CN$ (5.35 mg, 160 μmol) in methanol (400 μl) was added and the mixture was stirred for 72 h at 20°C. The crude product was purified by FC (ethyl acetate–2-propanol–H_2O 4:4:3) and subsequent gel filtration (Sephadex LH-20®, H_2O–CH_3OH 1:4). The combined fractions were concentrated and freeze dried to give **3a** (77.8 mg, 78%). ^{1}H NMR (H,H COSY, D_2O, 400 MHz): Characteristic signals at δ=1.18 (s, CH_3-4^F), 1.34 (d, CH_3-6^C), 1.99, 2.05 (s, $NHCOCH_3^E$, s, $NHCOCH_3^C$), 3.21 (m, CH_2-2^I), 3.25 (dd, H-2^D), 4.18 (s, H-4^B, H-5^B), 4.40 (s, H-5^F), 4.95 (m, H-3^F), 5.76 (m, H-1^F), J_{5C-6C}=5.9 Hz, J_{2D-3D}=8.3 Hz, J_{1D-2D}=8.3 Hz. ^{13}C NMR (50 MHz, D_2O): δ=15.01 (CH_3-4^F), 16.90 (CH_3-6^C), 22.59, 22.67 ($NHCOCH_3^E$, $NHCOCH_3^C$), 39.66 (C-2^I), 55.27, 55.75 (C-2^E, C-2^C), 61.06 (C-6^D), 66.09 (CH_2-1^I), 67.18 (m, C-3^H), 69.15, 69.97, 70.83, 71.35, 72.24, 72.44, 72.88, 73.08, 73.28, 73.38, 73.69, 74.49, 75.07, 76.03, 76.24, 76.77, 76.96 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-3^C, C-5^F, C-6^E, C-5^B, C-3^E, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D), 80.01 (C-4^E), 80.95 (d, C-2^H), 83.31 (C-4^C), 94.66 (d, C-1^F), 101.45, 102.46, 102.84, 103.45 (C-1^C, C-1^E, C-1^B, C-1^D), 158.39 ($OCONH_2^F$), 172.94,

173.37 ($CONH_2^B$, $CONH_2^F$), 174.48, 174.78 ($NHCOCH_3^E$), ($NHCOCH_3^C$), 176.66 (C-1^H). ^{31}P NMR (81 MHz, D_2O): $\delta=-2.18$. $C_{41}H_{69}N_6O_{32}P$ (1188.99, 1188.36), FAB MS: m/z=1189.2 $[M+H]^+$, 1211.2 $[M+Na]^+$. ESI MS (negative mode): m/z=1187.36548 (1187.36212) $[M-H]^-$, 593.17654 (593.17742) $[M-2H]^{2-}$.

3.1.2. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-[2-(methylamino)ethoxy]-propionic acid (3b). To a solution of **2a** (104.8 mg, 88 μmol) in a mixture of phosphate buffer (400 μl, 50 mM) and methanol (800 μl) a solution of methylamine (13.7 mg, 440 μmol) in a mixture of water (400 μl) and methanol (400 μl) was added. The pH was corrected until pH 7.2 was reached. A solution of $NaBH_3CN$ (11.2 mg, 176 μmol) in methanol (100 μl) was added and the mixture was stirred for 72 h at 20°C. The reaction mixture was directly applied to a Sephadex LH-20® column (H_2O–CH_3OH 1:4). The combined fractions were concentrated in vacuo and the residue dissolved in water (1 ml) and liberated from a second equivalent of methylamine by DOWEX® (50 W X8, Na^+-form, water) ion exchange. The crude product was purified by FC (ethyl acetate–2-propanol–H_2O 4:4:3) and subsequent gel filtration (Sephadex LH-20®, H_2O–CH_3OH 1:4). The combined fractions were concentrated and freeze dried to give **3b** (69.7 mg, 66%). ^1H NMR (H,H COSY, D_2O, 400 MHz): Characteristic signals at δ=1.18 (s, CH_3-4^F), 1.33 (d, CH_3-6^C), 1.99, 2.05 (s, $NHCOCH_3^E$, s, $NHCOCH_3^C$), 2.73 (s, CH_3-3^I), 3.25 (dd, H-2^D), 4.18 (s, H-4^B, H-5^B), 4.40 (s, H-5^F), 4.46 (d, H-1^D), 4.96 (d, H-3^F), 5.76 (q, H-1^F), J_{5C-6C}=5.8 Hz, J_{1D-2D}=7.8 Hz, J_{2F-3F}=10.5 Hz, J_{1F-2F}=3.6 Hz, $^3J_{1F-P}$=5.8 Hz. ^{13}C NMR (50 MHz, D_2O): δ=15.14 (CH_3-4^F), 17.04 (CH_3-6^C), 22.73, 22.83 ($NHCOCH_3^E$, $NHCOCH_3^C$), 33.04 (CH_3-NH^I) 49.07 (CH_3-NH-*C*-2^I), 55.53, 55.95 (C-2^E, C-2^C), 61.28 (C-6^D), 65.07 (CH_2-1^I), 67.39 (d, C-3^H), 69.36, 70.20, 71.06, 71.58, 72.50, 72.67, 73.18, 73.30, 73.52, 73.62, 73.92, 74.72, 75.30, 76.28, 76.49, 77.01, 77.17 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-3^C, C-5^F, C-6^E, C-5^B, C-3^E, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D), 80.27 (C-4^E), 83.53 (C-4^C), 94.91 (d, C-1^F), 101.74, 102.70, 103.11, 103.74 (C-1^C, C-1^E, C-1^B, C-1^D), 158.74 ($OCONH_2^F$), 173.35, 173.77, 174.80, 175.17 ($CONH_2^B$, $CONH_2^F$, $NHCOCH_3^E$, $NHCOCH_3^C$), 176.94 (C-1^H). ^{31}P NMR (81 MHz, D_2O): $\delta=-1.08$. $C_{42}H_{71}N_6O_{32}P$ (1203.02, 1202.38) ESI MS (negative mode): m/z=1201.38127 (1201.37777) $[M-H]^-$, 600.18675 (600.18525) $[M-2H]^{2-}$.

3.1.3. (*R*)-2-[2-(2-Benzoylhydrazino)ethoxy]-3-({β-D-galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryl-oxy)-propionic acid (3c). To a solution of **2a** (50 mg, 42 μmol) in water (300 μl) a solution of benzoyl hydrazine (8.6 mg, 63 μmol) in water (50 μl) and methanol (50 μl) was added and the mixture was stirred for 30 min at 20°C. Afterwards a solution of $NaBH_3CN$ (2.6 mg, 42 μmol) in water (100 μl) was added and the mixture was stirred for 4 h at 20°C. The reaction mixture was directly applied to a Sephadex LH-20® column (H_2O–CH_3OH 1:4). The combined fractions were concentrated and freeze dried to give **3c** (32.8 mg, 59%). ^1H NMR (400 MHz, D_2O): Characteristic signals at δ=1.16 (s, CH_3-4^F), 1.29 (d, CH_3-6^C, signal doubling), 1.92, 1.99 (s, $NHCOCH_3^E$, s, $NHCOCH_3^C$, signal doubling), 4.12 (s, H-4^B, H-5^B), 4.31 (s, H-5^F), 4.93 (d, H-3^F), 5.70 (q, H-1^F), 7.40–7.50 (m, H-4^K, H-6^K), 7.50–7.60 (m, H-5^K), 7.65–7.70 (m, H-3^K, H-7^K). ^{13}C NMR (100 MHz, D_2O): δ=16.08 (CH_3-4^F), 17.99 (CH_3-6^C), 23.76 ($NHCOCH_3^E$, $NHCOCH_3^C$), 51.42 (C-2^I), 56.61–56.85 (C-2^E, C-2^C), 62.18 (C-6^D), 68.49 (C-1^I), 70.28, 71.15, 71.95, 72.49, 73.40, 73.56, 73.85, 74.17, 74.46, 74.53, 74.89, 75.60, 76.28, 77.16, 77.38, 77.85 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-6^E, C-5^B, C-3^E, C-2^F, C-3^C, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D, C-5^F), 81.19 (C-4^E), 82.38 (C-2^H), 84.40 (C-4^C), 95.76 (C-1^F), 102.63, 103.37, 104.00, 104.63 (C-1^C, C-1^E, C-1^B, C-1^D), 128.63 (C-4^K,C-6^K), 130.24 (C-3^K, C-7^K), 133.35 (C-2^K), 133.79 (C-5^K), 159.55 ($OCONH_2^F$), 171.14 (C-1^K), 174.13, 174.61, 175.56, 176.00 ($CONH_2^B$, $CONH_2^F$, $NHCOCH_3^E$, $NHCOCH_3^C$), 178.22 (C-1^H). ^{31}P NMR (81 MHz, D_2O): $\delta=-1.37$. $C_{48}H_{74}N_7O_{33}P$ (1308.13, 1307.40) ESI MS (negative mode): m/z=1306.38851 (1306.39924) $[M-H]^-$, 652.69648 (652.69594) $[M-2H]^{2-}$, FAB MS: m/z=1330.1 $[M+Na]^+$, 1308.2 $[M+H]^+$.

3.1.4. (*R*)-2-[2-(Benzyloxyamino)ethoxy]-3-({β-D-galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}-hydroxyphosphoryloxy)-propionic acid (3d). To a solution of **2a** (50 mg, 42 μmol) in water (300 μl) a solution of *o*-benzylhydroxylamine (9.9 mg, 63 μmol) in water (100 μl) was added and the mixture was stirred for 4 h at 20°C. Afterwards a solution of $NaBH_3CN$ (2.6 mg, 42 μmol) in water (100 μl) was added. The mixture was stirred for 12 h at 20°C. The mixture was directly applied to a Sephadex PD-10® column (H_2O). The combined fractions were concentrated and freeze dried to give **3d** (30.1 mg, 55%). ^1H NMR (400 MHz, D_2O): Characteristic signals at δ=1.12 (s, CH_3-4^F), 1.27 (m, CH_3-6^C, signal doubling), 1.93, 1.99 (s, $NHCOCH_3^E$, s, $NHCOCH_3^C$), 4.12 (s, H-4^B, H-5^B), 4.34 (s, H-5^F), 4.92 (d, H-3^F), 5.69 (q, H-1^F), 7.30–7.50 (m, H-3^K -H-7^K). ^{13}C NMR (APT, 100 MHz, D_2O): δ=14.27 (CH_3-4^F), 16.19 (CH_3-6^C), 21.88, 21.96 ($NHCOCH_3^E$, $NHCOCH_3^C$), 49.66 (C-2^I), 54.81–55.05 (C-2^E, C-2^C), 60.36 (C-6^D), 65.78 (C-1^I), 66.60 (C-3^H), 68.46, 69.30, 70.16, 70.69, 71.60, 71.76, 72.01, 72.38, 72.64, 72.70, 73.08, 73.80, 74.48, 74.79, 75.35, 75.58, 75.96 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^C, C-5^F, C-3^B, C-6^E, C-5^B, C-3^E, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D, C-1^K), 79.36 (C-4^E), 80.73 (C-2^H), 82.60 (C-4^C), 93.94 (C-1^F), 100.85, 101.58, 102.19, 102.82 (C-1^C, C-1^E, C-1^B, C-1^D), 128.06–129.09 (C-3^K, C-4^K, C-6^K, C-7^K), 129.75 (C-5^K), 136.22 (C-2^K), 157.76 ($OCONH_2^F$), 172.31, 172.80, 173.79, 174.19 ($CONH_2^B$, $CONH_2^F$, $NHCOCH_3^E$, $NHCOCH_3^C$), 176.41 (C-1^H). ^{31}P NMR (81 MHz, D_2O): $\delta=-1.58$. $C_{48}H_{75}N_6O_{33}P$ (1295.13, 1294.41), ESI MS (negative mode): m/z=1293.41920 (1293.40399) $[M-H]^-$, 646.19918 (646.19836) $[M-2H]^{2-}$, FAB MS: m/z=1317.2 $[M+Na]^+$, 1295.2 $[M+H]^+$.

3.1.5. (*R*)-2-[2-(4-Benzylpiperazino)ethoxy]-3-({β-D-

galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}-hydroxyphosphoryl-oxy)-propionic acid (3e)3e was prepared from **2a** (53.8 mg, 45 μmol) and 1-benzylpiperazine (23.9 mg, 135 μmol) as described for **4a**. Yield: 53.8 mg (54%). ^{1}H NMR (H,H COSY, 400 MHz, D_2O): Characteristic signals at δ=1.17 (s, CH_3-4^F), 1.32 (d, CH_3-6^C), 1.98, 2.04 (s, NHCOCH_3^E, s, NHCOCH_3^C), 2.50–3.30 (bs, CH_2-1^K-CH_2-4^K), 3.25 (dd, H-2^D), 4.17 (s, H-4^B, H-5^B), 4.37 (s, H-5^F), 4.46 (d, H-1^D), 4.94 (d, H-3^F), 5.76 (q, H-1^F), 7.35–7.45 (m, H-2^L-H-6^L), J_{5C-6C}=5.3 Hz, J_{2D-3D}=8.5 Hz, J_{1D-2D}=7.8 Hz, J_{2F-3F}=10.6 Hz, J_{1F-2F}=2.8 Hz, $^3J_{1F-P}$=6.4 Hz. ^{31}P NMR (81 MHz, D_2O): δ=−1.05. $C_{52}H_{82}N_7O_{32}P$ 1348.23 (1347.47), ESI MS (negative mode): *m/z*=1346.47069 (1346.46692) [M−H]$^-$, 672.73183 (672.72982) [M−2H]$^{2-}$, FAB MS: *m/z*=1370.3 [M+Na]$^+$, 1348.3 [M+H]$^+$.

3.1.6. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-[2-(tetradecylamino)ethoxy]-propionic acid (4a). To a solution of **2a** (51.3 mg, 43 μmol) in a mixture of phosphate buffer (400 μl, 50 mM) and methanol (800 μl) a solution of 1-tetradecylamine (36.8 mg, 172 μmol) in methanol (800 μl) was added. The pH of the mixture was adjusted with 1N acetic acid to 6.5 and a solution of $NaBH_3CN$ (5.5 mg, 86 μmol) in methanol (100 μl) was added. The mixture was stirred for 24 h at 20°C. The crude mixture was directly applied to a Sephadex® LH-20 column (H_2O–CH_3OH 1:4). Product fractions were concentrated and freeze dried. The residue was dissolved in water (1ml) and liberated from a second equivalent of 1-tetradecylamine by DOWEX® (50 W X8, Na^+-form, water) ion exchange. The crude product was purified by FC (ethyl acetate–2-propanol–H_2O 6:4:3) and subsequent gel filtration (Sephadex LH-20®, H_2O–CH_3OH 1:4). The combined fractions were concentrated and freeze dried to give **4a** (41 mg, 68%). ^{1}H NMR (H,H COSY, 600 MHz, D_2O): Characteristic signals at δ=0.78 (t, CH_3-14^K), 1.15 (s, CH_3-4^F), 1.17–1.29 (m, CH_2-3^K-CH_2-13^K), 1.30 (d, CH_3-6^C), 1.63 (CH_2-2^K), 1.95, 2.01 (s, NHCOCH_3^E, s, NHCOCH_3^C), 3.00 (CH_2-1^K), 3.23 (H-2^D), 4.14 (s, H-4^B, H-5^B), 4.38 (s, H-5^F), 4.43 (d, H-1^D), 4.94 (d, H-3^F), 5.73 (q, H-1^F), J_{5C-6C}=5.9 Hz, J_{1K-2K}=7.3 Hz, J_{2D-3D}=8.1 Hz, J_{1D-2D}=8.1 Hz, J_{2F-3F}=10.6 Hz, J_{1F-2F}=4.0 Hz, $^3J_{1F-P}$=5.9 Hz. ^{13}C NMR (APT, 50 MHz, CD_3OD-D_2O): δ=14.37 (CH_3-14^K), 16.11 (CH_3-4^F), 17.72 (CH_3-6^C), 23.31, 23.40 (NHCO*C*H_3^E, NHCO*C*H_3^C), 26.80, 27.25 (C-13^K, C-2^K), 29.81–30.25 (C-3^K-C-11^K), 32.57 (C-12^K), 56.20, 56.50 (C-2^E, C-2^C), 62.25 (C-6^D), 66.12 (C-1^I), 67.94 (d, C-3^H), 70.17–77.41 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-6^E, C-5^B, C-3^E, C-3^C, C-5^F, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D), 81.67 (C-4^E), 81.89 (C-2^H), 84.32 (C-4^C), 95.52 (C-1^F), 102.57, 103.44, 103.95, 104.33 (C-1^C, C-1^E, C-1^B, C-1^D), 159.03 (O*C*ONH_2^F), 173.94, 174.18, 174.44, 174.83 ($CONH_2^B$, $CONH_2^F$, NH*C*OCH_3^E, NH*C*OCH_3^C), 177.18 (C-1^H). ^{31}P NMR (81 MHz, CD_3OD-D_2O): δ=0.00. $C_{55}H_{97}N_6O_{32}P$ (1385.38, 1384.59), ESI MS (negative mode): *m/z*=1383.58127 (1383.58123) [M−H]$^-$, 691.28725 (691.28697) [M−2H]$^{2-}$, FAB MS: *m/z*=1407.6 [M+Na]$^+$, 1385.6 [M+H]$^+$.

3.1.7. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-[2-(pentadecylamino)ethoxy]-propionic acid (4b). 4b was prepared from **2a** (53.2 mg, 45 μmol) and 1-pentadecylamine (40.7 mg, 180 μmol) as described for **4a**. Yield: 34.1 mg (54%). ^{1}H NMR (H,H COSY, D_2O, 600 MHz): Characteristic signals at δ=0.71 (t, CH_3-15^K), 1.08 (s, CH_3-4^F), 1.10–1.20 (m, CH_2-3^K -CH_2-14^K), 1.23 (d, CH_3-6^C), 1.57 (m, CH_2-2^K), 1.89, 1.95 (s, NHCOCH_3^E, s, NHCOCH_3^C), 2.93 (m, CH_2-1^K), 4.08 (s, H-4^B, H-5^B), 4.31 (s, H-5^F), 4.37 (m, H-1^D), 4.88 (d, H-3^F), 5.67 (q, H-1^F). ^{13}C NMR (50 MHz, CD_3OD-D_2O): δ=14.29 (CH_3-15^K), 16.02 (CH_3-4^F), 17.74 (CH_3-6^C), 23.31, 23.40 (NHCO*C*H_3^E, NHCO*C*H_3^C), 26.79, 27.26 (C-14^K, C-2^K), 29.92–31.68 (C-3^K-C-12^K), 32.57 (C-13^K), 56.30, 56.50 (C-2^E, C-2^C), 62.19 (C-6^D), 70.11, 71.15, 71.84, 72.27, 73.35, 73.58, 73.69, 74.13, 74.53, 74.94, 75.47, 76.01, 77.18, 77.33 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-3^C, C-6^E, C-5^B, C-3^E, C-5^F, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D, broad signals), 81.62 (C-4^E), 84.32 (C-4^C), 95.51, 95.71 (d, C-1^F), 102.55, 103.00, 103.97, 104.32 (C-1^C, C-1^E, C-1^B, C-1^D), 159.04 (O*C*ONH_2^F), 173.98, 174.15, 174.62, 174.93 ($CONH_2^B$, $CONH_2^F$, NH*C*OCH_3^E, NH*C*OCH_3^C). ^{31}P NMR (81 MHz, CD_3OD-D_2O): δ=1.04. $C_{56}H_{99}N_6O_{32}P$ (1399.41, 1398.60), ESI MS (negative mode): *m/z*=1397.59338 (1397.59688) [M−H]$^-$, 698.29649 (698.29480) [M−2H]$^{2-}$ FAB MS: *m/z*=1399.8 [M+H]$^+$.

3.1.8. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-[2-(hexadecylamino)ethoxy]-propionic acid (4c). 4c was prepared from **2a** (55.6 mg, 46 μmol) and 1-hexadecylamine (45.2 mg, 184 μmol) as described for **4a**. Yield: 42.1 mg (63%). ^{1}H NMR (H,H COSY, 600 MHz, D_2O): Characteristic signals at δ=0.76 (bs, CH_3-16^K), 1.12 (s, CH_3-4^F), 1.10–1.25 (m, CH_2-3^K-CH_2-15^K), 1.28 (d, CH_3-6^C), 1.61 (m, CH_2-2^K), 1.93, 1.99 (s, NHCOCH_3^E, s, NHCOCH_3^C), 2.98 (m, CH_2-1^K), 3.20 (bs, H-2^D), 4.12 (s, H-4^B, H-5^B), 4.93 (d, H-3^F), 5.70 (m, H-1^F), J_{5C-6C}=5.5 Hz, J_{2F-3}=8.8 Hz. ^{13}C NMR (APT, 150 MHz, D_2O): δ=14.36 (CH_3-16^K), 15.12 (CH_3-4^F), 17.06 (CH_3-6^C), 22.83, 23.08 (NHCO*C*H_3^E, NHCO*C*H_3^C), 26.26, 27.02 (C-15^K, C-2^K), 29.64–30.38 (C-3^K-C-13^K), 32.41 (C-14^K), 47.72, 48.07 (C-2^I, C-1^K), 55.66–55.91 (C-2^E, C-2^C), 61.24 (C-6^D), 65.70 (d, C-3^H), 67.42 (bs, C-1^I), 69.32, 70.17, 71.01, 71.54, 72.47, 72.61, 72.85, 73.00, 73.22, 73.49, 73.58, 73.93, 74.66, 75.34, 76.20, 76.42, 76.85 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-6^E, C-5^B, C-3^C, C-5^F, C-3^E, C-5^F, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D, broad signals), 80.22 (C-4^E), 83.47 (C-4^C), 97.76 (C-1^F), 101.68, 102.40, 103.04, 103.67 (C-1^C, C-1^E, C-1^B, C-1^D), 158.59 (O*C*ONH_2^F), 173.14, 173.63, 174.78, 175.01 ($CONH_2^B$, $CONH_2^F$, NH*C*OCH_3^E, NH*C*OCH_3^C). ^{31}P NMR (81 MHz,

D_2O): $\delta=-1.05$. $C_{57}H_{101}N_6O_{32}P$ (1413.44, 1412.62), ESI MS (negative mode): m/z=1411.60898 (1411.61253) $[M-H]^-$, 705.30412 (705.30262) $[M-2H]^{2-}$, FAB MS: m/z=1413.8 $[M+H]^+$.

3.1.9. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-[2-(octadecylamino)ethoxy]-propionic acid (4d). **4d** was prepared from **2a** (54.3 mg, 46 μmol) and 1-octadecylamine (49.3 mg, 184 μmol) as described for **4a**. Yield: 16.9 mg (25%). ^{1}H NMR (H,H COSY, 600 MHz, D_2O): Characteristic signals at δ=0.73 (t, CH_3-18^K), 1.08 (s, CH_3-4^F), 1.05–1.30 (m, CH_2-3^K- CH_2-17^K), 1.24 (d, CH_3-6^C), 1.57 (m, CH_2-2^K), 1.89, 1.95 (s, NHCOCH_3^E, s, NHCOCH_3^C), 3.15 (m, H-2^D), 4.08 (s, H-4^B, H-5^B), 4.37 (m, H-1^D), 4.89 (d, H-3^F), 5.64 (m, H-1^F), J_{2F-3F}=9.9 Hz. ^{31}P NMR (81 MHz, D_2O): $\delta=-1.08$. $C_{59}H_{105}N_6O_{32}P$ (1441.49, 1440.65), FAB MS: m/z=1464.0 $[M+Na]^+$, 1441.9 $[M+H]^+$.

3.1.10. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-[2-(methyloctadecylamino)ethoxy]-propionic acid (4e). To a solution of **2a** (106.4 mg, 89 μmol) in a mixture of phosphate buffer (400 μl, 50 mM), methanol (600 μl) and THF (600 μl) a solution of *N*-methyl-1-octadecylamine (50.7 mg, 178 μmol) in methanol (600 μl) and THF (600 μl) was added. The pH of the mixture was adjusted with 1N acetic acid to 7.0 and a solution of $NaBH_3CN$ (11.2 mg, 178 μmol) in methanol (100 μl) was added. The mixture was stirred for 36 h at 20°C. The crude mixture was directly applied to a Sephadex® LH-20 column (H_2O–CH_3OH 1:4). Product fractions were concentrated and freeze dried. The residue was dissolved in water (1ml) and liberated from a second equivalent of *N*-methyl-1-octadecylamine by DOWEX® (50 W X8, Na^+-form, water) ion exchange. The crude product was purified by FC (ethyl acetate–2-propanol–H_2O 6:4:2) and subsequent gel filtration (Sephadex LH-20®, H_2O–CH_3OH 1:4). The combined fractions were concentrated and freeze dried to give **4e** (11.8 mg, 9%). ^{1}H NMR (H,H COSY, 600 MHz, CD_3OD): Characteristic signals at δ=0.91 (t, CH_3-18^K), 1.15 (s, CH_3-4^F), 1.17–1.28 (m, CH_2-3^K-CH_2-17^K), 1.31 (d, CH_3-6^C), 1.70 (bs, CH_2-2^K), 1.90, 1.96 (s, NHCOCH_3^E, s, NHCOCH_3^C), 2.84 (bs, CH_3-19^K), 3.04 (bs, CH_2-1^K), 3.10 (dd, H-2^D), 4.34 (d, H-1^D), 5.12 (d, H-3^F), 6.00 (m, H-1^F), J_{5C-6C}=6.0 Hz, J_{2D-3D}=8.0 Hz, J_{1D-2D}=8.3 Hz, J_{2F-3F}=10.5 Hz. ^{13}C NMR (APT, 150 MHz, D_2O): δ=14.41 (CH_3-18^K), 15.59 (CH_3-4^F), 17.26 (CH_3-6^C), 22.97, 23.08 (NHCOCH_3^E, NHCOCH_3^C, C-17^K), 23.84, 26.90 (C-2^K, C-3^K), 29.55–30.31 (C-4^K-C-15^K), 32.39 (C-16^K), 39.68 (CH_3-19^K) 55.45–55.67 (C-2^E, C-2^C), 55.89 (C-1^K), 57.17 (C-2^I), 61.30 (C-6^D), 69.36, 70.17, 70.98, 71.52, 72.46, 72.78, 73.08, 73.59, 73.90, 74.77, 75.22, 76.32, 76.50, 76.97, 77.05 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-6^E, C-5^B, C-3^E, C-3^C, C-5^F, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D), 80.27 (C-4^E), 83.49 (C-4^C), 94.95 (C-1^F), 101.70, 102.66, 103.15, 103.59 (C-1^C, C-1^E, C-1^B, C-1^D), 158.43 (O$CONH_2^F$), 173.16–174.82 ($CONH_2^B$, $CONH_2^F$, NH$COCH_3^E$, NH$COCH_3^C$). ^{31}P NMR (81 MHz, CD_3OD): $\delta=-1.60$. $C_{60}H_{107}N_6O_{32}P$ (1455.52, 1454.67) ESI MS (negative mode): m/z=1453.66183 (1453.65948) $[M-H]^-$, 726.32800 (726.32610) $[M-2H]^{2-}$, FAB MS: m/z=1478.1 $[M+Na]^+$, 1456.0 $[M+H]^+$.

3.2. Synthesis of the [^{15}N]phytylphthalimides

Diethyl azodicarboxylate (855 μl, 4.35 mmol) was added dropwise in the dark to a solution of phytol (1 g, 3.37 mmol, commercially available from Aldrich® as a 60:40 mixture of *E*/*Z*-isomers), [^{15}N]phthalimide (644 mg, 4.35 mmol) and triphenylphosphine (1.141 g, 4.35 mmol) in THF (10 ml). The mixture was stirred for 12 h at 20°C and water (60 ml) was added. The aqueous phase was extracted with *n*-hexane (4×50 ml). The combined organic extracts were washed with brine, dried ($MgSO_4$) and filtered. The filtrate was concentrated and the residue was separated by FC (toluene–*n*-hexane 1:1) to give (*E*)-[^{15}N]phytylphthalimide (788.9 mg) and (*Z*)-[^{15}N]phytylphthalimide (306.3 mg). The combined yield was 76%.

3.2.1. (*E*)-[^{15}N]Phytylphthalimide. ^{1}H NMR (NOEDIF, 300 MHz, $CDCl_3$): δ=0.81–0.86 (s, CH_3-16^A-CH_3-19^A), 0.90–1.40 (m, CH_2-5^A, CH_2-6^A-CH_2-8^A, CH_2-10^A-CH_2-12^A, CH_2-14^A), 1.82 (s, CH_3-20^A), 1.95 (dd, CH_2-4^A), 4.28 (d, CH_2-1^A), 5.26 (t, CH-2^A), 7.70 (m, H-4^B, H-5^B), 7.83 (m, H-3^B, H-6^B), J_{2A-3A}=6.1 Hz, J_{H4-H5}=7.5 Hz, J_{H1-H2}=7.1 Hz, J_{H2-H1}=7.1 Hz. ^{13}C NMR (75 MHz, $CDCl_3$): δ=16.67 (C-20^A), 20.00–20.12 (C-18^A, C-19^A, isomerism leads to signal splitting), 23.01, 23.10 (C-16^A, C-17^A), 24.83 (C-9^A), 25.18 (C-13^A), 25.43 (C-5^A), 28.35 (C-15^A), 33.01, 33.02 (C-7^A, isomerism leads to signal splitting), 33.13, 33.15 (C-11^A, isomerism leads to signal splitting), 36.13, 36.25, 37.02, 37.12 (d, ^{15}N-C-1^A), $J_{15N-C1(A)}$=9.3 Hz, 37.67, 37.72, 37.75, 37.76, 37.79 (C-6^A, C-8^A, C-10^A, C-12^A), 39.74 (C-14^A), 40.20 (C-4^A), 118.07 (C-2^A), 123.51 (C-4^B, C-5^B), 132.67, 132.77 (C-2^B, C-7^B), 134.15 (C-3^B, C-6^B), 141.47, 141.49 (C-3^A), 168.44, 168.61 (d, ^{15}N-CO-1^B, ^{15}N-CO-8^B), $J_{15N-C1-C8(B)}$=13.1 Hz. $C_{28}H_{43}{}^{15}NO_2$ (426.65, 426.32), FAB MS: m/z=427.4 $[M+H]^+$.

3.2.2. (*Z*)-[^{15}N]Phytylphthalimide. ^{1}H NMR (NOEDIF, 300 MHz, $CDCl_3$): δ=0.84–0.88 (s, CH_3-16^A-CH_3-19^A), 0.95–1.50 (m, CH_2-5^A, CH_2-6^A-CH_2-8^A, CH_2-10^A-CH_2-12^A, CH_2-14^A), 1.70 (s, CH_3-20^A), 2.21 (dd, CH_2-4^A), 4.27 (d, CH_2-1^A), 5.28 (t, CH-2^A), 7.69 (m, H-4^B, H-5^B), 7.83 (m, H-3^B, H-6^B). ^{13}C NMR (75 MHz, $CDCl_3$): δ=19.62, 19.69, 19.76 (C-18^A, C-19^A, isomerism leads to signal splitting), 22.63, 22.73 (C-16^A, C-17^A), 23.32 (C-20^A), 24.47 (C-9^A), 24.82 (C-13^A), 25.53 (C-5^A), 27.98 (C-15^A), 32.18 (C-7^A, C-11^A), 32.73, 32.75, 32.78 (C-4^A, isomerism leads to signal splitting), 35.51, 35.63, 36.93, 37.03 (d, ^{15}N-C-1^A), $J_{15N-C1(A)}$=9.3 Hz, 37.30, 37.34, 37.40, 37.46, 37.79 (C-6^A, C-8^A, C-10^A, C-12^A), 39.38 (C-14^A), 118.47 (C-2^A), 123.13 (C-4^B, C-5^B), 132.27, 132.37 (C-2^B, C-7^B), 133.77 (C-3^B, C-6^B), 141.26, 141.28 (C-3^A), 168.01, 168.18 (d, ^{15}N-CO-1^B, ^{15}N-CO-8^B), $J_{15N-C1-C8(B)}$=13.1 Hz. $C_{28}H_{43}{}^{15}NO_2$ (426.65, 426.32), FAB MS: m/z=427.3 $[M+H]^+$.

3.2.3. (*E*)-[^{15}N]Phytylamine. To a solution of (*E*)-[^{15}N]phytylphthalimide (788.9 mg, 1.85 mmol) in ethanol

(5 ml) hydrazine hydrate (269.1 μl, 5.55 mmol) was added dropwise and stirred for 2 h. The solution was filtered and the filtrate was concentrated. The residue was purified by FC (CH_2Cl_2–CH_3OH–NH_3 conc. 10:1:0.1) to give *E*-phytylamine **36** (451 mg, 82%). ^1H NMR (H,H COSY, 300 MHz, D_2O): δ=0.83–0.86 (s, CH_3-16-CH_3-19), 0.95–1.40 (m, CH_2-5, CH_2-6-CH_2-8, CH_2-10-CH_2-12, CH_2-14), 1.61 (s, CH_3-20), 1.95 (dd, CH_2-4), 3.27 (d, CH_2-1), 5.24 (t, CH-2), J_{H4-H5}=7.3 Hz, J_{H1-H2}=6.3 Hz, J_{H2-H1}=6.7 Hz. ^{13}C NMR (75 MHz, D_2O): δ=16.02 (C-20), 19.68, 19.74, 19.76 (C-18, C-19, isomerism leads to signal splitting), 22.63, 22.73 (C-16, C-17), 24.48 (C-9), 24.81 (C-13), 25.25, 25.26 (C-5), 27.98 (C-15), 32.70 (C-7, isomerism leads to signal splitting), 32.78, 32.80 (C-11, isomerism leads to signal splitting), 36.68, 36.78, 36.88, 37.30, 37.34, 37.39, 37.44 (C-6, C-8, C-10, C-12), 39.38 (C-14), 39.47, 39.51 (d, ^{15}N-C-1), J_{15N-C1}=3.2 Hz, 39.88 (C-4), 125.17 (C-2), 137.16 (C-3). $C_{20}H_{41}{}^{15}N$ (229.55, 229.32), FAB MS: *m/z*=300.3 $[M+D]^+$, H/D exchange experiment, 297.3 $[M+H]^+$.

3.2.4. (*Z*)-[^{15}N]Phytylamine. (*Z*)-[^{15}N]Phytylamine was prepared as described for the (*E*)-isomer. Yield: 193 mg (90%). ^1H NMR (H,H COSY, 300 MHz, D_2O): δ=0.84–0.87 (s, CH_3-16-CH_3-19), 0.95–1.45 (m, CH_2-5, CH_2-6-CH_2-8, CH_2-10-CH_2-12, CH_2-14), 1.69 (s, CH_3-20), 2.00 (dd, CH_2-4), 3.25 (d, CH_2-1), 5.24 (t, CH-2), J_{H4-H5}=7.4 Hz, J_{H1-H2}=6.6 Hz, J_{H2-H1}=6.8 Hz. ^{13}C NMR (75 MHz, D_2O): δ=19.64, 19.69, 19.75 (C-18,19, isomerism leads to signal splitting), 22.63, 22.73 (C-16,17), 23.39 (C-20), 24.46 (C-9), 24.80, 24.81 (C-13), 25.61 (C-5), 27.98 (C-15), 32.09 (C-4), 32.68, 32.70 (C-7, isomerism leads to signal splitting), 32.77, 32.78 (C-11, isomerism leads to signal splitting), 36.88, 36.97, 37.29, 37.34, 37.38, 37.43 (C-6, C-8, C-10, C-12), 39.34 (d, ^{15}N-C-1), J_{15N-C1}=4.5 Hz, 39.37 (C-14), 126.20 (C-2), 137.28 (C-3). $C_{20}H_{41}{}^{15}N$ (296.55, 296.32), FAB MS: *m/z*=300.3 $[M+D]^+$, H/D exchange experiment, 297.3 $[M+H]^+$.

3.2.5. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-[2-(*rac*-phytylamino)ethoxy]-propionic acid (4g). To a solution of **2a** (65.2 mg, 55 μmol) in a mixture of phosphate buffer (400 μl, 50 mM) and methanol (800 μl) a solution of [^{15}N]-*Z*-phytylamine (48.8 mg, 165 μmol) in methanol (400 μl) was added. The pH of the mixture was adjusted with 1N acetic acid to 7.0 and a solution of $NaBH_3CN$ (6.9 mg, 110 μmol) in methanol (100 μl) was added. The mixture was stirred for 48 h at 20°C. The crude mixture was directly applied to a Sephadex® LH-20 column (H_2O–CH_3OH 1:4). Product fractions were concentrated and freeze dried. The residue was dissolved and liberated from a second equivalent of [^{15}N]-*Z*-phytylamine by DOWEX® (50 W X8, Na^+-form, water) ion exchange. The crude product was purified by FC (ethyl acetate–2-propanol–H_2O 6:4:2) and subsequent gel filtration (Sephadex LH-20®, H_2O–CH_3OH 1:4). The combined fractions were concentrated and freeze dried to give **4g** (6.7 mg, 8%). ^1H NMR (H,H COSY, 600 MHz, CD_3OD): Characteristic signals at δ=0.81, 0.82, 0.83, 0.84 (m, CH_3-16^K, CH_3-17^K, CH_3-18^K, CH_3-19^K), 1.17 (s, CH_3-4^F), 1.00–1.35 (m, CH_2-6^K, CH_2-8^K, CH_2-10^K, CH_2-12^K, CH_2-14^K) 1.34 (d, CH_3-6^C), 1.76 (s, CH_3-20^K), 1.94, 2.00 (s, $NHCOCH_3{}^E$, s, $NHCOCH_3{}^C$), 2.09 (m, CH_2-4^K), 5.07 (d, H-3^F), 5.35 (t, CH=C(CH_3)-2^K), 5.97 (m, H-1^F), J_{5C-6C}=5.8 Hz, J_{2F-3F}=10.4 Hz, J_{1K-2K}=6.8 Hz. ^{31}P NMR (81 MHz, CD_3OD): δ=−0.48. $C_{61}H_{107}N_5{}^{15}NO_{32}P$ (1468.53, 1467.66), ESI MS (negative mode): *m/z*=1466.66251 (1466.65596) $[M-H]^-$, 732.82489 (732.82257) $[M-2H]^{2-}$, FAB MS: *m/z*=1490.6 $[M+Na]^+$, 1468.5 $[M+H]^+$.

3.2.6. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-[2-(*rac*-phytylamino)ethoxy]-propionic acid (4f). 4f was prepared from **2a** (54.3 mg, 46 μmol) and [^{15}N]-*E*-phytylamine (27.1 mg, 92 μmol) as described for **4g**. Yield: 13.2 mg (19%). ^1H NMR (H,H COSY, 600 MHz, CD_3OD): Characteristic signals at δ=0.80, 0.82, 0.83 (m, CH_3-16^K, CH_3-17^K, CH_3-18^K, CH_3-19^K), 1.18 (s, CH_3-4^F), 1.00–1.35 (m, CH_2-6^K, CH_2-8^K, CH_2-10^K, CH_2-12^K, CH_2-14^K) 1.35 (d, CH_3-6^C), 1.71 (s, CH_3-20^K), 1.94, 2.00 (s, $NHCOCH_3{}^E$, s, $NHCOCH_3{}^C$), 2.04 (m, CH_2-4^K), 5.06 (d, H-3^F), 5.35 (m, CH=C(CH_3)-2^K), 5.96 (m, H-1^F), J_{5C-6C}=5.8 Hz, J_{2F-3F}=10.5 Hz. ^{31}P NMR (81 MHz, CD_3OD): δ=−0.48. $C_{61}H_{107}N_5{}^{15}NO_{32}P$ (1468.53, 1467.66), ESI MS (negative mode): *m/z*=732.82633 (732.82257) $[M-2H]^{2-}$, FAB MS: *m/z*=1468.6 $[M+H]^+$.

3.2.7. (*R*)-2-[2-(Dihexylamino)ethoxy]-3-({β-D-galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}-hydroxyphosphoryloxy)-propionic acid (5a). 5a was prepared from **2a** (60 mg, 50 μmol) and dihexylamine (37.4 mg, 200 μmol) as described for **4a**. Yield: 26 mg (38%). ^1H NMR (H,H COSY, 400 MHz, D_2O): Characteristic signals at δ=0.77 (t, CH_3-6^K, CH_3-6^L), 1.12 (s, CH_3-4^F), 1.18–1.32 (m, CH_2-3^K-CH_2-5^K, CH_2-3^L-CH_2-5^L) 1.28 (d, CH_3-6^C), 1.61 (m, CH_2-2^K, CH_2-2^L), 1.92, 1.99 (s, $NHCOCH_3{}^E$, s, $NHCOCH_3{}^C$), 3.08 (m, CH_2-1^K, CH_2-1^L), 3.19 (dd, 2-H^D), 4.11 (s, H-4^B, H-5^B), 4.34 (s, H-5^F), 4.40 (d, 1-H^D), 4.91 (d, H-3^F), 5.70 (m, H-1^F), J_{5C-6C}=5.6 Hz, J_{2D-3D}=8.1 Hz, J_{1D-2D}=7.8 Hz, J_{2F-3F}=10.6 Hz. ^{13}C NMR (APT, HETCOR, 50 MHz, D_2O): δ=13.41 (CH_3-6^K, CH_3-6^L), 14.98 (CH_3-4^F), 16.82 (CH_3-6^C), 21.89 (C-5^K, C-5^L), 22.50–22.58 ($NHCOCH_3{}^E$, $NHCOCH_3{}^C$), 22.63–22.82 (C-2^K, C-2^L), 25.59 (C-3^K, C-3^L), 30.61 (C-4^K, C-4^L), 52.80 (C-1^K, C-1^L), 53.41 (C-2^I), 55.42–55.68 (C-2^E, C-2^C), 61.02 (C-6^D), 63.84 (C-1^I), 66.97 (C-3^H), 69.08–76.80 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-3^C, C-5^F, C-6^E, C-5^B, C-3^E, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D), 79.98 (C-4^E), 80.83 (C-2^H), 83.22 (C-4^C), 94.61 (C-1^F), 101.41, 102.33, 102.80, 103.41 (C-1^C, C-1^E, C-1^B, C-1^D), 158.33 ($OCONH_2{}^F$), 173.08, 173.38 ($CONH_2{}^B$, $CONH_2{}^F$), 174.29, 174.76 ($NHCOCH_3{}^E$, $NHCOCH_3{}^C$), 176.58 (C-1^H). ^{31}P NMR (81 MHz, D_2O): δ=−1.06. $C_{53}H_{93}N_6O_{32}P$ (1357.33, 1356.56), ESI MS (negative mode): *m/z*=1355.54970 (1355.54992) $[M-H]^-$, 677.27124 (677.27132) $[M-2H]^{2-}$.

3.2.8. (*R*)-2-[2-(Dioctylamino)ethoxy]-3-({β-D-galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}-hydroxyphosphoryloxy)-propionic acid (5b). 5b was prepared from **2a** (60 mg, 50 μmol) and dioctylamine (48.8 mg, 200 μmol) as described for **4a**. Yield: 22.0 mg (31%). ^{1}H NMR (H,H COSY, 400 MHz, D_2O–CD_3OD): Characteristic signals at δ=0.83 (t, CH_3-8^K, CH_3-8^L), 1.19 (s, CH_3-4^F), 1.20–1.38 (m, CH_2-3^K-CH_2-7^K, CH_2-3^L-CH_2-7^L) 1.35 (d, CH_3-6^C), 1.69 (m, CH_2-2^K, CH_2-2^L), 1.96, 2.02 (s, NHCOCH_3^E, s, NHCOCH_3^C), 3.11 (m, CH_2-1^K, CH_2-1^L), 3.17 (dd, 2-H^D), 4.04 (s, H-4^B, H-5^B), 5.01 (d, H-3^F), 5.87 (q, H-1^F), J_{5C-6C}=6.0 Hz, J_{2D-3D}=8.5 Hz, J_{2F-3F}=10.2 Hz, J_{1F-2F}=3.5 Hz, $^3J_{1F-P}$=5.7 Hz. ^{13}C NMR (APT, 100 MHz, D_2O–CD_3OD): δ=14.41 (CH_3-8^K, CH_3-8^L), 16.42 (CH_3-4^F), 17.83 (CH_3-6^C), 23.42, 23.44, 23.51 (NHCO*C*H_3^E, NHCO*C*H_3^C, C-7^K, C-7^L), 24.11 (C-2^K, C-2^L), 27.49 (C-3^K, C-3^L), 29.99, 30.01 (C-4^K, C-4^L, C-5^K, C-5^L), 32.71 (C-6^K, C-6^L), 53.73 (C-1^K, C-1^L), 55.14 (C-2^I), 56.31, 56.64 (C-2^E, C-2^C), 62.62 (C-6^D), 65.06 (C-1^I), 68.19 (d, C-3^H), 70.01, 70.42, 71.62, 72.12, 72.48, 73.56, 73.70, 74.05, 74.31, 74.89, 74.96, 75.79, 75.85, 77.71, 77.79, 78.91, 79.00 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-3^C, C-5^F, C-6^E, C-5^B, C-3^E, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D), 81.81 (C-2^H), 82.09 (C-4^E), 84.63 (C-4^C), 95.86 (d, C-1^F), 102.91, 103.81, 104.32, 104.44 (C-1^C, C-1^E, C-1^B, C-1^D), 159.04 (O*C*ONH_2^F), 173.88, 174.03, 174.44, 174.70 ($CONH_2^B$, $CONH_2^F$, NH*C*OCH_3^E, NH*C*OCH_3^C), 177.30 (C-1^H). ^{31}P NMR (81 MHz, D_2O–CD_3OD): δ=−0.36. $C_{57}H_{101}N_6O_{32}P$ (1413.44, 1412.62), ESI MS (negative mode): *m/z*=1411.61048 (1411.61253) $[M-H]^-$, 705.30134 (705.30262) $[M-2H]^{2-}$, FAB MS: *m/z*=1457.7 $[M+2Na-H]^+$, 1435.5 $[M+Na]^+$, 1413.4 $[M+H]^+$.

3.2.9. (*R*)-2-[2-(Didodecylamino)ethoxy]-3-({β-D-galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}-hydroxyphosphoryloxy)-propionic acid (5c). To a solution of **2a** (101.5 mg, 85 μmol) in a mixture of phosphate buffer (400 μl, 50 mM), methanol (800 μl) and THF (800 μl) a solution of didodecylamine (60.4 mg, 170 μmol) in a mixture of methanol (400 μl) and THF (400 μl) was added. The pH of the mixture was adjusted with 1N sodium hydroxide to pH 7.0 and a solution of $NaBH_3CN$ (10.8 mg, 170 μmol) in methanol (100 μl) was added. The mixture was rapidly stirred for 72 h at 20°C. The crude mixture was filtered and directly applied to a Sephadex® LH-20 column (H_2O–CH_3OH 1:4). Product fractions were concentrated and freeze dried. The residue was dissolved in water (1 ml) and liberated from a second equivalent of didodecylamine by DOWEX® (50 W X8, Na^+-form, water) ion exchange. The crude product was purified by FC (ethyl acetate–2-propanol–H_2O 6:4:2) and subsequent gel filtration (Sephadex LH-20®, H_2O–CH_3OH 1:4). The combined fractions were concentrated and freeze dried to give **5c** (7 mg, 5%). ^{1}H NMR (H,H COSY, 400 MHz, CD_3OD): Characteristic signals at δ=0.84 (t, CH_3-12^K, CH_3-12^L), 1.19 (s, CH_3-4^F), 1.20–1.40 (m, CH_2-3^K-CH_2-11^K, CH_2-3^L-CH_2-11^L) 1.35 (d, CH_3-6^C), 1.60–1.75 (m, CH_2-2^K, CH_2-2^L), 1.93, 1.99 (s, NHCOCH_3^E, s, NHCOCH_3^C), 2.90–2.93, 3.10–3.15 (CH_2-1^K, CH_2-1^L), 3.98 (s, H-5^F), 4.10 (s, H-4^B, H-5^B), 5.06 (d, H-3^F), 5.91 (m, H-1^F), J_{2F-3F}=11 Hz. ^{31}P NMR (81 MHz, D_2O–CD_3OD): δ=−1.69. $C_{65}H_{117}N_6O_{32}P$ (1525.64, 1524.74), ESI MS (negative mode): *m/z*=1523.73461 (1523.73774) $[M-H]^-$, 761.36439 (761.36522) $[M-2H]^{2-}$, FAB MS: *m/z*=1525.7 $[M+H]^+$.

3.2.10. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-[2-(4-octylanilino)ethoxy]-propionic acid (6a). To a solution of **2a** (51.4 mg, 43 μmol) in a mixture of phosphate buffer (400 μl, 50 mM) and methanol (800 μl) a solution of octylaniline (26.6 mg, 132 μmol) in methanol (800 μl) was added. The pH of the mixture was adjusted with 1N sodium hydroxide to pH 7.0 and a solution of $NaBH_3CN$ (5.5 mg, 86 μmol) in methanol (100 μl) was added. The mixture was stirred for 48 h at 20°C. The crude mixture was directly applied to a Sephadex® LH-20 column (H_2O–CH_3OH 1:4). Product fractions were concentrated and freeze dried. The crude product was purified by FC (ethyl acetate–2-propanol–H_2O 6:4:2) and subsequent gel filtration (Sephadex LH-20®, H_2O–CH_3OH 1:4). The combined fractions were concentrated and freeze dried to give **6a** (42.2 mg, 71%). ^{1}H NMR (H,H COSY, 600 MHz, D_2O): Characteristic signals at δ=0.77 (t, CH_3-10^L), 1.14 (s, CH_3-4^F), 1.15–1.25 (m, CH_2-3^L-CH_2-7^L), 1.29 (d, CH_3-6^C), 1.50 (m, CH_2-2^L), 1.95, 2.01 (s, NHCOCH_3^E, s, NHCOCH_3^C), 2.49 (dd, CH_2-1^L), 3.22 (dd, H-2^D), 3.30 (m, H-4^D), 3.49 (m, H-3^D), 3.96 (bs, H-3^B), 4.37 (s, H-4^B, H-5^B), 4.37 (s, 5-H^F), 4.43 (d, H-1^D), 4.95 (d, H-3^F), 5.71 (bs, H-1^F), 6.93 (bs, H-2^K, H-6^K), 7.14 (bs, H-3^K, H-5^K), J_{5C-6C}=6.2 Hz, J_{1L-2L}=7.0 Hz, J_{2D-3D}=8.1 Hz, J_{1D-2D}=7.0 Hz, J_{2F-3F}=9.9 Hz. ^{13}C NMR (100 MHz, CD_3OD-D_2O): δ=13.94 (CH_3-8^L), 15.14 (CH_3-4^F), 16.87 (CH_3-6^C), 22.55–22.69 (NHCO*C*H_3^E, NHCO*C*H_3^C), 29.06–31.71 (C-2^L-C-7^L), 35.07 (C-1^L), 55.50–55.74 (C-2^E, C-2^C), 61.09 (C-6^D), 69.15–76.91 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-3^C, C-5^F, C-6^E, C-5^B, C-3^E, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D, C-2^H, broad signals), 80.18 (C-4^E), 83.32 (C-4^C), 94.67 (C-1^F), 101.52, 102.38, 102.88, 103.49 (C-1^C, C-1^E, C-1^B, C-1^D), 121.88 (C-2^K, C-6^K), 130.16 (C-3^K, C-5^K), 134.93 (C-1^K), 158.37 (O*C*ONH_2^F), 173.02–176.09 ($CONH_2^B$, $CONH_2^F$, NH*C*OCH_3^E, NH*C*OCH_3^C). ^{31}P NMR (81 MHz, D_2O): δ=−2.01. $C_{55}H_{89}N_6O_{32}P$ (1377.32, 1376.52), ESI MS (negative mode): *m/z*=1375.52081 (1375.51862) $[M-H]^-$, 687.25660 (687.25567) $[M-2H]^{2-}$, FAB MS: *m/z*=1377.5 $[M+H]^+$.

3.2.11. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-[2-(4-decylanilino)ethoxy]-propionic acid (6b). 6b was prepared from **2a** (55.1 mg, 46 μmol) and 4-decylaniline (32.5 mg, 138 μmol) as described for **6a**. Yield: 21.3 mg (32%). ^{1}H NMR (H,H COSY, 600 MHz, D_2O): Characteristic signals at δ=0.70 (t, CH_3-10^L), 1.06 (s, CH_3-4^F), 1.05–1.25 (m, CH_2-3^L- CH_2-9^L), 1.21 (d, CH_3-6^C), 1.41 (m,

CH_2-2^L), 1.88, 1.94 (s, $NHCOCH_3^E$, s, $NHCOCH_3^C$), 2.39 (dd, CH_2-1^L), 3.16 (m, H-2^D), 4.07 (s, H-4^B, H-5^B), 4.35 (d, H-1^D), 4.89 (d, H-3^F), 5.64 (m, H-1^F), 6.70 (d, H-2,6^K), 7.00 (d, H-3,5^K), J_{5C-6C}=5.2 Hz, J_{1L-2L}=6.3 Hz, J_{1D-2D}=7.8 Hz, J_{2F-3F}=8.4 Hz, J_{2K-3K}=J_{5K-6K}=7.3 Hz. ^{13}C NMR (100 MHz, CD_3OD-D_2O): δ=14.38 (CH_3-10^L), 16.04 (CH_3-4^F), 17.69 (CH_3-6^C), 23.27–23.39 ($NHCOCH_3^E$, $NHCOCH_3^C$, C-9^L), 29.65–30.08 (C-3^L-C-7^L), 31.98 (C-2^L), 32.49 (C-8^L), 35.82 (C-1^L), 56.19, 56.46 (C-2^E, C-2^C), 62.19 (C-6^D), 70.13–78.35 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-3^C, C-5^F, C-6^E, C-5^B, C-3^E, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D, C-2^H, broad signals), 81.51 (C-4^E), 84.27 (C-4^C), 95.54 (C-1^F), 102.55, 103.35, 103.90, 104.31 (C-1^C, C-1^E, C-1^B, C-1^D), 122.19 (C-2^K, C-6^K), 130.92 (C-3^K, C-5^K), 141.20 (C-1^K), 159.05 ($OCONH_2^F$), 173.98–174.88 ($CONH_2^B$, $CONH_2^F$, $NHCOCH_3^E$, $NHCOCH_3^C$). ^{31}P NMR (81 MHz, D_2O): δ=−2.05. $C_{57}H_{93}N_6O_{32}P$ (1405.37, 1404.58), ESI MS (negative mode): *m/z*=1403.54895 (1403.54992) $[M-H]^-$, 701.27149 (701.27132) $[M-2H]^{2-}$, FAB MS: *m/z*=1405.7 $[M+H]^+$.

3.2.12. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-[2-(4-dodecylanilino)ethoxy]-propionic acid (6c). 6c was prepared from **2a** (53.4 mg, 45 μmol) and 4-dodecylaniline (23.5 mg, 90 μmol) as described for **6a** (72 h reaction time). Yield: 20.1 mg (31%). ^{1}H NMR (H,H COSY, 600 MHz, D_2O): Characteristic signals at δ=0.77 (t, CH_3-10^L), 1.13 (s, CH_3-4^F), 1.10–1.28 (m, CH_2-3^L-CH_2-11^L), 1.28 (d, CH_3-6^C), 1.48 (m, CH_2-2^L), 1.95, 2.01 (s, $NHCOCH_3^E$, s, $NHCOCH_3^C$), 2.45 (dd, CH_2-1^L), 3.22 (m, H-2^D), 4.14 (s, H-4^B, H-5^B), 4.37 (s, H-5^F), 4.43 (d, H-1^D), 4.96 (d, H-3^F), 5.71 (m, H-1^F), 6.78 (d, H-2^K, H-6^K), 7.08 (d, H-3^K, H-5^K), J_{5C-6C}=5.5 Hz, J_{1L-2L}=7.0 Hz, J_{1D-2D}=7.7 Hz, J_{2F-3F}=9.9 Hz, J_{2K-3K}=J_{5K-6K}=7.3 Hz. ^{13}C NMR (100 MHz, CD_3OD-D_2O): δ=14.44 (CH_3-12^L), 15.51 (CH_3-4^F), 17.21 (CH_3-6^C), 23.09 ($NHCOCH_3^E$, $NHCOCH_3^C$, C-11^L), 29.87–30.28 (C-3^L-C-9^L), 32.38 (C-2^L, C-10^L), 35.48 (C-1^L), 46.12 (C-2^I), 55.43–55.91 (C-2^E, C-2^C), 61.23 (C-6^D), 67.46 (C-1^I), 69.32–76.42 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-6^E, C-5^B, C-3^E, C-3^C, C-5^F, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D, C-2^H, broad signals), 80.01 (C-4^E), 83.35 (C-4^C), 94.78 (C-1^F), 101.66, 102.63, 103.04, 103.54 (C-1^C, C-1^E, C-1^B, C-1^D), 117.02 (C-2^K, C-6^K), 129.32 (C-3^K, C-5^K), 135.60 (C-4^K), 143.71 (C-1^K), 158.59 ($OCONH_2^F$), 173.15–174.66 ($CONH_2^B$, $CONH_2^F$, $NHCOCH_3^E$, $NHCOCH_3^C$). ^{31}P NMR (81 MHz, D_2O): δ=−1.05. $C_{59}H_{97}N_6O_{32}P$ (1433.43, 1432.59), FAB MS: *m/z*=1433.9 $[M+H]^+$.

3.2.13. (*R*)-2-[2-(Benzhydrylamino)ethoxy]-3-({β-D-galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}-hydroxyphosphoryloxy)-propionic acid (6d). 6d was prepared from **2a** (55.2 mg, 46 μmol) and benzhydrylamine (17 mg, 92 μmol) as described for **6a**. Yield: 43 mg (62%). ^{1}H NMR (H,H COSY, 600 MHz, D_2O): Characteristic signals at δ=1.09 (s, CH_3-4^F), 1.24 (d, CH_3-6^C, signal doubling), 1.89, 1.97 (s, $NHCOCH_3^E$, s, $NHCOCH_3^C$, signal doubling), 3.18 (dd, H-2^D), 3.91 (s, H-4^B, H-5^B), 4.33 (s, H-5^F), 4.37 (d, H-1^D), 4.89 (d, H-3^F), 5.66 (q, H-1^F), 7.10–7.35 (m, H-3^K-H-7^K, m, H-3^L-H-7^L), J_{5C-6C}=6.0 Hz, J_{2D-3D}=8.1 Hz, J_{1D-2D}=8.1 Hz, J_{2F-3F}=10.5 Hz, J_{1F-2F}=3.6 Hz, $^3J_{1F-P}$=5.6 Hz. ^{13}C NMR (50 MHz, D_2O): δ=14.99 (CH_3-4^F), 16.89 (CH_3-6^C, signal doubling), 22.56–22.67 ($NHCOCH_3^E$, $NHCOCH_3^C$), 40.81 (C-2^I), 51.17 (C-1^K), 55.43–55.78 (C-2^E, C-2^C), 61.06 (C-6^D), 65.14 (C-1^I), 67.10 (C-3^H), 69.14–76.73 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-3^C, C-5^F, C-6^E, C-5^B, C-3^E, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D), 80.03 (C-4^E), 80.94 (C-2^H), 83.26 (C-4^C), 94.57 (d, C-1^F), 101.45, 102.45, 102.85, 103.46 (C-1^C, C-1^E, C-1^B, C-1^D), 122.38, 126.70, 129.10, 130.71, 134.33 (C-3^K-C-7^K, C-3^L-C-7^L), 141.46, 142.68 (C-2^K, C-2^L), 158.37 ($OCONH_2^F$), 173.00, 173.40, 174.45, 174.77 ($CONH_2^B$, $CONH_2^F$, $NHCOCH_3^E$, $NHCOCH_3^C$). ^{31}P NMR (81 MHz, D_2O): δ=−0.73. $C_{54}H_{79}N_6O_{32}P$ (1355.23, 1354.44), ESI MS (negative mode): *m/z*=1353.44065 (1353.44037) $[M-H]^-$, 676.21631 (676.21655) $[M-2H]^{2-}$, FAB MS: *m/z*=1377.2 $[M+Na]^+$, 1355.3 $[M+H]^+$.

3.2.14. (*R*)-2-{2-[4-(Benzyloxy)anilino]ethoxy}-3-({β-D-galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}-hydroxyphosphoryloxy)-propionic acid (6e). 6e was prepared from **2a** (55.2 mg, 46 μmol) and 4-benzyloxyaniline (21.9 mg, 92 μmol) as described for **6a**. Yield: 31.2 mg (51%). ^{1}H NMR (H,H COSY, 600 MHz, D_2O): Characteristic signals at δ=1.10 (s, CH_3-4^F), 1.24–1.27 (d, CH_3-6^C, signal doubling), 1.95–2.01 (s, $NHCOCH_3^E$, s, $NHCOCH_3^C$, signal doubling), 3.18 (dd, H-2^D), 4.10 (s, H-4^B, H-5^B), 4.33 (s, H-5^F), 4.38 (d, H-1^D), 4.89 (m, H-3^F), 5.06 (OCH_2-1^L), 5.68 (m, H-1^F), 7.01 (d, H-2^K, H-6^K), 7.18 (d, H-3^K, H-5^K), 7.30 (m, H-5^L), 7.34 (m, H-3^L, H-7^L), 7.38 (m, H-4^L, H-6^L), J_{2D-3D}=7.7 Hz, J_{1D-2D}=7.3 Hz, J_{2K-3K}=J_{5K-6K}=7.7 Hz. ^{13}C NMR (50 MHz, D_2O): δ=14.98 (CH_3-4^F), 16.89 (CH_3-6^C), 22.67 ($NHCOCH_3^E$, $NHCOCH_3^C$), 55.54–55.78 (C-2^E, C-2^C), 61.10 (C-6^D), 69.17–76.24 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-6^E, C-5^B, C-3^E, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-3^C, C-5^D, C-3^D, C-2^H, broad signals), 80.11 (C-4^E), 83.30 (C-4^C), 94.60 (C-1^F), 101.45, 102.28, 102.87, 103.49 (C-1^C, C-1^E, C-1^B, C-1^D), 116.96 (C-2^K, C-6^K), 122.58 (C-3^K, C-5^K), 128.39 (C-4^L, C-6^L), 129.09 (C-3^L, C-7^L), 158.40 ($OCONH_2^F$), 173.01, 173.42, 174.23, 174.80 ($CONH_2^B$, $CONH_2^F$, $NHCOCH_3^E$, $NHCOCH_3^C$). ^{31}P NMR (81 MHz, D_2O): δ=−0.67. $C_{54}H_{79}N_6O_{33}P$ (1371.23, 1370.44), ESI MS (negative mode): *m/z*=1391.41557 (1391.41723) $[M+Na-2H]^-$, 1369.44119 (1369.43529) $[M-H]^-$, 684.21463 (684.21401) $[M-2H]^{2-}$, FAB MS: *m/z*=1415.3 $[M+2Na-H]^+$, 1393.3 $[M+Na]^+$, 1371.3 $[M+H]^+$.

3.2.15. (*R*)-2-[2-(Biphenyl-4-ylamino)ethoxy]-3-({β-D-galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}-hydroxyphosphoryloxy)-propionic acid (6f). 6f

was prepared from **2a** (51.8 mg, 44 μmol) and 4-aminobiphenyl (22.1 mg, 132 μmol) as described for **6a**. Yield: 42.5 mg (58%). ^{1}H NMR (200 MHz, D_2O): Characteristic signals at δ=1.12 (s, CH_3-4^F, signal doubling), 1.24 (d, CH_3-6^C, signal doubling), 1.92, 1.97 (s, NHCOCH_3^E, s, NHCOCH_3^C, signal doubling), 3.20 (dd, H-2^D), 4.36 (s, H-5^F), 4.39 (d, H-1^D), 4.92 (d, H-3^F), 5.72 (q, H-1^F), 7.30–7.75 (m, H-2^K-H-6^K, m, H-2^L-H-6^L), J_{5C-6C}=6.0 Hz, J_{2D-3D}=8.1 Hz, J_{1D-2D}=8.5 Hz, J_{2F-3F}=10.6 Hz, J_{1F-2F}=3.9 Hz, $^3J_{1F-P}$=6.3 Hz. ^{13}C NMR (APT, 100 MHz, D_2O): δ=14.23 (CH_3-4^F), 16.11 (CH_3-6^C), 21.84, 21.92 (NHCO*C*H_3^E, NHCO*C*H_3^C), 52.52 (?), 54.67, 54.94 (C-2^E, C-2^C), 60.28 (C-6^D), 65.71 (C-2^I), 66.47 (d, C-3^H), 68.39 (+), 68.87, 69.22, 69.72, 70.09, 70.69, 71.31, 71.49, 71.56, 71.71, 71.96, 72.31, 72.58 (+), 72.64, 72.98, 73.60, 73.74, 74.39, 75.31, 75.52, 75.90, 75.99 (C-1^I, C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-3^C, C-5^F, C-6^E, C-5^B, C-3^E, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D), 79.31 (C-4^E), 80.44 (d, C-2^H), 82.47 (C-4^C), 93.87 (d, C-1^F), 100.71, 101.52, 102.10, 102.74 (C-1^C, C-1^E, C-1^B, C-1^D), 119.12 (C-2^K, C-6^K), 126.15 (C-3^K, C-5^K), 127.14 (C-4^L), 127.81 (C-3^L, C-5^L), 128.73 (C-2^L, C-6^L), 139.15 (C-1^L), 157.66 (O*C*ONH_2^F), 169.54 (?), 172.27, 172.68, 173.69, 174.05 (CONH_2^B, CONH_2^F, NH*C*OCH_3^E, NH*C*OCH_3^C), 176.04 (C-1^H). ^{31}P NMR (81 MHz, D_2O): δ=−0.88. $C_{53}H_{77}N_6O_{32}P$ (1341.20, 1340.43), ESI MS (negative mode): *m/z*=1339.42427 (1339.42472) $[M-H]^-$, 669.20894 (669.20872) $[M-2H]^{2-}$, FAB MS: *m/z*=1341.1 $[M+H]^+$.

3.2.16. (*R*)-2-[2-(Terphenyl-4-ylamino)ethoxy]-3-({β-D-galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}-hydroxyphosphoryloxy)-propionic acid (6g). To a solution of **2a** (103.8 mg, 87 μmol) in a mixture of phosphate buffer (400 μl, 50 mM) and THF (800 μl) a solution of 4-aminoterphenyl (55.9 mg, 176 μmol) in a mixture of methanol (400 μl) and THF (400 μl) was added. The pH of the mixture was adjusted with 1N sodium hydroxide to pH 7.0 and a solution of $NaBH_3CN$ (11.1 mg, 174 μmol) in methanol (100 μl) was added. The reaction mixture was sonicated for 60 min and rapidly stirred for 72 h at 20°C. The crude mixture was directly applied to a Sephadex® LH-20 column (H_2O–CH_3OH 1:4). Product fractions were concentrated and freeze dried. The crude product was purified by FC (ethyl acetate–2-propanol–H_2O 6:4:2) and subsequent gel filtration (Sephadex LH-20®, H_2O–CH_3OH 1:4). The combined fractions were concentrated and freeze dried to give **6g** (12.9 mg, 10%). ^{1}H NMR (H,H COSY, 600 MHz, D_2O): Characteristic signals at δ=1.12 (s, CH_3-4^F), 1.23 (d, CH_3-6^C), 1.96, 1.98 (s, NHCOCH_3^E, s, NHCOCH_3^C), 3.22 (dd, H-2^D), 4.41 (d, H-1^D), 4.97 (d, H-3^F), 5.71 (m, H-1^F), 6.94 (d, H-2^K, H-6^K), 7.38 (dd, H-4^M), 7.48 (dd, H-3^M, H-5^M), 7.61 (d, H-3^K, H-5^K), 7.70 (d, H-2^M, H-6^M), 7.73 (s, H-2^L, H-3^L, H-5^L, H-6^L), J_{5C-6C}=5.9 Hz, J_{2D-3D}=8.1 Hz, J_{1D-2D}=7.7 Hz, J_{2F-3F}=10.6 Hz, J_{2K-3K}=J_{5K-6K}=7.7 Hz, J_{4M-5M}=7.3 Hz, J_{2M-3M}=J_{5M-6M}=7.3 Hz. ^{13}C NMR (150 MHz, D_2O): δ=15.26 (CH_3-4^F), 17.00 (CH_3-6^C), 22.87 (NHCO*C*H_3^E, NHCO*C*H_3^C), 43.83 (C-2^I) 55.53, 55.78 (C-2^E, C-2^C), 61.08 (C-6^D), 67.42 (C-1^I), 68.86, 68.92, 69.16, 69.31, 69.96, 70.85, 70.99, 71.38, 72.40, 72.50, 72.59, 73.04, 73.23, 73.51, 73.80, 74.51, 75.33, 76.24, 76.41 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-3^C, C-5^F, C-6^E, C-5^B, C-3^E, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D), 80.15 (C-4^E), 81.57 (d, C-2^H), 83.15 (C-4^C), 94.67 (C-1^F), 101.47, 102.35, 102.88, 103.53 (C-1^C, C-1^E, C-1^B, C-1^D), 114.89 (C-2^K, C-6^K), 126.41 (C-3^K, C-5^K), 126.74 (C-2^M, C-6^M), 127.44 (C-3^L, C-5^L), 127.81 (C-2^L, C-6^L, C-4^M), 129.23 (C-4^K), 129.62 (C-3^M, C-5^M), 137.96 (C-4^L), 139.32 (C-1^M), 139.77 (C-1^L), 148.41 (C-1^K), 158.53 (O*C*ONH_2^F), 173.07, 173.34, 174.43, 174.73 (CONH_2^B, CONH_2^F, NH*C*OCH_3^E, NH*C*OCH_3^C), 177.23 (C-1^H). ^{31}P NMR (81 MHz, D_2O): δ=−1.06. $C_{59}H_{81}N_6O_{32}P$ (1417.30, 1416.46), ESI MS (negative mode): *m/z*=1437.44264 (1437.43797) $[M+Na-2H]^-$, 1415.45998 (1415.45602) $[M-H]^-$, 718.21609 (718.21609) $[M+Na-3H]^{2-}$, 707.22336 (707.22437) $[M-2H]^{2-}$, FAB MS: *m/z*=1461.6 $[M+2Na-H]^+$, 1439.4 $[M+Na]^+$, 1417.4 $[M+H]^+$.

3.2.17. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-[2-(5α-pregnan-3α-ylamino)ethoxy]-propionic acid (7a). **7a** was prepared from **2a** (50 mg, 42 μmol) and 3α-amino-5α-pregnane (28.6 mg, 84 μmol) as described for **4a**. Yield: 15.7 mg (25%). ^{1}H NMR (H,H COSY, 600 MHz, D_2O): Characteristic signals at δ=0.40–1.90 (m, CH_x-1^K-CH_x-21^K, broad signals), 1.10 (s, CH_3-4^F), 1.26 (d, CH_3-6^C), 1.91, 1.98 (s, NHCOCH_3^E, s, NHCOCH_3^C), 3.18 (m, H-2^D), 4.10 (s, H-4^B, H-5^B), 4.33 (s, H-5^F), 4.39 (d, H-1^D), 4.91 (d, H-3^F), 5.05–5.06 (m, ?), 5.67 (q, H-1^F), J_{5C-6C}=6.1 Hz, J_{1D-2D}=7.7 Hz, J_{2F-3F}=10.1 Hz, J_{1F-2F}=4.0 Hz, $^3J_{1F-P}$=6.1 Hz. ^{13}C NMR (APT, 100 MHz, D_2O): δ=11.81 (CH_3-18^K), 12.89 (CH_3-21^K), 13.70 (CH_3-19^K), 16.19 (CH_3-4^F), 17.72 (CH_3-6^C), 21.35 (C-11^K), 23.41 (NHCO*C*H_3^E, NHCO*C*H_3^C), 23.93 (C-20^K), 25.25 (C-15^K), 28.90, 30.25, 32.49, 32.72 (C-16^K, C-6^K, C-2^K, C-8^K), 36.39 (C-7^K), 36.72 (C-10^K, C-1^K), 38.92 (C-12^K), 40.13 (C-5^K), 42.97 (C-1^I, C-13^K), 46.94 (C-2^K), 54.08, 55.11 (C-17^K, C-9^K), 55.91–55.05 (C-2^E, C-2^C), 56.48, 56.91 (C-14^K, C-3^K), 62.29 (C-6^D), 66.71 (C-1^I), 67.97 (C-3^H), 70.15, 71.25, 71.85, 72.28, 73.38, 73.62, 73.87, 74.19 (+), 74.63, 75.49, 75.71, 75.91, 77.29, 77.41, 78.72 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-6^E, C-5^B, C-3^E, C-3^C, C-5^F, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D), 81.65 (C-4^E), 81.82 (C-2^H), 84.30 (C-4^C), 95.60 (C-1^F), 102.59, 103.58, 103.96, 104.33 (C-1^C, C-1^E, C-1^B, C-1^D), 159.00 (O*C*ONH_2^F), 173.96–174.88 (CONH_2^B, CONH_2^F, NH*C*OCH_3^E, NH*C*OCH_3^C). ^{31}P NMR (161 MHz, CD_3OD-D_2O): δ=−0.18. $C_{62}H_{103}N_6O_{32}P$ (1475.49, 1474.63), ESI MS (negative mode): *m/z*=(1473.62318, 1473.62818) $[M-H]^-$, 736.30892 (736.31045) $[M-2H]^{2-}$, FAB MS: *m/z*=1475.8 $[M+H]^+$.

3.2.18. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-{2-[(1*H*,1*H*-pentadecafluorooctyl)-amino]-ethoxy}-propionic acid (7b). 7b was prepared from **2a** (50 mg, 42 μmol) and

1H,1H-pentadecafluorooctylamine (33.6 mg, 84 μmol) as described for **4a**. Yield: 6.5 mg (10%). ^{1}H NMR (H,H COSY, 400 MHz, D_2O): Characteristic signals at δ=1.14 (s, CH_3-4^F), 1.29 (d, CH_3-6^C), 1.94, 2.01 (s, $NHCOCH_3^E$, s, $NHCOCH_3^C$), 2.87 (m, CH_2-2^I), 3.21 (dd, H-2^D), 4.14 (s, H-4^B, H-5^B), 4.37 (s, H-5^F), 4.43 (d, H-1^D), 4.94 (d, H-3^F), 5.70 (m, H-1^F), J_{5C-6C}=4.9 Hz, J_{2D-3D}=8.5 Hz, J_{1D-2D}=7.8 Hz, J_{2F-3F}=10.6 Hz. ^{19}F NMR (188 MHz, D_2O): δ=−48.57 (CF_2-7^K), −45.85, −45.11, −44.42, −44.16 (CF_2-3^K, CF_2-4^K, CF_2-5^K, CF_2-6^K), −40.16 (CF_2-2^K), −3.54 (CF_3-8^K). ^{31}P NMR (161 MHz, D_2O): δ=−1.28. $C_{49}H_{70}N_6O_{32}F_{15}P$ (1571.07, 1570.35), ESI MS (negative mode): *m/z*=1569.35421 (1569.34600) $[M-H]^-$, 784.17030 (784.16936) $[M-2H]^{2-}$, MS FAB *m/z*=1571.8 $[M+H]^+$.

3.3. IAM chromatography

HPLC columns (30 cm×0.46 cm) containing the PC.DD IAM stationary phase were purchased from Regis Technologies Inc. The IAM studies were performed at 25°C. The injection volume was 10 μl of a phosphate buffer (0.01 M, pH 7.0) solution of the analyte (0.1 mg/ml). Elution was performed with a stepwise gradient of acetonitrile-phosphate buffer (0.01M, pH 7.0) and extrapolated for pure buffer. The flow rate was 1 ml min^{-1}. For detection a photodiode array detector was used.

Acknowledgements

We wish to thank Dr Françis Maquin for his support. The Leipzig group gratefully acknowledges financial support by the Deutsche Forschungsgemeinschaft (Innovationskolleg 'Chemisches Signal und biologische Antwort') and the Fonds der Chemischen Industrie. S. V. wishes to thank Hoechst Marion Roussel for a fellowship.

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