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STRUCTURES OF SOME MOENOMYCIN ANTIBIOTICS - INHIBITORS OF PEPTIDOGLYCAN BIOSYNTHESIS

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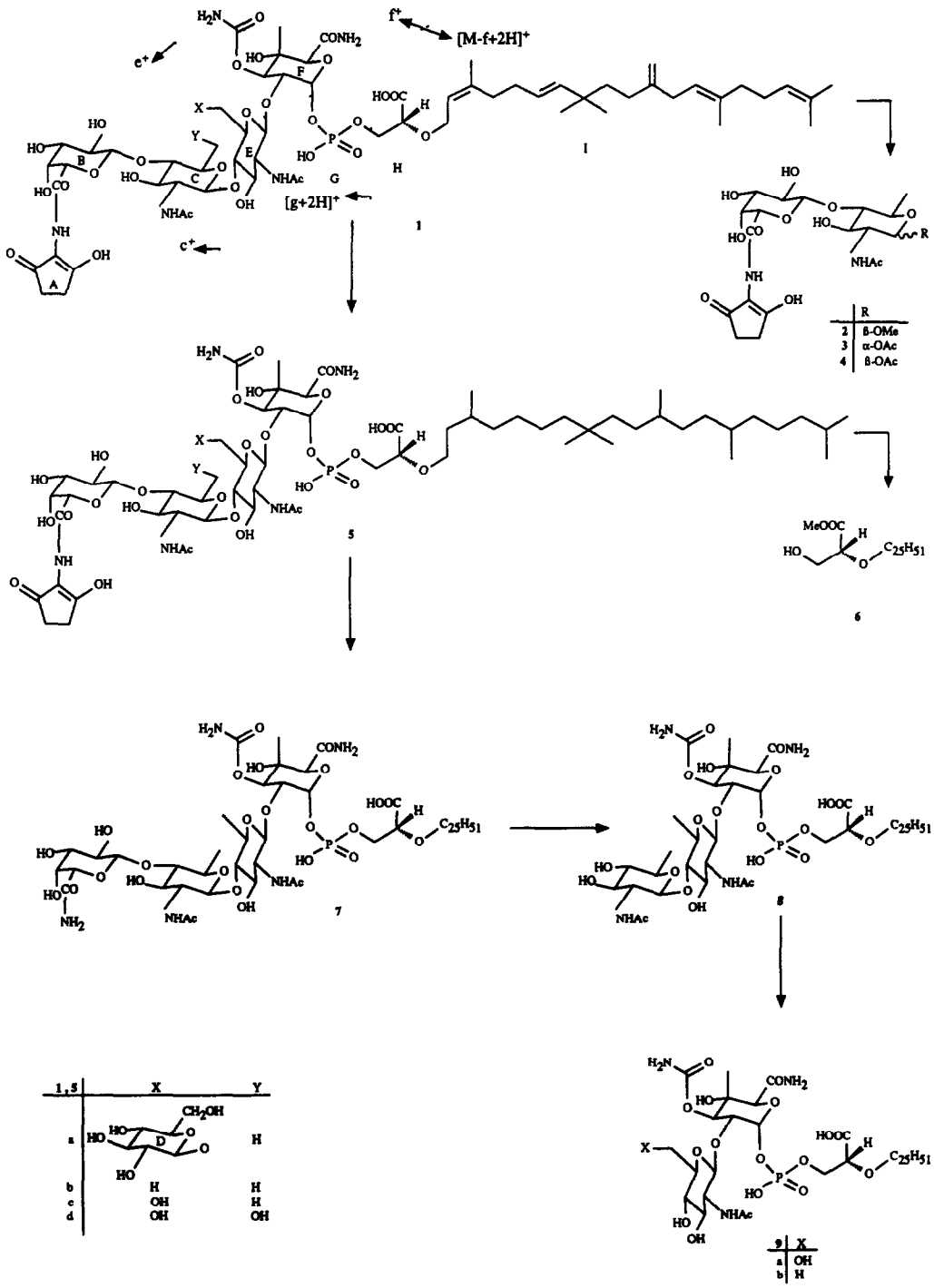
Abstract - Isolation, structural assignment, and antibiotic efficiency of the new moenomycin antibiotics C_3 (**1b**) and C_4 (**1c**) is decribed The previously published structure of pholipomycin (**1d**) is modified

Introduction

The family of moenomycin-type antibiotics includes the components of the flavomycin^R complex, the prasinomycins, diumycins (marcarbomycins), 11837 R P , 8036 R.P (quebemecin), 19402 R P , ensachomycin, prenomycin, teichomycin, and pholipomycin [1] They are very efficient inhibitors of the bacterial cell wall peptidoglycan biosynthesis (*in vivo* activity mainly against *gram*-positive bacteria) Work by van Heijenoort and coworkers has demonstrated that moenomycin A interacts with the enzyme(s) that catalyses the formation of the linear peptidoglycan strands from a membrane disaccharide phospholipid intermediate [2] Structurally, the moenomycin-type antibiotics are rather complex Until now, only the structure of moenomycin A (**1a**) seems to be firmly established [3] Practically nothing is known on the biosynthesis of **1a** and related antibiotics We reasoned that from the structures of further members of the family at least some hints might be gained on how the rather complicated array of building blocks is assembled in the course of the biosynthesis Even in the days of highly developed tools, the structure elucidation of these compounds is by no means simple X-ray analysis cannot be used, since (at the best of our knowledge) none of the moenomycin antibiotics has ever been obtained crystalline More importantly, ^{1}H NMR spectroscopy is usually of litte help In most instances very broad peaks are observed, probably the result of the well-known association of these compounds in aqueous solution[1] and of other time-dependent phenomena.

In the present paper we describe the structure elucidation of the new flavomycin^R component moenomycin C_3 and propose a structure for moenomycin C_4 Included is some work on pholipomycin The structure of this antibiotic was previously elucidated, relying on the moenomycin A work [4] In view of the recent


[M-f+2H]+
[g+2H]+
1
I
5
6
7
8
R
2 β-OMe
3 α-OAc
4 β-OAc
1,5 X Y
a H
b H H
c OH H
d OH OH
9 X
a OH
b H

modification of the moenomycin A structure[3] (unit F, see formula **1a**, is derived from the amide of moenuronic acid rather than from the free acid, as originally assumed) it seemed probable that the structure of pholipomycin also needed some modification

Isolation of Moenomycins C_3 and C_4

TLC plates of the trade product flavomycin^R (solvent system 1-propanol - 2N NH_3 70 30) show two spots for moenomycin A (**1a**) and the less polar moenomycin C (ratio 3:1).[5] Reversed-phase HPLC has now demonstrated moenomycin C to be a mixture of several components Careful chromatographic separation of this mixture has led to the isolation of two new antibiotics which were named moenomycin C_3 and C_4 (according to their retention times[6]).

Spectral Properties of Moenomycin C_3

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.[3] Application of these rules to moenomycin C_3 clearly indicated its structure to differ from that of moenomycin A by the absence of both the glucose unit D and the OH group at C-6 of the amino sugar moiety E, see formula **1b** The ^{13}C NMR spectrum of moenomycin C_3 was very informative showing 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 β = 56 2 and 57.2), and the $^{31}P,^{13}C$ coupling in the vicinity of the phosphate group. The assignments are collected in the Experimental

Chemical Degradation of Moenomycin C_3 (**1b**)

Treatment of moenomycin C_3 with trifluoroacetic acid (5h at 60°C), a method known to cleave the ß-glycosidic bonds of 2α-acetylamino-2-deoxy sugars rather specifically,[7] followed by (i) trapping the intermediate oxazolinium ion with methanol, (ii) acetylation, and (iii) chromatographic separations furnished **2** along with a 3 2 mixture (^1H NMR) of **3** and **4**, from which pure **3** was obtained by crystallization. The structure of these compounds was rigorously established by comparison with the products that were obtained when moenomycin A (**1a**) was submitted to the same conditions [8]

Hydrogenation of **1b** in 33 1 methanol-acetic acid (Pt catalyst)[9] provided decahydromoenomycin C_3 (**5b**) in 95% yield Cleavage of **5b** under rather vigorous conditions with trifluoroacetic acid (16.5 h at 60°C), followed by ester formation with 1 5% HCl in methanol, and separations provided the known compound **6**[10] in 47% overall yield This part of the work proves, that moenomycin C_3 contains units A-B-C and H-I

5b was then submitted to the stepwise degradation developed for **5a** [9] Thus, oxidation of **5b** with $K_3[Fe(CN)_6]$ in 0 37 mol/l K_2CO_3 solution removed unit A selectively to give **7** in 53% yield From **7** the galacturonic acid moiety B was degraded by (i) diol cleavage with $NaIO_4$ in 50 % acetic acid containing sodium acetate (5h at 40°C), (ii) reversed-phase chromatography, (iii) treatment with N,N-dimethylhydrazine in 2-propanol - 1 mol/l H_2SO_4 (3h at 85°C), and (iv) chromatographic separations to furnish **8** in 54% yield A second degradation cycle in which ammonia was used instead of N,N-dimethylhydrazine converted **8** into **9b** (33%)

Spectroscopic Properties of the Moenomycin C_3 Degradation Products

Positive ion FAB spectra of compounds **5b**, **7**, **8**, and **9b** have been obtained fully in accord with the proposed structures (see Experimental) The ^{13}C chemical shifts for compounds **7**, **8** and **9b** (see Table 1) were assigned making use of the DEPT sequence and by comparison with known moenomycin A degradation products. The presence of two 6-deoxy sugars in 7 carrying ether substituent at C-4 is indicated by the two low-field signals at δ = 86 1 and δ = 84 3 [11] In **8** one and in **9b** both these signals are absent. This result seems only to be consistent with a 1∞ 4 linkage between units C and E. For the first time under carefully selected experimental conditions (solvent $CDCl_3$-CD_3OD-D_2O 18:11 2 7) informative ^1H NMR spectra of moenomycin-type compounds have been obtained The results are collected in Table 2. Assignments are based on H/H COSY From the coupling between the ring protons of unit E (see Table 2) gluco configuration, $^4C_1$(D) conformation and a ß-glycosidic linkage to unit F may be deduced.[12] Similarly, J = 8 3 Hz for the coupling between 1-H and 2-H of the quinovosamine unit C in **8** is indicative of a ß-glycosidic bond. From $J_{2,3}$ (F) ≈ 10 5 Hz it may be concluded that the moenuronic acid moiety F also adopts the $^4C_1$(D) conformation

Table 1 ^{13}C NMR spectral data of compounds **7**, **8**, and **9b** (δ values)

7[a]	8[b]	9b[b]	assignment	unit
173 4	-	173 1	C-1	H
172 1	172 2	172 3	C-6	F
171 1	172 1	-	$NHCOCH_3$	C
170 5	-	-	C-6	B
170 0	172 0	172 3	$NHCOCH_3$	E
156 9	157 2	?	$OCONH_2$	F
103 8	-	-	C-1	B
102 1	101 7	102 0	C-1	E
101 5	101 7	-	C-1	C
93 8	94 6	94 6	C-1	F
86 1	85 8	75 1	C-4	E
84 3	74 9	-	C-4	C
80 8	77 6	77 8	C-2	H
76 0	76 7	75 7	C-2	F
74 6	73 4	-	C-3	C
74 3	-	-	C-3	B
74 3	74 1	74 3	C-3	F
72 9	72 4	72 5	C-4	F
72 5	71 7	71 5	C-5	E
72 1	72 0	72 0	C-5	F
71 7	71 6	73 3	C-3	E
70 8	-	-	C-5	B
70 2	-	-	C-2	B
69 8	-	-	C-4	B
68 9	69 7	-	C-5	C
67 5	69 3	69 3	C-1	I
66 3	66 0	66 0	C-3	H
55 8	55 3	-	C-2	C
55 2	54 8	55 5	C-2	E
?	?	36 0	C-2	I
24 5	22 0	22 0	$NHCOCH_3$	E
24 4	21 7	21 9	$NHCOCH_3$	C
?	12 2	16 7	C-6	E

[a] in DMSO-d_6 [b] in $CDCl_3$-CD_3OD-D_2O 18 11 2 7 + trifluoroacetatic acid (6 µl/mL)

Table 2. ^{1}H NMR spectral data of **8**, **9b**, and moenomycin A degradation product **9a** (δ and J values)[a]

8	9b	9a	assignment	unit
4 17			1-H	C
3 43			2-H	C
3 12			3-H	C
2 83			4-H	C
3 10			5-H	C
1 10			CH_3-6	C
1 72			$NHCOCH_3$	
$J_{1,2}$=8 3Hz $J_{2,3}$=10 5Hz $J_{3,4}$=8 9Hz $J_{4,5}$=8 9Hz				
4 17	4 17	4 20	1-H	E
3 39	3 36	3 25	2-H	E
3 28	3 16	3 12	3-H	E
2 86	2 82	2 98	4-H	E
3 14	3 06	2 95	5-H	E
-	-	3 37, 3 50	6-H, 6-H'	-
?	1 05	-	CH_3-6	E
1 72		1 67	$NHCOCH_3$	
$J_{1,2}$=8 3Hz $J_{3,4}/J_{4,5}$=8 0Hz/ 10 0Hz	$J_{1,2}$=8 3Hz $J_{2,3}$=9 0Hz $J_{3,4}$=9 0Hz $J_{4,5}$=9.0Hz	$J_{1,2}$= 8 6Hz $J_{2,3}$=10 0Hz $J_{3,4}$=10 0Hz $J_{4,5}$=10 0Hz $J_{5,6'}$= 5 8Hz $J_{5,6}$= 2 0Hz $J_{6,6'}$=13 0Hz		
5 49	5 47	5 63	1-H	F
3 31	3 33	3 33	2-H	F
4 75	4 75	4 69	3-H	F
4 08	4 10	4 00	5-H	F
?	0 96	?	CH_3-6	F
$J_{2,3}$=10 5Hz	$J_{2,3}$=10 8Hz	$J_{1,2}$= 3 5Hz $J_{1,P}$= 8 0Hz $J_{2,3}$=10 8Hz		
3 83	3 83	3 75	2-H	H
3 89-3 97	3 91,3 94	3 82,3 86 $J_{2,3}$=3 5Hz $J_{2,3'}$=8 0Hz	CH_2-3	H
3 16, 3 20	3 25, 3 39	3 17, 3 31	CH_2-1	I
1 03, 1 07	1 27, 1 39	1 10, 1 32	CH_2-2	I

[a] in $CDCl_3$-CD_3OD-D_2O 18 11 2 7

Moenomycin C_4

From the FAB MS (for details, see Experimental) it was obvious that moenomycin C_4 contained one more oxygen than moenomycin C_3 (**1b**) Fragmentation e (see formula 1) led to an ion that was 16 mass units larger than in the case of **1b** wheras fragmentation c was the same as found previously in moenomycin A (**1a**). Thus the extra oxygen (with respect to **1b**) is located in unit E. We propose, therefore, structure **1c** for moenomycin C_4. This structure is well in accord with the ^{13}C NMR spectrum (see Experimental), but it has not rigorously been proven

Pholipomycin

As was already mentioned above, in view of the recent structural revision of moenomycin A it seemed very probable that unit F of pholipomycin also contains the amide of moenuronic acid rather than the free uronic acid And indeed, a careful mass determination of the M+K ion of both pholipomycin and its decahydro derivative has unambigously shown that the molecular mass of pholipomycin is one mass unit less than the structure with moenuronic acid in its free acid form requires Thus, as in the case of all the other moenomycins, pholipomycin has a uronamide unit F and should be represented by formula **1d**

Biological activity of moenomycins C_3 and C_4

The minimum inhibitory concentrations (MIC) of moenomycins C_3 (**1b**) and C_4 against various microorganisms have been determined by a serial two-fold agar dilution method (Müller Hinton Agar) In addition, inhibition of the UDP-N-acetylmuramyl pentapeptide-dependent incorporation of [^{14}C]UDP-N-acetylglucosamine into cross-linked high-molecular weight peptidoglycan was studied with a slightly modified[13] version of the assay described by Izaki, Matsuhashi, and Strominger[14]

Table 3 Minimal inhibitory concentrations (in g/L) of moenomycins C_3 (**1b**), C_4 (**1c**), and A (**1a**, for comparison) against various test organisms

test organism	1b	1c	1a
Staph aureus SG 511	0 10	0 05	0 05
Strept pyogenes 77	< 0 01	< 0 01	< 0 01
Strept pyogenes 308	25	6 25	25
Pseud aerug 1771	50	25	100
Pseud aerug 1771M	1 56	1 56	3 13
E coli DC 2	25	25	50
Salm typhimurium	100	100	> 100
Klebs aerog 1082E	100	100	> 100
Ent cloacae 1321E	1 56	0 78	1 56

Table 4 Effect of moenomycins C_3 (**1b**), C_4 (**1c**), and A (**1a**, for comparison) on the *in-vitro* UDP-N-acetylmuramyl pentapeptide-dependent incorporation of [^{14}C]UDP-N-acetylglucosamine into cross-linked high-molecular weight peptidoglycan

concentration (mg/L)	% inhibition		
	1b	1c	1a
1	85	85	88
0 1	63	60	63
0 01	0	0	0

Finally, the inhibitory effect of **1b** and **1c** directly on the transglycosylation reaction was determined by the *in vitro* assay developed earlier in one of our laboratories[15] which uses a particulate fraction from E.coli and as substrate the lipid intermediate which is the immediate precursor of uncross-linked peptidoglycan. The results are summarized in Tables 3 - 5.

Table 5 Effect of moenomycins A (**1a**, for comparison), C_3 (**1b**) and C_4 (**1c**) on the *in-vitro* formation of peptidoglycan by transglycosylation[a]

final concentration (μg/mL)	% inhibition		
	1b	**1c**	**1a**
10	100	100	100
1	100	100	100
0 1	91	86	78

[a]Assays were carried out as described previously[15]

Discussion

Like moenomycin A (**1a**), the new moenomycins **1b** and **1c** are *in-vitro* highly active only against gram-positive bacteria (see Table 3). As far as their inhibitory effect on the transglycosylation reaction is concerned, **1a**, **1b**, and **1c** possess practically the same inhibitory efficiency (Table 5). This is well in agreement with the previously established structure-activity relations which state, that only units E-F-G-H-I are essential for full transglycosylase inhibiting activity From the results collected in Table 4 one may arrive at the same conclusion

The structures of the moenomycin antibiotics described above seem to shed some light on biosynthetic events Obviously, first the whole complicated structure is assembled and is subsequently modified. One is tempted to speculate that pholipomycin (**1d**) is the precursor of the other members of the group which could be formed from **1d** by glucosidation and deoxygenations, respectively

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) Solvent evaporations were performed in vacuo at 40°C using a rotatory evaporator, lyophilizations Leybold-Heraeus GT2. Solvents were purified by standard techniques The instrumentation used was ^{1}H 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): 40 0 cm x 4 5 cm glass tubes, 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)[16] 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 refers to ^{12}C, ^{1}H, ^{16}O, ^{14}N, ^{31}P (mono-isotopic masses), the second was calculated using the International Atomic Masses

Isolation of moenomycins C_3 and C_4

Silica gel chromatography (300g SiO_2, Grace 60-200 μm) of moenomycin complex (Flavomycin^R, 12 0 g) performed as described previously[17] provided moenomycin C complex (1 05 g) and pure moenomycin A (1 87 g) A moenomycin C mixture (1 35 g) 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 1 of the solvent mixture then 15 mL fractions were taken Fractions 52-68 contained moenomycin C_3 (130 mg) and fractions 69-80 (173 mg) moenomycins C_3 (55%) and C_4 (38%) The latter fraction was further purified under the same conditions and furnished pure moenomycin C_4 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

Moenomycin C_3 (1b)

UV [18] λmax (ε) in methanol 259 (21000), in methanol + HCl 243 (12000) - ^{13}C NMR ((100 6 MHz, D_2O-CD_3OD 1 1, DEPT) δ = 16 10 (CH_3), 16 29 (CH_3), 17 80 (CH_3), 17 84 (CH_3), 17 95 (CH_3), 23 15 (CH_3), 23.20 (CH_3), 23 33 (CH_3), 23 38 (CH_3) 23 97 (CH_3), 25 96 (CH_3), 27 59 (CH_2), 27 82 (CH_3, C-23^I, C-24^I), 31 47 (CH_2), 32 21 (CH_2), 32 60 (CH_2), 33 40 (CH_2), 35 89 (C-12^I), 36 39 (C-8^I), 40 80 (C-15^I), 42 77 (C-9^I), 56 24 (C-2^E), 57 19 (C-2^C), 67 04 (C-1^I), 68 63 (C-3^H), 70 66 (CH), 71 44 (CH), 72 28 (CH), 72 65 (CH), 73 48 (CH), 73 86 (CH), 74 22 (CH), 75 99 (CH), 76 52 (CH), 78 37 (C-2^F), 80 51 (C-2^H), 84 60 (C-4^C), 87 72 (C-4^E), 95 91 (C-1^F), 103 42 (C-1^C), 103 93 (C-1^E), 104 70 (C-1^B), 109 27 (C-22^I), 111 29 (C-2^A), 123 09 (C-13^I), 123 44 (C-2^I), 125 26 (C-17^I), 126 83 (C-6^I), 132 24 (C-18^I), 137 30 (C-14^I), 141 23 (C-3^I), 141 45 (C-7^I), 151 02 (C-11^I), 159 20 ($OCONH_2$), 159 23, 170 23 (C-6^B), 173 82 (C-6^F), 173 92, 174 46 ($NHCOCH_3$), 174 56 ($NHCOCH_3$), 177 05 (C-1^H), 199 54 (C-1^A, C-3^A) - $C_{63}H_{98}N_5O_{28}P$ (1403 6, 1404 5), FAB MS (matrix lactic acid) 1480 4 ([M+2K-H]$^+$, 1442 4 ([M+K]+), 916 2 ([f-H+K]+), 684 1 ([e-H+K]+)

Decahydromoenomycin C_3 (5b)

1b (51 2 mg, 36 μmol) and PtO_2 (12 8 mg) in 30 1 methanol-acetic acid (5 2 mL) were stirred under hydrogen for 3d at 20°C Progress of the reaction was monitored by HPLC (5 μm RP-18, methanol-water-acetonitril (6 3 1), and by TLC (chloroform-methanol-water 18 11 2 7) After filtration, solvent evoparation and lyophilization the reaction product war purified by reversed-phase LC (20 g HP 20, gradient water - methanol) to give pure **5a** (49 1 mg), 95 %) - δ = 56 38 / 57 41 (C-2^E and C-2^C), 68 52 (C-1^I), 70 14 (C-3^H), 70 70, 71 59, 72 41, 72 75, 73 58, 73 78, 73 98, 74 21, 74 47, 75 92, 76 77, 78 60, 84 74 (C-4^C), 87 80 (C-4^E), 96 12 (C-1^F), 103 55 / 104 11 / 104 80 (C-1^C, C-1^E, C-1^B), 111 62 (C-2^A), 159 16 ($OCONH_2$), 170 21 (C-6^B), 173 60, 174 20, 174 75 (2x$NHCOCH_3$, C-6^F, C-1^H), 198 92 C-1^A, C-3^A) - $C_{63}H_{108}N_5O_{28}P$ (1413 7, 1414 5), FAB MS (matrix methanol - nitrobenzylalcohol) 1458 7 ([M+2Na-H]+), 1436 6 ([M+Na]+), 1020 3 ([g+2Na]+), 900 3 (f+Na-H]+), 668 3 ([e+Na-H]+), 559 4 ([M-f+Na+H]+), 459 ([c]+)

Methyl (R)-2-(3,8,8,11,14,18-hexamethylnonadecyloxy-3-hydroxypropionate (6)

In a 1 mL sealed vessel a mixture of **5b** (49 1 mg, 34 7 μmol) and trifluoroacetic acid (TFA, 0 25 mL) was stirred at 60°C for 16 5 h After TFA removal (stream of argon) the dark brown residue was treated with 1 5 per cent HCl in methanol at 70°C for 4h After dilution with CH_2Cl_2, and solvent evaporation the residue was taken up in CH_2Cl_2 (6 mL) Washing with 5 per cent aq $NaHCO_3$ solution (2 x 1 mL), H_2O (2 x 1 mL), drying, and solvent evaporation, followed by LC (2 5 g SiO_2, $CHCl_3$-MeOH 100 1) of the residue (13 4 mg) yielded pure **6** (7 6mg, 46%), identical with a sample obtained from **1a** - ^1H NMR (80 MHz, $CDCl_3$) δ = 0 80-1 60 (signals of unit I), 3 80 (s, OCH_3), 3 82-4 10 (2-H^H, CH_2-3^H, CH_2-1^I) - $C_{29}H_{58}O_4$ (470 4, 470 8), EI-MS 470 (M+), 452 ([M-H_2O]+), 440 ([$C_{28}H_{56}O_3$]+), 411 ([M-$COOCH_3$]+), 349 ([$C_{25}H_{49}$]+), 273 ([M-$C_{14}H_{29}$]+), 239, 238, 169, 121 ([$C_4H_9O_4$]+)

Degradation of **1b** with trifluoroacetic acid[19]

a) A mixture of **1b** (300 1 mg, 0 21 mmol) and trifluoroacetic acid (1 5 mL) was heated to 60°C for 5h in a sealed vessel Most of the trifluoroacetic acid was then removed by blowing argon over the mixture The residue was dissolved in dry methanol and the solution was allowed to stand at 40°C for 17 h After solvent evaporation and LC (25 g SiO_2, chloroform-methanol-water 18 11 2 7) the fractions containing compounds with Rf values in the range of 0 1 (287 6 mg, TLC chloroform-methanol-water 18 11 2 7) were collected, evaporated to dryness, and the residue was dissolved in 1 1 pyridine-acetic anhydride (6 mL) and left at 20°C for 24 h Solvent evaporation, lyophilization, and MPLC (column B, chloroform-methanol-water 30 5 0 5) gave a fraction (21 2 mg) which according to the 400 MHz ^1H NMR spectrum was a mixture of **3** and **4**, and a fraction consisting of the more polar compound **2** (27 4 mg) Both fractions were rechromatographed under the same conditions (using column A) **3** and **4** which could not be separated chromatographically but yielded pure **3** (10 4 mg) on crystallization from hexanes-ethyl acetate

b) Moenomycin A (**1a**) was degraded under the same conditions, to give **2** and **3** which were identical with the samples obtained from **1b**

Methyl 4-O-[2,3,4,-tri-O-acetyl-ß-D-galactopyranosyluron-N-(1-hydroxy-3-oxo-1-cyclopenten-2-yl)amide]-(1-4)-2-acetamido-3-O-acetyl-2,6-dideoxy-ß-D-glucopyranoside (**2**)
M p. of the sample obtained from moenomycin C_3 (**1b**) 241-243°C (from hexanes-ethanol), m p of the sample obtained from **1a** 241-243°C (from hexanes-ethanol) - For ^{1}H NMR and mass spectra, see ref [8a, 8b]

O-2,3,4-Tri-O-acetyl-ß-D-galactopyranosyluron-N-(1-hydroxy-3-oxo-1-cyclopenten-2-yl)amide -(1-4)-2-acetamido-1,3-di-O-acetyl-2,6-dideoxy-α-D-glucopyranose (**3**).
M p of the sample obtained from moenomycin C_3 265-267°C (from hexanes-ethanol), m p of the sample obtained from **1a** 265-266°C (from hexanes-ethanol) - For ^{1}H NMR and mass spectra, see ref [8a, 8b]

2-0-{2-Acetylamino-4-O-[2-acetylamino-4-O-((5S)-5-carbamoyl-ß-L-arabinopyranosyl)-2,6-dideoxy-ß-D-glucopyranosyl]-2,6-dideoxy-ß-D-glucopyranosyl}-3-O-carbamoyl-1-O-{[(S)-2-carboxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxyl-hydroxyphosphoryl}-4-C-methyl-α-D-glucopyranuronamide (**7**).
5b (362 3 mg, 0 26 mmol) was degraded with $K_3[Fe(CN)_6]$ (841 2 mg, 2 49 mmol) essentially as described in ref [9] for **5a** MPLC (2 x column B, chloroform-methanol-water 18 11:2 7) gave pure **7** (179 0 mg, 53 %) - ^{13}C NMR (62 9 MHz, DMSO-d_6, DEPT) δ = 24 4 ($NHCOCH_3$), 24 5 ($NHCOCH_3$), 55 2 (C-2^E), 55 8 (C-2^C), 66 3 (C-3^H), 67 5 (C-1^I), 68 9 (C-5^C), 69 8 (C-4^B), 70 2 (C-2^B), 70 8 (C-5^B), 71 7 (C-3^E), 72 1 (C-5^F), 72 5 (C-5^E), 72 9 (C-4^F), 74 3 (C-3^B, C-3^F), 74 6 (C-3^C), 76 0 (C-2^F), 80 8 (C-2^H), 84 3 (C-4^C), 86 1 (C-4^E), 93 8 (C-1^F), 101 5 (C-1^C), 102 1 (C-1^E), 103 8 (C-1^B), 156 9 ($OCONH_2$), 170 0 ($NHCOCH_3$), 170 5 (C-6^B), 171 1 ($NHCOCH_3$), 172 1 (C-6^F), 173 4 (C-1^H) - $C_{58}H_{104}N_5O_{26}P$ (1317 7, 1318 5), FAB MS (glycerol) 1385 ([M+3Na-2H]+), 1379 ([M+Na+K-H]+), 1363 ([M+2Na-H]+), 1341 ([M+Na]+), 1319 ([M+H]+), 804 ([f+Na-H]+), 597 ([M-f+Na+K]+), 581 ([M-f+2Na]+), 572 ([e+Na-H]+), 559 ([M-f+Na+H]+)

2-0-{2-Acetylamino-4-O-[2-acetylamino-2,6-dideoxy-ß-D-glucopyranosyl]-2,6-dideoxy-ß-D-glucopyranosyl}-3-O-carbamoyl-1-O-{[(S)-2-carboxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxyl-hydroxyphosphoryl}-4-C-methyl-α-D-glucopyranuronamide (**8**).
7 (189 8 mg, 0 14 mmol) was degraded with (i) $NaIO_4$ and then with dimethylhydrazine exactly as described in ref [9] Two chromatographic separations· a) LC (8 g SiO_2, chloroform-methanol-water 10 6 1), b) MPLC (column B, the same solvent system) gave pure **8** (89 7 mg, 54 %) - $C_{52}H_{95}N_4O_{21}P$ (1142 6, 1143 3), FAB MS (TEA) 1204 ([M+Na+K-H]+), 1188 ([M+2Na-H]+), 1182 ([M+K)+), 1166 ([M+Na]+), 1144 ([M+H]+), 629 ([f+Na-H]+), 597 ([M-f+Na+K]+), 581 [M-f+2Na]+), 559 ([M-f+Na+H]+), 397 ([e+Na-H]+), 375 ([e]+)

2-O-(2-Acetylamino-2,6-dideoxy-ß-D-glucopranosyl)-3-O-carbamoyl-1-O-{[(S)-2-carboxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxyl-hydroxyphosphoryl}-4-C-methyl-α-D-glucopyranuronamide (**9b**)
8 (89 0 mg, 77.9 μmol) was degraded with (i) $NaIO_4$, (ii) 25 per cent aqueous NH_3 exactly as described in ref [9] MPLC (column A, chloroform-methanol-water 18 11 2 7) provided pure **9a** (24 9 mg, 33 %).- $C_{44}H_{82}N_3O_{17}P$ (955 5, 956 1), FAB MS (matrix nitrobenzylalcohol) 1022 5 ([M+3Na-2H]+), 1000 6 ([M+2Na-H]+), 581 (M-f+2Na]+), 559 3 ([M-f+Na+H]+), 442 2 ([f+Na-H]+).

Moenomycin C_4 (**1c**)
UV λmax (ε) in methanol 259 (21000), in methanol + HCl 243 nm (12000) - ^{13}C NMR (100 6 MHz,CD_3OD at 50°C, DEPT) δ = 16 16 (CH_3), 16 39 (CH_3), 17 76 (CH_3), 18 15 (CH_3), 23 20 (CH_3), 23 34 (CH_3), 23 93 (CH_3), 25 84 (CH_3), 27 74 (CH_2), 27 89 (CH_3, C-23^I, C-24^I), 31 68 (CH_2), 32 37 (CH_2), 32 59 (CH_2), 33 53 (CH_2), 35 97 (C-12^I), 36 42 (C-8^I), 40 79 (C-15^I), 42 94 (C-9^I), 56 20 (C-2^E), 57 49 (C-2^C), 61 06 (C-6^C), 67 07 (C-1^I), 68 28 (C-3^H), 70 78 (CH), 71 86 (CH), 72 40 (CH), 72 78 (CH), 73 82 (CH), 74 08 (CH), 74 52 (CH), 76 14 (CH), 76 78 (CH), 78 11 (CH), 79 56 (C-2^F), 81 55 (C-2^H), 84.93 (C-4^C), 95 91 (C-1^F), 103.14 (C-1^C), 104 38 (C-1^E), 104 82 (C-1^B), 109 24 (C-22^I), 111 55 (C-2^A), 123 15 (C-13^I), 123 49 (C-2^I), 125.38 (C-17^I), 126 86 (C-6^I), 132.17 (C-18^I), 137 33 (C-14^I), 141 62 (C-3^I, C-7^I), 151 15 (C-11^I), 159 50 ($OCONH_2$), 169 85 (C-6^B), 173 80 (C-6^F), 174 34 (2x$NHCOCH_3$), 177 62 (C-1^H), 192 44 (C-1^A, C-3^A).- $C_{63}H_{98}N_5O_{29}P$ (1419 6, 1420 5), FAB MS (matrix· lactic acid) 1496 3 ([M+2K-H+), 1458.4 ([M+K]+), 932 1 ([f-H+K]+), 700 1 ([e-H+K]+), 603 1 ([M-f+2K]+), 565 2 ([M-f+H+K]+), 497 0 ([c-H+K]+), 459 1 ([c]+), 417 ([c-42]+).

Pholipomycin (1d)

1d was purified by preparative HPLC (10 µm RP 18, methanol-acetonitrile-0.02% phosphate buffer (pH 7 8) 10:2·5, followed by LC (HP 20 (17g), elution with water and methanol) The methanol fraction contained 1d.- ^{13}C NMR ((100 6 MHz, CD_3OD) δ = 16 12, 16 37, 17.79, 23.11, 23 30, 23 97, 25.95, 27.72, 27 86, 30.44, 30.68, 30 77, 31 70, 32 34, 32 70, 33.54, 35.94, 36.46, 40 89, 42 89, 56 24 / 57.22 (C-2^E and C-2^C), 61 21 / 61.51 (C-6^C and C-6^E), 67.11, 68.92, 70 80, 72.17, 73 59, 74.07, 74.20, 74 33, 74 40, 75.97, 76.03, 76 91, 76.96, 79.13, 79 56, 79 64, 80.92, 81 23, 96.03 (C-1^F), 103.32 / 104 15 / 104 49 (C-1^C, C-1^E, C-1^B), 109.24 (C-22^I), 111.18 (C-2^A), 123 50, 125 36, 126 94, 132 20, 137 33, 140 76, 141 48, 151 08, 159 43 ($OCONH_2$), 170 12, 173 80, 174 42, 174.51, 177 70, 200.00 (C-1^A, C-3^A).- $C_{63}H_{98}N_5O_{30}P$ (1435 6, 1436.5), FAB MS (narrow voltage scan, resolution 7300, internal reference· PEG-1540,, matrix. nitrobenzylalkohol) m/z (mean of 3 measurements, S ± 0.002) = 1474 57 ([M + K]+, calc 1474 57).-

Decahydropholipomycin (5d)

To 1d (13 0 mg) and PtO_2 (4 mg) were added methanol (1 5 mL) and acetic acid (49 µl), and the mixture was stirred under H_2 for 135 min. Filtration, solvent evaporation, and preparative HPLC (RP 18, methanol-water-acetonitril 6 2 1) furnished 5d (9 4 mg) - $C_{63}H_{108}N_5O_{30}P$ (1445 7, 1446 5), FAB MS (conditions as described for 1d)· 1484 65 ([M + K]+, calc 1484 65)

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