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A Structurally and Biogenetically Interesting Moenomycin Antibiotic

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Abstract- Isolation and structure elucidation of a new moenomycin antibiotic (A_{12}, **1a**) is reported that differs from moenomycin A by lack of the branching methyl group and by the configuration at C-4 of unit F. The smallest antibiotically active degradation product of **1a** is the *trisaccharide* derivative **3a.** This observation is in contrast to structure activity relations in the moenomycin A series where it was found that *disaccharide* **4b** is fully active. An explanation is offered for this difference.

Introduction

Moenomycin A (**1b**) is the prototype of a slowly growing group of antibiotics that are unique both structurally and with regard to their mode of action.[1] **1b** contains besides ordinary components (B, C, D, E, G, H in formula **1**) units which have been found only rarely in nature (A) or have never been encountered in compounds other than the moenomycin antibiotics (F, I). Neither has the biosynthesis of units F and I been elucidated nor is known how the rather complicated array of building blocks is assembled in the course of the biosynthesis. Until now, a biosynthetic precursor of moenomycin-type antibiotics has never been found. This is probably not too surprizing in view of the analytical methods used in the isolation of the moenomycins. The almost linear structure of the moenomycins demands that intermediates containing both units A and I appear at a rather late stage of the biosynthesis. Intermediates lacking unit A would escape the chromatographic separations (UV monitoring). Intermediates lacking unit I would be very polar compounds and probably not be isolated with the usual methods.[2] This explains that in the Flavomycin® complex until now only compounds have been detected that differ solely in the „periphery".[3,4] Figure 1 shows the HPLC chromatogram of the moenomycin complex. With one exception, all (UV visible) compounds of this mixture have been isolated and structurally elucidated. The structure of the last unknown component is described below.

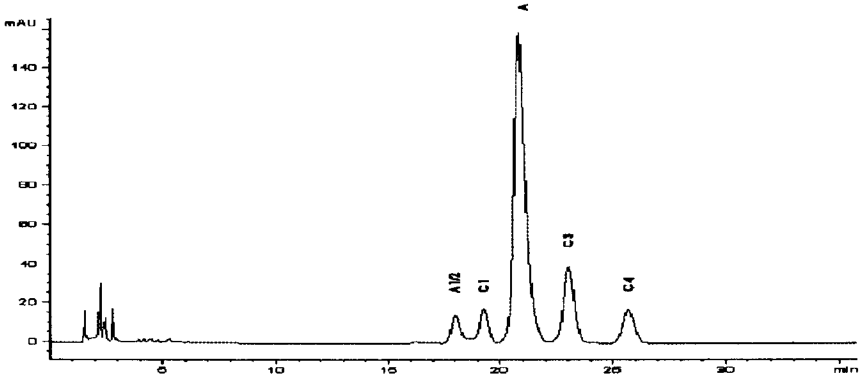


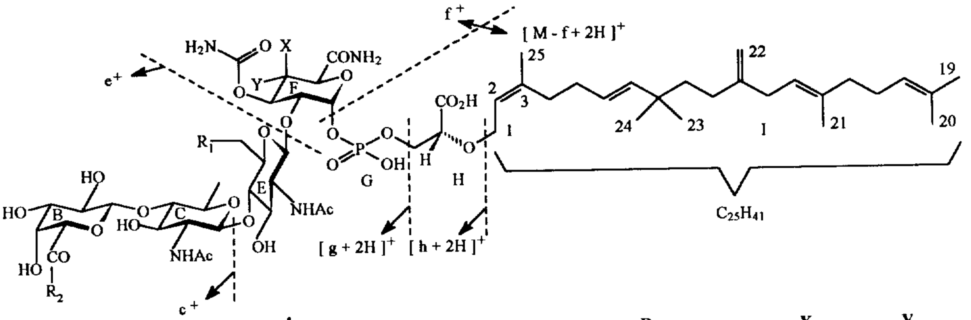
Figure 1. Separation of the moenomycin complex by HPLC (for conditions, see Experimental)

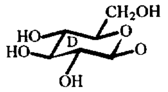
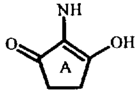
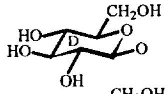
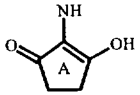
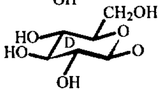
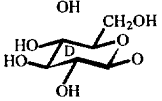
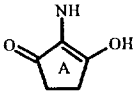
Isolation and structure elucidation of moenomycin A_{12}.

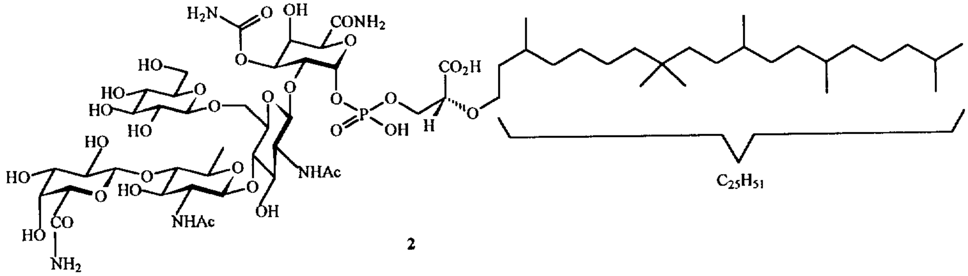
Moenomycin A_{12} (about 5% of the complex) was isolated by careful and time-consuming chromatographic separations. The FAB and ESI mass spectra indicated that the molecular mass of moenomycin A_{12} is 14 mass units smaller than that of moenomycin A (**1b**) what was taken as a hint that the branching methyl group in unit F of **1b** was lacking in moenomycin A_{12} as in moenomycin C_1 (**1e**).[3] This assumption was corroborated by appearance of the characteristic FAB MS fragments which are indicated in formula **1**. The ^{13}C NMR spectrum displayed the expected signals of the lipid moiety I, of four glycosidic carbon signals (a broad one at $\delta = 96.1$, C,P coupling), two C-2 signals of N-acetylamino sugars, and the very characteristic signal of the urethane grouping at $\delta = 158.7$.[1] In addition, the CD spectrum was indicative of the presence of the chromophore unit A.[2] As usual, ^{1}H NMR spectra were of little use. Of main interest was the configuration at C-4 of unit F which was established by a degradative correlation. Recently, we have demonstrated that part A of **1b** can selectively be removed by oxidation.[2] Employing this method to the degradation of moenomycin A_{12} led to the formation of **1c** in 63% yield.

At this stage, we studied whether the Barry degradation[5] can also be performed in the presence of the unsaturated lipid part. Thus, **1d** was submitted to (i) periodate cleavage and (ii) treatment with N,N-dimethylhydrazine/H^+ whereupon moenomycin A trisaccharide analogue **3b** was obtained in 23% yield.

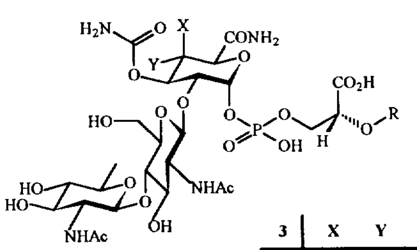
In the moenomycin A_{12} series the lipid part was hydrogenated (**1c** $\rightarrow$ **2**, 93%). Barry degradation of **2** as described above provided **3a** in 34% yield. One further degradation cycle[5] - (i) $NaIO_4$, (ii) NH_3 - gave the disaccharide derivative **4a** which proved identical in all respects (see Experimental) with a sample that was



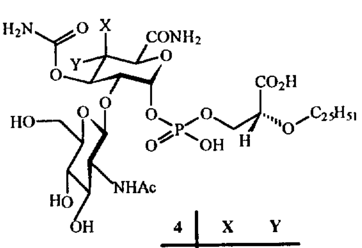
1	R₁	R₂	X	Y
a			OH	H
b			CH₃	OH
c		NH₂	OH	H
d		NH₂	CH₃	OH
e	H		OH	H



2



3	X	Y	R
a	OH	H	$C_{25}H_{51}$
b	CH₃	OH	$C_{25}H_{41}$



4	X	Y
a	OH	H
b	CH₃	OH

Scheme 1.

previously obtained by synthesis.[6] Most specifically, the 3-H^F signal was a doublet of doublets with one large coupling constant ($^3J_{2,3}$ = 10 Hz) and a small one (identified by ^{1}H,^{1}H COSY) indicative of the D-galacto configuration in unit F.

Antibiotic activity of moenomycin A_{12} (1a), its degradation products 2, 3a, 4a, and of moenomycin A analogue 3b.

The minimum inhibitory concentrations (MIC) 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 A_{12} like the other moenomycins is mainly active against *gram-positive* bacteria. When compared with moenomycin A (**1b**), moenomycin A_{12} is of distinct lower activity against *Staph. aureus*, an observation that parallels recent findings with moenomycin C_1 (**1e**).[3] As found for other moenomycins, in the series of the degradation products the *in-vivo* activity against *Staph. aureus* slowly decreases. Degradation product **4a** is antibiotically inactive.[6]

The inhibitory effect of **1a** and a number of degradation products directly on the transglycosylation reaction was determined by the *in-vitro* assay developed earlier[7] 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 A_{12} (**1a**) is an as active inhibitor of the transglycosylation reaction as moenomycin A (**1b**) itself. In addition, degradation products **2**, **3a**, and **3b** are fully active. Practically identical results were obtained with another *in-vitro* test, inhibition of the UDP-N-acetylmuramyl pentapeptide-dependent incorporation of [^{14}C]UDP-N-acetyl-glucosamine into cross-linked high-molecular weight peptidoglycan (slightly modified[8] version of the assay described by Izaki, Matsuhashi, and Strominger.[9]) The results are collected in Table 3. In this test **4a** was shown to be inactive.[6]

Table 1. Minimum inhibitory concentrations (in µg/mL) of moenomycin A_{12} (**1a**), its degradation products **2**, **3a**, **4a**, and of moenomycin A (**1b**, for comparison) against various test organisms.

test organism	**1b**	**1a**	**2**	**3a**	**4a**
Staph. aureus SG 511	0.025	0.195	1.563	6.25	>50
Staph. aureus 503	0.049	0.391	1.563	6.25	>50
Strept. pyogenes A77	<0.002	<0.002	0.391	0.391	3.125
Pseud. aerug. 1771m	3.125	3.125	25	25	>50
E. coli DC 2	12.5	50	>100	>100	>50

Table 2. Effect of some moenomycin-type compounds on the *in-vitro* formation of uncrosslinked peptidoglycan by transglycosylation.

final concentration (μg/mL)	%-inhibition				
	1b	**1a**	**2**	**4a**	**3b**
1	100	100	96	100	100
0.1	78	83	29	20	40

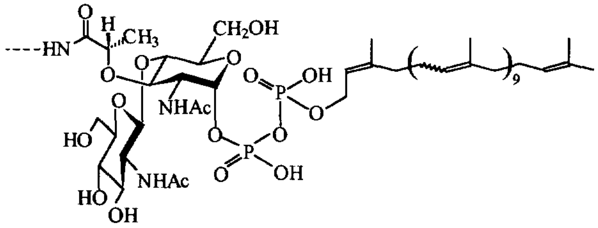
Table 3. Effect of moenomycin A_{12} and some analogues on the *in-vitro* UDP-N-acetylmuramyl pentapeptide-dependent incorporation of [^{14}C]UDP-N-acetylglucosamine into cross-linked high-molecular weight peptidoglycan.

final concentration (μg/mL)	%-inhibition					
	1b	**1a**	**2**	**3a**	**4a**	**3b**
10	87	89	88	89	36	96
1	84	93	86	91	0	91
0.1	41	53	71	76	0	57

Discussion

Comparing the structure-activity relations allows to divide the moenomycins into two groups. Moenomycins A_{12} (**1a**) and C_1 (**1e**) with D-galacto configuration in unit F and lacking the 4-C-methyl form one group. They are *in-vivo* less active against *gram-positive* bacteria than the other known moenomycins (A (**1b**), C_3, C_4, pholipomycin with a D-glucuronic-derived unit F).[4] The *in-vitro* inhibiting activity against the transglycosylating enzyme obtained from *E. coli* is, however, similar for all moenomycin antibiotics. In the series of degradation products with a D-glucuronic-derived unit F disaccharide degradation products such as **4b** are active both *in-vivo* and *in-vitro*,[1,10] whereas in the series with a D-galacturonic-derived unit F it is a trisaccharide analogue such as **3a** which is the smallest active compound[11,12] and the disaccharides of type **4a** are inactive.[6,11] It has been shown that the moenomycin antibiotics are competitive inhibitors of the peptidoglycan biosynthesis competing probably with the membrane disaccharide precursor **5** for the binding site at the enzyme.[13] In view of the striking structural similarity between **4b** and **5** it is tempting to speculate that the equatorial OH group at C-4 in unit F of **4b** is involved in binding to the enzyme (as a hydrogen bond acceptor). In moenomycins A_{12}, C_1 and the antibiotically active degradation products derived therefrom this OH group may be substituted in its function by a hydroxyl group of unit C. Experiments aimed at probing this hypothesis are underway.

Isolation of moenomycins A_{12} and C_1 alongside with their counterparts carrying the 4-C-methyl group in unit F strengthens the assumption expressed previously[3] that the branching methyl group is introduced at a late stage of the biosynthesis.



5

Scheme 2.

Experimental

General

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: ^{1}H NMR: AM 400 (Bruker), AMX 300 (Bruker); ^{13}C NMR: AM 400 (Bruker, at 100.6 MHz, T = 303 K), AMX 300 (Bruker, at 75.5 MHz, T = 293 K), in solvent mixtures the methanol signal ($\delta = 49.05$) was used as internal standard; ^{31}P NMR: AMX 300 (Bruker, at 121.4 MHz); FAB MS: VG AUTOSPEC; LC (preparative gravitational liquid chromatography): silica gel (ICN Biomedicals Silica 63-100 μm); MPLC (medium-pressure liquid chromatography): 400 mm · 10 mm glass tubes (column A), 50 μm silica gel (Amicon), or RP-18, 40-63 μm, LiChroprep® (Merck, precolumn with silical gel 60 silanised, 63-200 μm, Merck), 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 with a 2.22 mol/L H_2SO_4 solution which contained $Ce(SO_4)_2{\cdot}4H_2O$ (10 g/L) and $H_3[PO_4(Mo_3O_9)_4]{\cdot}H_2O$ (25 g/L)[14] 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, ^{1}H, ^{16}O, ^{14}N, ^{31}P (monoisotopic 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, 12.25 mmol) 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 6.35 mL). Only freshly prepared solutions were used.

Moenomycin A$_{12}$ (1a)

Moenomycin complex (Flavomycin®) was separated by LC as described earlier[4] (SiO$_2$ (Grace 35-70 µm), 16 kg, elution with 2-propanol-2 mol/L NH$_3$ 8:2) to give the C components (102 g), pure moenomycin A (54 g) and a mixture of moenomycins A and A$_{12}$ (240 g). 90 g of the latter mixture were separated by eight repeated HPLC separations (AMICON K-Prime 3000 prep. LC, 200 mm · 220 mm, MERCK LiChrospher RP-18, 12 µm, 4 kg), elution with acetonitril-0.6 mol/L phosphate buffer (pH 8) 37:63 to give after desalting (by reversed-phase chromatography) pure moenomycin A$_{12}$ (2 g). Analytical HPLC: RP 18, elution with a 80:20 mixture of solution A and acetonitrile. (Solution A: Sodium heptanesulfonate (3 g), K$_2$HPO$_4$ (15.5 g), KH$_2$PO$_4$ (1.0 g) and water, final volume: 1 L.) - ^{13}C NMR (CD$_3$OD-D$_2$O 1:1): δ = 201.4 (C-1^{A}, C-3^{A}); 177.4, 175.0, 173.5, 170.5 (NH$\underline{\mathrm{C}}$OCH$_3^{\mathrm{C,\,E}}$, C-6$^{\mathrm{B,\,F}}$); 158.7 (O$\underline{\mathrm{C}}$ONH$_2^{\mathrm{F}}$); 150.3 (C-11^{I}); 141.9 (C-3^{I}); 141.3 (C-7^{I}); 136.9 (C-14^{I}); 131.9 (C-18^{I}); 126.7, 125.3, 123.2, 122.4 (C-2^{I}, C-6^{I}, C-13^{I}, C-17^{I}); 110.7 (C-2^{A}); 109.7 (C-22^{I}); 104.2, 104.1, 102.5 (C-1$^{\mathrm{B,\,C,\,D,\,E}}$); 96.1 (C-1^{F}); 84.5 (C-4^{C}); 80.8-66.7 (unassigned signals); 61.9 (C-6^{D}); 56.7, 55.7 (C-2$^{\mathrm{C,\,E}}$); 42.7, 40.7 (C-9^{I}, C-15^{I}); 36.3, 35.8 (C-8^{I}, C-12^{I}); 33.1, 32.5, 32.3 (C-4^{I}, C-5^{I}, C-10^{I}); 31.5, 28.0, 27.6, 26.4, 24.2, 23.4, 22.9 (C-16^{I}, C-19^{I}, C-23^{I}, C-24^{I}, C-25^{I}, NHCO$\underline{\mathrm{C}}$H$_3^{\mathrm{C,\,E}}$); 18.3, 17.8, 16.6 (C-20^{I}, C-21^{I}, C-6^{C}).- $C_{68}H_{106}N_5O_{34}P$ (1568.58, 1567.65), FAB MS (matrix: lactic acid) m/z = 1644.6 ([M+2K-H]$^+$); 1606.6 ([M+K]$^+$); 1590.6 ([M+Na]$^+$); 1568.6 ([M+H]$^+$); 1080.3 ([f+K-H]$^+$); 1042.4 ([f]$^+$); 862.3 ([e+K-H]$^+$). ES MS m/z = 1568.5 ([M+H]$^+$); 1228.1 ([h+2H]$^+$); 1041.7 ([f]$^+$).- CD (water): λ_{max} (Δε) = 276.4 nm (-3.89); 255.8 nm (6.27).

2-O-{2-Acetamido-4-O-[2-acetamido-4-O-((5S)-5-carbamoyl-ß-L-arabinopyranosyl)-2,6-dideoxy-ß-D-glucopyranosyl]-2-deoxy-6-O-ß-D-glucopyranosyl-ß-D-glucopyranosyl}-3-O-carbamoyl-1-O-{[(R)-2-carboxy-2-((2Z, 6E, 13E)-3,8,8,14,18-pentamethyl-11-methylene-nonadeca-2,6,13,17-tetraen-1-yloxy)-ethoxy]-hydroxyphosphoryl}-α-D-galactopyranuronamide (1c)

To **1a** (299.5 mg, 191 µmol), dissolved in water (20 mL) solutions of K$_2$CO$_3$ (1.5338 g, 11.098 mmol) in water (5 mL) and K$_3$[Fe(CN)$_6$] (1.2586 g, 3.823 mmol) in water (5 mL) were added at 0°C. The mixture was stirred for 30 min at 0°C, then 1.5 h at 40°C. Progress of the reaction was monitored by TLC (chloroform - methanol - water 18:11:2.7).[15] The inorganic salts were removed by reversed-phase chromatography (HP 20, 60 g, elution with water (150 mL) and methanol (500 mL)). After solvent evaporation and lyophilization the mixture was separated by LC (SiO$_2$, 2-propanol - 25 per cent NH$_3$ - water 10:3:0.5). After evaporation and lyophilization pure **1c** was obtained (175.9 mg, 62%).- ^{13}C NMR (D$_2$O-CD$_3$OD 1:1): δ = 175.6, 175.0, 174.0, 173.6 (NH$\underline{\mathrm{C}}$OCH$_3^{\mathrm{C,\,E}}$, C-6$^{\mathrm{F,\,B}}$); 158.6 (O$\underline{\mathrm{C}}$ONH$_2^{\mathrm{F}}$); 150.1 (C-11^{I}); 142.1 (C-3^{I}); 141.3 (C-7^{I}); 136.9 (C-14^{I}); 131.8 (C-18^{I}); 126.6, 125.3, 123.2, 122.1 (C-2^{I}, C-6^{I}, C-13^{I}, C-17^{I}), 109.7 (C-22^{I}); 103.9, 102.4 (C-1$^{\mathrm{B,\,C,\,D,\,E}}$); 96.0 (C-1^{F}); 84.2 (C-4^{C}); 80.9 - 67.2 (unassigned signals); 62.0 (C-6^{D}); 56.5 (C-2$^{\mathrm{C,\,E}}$); 42.6, 40.7 (C-9^{I}, C-15^{I}); 36.3, 35.8 (C-8^{I}, C-12^{I}); 33.1, 32.5, 32.3 (C-4^{I}, C-5^{I}, C-10^{I}); 28.0, 27.6, 26.4, 24.2, 23.5, 23.1 (C-16^{I}, C-19^{I}, C-23^{I}, C-24^{I}, C-25^{I}, NHCO$\underline{\mathrm{C}}$H$_3^{\mathrm{C,\,E}}$); 18.4, 17.8, 16.2 (C-20^{I}, C-21^{I}, C-6^{C}).- $C_{63}H_{102}N_5O_{32}P$ (1472.49, 1471.63), FAB MS (matrix: lactic acid): m/z = 1510.3 ([M+K]$^+$); 1494.4 ([M+Na]$^+$); 1472.4 ([M+H]$^+$); 946.2 ([f]$^+$); 728.1 ([e]$^+$); 363.1 ([c]$^+$).

2-O-{2-Acetamido-4-O-[2-acetamido-4-O-((5S)-5-carbamoyl-ß-L-arabinopyranosyl)-2,6-dideoxy-ß-D-glucopyranosyl]-2-deoxy-6-O-ß-D-glucopyranosyl-ß-D-glucopyranosyl}-3-O-carbamoyl-1-O-{[(R)-2-carboxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy]-hydroxyphosphoryl}-α-D-galacto-pyranuronamide (2)

A mixture of **1c** (175.9 mg, 119 µmol), methanol (17.5 mL), PtO$_2$·H$_2$O (46.7 mg), and acetic acid (0.6 mL) was stirred under H$_2$ at normal pressure and 20°C (95 h). Filtration and solvent evaporation yielded **2a** (165.3 mg, 93 %).- ^{13}C NMR (D$_2$O-CD$_3$OD 1:1): δ = 174.8, 174.5, 173.8 (NH$\underline{\mathrm{C}}$OCH$_3^{\mathrm{C,\,E}}$, C-6^{F}, C-6^{B}); 158.7

($O\underline{C}ONH_2^F$); 103.7, 103.3, 102.3 ($C\text{-}1^{B, C, D, E}$); 95.7 ($C\text{-}1^F$); 84.1, 81.0 ($C\text{-}4^{C, E}$); 77.1-69.4 (unassigned signals); 61.9 ($C\text{-}6^D$); 56.3 ($C\text{-}2^{C, E}$); 43.1; 40.3; 38.3; 38.1; 35.3; 34.7; 33.9; 33.2; 32.1; 31.8; 31.0; 30.4; 28.9; 25.7; 25.65; 25.3; 23.6; 23.5; 20.8; 20.7; 20.6; 20.55; 20.1; 17.7/17.5.- $C_{63}H_{112}N_5O_{32}P$ (1482.55, 1481.70), FAB MS (matrix: lactic acid): m/z = 1520.4 ($[M+K]^+$); 1504.5 ($[M+Na]^+$); 1482.5 ($[M+H]^+$); 1066.4 ($[g+Na+H]^+$); 946.4 ($[f]^+$); 728.4 ($[e]^+$).

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

To a solution of **2a** (146.7 mg, 99 μmol) in the smallest possible amount of water the hot (60°C, see General) $NaIO_4$ solution (1.8 mL) was added, and the mixture was stirred in the dark for 4 h at 40°C. Inorganic salts were removed by reversed-phase chromatography (HP 20, 60 g, elution with water (400 mL) and methanol (1250 mL)). The pH of the eluate was first 2.5 and then slowly raised to 5.5. Aqueous fractions with pH 5.5 and higher and methanolic fractions were combined. Solvents were removed by distillation and subsequent lyophilization. The residue (101.8 mg) was dissolved in as little water as possible. To this solution the N,N - dimethylhydrazine solution (see General, 0.38 mL) was added, and the mixture was stirred at 85°C for 4 h. After cooling to 20°C inorganic salts were removed by reversed-phase chromatography (HP 20, 60 g, elution with water (700 mL) and methanol (1000 mL)). From the methanolic eluate after solvent evaporation and lyophilization a crude degradation product (94.3 mg) was obtained which yielded after three separation steps [(i) LC (SiO_2, 8 g); (ii),(iii) MPLC (column A), elution with chloroform-methanol-water 10:6:1] pure **3a** (38.8 mg, 34%).- ^{13}C NMR ($CDCl_3\text{-}CD_3OD\text{-}D_2O$ 18:11:2.7; DEPT; C,H COSY):[16] δ = 177.4 (b), 174.2, 173.5, 172.8 ($C\text{-}1^H$, $C\text{-}6^F$, $NH\underline{C}OCH_3^{C, E}$); 158.5 ($O\underline{C}ONH_2^F$); 103.6 ($C\text{-}1^E$); 102.7 ($C\text{-}1^C$); 96.0 (b, $C\text{-}1^F$); 81.0 ($C\text{-}4^E$); 76.2 ($C\text{-}4^C$); 75.2; 74.7; 73.3; 73.1; 72.4 (b); 71.7; 69.8-70.0 ($C\text{-}1^I$); 68.9 ($C\text{-}5^F$); 67.4 (b, $C\text{-}3^H$); 60.6 (b, $C\text{-}6^E$); 56.6 ($C\text{-}2^E$); 55.7 ($C\text{-}2^C$); 43.1-33.9 (unassigned signals); 33.3 ($C\text{-}8^I$); 32.0-25.3 (unassigned signals); 23.4, 23.3 ($NHCO\underline{C}H_3^{C, E}$); 20.7-20.1 (unassigned signals); 17.9 ($C\text{-}6^C$).- ^1H NMR (400 MHz, $CDCl_3\text{-}CD_3OD\text{-}D_2O$ 18:11:2.7, T = 316 K, lyophilization of the sample 6 times with D_2O prior to spectral analysis, H,H COSY, 300 MHz, T = 325 K): δ = 5.65 ($w_{1/2} \approx 30$ Hz, $1\text{-}H^F$); 4.76 ($w_{1/2} \approx 30$ Hz, $3\text{-}H^F$); 4.31 (d, $J_{1,2} = 8$ Hz), 4.26 (d, $J_{1,2} = 8$ Hz) ($1\text{-}H^{C, E}$); 4.17 (broadened, s); 3.77 (d, $J_{2,3} \approx 10$ Hz, $2\text{-}H^F$); 3.62-3.46; 3.45-3.35; 3.35-3.11; 2.89 (t, $J_{3,4} \approx J_{4,5} \approx 9$ Hz, $4\text{-}H^C$); 1.79 ($2xNHCOC\underline{H}_3$).- ^{31}P NMR ($CDCl_3\text{-}CD_3OD\text{-}D_2O$ 18:11:2.7, external standard: H_3PO_4): δ = -1.83, $w_{1/2} \approx 80$ Hz.- $C_{51}H_{93}N_4O_{22}P$ (1145.29, 1144.60), FAB MS (matrix: lactic acid): m/z = 1189.7 ($[M+2Na-H]^+$); 1183.7 ($[M+K]^+$); 1167.7 ($[M+Na]^+$); 1145.7 ($[M+H]^+$); 729.3 ($[g+Na+H]^+$); 631.3 ($[f+Na-H]^+$); 609.3 ($[f]^+$).

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

To a solution of **3a** (25.6 mg, 22 μmol) in water (4 mL) the hot (60°C, see General) $NaIO_4$ solution (0.24 mL) was added and the mixture was stirred in the dark at 20°C for 10 h. Excess $NaIO_4$ was destroyed with ethylene glycol (9 μL, 1 h at 20°C). 25 per cent aqueous NH_3 (1.7 mL) was added in 4 portions at 0°C and the mixture was stirred at 20°C for 36.5 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, 20 g, elution with water (450 mL) and methanol (750 mL); (ii) MPLC of the compounds of the methanolic fractions (column A, chloroform-methanol-water 10:6:1)) yielded after solvent evaporation and lyophilization slightly impure **4a** (13.3 mg). Further separation (MPLC (iii) column A, 2-propanol-2 mol/L NH_3 7:3, (iv) column A, 2-propanol-2 mol/L NH_3 10:3, (v) column A, 2-propanol-2 mol/L NH_3 13:3, (vi) column of RP-18, methanol-water-acetonitrile 8:2:4) gave slightly impure **4a** (3.9 mg), and a pure specimen of **4a**

(6.1 mg, 29%).- ^{13}C NMR ($CDCl_3$-CD_3OD-D_2O 18:13:2.7):[17,18] δ = 177.5 (b), 174.4 (b), 173.2 (C-1^{H}, C-6^{F}, NH$\underline{C}$OCH$_3^{E}$); 158.3 (OCONH$_2^{F}$); 103.5 (C-1^{E}); 95.8 (C-1^{F}); 76.8 (C-2^{H}); 75.9 (C,P coupling, C-2^{F}); 74.8, 72.2, 71.7, 71.0 (C,P coupling), 70.7, 69.6 (C,P coupling) (C-1^{I}, C-3^{E,F}, C-4^{E,F}, C-5^{E}); 68.8 (C-5^{F}); 67.5 (C,P coupling, C-3^{H}); 61.5 (C-6^{E}); 56.5 (C-2^{E}); 33.1 (C-8^{I}).- ^{1}H NMR (400 MHz, $CDCl_2$-CD_3OD-D_2O 18:13:2.7, T = 316 K, lyophilization of the sample 5 times with D_2O prior to spectral analysis, H,H COSYDQF): δ = 5.65 ($w_{1/2} \approx$ 18 Hz, 1-H^{F}); 4.75 (broadened d, $J_{2,3}$ = 10 Hz, a second coupling was identified by H,H COSYDQF, 3-H^{F}).- $C_{43}H_{80}N_3O_{18}P$ (958.09, 957.52), FAB MS (matrix: lactic acid): m/z = 1018.2 ($[M+Na+K-H]^+$); 1002.2 ($[M+2Na-H]^+$); 996.2 ($[M+K]^+$); 980.3 ($[M+Na]^+$); 958.3 ($[M+H]^+$); 559.2 ($[M-f+Na+H]^+$); 542.0 ($[g+Na+H]^+$); 444.0 ($[f+Na-H]^+$); 422.1 ($[f]^+$).

The **4a** sample obtained from **2a** by degradation and the specimen obtained previously by synthesis[6] had identical R_f values using the following TLC systems: chloroform-methanol-water 18:11:2.7, chloroform-methanol-water 10:6:1 (2 x developed), 1-butanol-pyridine-acetic acid-water 43:33:3:21, 1-butanol-pyridine-water 6:4:3, ethyl acetate-pyridine-water 10:4:3, ethyl acetate-2-propanol-pyridine-water 25:22:14:14, 2-propanol-NH_3 (25 per cent)-water 10:3:1.5 and 6:3:1, 2-propanol-2 mol/L NH_3 7:3, ethanol-NH_3 (25 per cent)-water 150:1:20.

2-O-{2-Acetamido-4-O-[2-acetamido-2,6-dideoxy-ß-D-glucopyranosyl]-2-deoxy-ß-D-glucopyranosyl}-3-O-carbamoyl-1-O-{[(R)-2-carboxy-2-((2Z, 6E, 13E)-3,8,8,14,18-pentamethyl-11-methylene-nonadeca-2,6,13,17-tetraen-1-yloxy)-ethoxy]-hydroxyphosphoryl}-4-C-methyl-α-D-glucopyranuronamide (3b)

To a solution of **1d** (142 mg, 96 µmol) in the smallest possible amount of water the hot (60°C, see General) $NaIO_4$ solution (1.7 mL) was added, and the mixture was stirred in the dark for 4.5 h at 40°C. Inorganic salts were removed by reversed-phase chromatography (HP 20, 60 g, elution with water (400 mL) and methanol (1050 mL)). The pH of the eluate was 3 at the beginning and then slowly raised to 5. Methanolic fractions were combined. Solvents were removed by distillation and subsequent lyophilization. The residue (112.4 mg) was dissolved in as little water as possible. To this solution the N,N-dimethylhydrazine solution (see General, 0.4 mL) was added, and the mixture was stirred at 85°C for 5 h. After cooling to 20°C inorganic salts were removed by reversed-phase chromatography (HP 20, 60 g, elution with water (650 mL) and methanol (1100 mL)). From the methanolic eluate after solvent evaporation and lyophilization a crude degradation product (84.9 mg) was obtained which yielded after two separation steps [(i) LC (SiO_2, 8 g), (ii) MPLC (column A), elution with chloroform-methanol-water 10:6:1] pure **3b** (25.5 mg, 23%).- ^{13}C NMR ($CDCl_3$-CD_3OD-D_2O 18:11:2.7): δ = 174.4, 174.1, 173.9 (C-1^{H}, C-6^{F}, NH$\underline{C}$OCH$_3^{C,E}$); 159.0 (O$\underline{C}$ONH$_2^{F}$); 150.9 (C-11^{I}); 142.1 (C-3^{I}); 141. 6 (C-7^{I}); 137.3 (C-14^{I}); 132.2 (C-18^{I}); 126.3 (C-6^{I}); 125.2 (C-17^{I}); 123.0 (C-2^{I}); 121.9 (C-13^{I}); 109.2 (C-22^{I}); 103.4 (C-1^{E}); 102.8 (C-1^{C}); 95.5 (C-1^{F}); 81.1 (C-4^{E}); 78.4; 76.3; 75.5; 75.3; 74.9; 73.9; 73.3; 73.2; 73.1; 67.3 (b), 66.9 (b) (C-3^{H}, C-1^{I}); 60.6 (C-6^{E}); 56.6, 55.8 (C-2^{C,E}); 42.5 (C-9^{I}); 40.7 (C-15^{I}); 36.4 (C-8^{I}); 35.8 (C-12^{I}); 33.3 (C-4^{I}); 32.6, 32.3 (C-5^{I}, C-10^{I}); 28.0 (C-23^{I}, C-24^{I}); 27.5 (C-16^{I}); 26.4 (C-19^{I}); 24.4 (C-25^{I}); 23.4, 23.3 (NHCO$\underline{C}$H$_3^{C,E}$); 18.4, 17.9 (C-20^{I}, C-6^{C}); 16.7, 16.2 (C-21^{I}, CH$_3$ of C-4^{F}).- $C_{52}H_{85}N_4O_{22}P$ (1149.23, 1148.54), FAB MS (matrix: lactic acid): m/z = 1149.5 ($[M+H]^+$); 831.2 ($[h+Na+H]^+$); 645.1 ($[f+Na-H]^+$).

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[16] b: broad signal.

[17] The chemical shifts of $C\text{-}2^H$, $C\text{-}2^F$, $C\text{-}1^1$ reported in ref.[6] had to be corrected.

[18] Only signals which could be assigned are reported; b: broad signal.

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