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THE FIRST MOENOMYCIN ANTIBIOTIC WITHOUT THE METHYL-BRANCHED URONIC ACID CONSTITUENT. - UNEXPECTED STRUCTURE ACTIVITY RELATIONS

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Abstract- Isolation and structure elucidation of a new moenomycin antibiotic (C_1, **1e**) that lacks the branching methyl group in the 4-position of unit F are reported. The smallest antibiotically active degradation product of **1e** is the *trisaccharide* derivative **3.** This observation is in contrast to structure activity relations in the moenomycin A series where it was found that *disaccharide* **4a** is fully active.

Introduction

Moenomycin A and related compounds are a group of unique antibiotics.[1] All of them seem to contain an oligosaccharide part, phosphoric acid, and a lipid unit which may be either moenocinol (see unit I in formula **1**) or diumycinol, an isomer of moenocinol with one six-membered ring. Some of these antibiotics carry a so-called chromophore moiety (unit A in **1**) which is lacking in others which may contain glycine instead.[2] Until now the full structures of only moenomycins A (**1a**), C_3 (**1b**), C_4 (**1c**), and of pholipomycin (**1d**) have been established.[3] They can be devided into two classes, depending on whether unit E carries a glucose moiety as in moenomycin A (**1a**) or not (cf. **1b**, **1c**, **1d**).

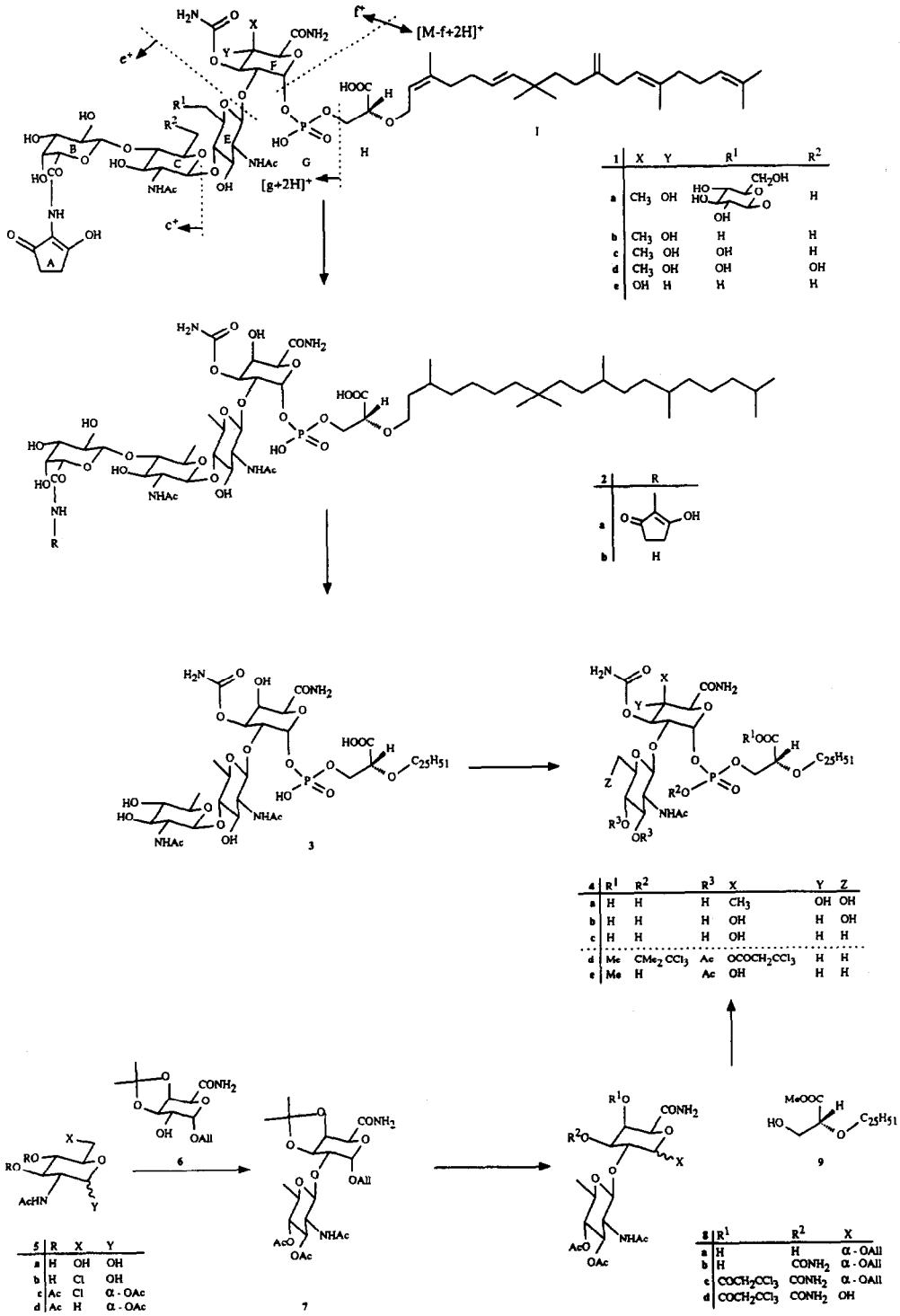
For moenomycin A it has been shown that its antibiotic activity originates from its interference with penicillin-binding protein 1b (PBP 1b). It is the *transglycosylase* activity of this bifunctional enzyme that is inhibited by moenomycin.[4] Thus, the moenomycin antibiotics belong to the rare compounds known to inhibit the transglycosylation reaction, one of the key steps in the formation of high-molecular peptidoglycan from a disaccharide precursor.[5]

From degradation work it is known that units E-F-G-H-I of **1a** are responsible for the inhibiting interaction of moenomycin with PBP 1b. More specifically, compound **4a** is the smallest degradation product of **1a** with full PBP 1b inhibiting activity.[6,7] Recently, we accomplished to synthesize **4b**, which differs from **4a** only by the lack of the methyl group at C-4 and the configuration of unit F (D-galacto configuration rather than D-gluco). **4b** was found to be devoid of antibiotic activity.[8] This result stresses the high specificity of the interaction of moenomycin antibiotics **1a-1d** and degradation products such as **4a** with the binding site at the transglycosylase that forms the basis of the antibiotic activity. Until now it is unclear, whether it is the equatorial hydroxyl, the axial methyl group at $C\text{-}4^F$, or the combination of both structural features that is responsible for this striking effect on the structure activity relations. In view of these results, it was very exciting, when a new moenomycin antibiotic was isolated the molecular mass of which was found to differ from that of moenomycin C_3 (**1b**) by 14 mass units. Structure and properties of this new antibiotic (moenomycin C_1) are the subject of the present publication.

Structure elucidation of moenomycin C_1

A careful analysis of the positive ion FAB mass spectra of moenomycin A (**1a**) and degradation products derived thereof has revealed that all structurally relevant fragments can be assigned as summarized in formula **1**: Cleavage of the glycosidic bonds of the pyranose units C, E, and F gives rise to the formation of cations c^+, e^+, f^+, stabilized by the respective pyranose oxygens. Cleavage of either phosphoric acid diester bond yields the protonized phosphoric monoesters $[M\text{-}f+2H]^+$ and $[g+2H]^+$, respectively.[7] When the spectra of moenomycins C_1 and C_3 were compared, both displayed a signal at m/z 686.2 = $[e+Na\text{-}H]^+$, whereas the $[g+2\ Na]^+$ ion gave rise to peaks at m/z = 1020.2 in moenomycin C_3 and m/z = 1006.2 in C_1, respectively. This result clearly demonstrated that in moenomycins C_1 and C_3 unit F differs by 14 mass units which was taken as a hint that the branching 4-methyl group is lacking in moenomycin C_1. The ^{13}C NMR spectrum of moenomycin C_1 showed the presence of the moenocinol unit I, the chromophore part A, the carbamoyl group, four sugar units (well separated anomeric carbon signals), two of them being 2-N-acetylamino-2-deoxy sugars (C-2 signals at δ = 56.2 and 57.2), and the $^{31}P,^{13}C$ coupling in the vicinity of the phosphate group. The signals at δ = 85.03 and 87.87 are assumed to correspond to the C-4 carbons in the 6-deoxy sugar units C and E.[9] The assignments are collected in the Experimental. In order to gain further insight into the structure of unit F in moenomycin C_1, we performed the stepwise degradation that was developed for the moenomycin antibiotics.[6] Thus, (i) hydrogenation (**1e**→**2a**), (ii) $K_3[Fe(CN)_6]$ oxidation (**2a**→**2b**), (iii) diol cleavage of **2b** with $NaIO_4$, followed by treatment with N,N-dimethylhydrazine (Barry degradation[10]) yielded **3**. Under carefully selected conditions (solvent: $CDCl_3$-CD_3OD-D_2O 18:11:2.7, T = 315 K) the ^1H NMR spectrum of **3** was very informative inasmuch as it exhibited two doublets for $1\text{-}H^C$ and $1\text{-}H^E$ ($J_{1,2} \approx 8$ Hz), the signals of $1\text{-}H^F$ and $2\text{-}H^F$ ($J_{2,3} \approx 9.5$ Hz), and most significantly, the $3\text{-}H_F$ signal as a broadened doublet at δ = 4.66 with $J_{2,3} \approx 9.5$ Hz, and $J_{3,4} \approx 1.5$ Hz, and the $5\text{-}H^F$ signal as a broadened singlet. These spectral data seemed only to be consistant with unit F adopting the $^4C_1$ conformation, the 2- and the 3-substituents being in an equatorial and the 4-OH group in an axial position. Thus, we were led to the conclusion that unit F is derived from D-galactopyranuronic acid.

3 was then further degraded by diol cleavage followed by treatment with ammonia[11] to yield **4c**. FAB and ^{13}C NMR spectra of **4c** were in accord with the proposed structure, the ^1H NMR spectrum was of lower


[M-f+2H]+
[g+2H]+
1
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9

quality than that of **3** but was in agreement with the configurational assignment in unit F as discussed above.

After acid-catalyzed cleavage of **4c** in methanolic solution the reaction products were trimethylsilylated and then compared by GLC with the products obtained from D-glucuronic acid and D-galacturonic acid under the same conditions. Beyond doubt the peaks obtained from **4c** corresponded to those obtained from D-galacturonic acid. The latter were identified as the silyl derivatives of methyl (methyl α- and ß-D-galactofuranosid)uronates and the corresponding pyranosiduronates. Reference samples were prepared by known methods.[12]

Conclusive evidence for the D-galacto configuration of unit F in **4c** was obtained from an unambiguous synthesis of **4c** which followed the pathway recently developed for **4b**.[8] The quinovosamine derived building block was prepared[13] from **5a** making use of a slightly modified version of the procedure recently reported by Belkhouya et al.[14] Thus, 2-acetamido-2-deoxy-D-glucose (**5a**) was converted into **5b** by treatment with triphenylphosphine and carbon tetrachloride. Acetylation and subsequent dehalogenation with tributyltin hydride[15] furnished **5d**.[16] This compound was in turn coupled to the known galacturonamide derivative **6**[8] employing the oxazolin method.[17,18] After removal of the acetonide group (**7**→**8a**) the carbamoyl group was introduced via the tributyltin ether[6] (**8a**→**8b**). Two further protecting group manipulations, (i) conversion of the 4^F-OH group into the 2,2,2-trichloroethoxycarbonyl derivative (**8b**→**8c**),[19] and removal of the allyl group with Corey´s two-step procedure[20] (isomerisation of the allyl into the propenyl ether and subsequent cleavage with $HgCl_2$ - HgO in acetone-water) provided the desired disaccharide building block **8d**. For the construction of the phosphoric acid diester grouping we used the Ugi variant[21] of the phosphite methodology.[22] Thus, the sequence (i) treatment of 2,2,2-trichloro-1,1-dimethylethyl dichlorophosphite with two equivalents of 1H-1,2,4-triazole, (ii) reaction of the thus prepared reagent with **8d**, (iii) subsequent reaction with the moenomycin-derived building block **9**,[8] and (iv) oxidation of the intermediate phosphite triester with bis(trimethylsilyl)peroxide[23] furnished the phosphate triester **4d** (mixture of stereoisomers). Removal of the protecting groups containing the trichloroethyl unit was achieved under the Imai conditions[24] with freshly prepared Zn-Cu couple[8] to provide **4e**. Finally, hydrolysis of the ester groups converted **4e** into **4c**, which proved identical (^1H and ^{13}C NMR, FAB MS, and TLC behaviour in many solvent systems) with the specimen obtained from moenomycin C_1 by degradation.

Antibiotic Activity of Moenomycin C_1 and its Degradation Products 2a, 2b, 3, and 4c

The minimum inhibitory concentrations (MIC) of moenomycin C_1 (**1e**) and a number of degradation products derived thereof against various microorganisms have been determined by a serial two-fold agar dilution method (Müller Hinton Agar). The results (see Table 1) demonstrate that moenomycin C_1 like the other moenomycins is mainly active against *gram-positive* bacteria. When compared with moenomycin A (**1a**), moenomycin C_1 is of distinct lower activity against *Staph. aureus*. In the series of the degradation products the *in-vivo* activity against *Staph. aureus* slowly decreases, an observation also made in the moenomycin series.[6,7] Degradation product **4c** is antibiotically inactive.

Table 1. Minimum inhibitory concentrations (in mg/L) of moenomycin C_1 (**1e**), its degradation products **2a**, **2b**, **3**, **4c**, and of moenomycin A (**1a**, for comparison) against various test organisms.

test organism	**1a**	**1e**	**2a**	**2b**	**3**	**4c**
Staph. aureus SG 511	0.05	0.391	1.56	1.56	6.25	>100
Staph.aureus 503	0.05	0.391	1.56	1.56	6.25	>100
Strept. pyogenes A77	<0.01	<0.002	0.025	0.195	0.781	3.13
Bac.subtilis		25	>100	>100	>100	>100
Pseud. aerug. 1771m	6.25	3.13	100	50	25	>100
E. coli DC 2	50	50	>100	>100	>100	>100

The inhibitory effect of **1e** and a number of degradation products directly on the transglycosylation reaction was determined by the *in vitro* assay developed earlier in one of our laboratories[25] using a crude extract from an over producer *E.coli JA200 plc19-19* and as substrate the lipid intermediate which is the immediate precursor of uncross-linked peptidoglycan. The results (see Table 2) demonstrate that in this *in-vitro* system moenomycin C_1 (**1e**) is an as active inhibitor of the transglycosylation reaction as moenomycin A (**1a**) itself. In addition, degradation products **2a**, **2b**, and **3** are fully active inhibitors of the transglysosylating enzyme. However, in contrast to the moenomycin A series the disaccharide degradation product **4c** is inactive.

Table 2. Effect of moenomycins A (**1a**, for comparison), C_1 (**1e**), and degradation products **2a**, **2b**, **3**, **4c** on the *in-vitro* formation of uncross linked peptidoglycan by transglycosylation

final concentration (μg/mL)	% inhibition					
	1a	**1e**	**2a**	**2b**	**3**	**4c**
10	100	100	100	100	100	85
1	100	100	100	100	100	0
0.1	78	83	35	48	51	0

Discussion

For the first time a moenomycin antibiotic has been isolated that lacks the branching methyl group in the 4-position of unit F. This new compound (moenomycin C_1) is *in-vivo* less active against *gram-positive* bacteria than the other known moenomycins (A, C_3, C_4, pholipomycin), wheras the *in-vitro* inhibiting activity against the transglycosylating enzyme obtained from *E.coli* is similar for all moenomycin antibiotics. A stepwise degradation of moenomycin C_1 coupled with an investigation of the biological activity of the degradation products has revealed a very interesting observation: Whereas in the series of compounds with the C-4^F methyl group the disaccharide degradation products such as **4a** are active both *in-vivo* and *in-vitro*, moenomycin C_1 disaccharide degradation product **4c** is antibiotically inactive. The last-mentioned results confirms our recent finding that the synthetic product **4b** is devoid of antibiotic activity.[8] The reason for this difference in the structure activity relations in the two series which differ from each other solely by the methyl group and the configuration at C-4 in unit F is at present not understood. In any case, with trisaccharide **3** a compound has been identified that is antibiotically active and contains solely ordinary sugar components. Compounds of type **3** are of such a degree of complexity that they should be synthetically attainable with reasonable efforts.

Moenomycin C_1 is, of course, also highly interesting with regard to biosynthesis. Its isolation raises the question whether the complex array of building blocks of this and the traditional moenomycin antibiotics

(with the C-4-methyl group in unit F) is assembled in parallel or whether an antibiotic of the C_1 type is the precursor of the others, which would mean that the branching methyl group in unit F, that occurs in moenomycins A, C_3, C_4, and pholipomycin, is introduced at a late stage of the biosynthesis.

Experimental

General

O_2- or moisture-sensitive reactions were performed in oven-dried glassware under a positive pressure of argon. Liquids and solutions were transferred by syringe. Small-scale reactions were performed in Wheaton serum bottles sealed with aluminium caps with open top and Teflon-faced septum (Aldrich). Organic solvent evaporations were performed in vacuo at 40°C using a rotatory evaporator, water was removed by lyophilization using the Leybold-Heraeus GT2 apparatus. Solvents were purified by standard techniques.- The instrumentation used was: ^1H NMR: WP 80 (Bruker), AM 400 (Bruker); ^{13}C NMR: AM 400 (Bruker, at 100.6 MHz); EI MS: MAT CH5 (Varian); FAB MS: (i) MAT 731 (Varian) with a modified Saddle Field Source, (ii) VG AUTOSPEC, (iii), VG Analytical ZAB2-SEQ (BEQQ configuration); LC (preparative gravitational liquid chromatography): silica gel (ICN Biomedicals Silica 63-100); MPLC (medium-pressure liquid chromatography): 30.0 cm x 2.5 cm or 40.0 cm x 1.5 cm glass tubes (columns B and A, respectively), 50 μm silica gel (Amicon), Duramat pump (CfG), Thomachrom UV detector (Reichelt); analytical TLC: Merck precoated silica gel 60 F_{254} plates (0.2 mm), spots were identified under a UV lamp (Camag 29 200) and by spraying with a 2.22 mol/L H_2SO_4 solution which contained $Ce(SO_4)_2x4H_2O$ (10 g/L) and $H_3[PO_4(Mo_3O_9)_4]xH_2O$ (25 g/L)[26] and heating at 140°C. For crude reversed-phase separations polystyrene resin HP-20 (Mitsubishi) was used.- Carbon and proton numbering in the subunits (see NMR data) follows the moenomycin nomenclature (see formula 1). Two molecular masses are always communicated, the first was calculated using the International Atomic Masses, the second refers to ^{12}C, ^1H, ^{16}O, ^{14}N, ^{31}P (mono-isotopic masses).-Sodium metaperiodate solution for the diol cleavage reactions: A mixture of sodium metaperiodate (1.07 g, 5.0 mmol), sodium acetate trihydrate (1.38 g, 10.0 mmol), 50 per cent acetic acid (12.0 mL) was stirred at 80°C until a clear solution resulted. After cooling to 60°C the always freshly prepared solution was added to the diol to be cleaved.- N,N-Dimethylhydrazine solution for the Barry degradation: To a solution of N,N-dimethylhydrazine (0.94 mL) in 2-propanol (2.80 mL) 1 mol/L H_2SO_4 was added at 0°C until a pH of 4.5 was reached (about 6.40 mL). Only freshly prepared solutions were used.

Moenomycin C1 (1e)

1.35 g of a moenomycin C mixture isolated as described in ref.[3] was separated by preparative HPLC (Waters prep LC 500; Merck LiChroprep RP-18, 25-40 μm; mobile phase: methanol-acetonitrile-water 52:8:40; flow rate: 25 mL/min). First the column was washed with 1 L of the solvent mixture, then 15 mL fractions were taken. Fractions 14-38 contained moenomycin C_1 (128 mg), fractions 39-51 (86 mg) moenomycins C_1 (52%)and C_3 (45%), fractions 52-68 moenomycin C_3 (130 mg) and fractions 69-80 (173 mg) moenomycins C_3 (55%) and C_4 (38%). Analytical HPLC: Spherisorb ODS 5 μm, solvent system: methanol-acetonitrile-0.02% phosphate buffer (pH 7.8) 4:1:5; UV detection at 258 nm.- ^{13}C NMR (CD_3OD, DEPT): δ = 13.96 (CH_3); 16.12 (CH_3); 17.80 (CH_3); 17.91 (CH_3); 18.05 (CH_3); 20.70 (CH_2); 23.16 (CH_3); 23.32 (CH_3); 23.90 (CH_3); 24.75 (CH_3); 25.96 (CH_3); 27.70 (CH_2); 27.85 (C-23^I, C-24^I); 30.69 (CH_2); 32.32 (CH_2); 32.63 (CH_2); 33.45 (CH_2); 35.94 (C-12^I); 36.47 (C-8^I); 40.89 (C-15^I); 42.85 (C-9^I); 56.80, 57.54 (C-2^C, C-2^E); 67.46, 67.82 (C-1^I, C-3^H); 69.37 (CH); 70.66 (CH); 71.59 (CH); 72.20 (CH); 72.60 (CH); 72.71 (CH); 73.07 (CH); 73.63 (CH); 73.73 (CH); 74.36 (CH); 75.71 (C-2^F); 76.38 (CH); 79.06 (C-2^H); 85.03 (C-4^C); 87.87 (C-4^E); 95.65 (C-1^F); 103.39, 103.80 (C-1^C, C-1^E); 104.84 (C-1^B); 109.24 (C-22^I); 113.08 (C-2^A); 122.84 (C-13^I); 123.50 (C-2^I); 125.37 (C-17^I); 126.84 (C-6^I); 132.19 (C-18^I); 137.33 (C-14^I); 141.56 (C-7^I); 141.71 (C-3^I); 151.06 (C-11^I); 158.65 ($OCONH_2$); 170.52 (C-6^B); 173.53, 173.65, 173.79 (2x$NHCOCH_3$, C-6^F); 174.90 (C-1^H); 195.2 (C-1^A, C-3^A).- $C_{62}H_{95}N_5O_{28}P$ (1390.435, 1389.598), FAB MS (matrix: nitrobenzylalcohol): m/z = 1450.6 ([M+Na+K-H]+); 1434.5 ([M+2Na-H]+); 1412.6 ([M+Na]+); 1006.2 ([g+2Na]+); 886.3 ([f+Na-H]+); 668.2 ([e+Na-H]+); 459.2 ([c]+).

Decahydromoenomycin C1 (2a)

A mixture of moenomycin C1 (123.0 mg. 0.089 mmol), methanol (12.4 mL), $PtO_2 \cdot H_2O$ (37.2 mg), and acetic acid (0.46 mL) was stirred under H_2 at normal pressure and 20°C until HPLC control (5µm RP-18, methanol-water-acetonitrile 6:3:1) indicated completion of the reaction (after about 3 h.). Filtration, solvent evaporation, and MPLC (column B, RP-18, methanol-water-acetonitrile 6:3:1) yielded **2a** (84.6 mg, 69 %).- ^{13}C NMR (CD_3OD): δ = 200.0 (C-1^A, C-3^A); 177.8 (C-1^H); 174.1, 173.6, 173.5 (2x $NHCOCH_3$, C-6^F); 170.1 (C-6^B); 158.7 ($OCONH_2$); 111.1 (C-2^A); 104.8 (C-1^B); 104.0 (C-1^E); 103.5 (C-1^C); 96.6 (C-1^F); 87.7 (C-4^E); 84.6 (C-4^C); 82.8 (C-2^H); 75.8 (C-2^F); 70.0 (C-3^H); 68.9 (C-1^I); 57.4 (C-2^C); 56.4 (C-2^E).- $C_{62}H_{106}N_5O_{28}P$ (1400.514, 1399.676), FAB MS (matrix nitrobenzylalcohol): m/z = 1460.6 ([M+Na+K-H]+); 1444.5 ([M+2Na-H]+); 1422.7 ([M+Na]+); 1006.3 ([g+2Na]+); 886.3 ([f+Na-H]+); 668.3 ([e+Na-H]+); 646.3 ([e]+); 559.5 ([M-f+Na +H]+); 459.2 ([c]+).

2-O-{2-Acetamido-4-O-[2-acetamido-4-O-((5S)-5-carbamoyl-ß-L-arabinopyranosyl)-2,6-dideoxy-ß-D-gluco-pyranosyl]-2,6-dideoxy-ß-D-glucopyranosyl}-3-O-carbamoyl-1-O-{[(R)-2-carboxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxyl-hydroxyphosphoryl}-α-D-galactopyranuronamide (2b)

To a stirred solution of **2a** (84.5 mg, 0.060 mmol) in water (2.7 mL) were added at 0°C solutions of K_2CO_3 (136 mg, 0.986 mmol) in water (0.21 mL) and $K_3[(Fe(CN_6)]$ (409.9 mg, 1.245 mmol) in water (0.5 mL). After 30 min, the reaction mixture was allowed to warm to 20°C and was stirred at this temperature for 2.5 h. Inorganic salts were removed by reversed-phase chromatography {HP-20, 60 g, elution with water (600 mL) and then with methanol (1 L)}. Evaporation of the methanol fraction followed by lyophilization gave pure **2b** (80.6 mg, 100%).- ^{13}C NMR (DMSO-d_6): δ = 172.4 (C-1^H); 170.6, 169.2 (2 x $NHCOCH_3$, C-6^F, C-6^B); 156.4 ($OCONH_2$); 103.5 (C-1^B); 101.7 (C-1^C, C-1^E); 94.3 (C-1^F); 86.0 (C-4^E); 84.3 (C-4^C); 79.5 (C-2^H); 74.6 (C-3^C); 73.1; 72.5; 71.8; 71.5; 70.8; 70.3; 69.9; 69.0; 67.9; 67.4 (C-1^I ?); 65.4 (C-3^H?); 55.6 (C-2^C); 54.9 (C-2^E); 41.6; 38.6; 36.9; 36.6; 33.6; 33.2; 32.4; 32.2; 30.6; 30.3; 29.5; 29.1; 27.4; 27.2; 24.2; 23.0; 22.6; 22.5; 19.9; 19.8; 19.6; 17.3.- $C_{57}H_{102}N_5O_{26}P$ (1304.429, 1303.655), FAB MS (matrix: glycerol-DMSO), m/z: = 1305 ([M+H]+); 768 ([f]+); 550 ([e]+); 537 ([M-f+2H]+); 363 ([c]+); 176 ([b]+).

2-O-{2-Acetamido-4-O-[2-acetamido-2,6-dideoxy-ß-D-glucopyranosyl]-2,6-dideoxy-ß-D-glucopyranosyl}-3-O-carbamoyl-1-O-{[(R)-2-carboxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxyl-hydroxyphosphoryl}-α-D-galactopyranuronamide (3)

To a solution of **2b** (288.7 mg, 0.221 mmol) in the smallest possible amount of water the hot (60°C, see General) $NaIO_4$ solution (1.4 mL) was added, and the mixture was stirred in the dark for 3 h at 40°C. Inorganic salts were removed by reversed-phase chromatography [60 g HP-20, elution with water (600 mL) and methanol (1000 mL)]. The pH of the eluate was first 3.5 and then slowly raised to 6.0-6.5. Aqueous fractions with pH 5.5 and higher and the methanolic fractions were combined. Solvents were removed by distillation and subsequent lyophilization. The residue (221.1 mg) was dissolved in as little water as possible. To this solution the dimethylhydrazine solution (see General, 0.62 mL) was added, and the mixture was stirred at 80-85°C for 3.5 h. After cooling to 20°C inorganic salts were removed by reversed-phase chromatography [HP-20 (60 g), elution with water (600 mL) and methanol (1 L)]. From the methanolic eluate after solvent evaporation and lyophilization a crude degradation product (142.7 mg) was obtained which yielded after three separation steps, (i) LC, (SiO_2, 6g), (ii) MPLC (column B), (iii) MPLC (column A), elution with $CHCl_3$-methanol-water 10:6:1, pure **3** (97.0 mg, 50%).- ^{13}C NMR ($CDCl_3$-CD_3OD-D_2O 18:11:2.7): δ = 174.99 (C-1^H); 173.95 (C-6^F); 173.54; 173.08 (2x$NHCOCH_3$); 158.10 ($OCONH_2$); 103.26 (C-1^C); 102.82 (C-1^E); 95.76 (C-1^F); 87.03 (C-4^E); 80.0 (C-2^H); 78.36; 76.06 ; 75.01 (C-2^F); 74.57; 72.97; 72.84; 72.24; 71.92 (C-3^F); 70.91 (C-5^C); 70.00 (C-1^I); 66.96 (C-3^H); 56.50 (C-2^C); 55.74 (C-2^E).- ^1H NMR (400 MHz, $CDCl_3$-CD_3OD-D_2O 18:11:2.7, T = 315 K): δ = 5.42 ($w_{1/2} \approx 20$ Hz,1-H^F); 4.66 ($J_{2,3} \approx$ 9.5 Hz, $J_{3,4} \approx 1.5$ Hz, 3-H^F); 4.21 (d, $J_{1,2} = 8$ Hz) and 4.19 (d, $J_{1,2} = 8$ Hz, 1-H^C and 1-H^E); 4.14 (broadened s, 5-H^F); 3.73 ($J_{2,3} \approx 9.5$ Hz, 2-H^F); 3.50-3.20, 3.20-3.02, 2.90-2.80; 1.70 (NH-CO-C<u>H_3</u>).- $C_{51}H_{93}N_4O_{21}P$ (1129.288, 1128.607), FAB MS (matrix: DMSO-acetic acid-glycerol): m/z: 1168 ([M+K]+); 1152 ([M+Na]+); 1130 ([M+H]+); 615 ([f-H+Na]+); 593 ([f]+); 559 ([M-f+Na+H]+); 537 ([M-f+2H]+); 397 ([e-H+Na]+); 375 ([e]+); 188 ([c]+).

2-O-(2-Acetamido-2,6-dideoxy-ß-D-glucopranosyl)-3-O-carbamoyl-1-O-{[(R)-2-carboxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxyl-hydroxyphosphoryl}-α-D-galactopyranuronamide (4c)

To **3** (57.5mg, 0.051 mmol) the hot (60°C, see General) $NaIO_4$ solution (0.51 mL) was added and the mixture was stirred in the dark at 20°C for 3.5 h. Excess $NaIO_4$ was destroyed with ethylene glycol (14.8 µL, 1 h at 20°C). 25

per cent aqueous NH_3 (2.15 mL) was added at 0°C and the mixture was stirred at 20°C for 48 h. Concentration of the solution, followed by addition of 50 per cent acetic acid until pH 5.5 was reached and subsequent chromatographic separations [(i) HP-20, elution with water (300 mL) and methanol (500 mL); (ii) MPLC of the compounds of the methanolic fractions (column A, $CHCl_3$-methanol-water 18:11:2.7)] yielded after solvent evaporation and lyophilization pure **4c** (21.4 mg, 44%).- ^{13}C NMR ($CDCl_3$-CD_3OD-D_2O 18:11:2.7): δ = 173.18 (C-1^H); 171.91 (C-6^F); 171.22 ($NHCOCH_3$); 156.92 ($OCONH_2$); 102.08 (C-1^E); 94.73 (C-1^F); 77.18 (C-2^H); 74.94 (C-4^E); 73.84; 73.65 (C-3^E); 73.21 (C-2^F); 71.37 (C-5^E); 71.04; 70.71 (C-3^F); 68.83; 67.41 (C-1^I); 66.46; 65.84 (C-3^H); 55.39 (C-2^E).- ^1H NMR (400 MHz, $CDCl_3$-CD_3OD-D_2O 18:11:2.7, at 318K): δ = 5.37 (1-H^F); 4.63 ($J_{2,3}$ = 8 Hz, 3-H^F); 4.19 ($J_{1,2}$ = 7 Hz, 1-H^E); 4.08 (5-H^F); 3.72 ($J_{2,3}$ = 9.7 Hz, 2-H^F); 1.64 ($NHCOCH_3$).- $C_{43}H_{80}N_3O_{17}P$ (942.092, 941.523), FAB MS (matrix: nitrobenzylalcohol): m/z = 1002.6 ([M+Na+K-H]+); 986.6 ([M+2Na-H]+); 964.6 ([M+Na]+); 559.5 ([M-f+Na+H]+); 548.1 ([g+2Na]+); 428.2 ([f+Na-H]+).

Identification of unit F in **4c**.
A mixture containing **4c** (2.3 mg, 2.44 μmol), Dowex 50/H+ (0.186 g), methanol (0.5 mL) was stirred at 70°C (sealed vessel) for 99 h. After cooling to 20°C the ion exchange resin was filtered off and the solvent was removed in a stream of argon. The residue was redissolved in water and freeze-dried. LC (silica gel, $CHCl_3$-ethanol-petrol 1:1:3) was employed to enrich compounds with R_f values close to those obtained under similar conditions from D-glucuronic acid and D-galacturonic acid. This fraction (1 mg) after careful drying was dissolved in pyridine (2.5 mL) and treated with trimethylsilyl triflate (25μl, 0.138 mmol). The mixture was left at 20°C for 2 h and then directly analyzed by GLC (5m glas capillary column, Ø 0.28 mm, OV 17), carrier gas: H_2, temperature: 5 min 150°C, then 5°C/min → 220°C). Retention times for the galacturonic acid derived products 455 s [methyl (methyl ß-D-galactofuranosid)uronate], 502 s [methyl (methyl α-D-galactofuranosid)uronate], 669 s [methyl (methyl α-D-galactopyranosid)uronate], 670 s [methyl (methyl ß-D-galactopyranosid)uronate]; retention times for the glucuronic acid derived reaction products 586 s, 684 s, 737 s. According to this analysis **4c** contained galacturonic acid.

2-Acetamido-6-chloro-2,6-dideoxy-1,3,4-tri-O-acetyl-α-D-glucose (**5c**)
Triphenylphospin (128 mg, 488 μmol) was added at 0°C to a solution of 2-acetamido-2-deoxy-D-glucose (**5a**, 53.2 mg, 240 μmol) in pyridine (2 mL). After 5 min slowly CCl_4 (300 μl, 3.07 mmol) was added. The mixture was stirred for 10 min at 0°C and for 2.5 h at 50°C. After addition of methanol (2 mL) the mixture was stirred for 30 min at 50°C. Solvent evaporation, followed by lyophilization and LC (petrol-ethyl acetate-ethanol 1:1:0.7) yielded **5b** (17.5 mg, 30%). A solution containing **5b** (17 mg, 71 μmol), 4-dimethylaminopyridine (26 mg, 21 μmol), pyridine (1.0 mL), and acetic anhydride (0.5 mL) was stirred at 20°C for 4.5 h. Solvent evaporation, followed by lyophilization, LC (petrol-ethyl acetate-ethanol 1:1:0.3), and MPLC (petrol-ethyl acetate-ethanol 1:1:0.3) provided pure **5c** (18.4 mg, 70%, based on **5b**).-^1H NMR (400 MHz, $CDCl_3$): δ = 6.15 (d, 1-H); 4.50 (dt, 2-H); 5.25-5.28 (m, 3-H; 4-H); 4.00 (5-H); 3.50 and 3.60 (CH_2-6); 5.50 (d, NHAc); 2.00-2.10 (2s, 2 * $COCH_3$); 1.95 (s, $NHCOCH_3$); 2.20 (s, 1-OAc); $J_{1,2}$ = 4 Hz, $J_{NH,2}$ = 9 Hz, $J_{6,6'}$ = 12.5 Hz, $J_{5,6}$ =5.5 Hz, $J_{5,6'}$ = 3 Hz.- $C_{14}H_{20}ClNO_8$ 365.767, 365.088), FAB MS (matrix: lactic acid): m/z = 733/731 ([2M+H]+), 368/366 ([M+H]+, 308/306 ([M+H-AcOH]+), 248/246 ([M+H-2 AcOH]+), 188/186 ([M+H-3 AcOH]+).

2-Acetamido-2,6-dideoxy-1,3,4-tri-O-acetyl-α-D-glucose (**5d**)
A solution of **5c** (600 mg, 1.64 mmol), tributyltin hydride (1.28 mL, 4.78 mmol), and AIBN (182.2 mg, 1.11 mmol) in THF (25 mL) was stirred at 60°C for 3 h. After solvent evaporation the residue was extracted with petrol and with acetonitrile. The acetonitrile solution yielded after solvent evaporation and LC (petrol-ethyl acetate-ethanol 1:1:0.1) pure **5c** (455.1 mg, 83%).- ^1H NMR (400 MHz, $CDCl_3$): δ = 6.08 (d, 1-H); 4.42 (ddd, 2-H); 5.15 (dd, 3-H); 4.90 (t, 4-H); 3.80-3.90 (m, 5-H); 1.15 (d, CH_3-6); 1.92 (s, $NHCOCH_3$); 2.03 and 2.05 (2s, 2 * $OCOCH_3$); 2.15 (s, 1-OAc); 5.57 (d, NHAc); $J_{1,2}$ = 3.5 Hz, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6 Hz , $J_{NH,2}$ = 9 Hz.- $C_{14}H_{21}NO_8$ (331.32, 331.13), EI MS: m/z: 331 ([M]+·, 0.25); 288 (1.4); 272 (1.5); 271 (1.2); 156 (21.1); 114 (45); 72 (16); 43 (100).

2-Methyl-(3,4-di-O-acetyl-1,2,6-trideoxy-α-D-glucopyranosyl)-[1,2-d]-4-oxazoline
To a solution of **5d** (561.6 mg, 1.697 mmol) in 1,2-dichloroethane (5 mL) trimethylsilyl triflate (345 μl, 1.78 mmol) was added. The mixture was stirred at 60°C for 22 h. The solution was cooled to 20°C and after addition of triethylamine (1 mL) stirred at 20°C for 30 min. Solvent evaporation and LC (ethyl acetate-toluene-triethylamine

200:100:1) yielded a very sensitive compound (404.6 mg) which according to its ^{1}H NMR spectrum (80 MHz, $CDCl_3$): δ = 1.25 (d, $J_{5,6}$ = 6 Hz, CH_3-6); 1.70 (s, oxazoline-CH_3); 5.98 (d, $J_{1,2}$ = 7 Hz, 1-H) was the desired oxazolin.

Allyl 2-O-(2-acetamido-3,4-di-O-acetyl-2,6-dideoxy-β-D-glycopyranosyl)-3,4-O-isopropylidene-α-D-galacto-pyranosiduronamide (7)

To a solution of **6** (90.4 mg, 331.1 mmol) and camphorsulfonic acid (3.8 mg, 16.6 μmol) in CH_2Cl_2 (0.5 mL) the above oxazoline (22.4 mg, 82.8 μmol) was added. The mixture was stirred in a sealed vessel at 60°C for 3 h. Then, after 1 and 2 h further portions of the oxazoline (each time 22.4 mg, 82.8 μmol) were added and stirring at 60°C was continued for a total of 5 h. The reaction was stopped by addition (at 20°C) of triethylamine (50 μl). Solvent evaporation and LC ($CHCl_3$-ethanol-toluene 40:1:0.2) gave a fraction which was rechromatographed under the same conditions to furnish pure **7** (48.5 mg, 48%).- ^{1}H NMR (400 MHz, $CDCl_3$): *unit E*: δ = 4.90 (d, 1-H); 3.50 (m, 2-H); 5.23 (dd, 3-H); 4.78 (dd, 4-H); 3.73-3.83 (5-H and 2-H^F); 1.20 (d, CH_3-6); 5.76 (d, NHAc); 1.93 (s, NHCOCH_3); 2.00 (2 s, 2*COCH_3); $J_{1,2}$ = 8.5 Hz, $J_{2,3}$ = $J_{3,4}$ = 10 Hz , $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6 Hz, $J_{NH,2}$ = 8 Hz; *unit F*: 5.00 (d, 1-H); 3.73-3.83 (2-H and 5-H^E); 4.30 (dd, 3-H); 4.53 (dd, 4-H); 4.49 (d, 5-H); 6.47 and 5.90 (2 d, J = 3 Hz, CONH$_2$); 1.33 and 1.48 (2 s, 2* isopropylidene CH_3); $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 7 Hz, $J_{3,4}$ = 5.5 Hz, $J_{4,5}$ = 3 Hz; allyl signals at 4.03 (1-H); 4.12 (1-H'); 5.80-5.90 (2-H); 5.18 (3-H); 5.28 (3-H').- ^{13}C NMR ($CDCl_3$): δ = 171.07, 170.77 and 170.62 (NHCOCH$_3$ and 2*COCH$_3^E$); 169.89 (C-6^F); 133.43 (C-2^{allyl}); 118.20 (C-3^{allyl}); 109.75 (C(CH$_3$)$_2^F$); 101.12 (C-1^E); 97.77 (C-1^F); 77.45; 75.51; 73.87; 69.66 (C-1^{allyl}); 68.55; 55.33 (C-2^E); 26.68 and 28.47 (C(CH$_3$)$_2^F$); 20.97-23.55 (NHCOCH$_3$ and 2*COCH$_3^E$); 17.79 (C-6^E).- $C_{24}H_{36}O_{12}N_2$ (544.556, 544.223), FAB MS (matrix: lactic acid): m/z: 1089 ([2M+H]+); 545 ([M+H]+); 272 ([e]+).

Allyl 2-O-(2-acetamido-2,4-di-O-acetyl-2,6-dideoxy-β-D-glucopyranosyl)-α-D-galactopyranosiduronamide (8a)

A mixture of **7** (20.9 mg, 39.1 μmol) and aqueous acetic acid (20 per cent, 0.85 mL) was stirred at 60°C for 2 h. After solvent evaporation (codistillation with toluene), lyophilization and LC (petrol-ethyl acetate-ethanol 1:1:0.7) pure **8a** was obtained (15.0 mg, 76%).-^{1}H NMR (400 MHz, pyridine-d_5): *unit E*: δ = 5.38 (d, 1-H); 4.55 (2-H); 5.75 (t, 3-H); 5.10 (t, 4-H); 3.45-3.52 (5-H); 1.20 (d, CH_3-6); 9.20 (d, NHAc); 1.95-2.05 (3 s, NHCOCH_3 and 2*COCH_3); $J_{1,2}$ = 8.5 Hz, $J_{2,3}$ = $J_{3,4}$ = $J_{4,5}$ = 10 Hz, $J_{5,6}$ = 6 Hz, $J_{NH,2}$ = 8.5 Hz; *unit F*: 5.47 d (1-H); 4.58-4.68 (2-H, 3-H); 5.02 ($w_{1/2}$ = 8 Hz, 4-H); 4.80 (broad s, J ≈ 1 Hz, 5-H); 8.40 and 7.89 (2 broad s, CONH$_2$); 6.60 and 7.39 (2 broad s, 2 * OH); $J_{1,2}$ = 3.5 Hz; allyl group: 4.15 (1-H), 4.25 (1-H'); 5.90-6.00 (2-H); 5.00-5.30 (CH_2-3).-^{13}C NMR (DMSO-d_6): δ = 172.53, 170.76 (2 * COCH$_3^E$ and NHCOCH$_3^E$); 170.68; 169.95 (C-6^F); 134.92 (C-2^{allyl}); 116.94 (C-3^{allyl}); 103.35 (C-1^E); 99.17 (C-1^F); 78.87; 74.40; 74.10; 73.22; 71.47; 70.05; 69.91; 69.05 (C-1^{allyl}); 55.07 (C-2^E); 23.25 (NHCOCH$_3^E$); 20.60 and 20.66 (COCH$_3$); 17.74 (C-6^E).- $C_{21}H_{32}N_2O_{12}$ (504.491, 504.196), FAB MS (matrix: lactic acid): m/z 505 ([M+H]+); 272 ([e]+).

Allyl 2-O-(2-acetamido-3,4-di-O-acetyl-2,6-dideoxy-β-D-glucopyranosyl)-3-O-carbamoyl- α-D-galactopyranosid-uronamide (8b)

A mixture of **8a** (85.7 mg, 170 μmol), bis(tributyltin)oxide (50.3 μL, 94.9 μmol), and $CHCl_3$ (30 mL) was heated under reflux for 20 h. Water was continously removed by passing the condensed solvent through a layer of 4Å molecular sieves. After cooling to 0°C trichloroacetyl isocyanate (27.0 μL, 209 μmol) was added and the mixture was stirred at 0°C for 1.5 h. Excess of the reagent was destroyed by addition of methanol (2.4 mL). After solvent evaporation the residue was redissolved in methanol (25 mL), Zn dust (117 mg) was added, and the mixture was stirred at 20°C for 4 h. Filtration, washing the solid with methanol and methanol-water 1:1, evaporation and lyophilization of the combined liquid phases, followed by LC (petrol-$CHCl_3$-methanol 1:1:0.35) gave **8b** (69.4 mg, 80%).- ^{1}H NMR (400 MHz, pyridine-d_5): *unit E*, δ = 5.70 (d, 1-H); 4.02-4.20 (2-H); 5.97 (3-H); 3.50 (5-H); 1.18 (d, CH_3-6); 8.85 (d, NHAc); 2.15 (s, NHCOCH_3); 2.00 (s, 2 * COCH_3); $J_{1,2}$ = 8.5 Hz, $J_{2,3}$ = $J_{3,4}$ = 10.5 Hz, $J_{5,6}$ = 6 Hz, $J_{NH,2}$ = 8.5 Hz; *unit F*: 5.50 d (1-H); 4.90 (dd, 2-H); 5.82 (dd, 3-H); 4.85 (d, 5-H); 7.90 and 8.45 (2 broad s, CONH$_2$); 7.90 (broad s, OCONH$_2$); 7.20-7.40 (OH); $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.0 Hz, $J_{4,5}$ < 1 Hz; allyl group: 4.02-4.20 (1-H), 4.25 (1-H'), 5.88 (2-H), 4.34 (3-H), 5.05 (3-H').- ^{13}C NMR (DMSO-d_6): δ = 170.18, 169.85, 169.54, and 169.09 (NHCOCH$_3$, 2*COCH$_3$, C-6^F); 156.41 OCONH$_2$); 134.25 (C-2^{allyl}); 116.93 (C-3^{allyl}); 102.03 (C-1^E); 97.55 (C-1^F); 74.46; 73.26; 72.80; 72.27; 71.15; 71.11; 68.74; 67.69; 67.42; 60.25 (C-1^{allyl}); 53.21 (C-2^E); 22.64 (NHCOCH$_3^E$); 20.51 and 20.37 (2*COCH$_3$, 17.33 C-6^E).- $C_{22}H_{33}N_3O_{13}$ (547.516, 547.201), FAB MS (matrix: lactic acid), m/z: 1095 ([2M+H]+); 548 ([M+H]+); 272 ([e]+).

Allyl 2-O-(2-acetamido-3,4-di-O-acetyl-2,6-dideoxy-β-D-glucopyranosyl)-3-O-carbamoyl-4-O-(2,2,2-trichloroethoxy)carbonyl-α-D-galactopyranosiduronamide (**8c**)

To a solution of **8b** (104.4 mg, 191.2 μmol) in pyridine (18 mL) at 0°C trichloroethyl chloroformate (40 μl, 248 μmol) was added, and the mixture was stirred at 20°C for 14 h. An additional portion of trichloroethyl chloroformate (40 μL, 248 μmol) was added, and stirring continued for 3h. After addition of water (5 mL) solvents were removed by evaporation and lyophilization. LC (petrol-$CHCl_3$-methanol 1:1:0.5) furnished **8c** (120.3 mg, 87%).- ^{1}H NMR (400 MHz, pyridine-d_5): *unit E*: δ = 5.42 (d, 1-H); 4.04-4.10 (2-H); 6.00 (dt, 3-H); 4.90-5.10 (4-H); 3.60 (m, 5-H); 1.22 (d, CH_3-6); 8.95 (d, NHAc); 2.12 (s, NHCOCH_3); 2.00 (s, OCOCH_3); 2.02 (s, OCOCH_3); $J_{1,2}$ = 8 Hz, $J_{3,4}$, $J_{2,3}$ = 9.5 Hz and 10.5 Hz, $J_{5,6}$ = 2 Hz, $J_{NH,2}$ = 8 Hz; *unit F*: 5.46 (d,1-H); 4.45 (2-H); 6.00 (dd, 3-H); 6.55 (dd, 4-H); 4.90 (m, 5-H); 8.20 and 8.72 (2 broad s, CONH_2); 4.80 and 5.12 (AB, J_{gem} = 12 Hz, COOCH_2CCl_3); $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10 Hz, $J_{3,4}$ = 3.5 Hz, $J_{4,5}$ =1,5 Hz; allyl group: 3.98-4.01 (1-H); 4.02-4.20 (1-H'); 5.80-5.95 (2-H); 5.27 (3-H); ≈ 5.1 (3-H').-^{13}C NMR (pyridine-d_5): δ = 170.87, 170.48 170.03, 169.78 (3*COCH_3^E and CONH_2^F); 156.98 (OCONH_2), 154.31 (OC$OOCH_2CCl_3^F$); 134.33 (C-2^{allyl}); 117.39 (C-3^{allyl}); 101.84 (C-1^E); 98.66 (C-1^F); 95.27 (OCOOCH_2CCl_3^F); 77.16; 76.29; 75.52; 74.56; 72.63; 70.25; 69.98; 69.69; 69.37 (C-1^{allyl}); 56.54 (C-2^E); 20.61 and 23.33 (2*COCH_3 and NHCOCH_3); 17.71 (C-6^E).- $C_{25}H_{34}Cl_3N_3O_{15}$ (722.914, 721.106), FAB MS (matrix: lactic acid), m/z: 726, 724, 722 ([M+H]+); 272 ([e]+).

2-O-(2-Acetamido-3,4-di-O-acetyl-2,6-dideoxy-β-D-glucopyranosyl)-3-O-carbamoyl-4-O-(2,2,2-trichloroethoxy)-carbonyl-α-D-galactopyranuronamide (**8d**)

A mixture consisting of **8c** (50 mg, 69.5 μmol) tris(triphenylphosphine)rhodium-(I) chloride (freshly prepared, 6.8 mg, 7.1 μmol), DABCO (2.4 mg, 22.2 μmol), and ethanol (0.5 mL) was heated to 80°C for 2.5 h in a sealed vessel. Solid material was removed by filtration and the filtrate evaporated. The residue was redissolved in 9:1 acetone - water (5 mL) and treated with HgO (74.5 mg, 347.5 μmol) and $HgCl_2$ (74.5 mg, 280 μmol). The mixture was stirred at 20°C for 2.5 h. Solids were removed by filtration. Into the clear solution carefully gaseous H_2S was bubbled avoiding an excess of H_2S. The precipitates were removed by centrifugation. and the solid material was washed with acetone. The combined solutions were evaporated. LC ($CHCl_3$ - ethanol 5:1) yielded **8d** (30 mg, 63%).- ^{1}H NMR (400 MHz, pyridine-d_5): *unit E*, δ = 5.40 (d,1-H, probably overlapping with 5-H^F); 4.14 (2-H); 5.91 (dd, 3-H); 5.02 (t, 4-H); 3.55 (m, 5-H); 1.12 (d, CH_3-6); 8.85 (d, NHAc) 2.00 (2*OCOCH_3;) 2.15 (s, NHCOCH_3); $J_{1,2}$ = 8.5 Hz, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 9.5 Hz, $J_{5,6}$ = 6Hz, $J_{NH,2}$ = 8.5 Hz; *unit F*: 6.02 (d, 1-H); 4.57 (dd, 2-H); 6.24 (dd, 3-H); 6.67 (dd, 4-H); 5.39 (m, 5-H, probably overlapping with 1-H^E); 8.60 and 8.05 (2d, J = 3.5 Hz and 1.5 Hz, CONH_2); 4.85 and. 5.00 (AB, |J| = 12 Hz, OCOOCH_2CCl_3); $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.5 Hz, $J_{4,5}$ = 1.5 Hz.- ^{13}C NMR (pyridine-d_5): δ = 170.85 and 170.48 (2*OCOCH_3^E); 170.00 (C-6^F); 157.12 (OCONH_2); 154.41 (OC$OOCH_2CCl_3^F$); 102.10 (C-1^E); 95.26 (OCOOCH_2CCl_3^F); 93.55 (C-1^F); 77.06; 76.71; 76.52; 74.42; 72.78; 69.93; 69.82; 56.26; (C-2^E); 23.37 (NHCOCH_3^E); 20.56 and 20.54 (2*OCOCH_3^E); 17.54 (C-6^E).- $C_{22}H_{30}Cl_3N_3O_{15}$ (682.850, 681.074), FAB MS (matrix: lactic acid), m/z: 686, 684, 682 ([M+H]+); 272 ([e]+).

2-O-(2-Acetamido-3,4-di-O-acetyl-2,6-dideoxy-β-D-glucopyranosyl)-3-O-carbamoyl-4-O-(2,2,2-trichloroethoxy)-carbonyl-1-O-{[(R)-2-methoxycarbonyl-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxyl-(2,2,2-trichloro-1,1-dimethethoxy)-phosphoryl}-α-D-galactopyranuronamide (**4d**)

To a solution of 1H-1,2,4-triazole (8.4 mg, 119.2 μmol) in 1:4 pyridine-CH_2Cl_2 (370 μl) 2,2,2-trichloro-1,1-dimethylethyl dichlorphosphite (6.7 μl, 32.2 μmol) was added at 0°C. The mixture was stirred at 0°C for 1 h. **8d** (19.1 mg, 29.4 μmol), dissolved in 1:4 pyridine-CH_2Cl_2 (400 μl), was added and the reaction mixture stirred for 3h at 0°C. After addition of **9** (40.9 mg, 32.2 μmol) in three portions over a period of 2 h the mixture was stirred for 2 h at 0°C. Bis(trimethylsilyl)peroxide (9.0 μL, 41.2 μmol) was injected into the reaction flask and the stirred mixture was maintained at 20°C for 12 h. Solvent evaporation followed by LC (petrol-ethyl acetate-ethanol 2.5:1:0.5) furnished slightly impure **4d** (18.0 mg, 54%, based on **8d**).-^{1}H NMR (400 MHz, pyridine-d_5): *unit E*: δ = 5.90 (t, 3-H), 5.15 (t, 4-H), 9.08 (d, NHAc), $J_{2,3}$ = $J_{3,4}$ = $J_{4,5}$ = 9.5, $J_{2,NH}$ = 8.5 Hz; *unit F*: 6.53 (dd, 1-H), 4.55 (dt, 2-H); 5.98 (dd, 3-H); 6.65 (dd, 4-H); 8.05 and 8.90 (2 broad s's, CONH_2); 4.82 and 5.01 (AB, |J| = 12 Hz, OCOOCH_2CCl_3); 7.82 (broad s, OCONH_2), $J_{1,2}$ = 3.5 Hz, $J_{1,P}$ = 5.5, $J_{2,P}$ = 3.5 Hz, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.5 Hz, $J_{4,5}$ = 1.5 Hz; *unit H*: 3.78 (s, COOCH_3); *unit I*: 3.82-3.91 (CH_2-1).-^{13}C NMR (pyridine-d_5): δ = 157 (OCONH_2^F); 154 (OC$OOCH_2CCl_3^F$); 102.70 (C-1^E); 97.67 (C-1^E); 77.16; 75.74; 74.50; 73.03; 71.79; 70.13; 69.84; 68.87; 68.43; 55.62 (C-2^E); 52.18 (COOCH_3^H); 27.45 ((CH_3)$_2$C-CCl_3^G).

2-O-(2-Acetamido-3,4-di-O-acetyl-2,6-dideoxy-β-D-glucopyranosyl)-3-O-carbamoyl-1-O-{[(R)-2-methoxycarbonyl-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy]-hydroxy-phosphoryl}-α-D-galactopyranuronamide (4e)

To a solution of triester **4d** (16 mg, 116.7 μmol) in pyridine (0.7 mL) Zn-Cu couple (freshly prepared,12 mg) and 2,4-pentanedione (10 μl) were added and the mixture was stirred at 20°C for 4 h. Excess Zn-Cu couple was removed by filtration (washing with methanol). After solvent evaporation the residue was redissolved in 10:1 water-methanol (2.2 mL), and Zn^{2+} ions were removed by treatment with Dowex 50 W X 10 resin (H^+ form). Filtration, lyophilization, and LC ($CHCl_3$-ethanol 2:1) provided **4e** (7.5 mg, 25%).-^{13}C NMR (pyridine-d_5, rather broad signals), δ = 171.80 and 171.80 (2*$\underline{C}OCH_3^E$); 169.67 ($NH\underline{C}OCH_3^E$ and $\underline{C}ONH_2^F$); 157.80 ($O\underline{C}ONH_2^F$); 101.79 (C-1^E); 96.02 (C-1^F); 79.54 (C-2^H); 74.62; 73.66; 73.28; 69.93 (C-1^I); 69.71; 69.58; 68.67; 55.07 (C-2^E); 52.01 ($COO\underline{C}H_3^H$); 17.50 (C-6^E).- $C_{48}H_{86}N_3O_{19}P$ (1040.193, 1039.559), FAB MS (matrix: lactic acid), m/z: 1084.5 ([M+2Na-H]+); 1078.5 ([M+K]+); 1062.5 ([M+Na]+); 589.3 ([M-f+K+H]+); 573.4 ([M-f+Na+H]+), 512.1 ([f+Na-H]+), 272.1 ([e]+).

2-O-(2-Acetamido-2,6-dideoxy-β-D-glucopranosyl)-3-O-carbamoyl-1-O-{[(R)-2-carboxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy]-hydroxyphosphoryl}-α-D-glucopyranuronamide (4c).

A solution of **4e** (19.5 mg, 18.7 μmol) in 2:1 THF-water (bidist., 0.1 mL) was flushed with argon and then at 0°C 0.3 mol/L LiOH (278 μL, 84.15 μmol) was added. The mixture was stirred at 20°C for 2 h, then the reaction was stopped by addition of DOWEX 50 W X 2 resin (H^+ form). Stirring at 20°C for 30 min, filtration, lyophilization, and subsequent MPLC (2-propanol - 2 mol/L NH_3 7:3) yielded pure **4c** (6.1 mg, 35%). This sample and the specimen obtained from **1e** by degradation (vide supra) had identical R_f values when the following TLC developing systems were used: $CHCl_3$-methanol-water 18:11:2.7, $CHCl_3$-methanol-water 10:6:1 (2 x developed), 1-butanol-acetic acid-water 2:3:1, 1-butanol-pyridine-acetic acid-water 43:33:3:21, 1-butanol-pyridine-water 6:4:3, ethyl acetate-pyridine-water 10:4:3, 2-propanol-NH_3 (30 per cent)-water 10:3:1.5, 2-propanol - 2 mol/L NH_3 7:3.- ^1H NMR (400 MHz, $CDCl_3$-CD_3OD-D_2O 18:11:2.7, lyophilzation of the sample 4 times with D_2O prior to spectral analysis): δ = 4.71 (broadened doublet, $J_{2,3}$ = 10 Hz, 3-H^E); 2.88 ($w_{1/2}$ = 20 Hz, 4-H^E); 1.75 (s, $NHCOC\underline{H}_3$); 5.45 ($w_{1/2}$ = 20 Hz, 1-H^F); 3.79 (2-H^F).-^{13}C NMR ($CDCl_3$-CD_3OD-D_2O 18:11:2,7): δ = 172.10 ($NH\underline{C}OCH_3^E$, $\underline{C}ONH_2^F$, $\underline{C}OOH^H$); 157.02 ($O\underline{C}ONH_2$); 101.94 (C-1^E); 95.95 (C-1^F); 74.95; 73.71; 73.22; 71.68; 71.42; 71.05; 70.73; 69.63; 67.43 (C-5^F); 66.00 (C-3^H); 60.47; 55.63 (C-2^E); 16.55 (C-6^E).-$C_{43}H_{80}N_3O_{17}P$ (942.092, 941.523), FAB MS (matrix: lactic acid), m/z : 986.3 ([M+2Na-H]+); 980.3 ([M+K]+); 964.3 ([M+Na]+); 942.3 ([M+H]+); 559.3 ([M-f+Na+H]+); 428.0 ([f+Na-H]+); 406.0 ([f]+); 188.0 ([e]+).

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