Tetrahedron Vol. 43, No. 3, pp. 585 to 598, 1987
Printed in Great Britain.

0040-4020/87 $3.00+.00
Pergamon Journals Ltd.

MOENOMYCIN A:
MINIMUM STRUCTURAL REQUIREMENTS FOR BIOLOGICAL ACTIVITY

Peter Welzel [a*], Franz Kunisch [a], Frithjof Kruggel [a], Hermann Stein [a], Jürgen Scherkenbeck [a], Aranka Hiltmann [a], Helmut Duddeck [a], Dietrich Müller [a], John E.Maggio [b], Hans-Wolfram Fehlhaber [c], Gerhard Seibert [c], Yveline van Heijenoort [d], and Jean van Heijenoort [d*]

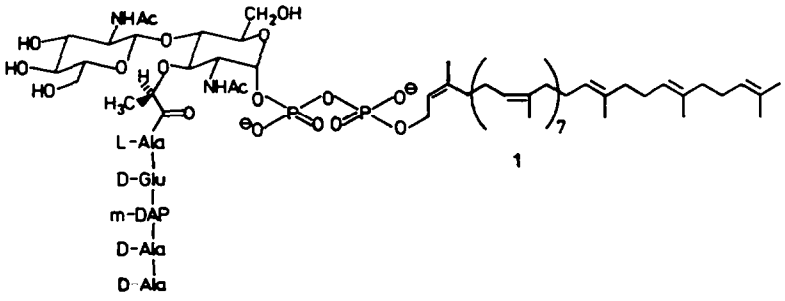
[a] Fakultät für Chemie der Ruhr-Universität, Postfach 102148, D-4630 Bochum (FRG)
[b] University Chemical Laboratory, Cambridge CB2 1EW (England)
[c] Hoechst AG, Postfach 800320, D-6230 Frankfurt 80 (FRG)
[d] Unite Associee du CNRS: Biochimie moleculaire et cellulaire, Bat. 432 Universite Paris-Sud, 91405 Orsay (France)

(*Received in Germany* 3 *November* 1986)

Abstract - A stepwise degradation of the oligosaccharide part of moenomycin A (2) was performed. The degradation products were assayed for antibiotic activity both *in vivo* and in an *E.coli* cell-free system. Units E, F, G, H, and I have been found to be essential for full biological (*in vitro*) activity. It is suggested that 2 is a competitive inhibitor of the peptidoglycan polymerase.

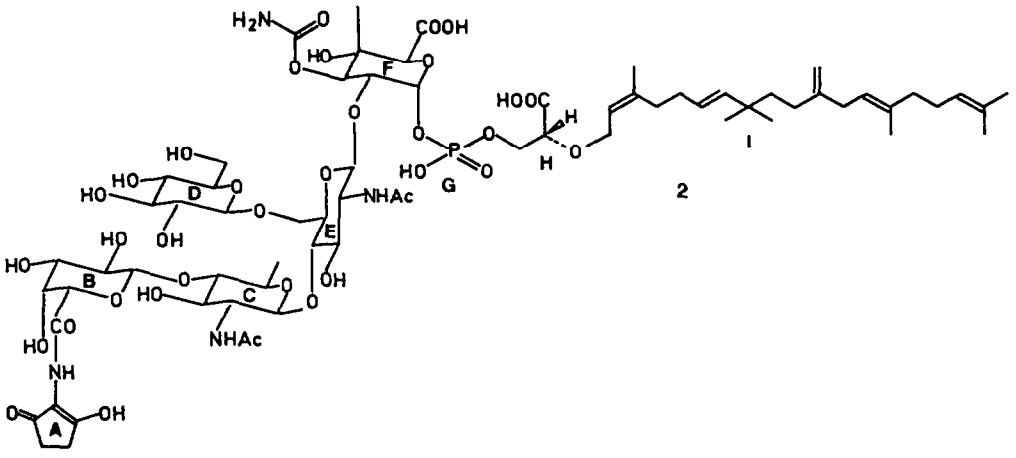
Introduction

Moenomycin and related antibiotics are known to inhibit the biosynthesis of peptidoglycan, the main structural component of the bacterial cell wall. [1] Peptidoglycan consists of glycan chains with peptide substituents that are crosslinked. The biosynthesis of bacterial cell wall peptidoglycan involves both cytoplasmic and membrane steps. An N-acetylglucosaminyl-N-acetylmuramyl-(pentapeptide)-pyrophosphoryl-undecaprenol of type 1 is the last membrane precursor prior to polymerization which proceeds by transglycosylation (formation of the linear glycan strands) and transpeptidation (crosslinking of the peptide subunits). [2] With cell-free systems from *Escherichia coli*, it was demonstrated that moenomycin inhibits the formation of the linear glycan strand of peptidoglycan from 1. [3] More specifically it has been established, that penicillin-binding protein 1b (PBP 1b) is the polymerase responsible for the transglycosylation reaction, and that moenomycin inhibits the polymerization reaction catalyzed by PBP 1b in a cell-free system and by purified PBP 1b. With both the cell-free system and purified PBP 1b, moenomycin has an inhibitory effect at concentrations between 10^{-8} and 10^{-7} M. [3-5] It was also found that the *in vitro* polymerization catalyzed by two other peptidoglycan polymerases of E.coli, PBP 1a and PBP 3, is also sensitive to moenomycin, or at least to a related antibiotic like macarbomycin. [6] Furthermore, it was observed that moenomycin could very efficiently promote the autolysis of *E.coli* cells. [7]



1

Moenomycin A is the main constituent of a complex of very similar compounds (moenomycin) which is used in animal nutrition (Flavomycin®). [1] On the basis of degradation and spectroscopic studies it was shown to have structure **2**. [8-10]



In the present publication we describe a systematic degradation of the oligosaccharide part of **2**. The degradation products were assayed for antibiotic activity both *in vivo* and in the *Escherichia coli* cell-free system. [3] This study was performed with the aim of defining the structural basis of the biological activity of **2**.

In designing a degradation scheme for **2** the following properties of **2** had to be taken into account:

a) **2** is soluble only in water and some very polar solvents such as methanol or DMSO. [11]

b) The allyl ether bond between units H and I is very acid-labile. The moenocinol part I can be cleaved off from the rest of the molecule and the remaining delipido moenomycin A was reported to be devoid of any antibiotic activity. [12]

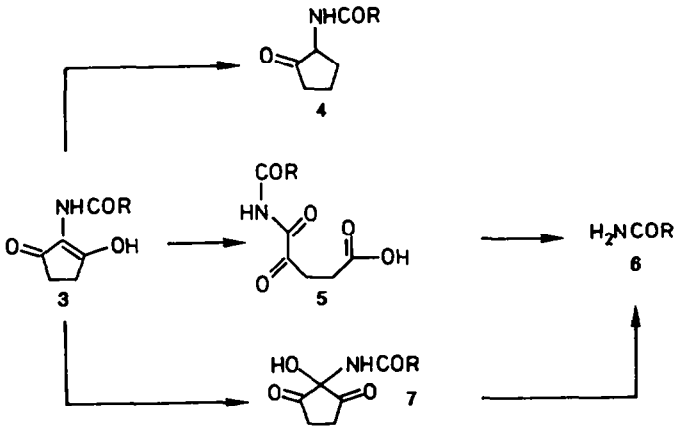
c) HCl-catalyzed hydrolysis degrades **2** completely into its components. [1] The uronic acid units B and F decompose under these conditions. [13,14,15]

d) The β-glycosidic linkages of the N-acetylated amino sugars can be cleaved rather specifically with trifluoroacetic acid. Degradation products containing units A-B-C, D-E, D-E-F-G-H, and F-G have been obtained using this method. [15,16]

e) Part I of **2** can be hydrogenated to furnish decahydromoenomycin A (**9**). [9]

f) Compounds of type **3** are not hydrogenated under neutral conditions but in acetic acid solution they furnish reduction products of type **4**. [13]

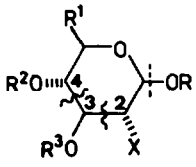
g) The enolic double bond in compounds of type **3** is cleaved by ozonolysis to give diacyl amines **5**, from which the α-keto acid unit can be removed selectively by solvolysis to furnish primary amides **6**. [13]



It was decided to base the stepwise degradation of **2** on the diol cleavage methodology. [17] Two different structural situations were encountered (see formula **8**):

a) Ring cleavage between C-2 and C-3. In this case the oxidation products are treated with N,N-dimethylhydrazine in acidic solution [18] to effect selective cleavage of the neighbouring acetal group (Barry degradation [19]),

b) Ring cleavage between C-3 and C-4. The acetal group of the cleaved ring is then degraded by β-elimination using NH_3 as base. [20]



8

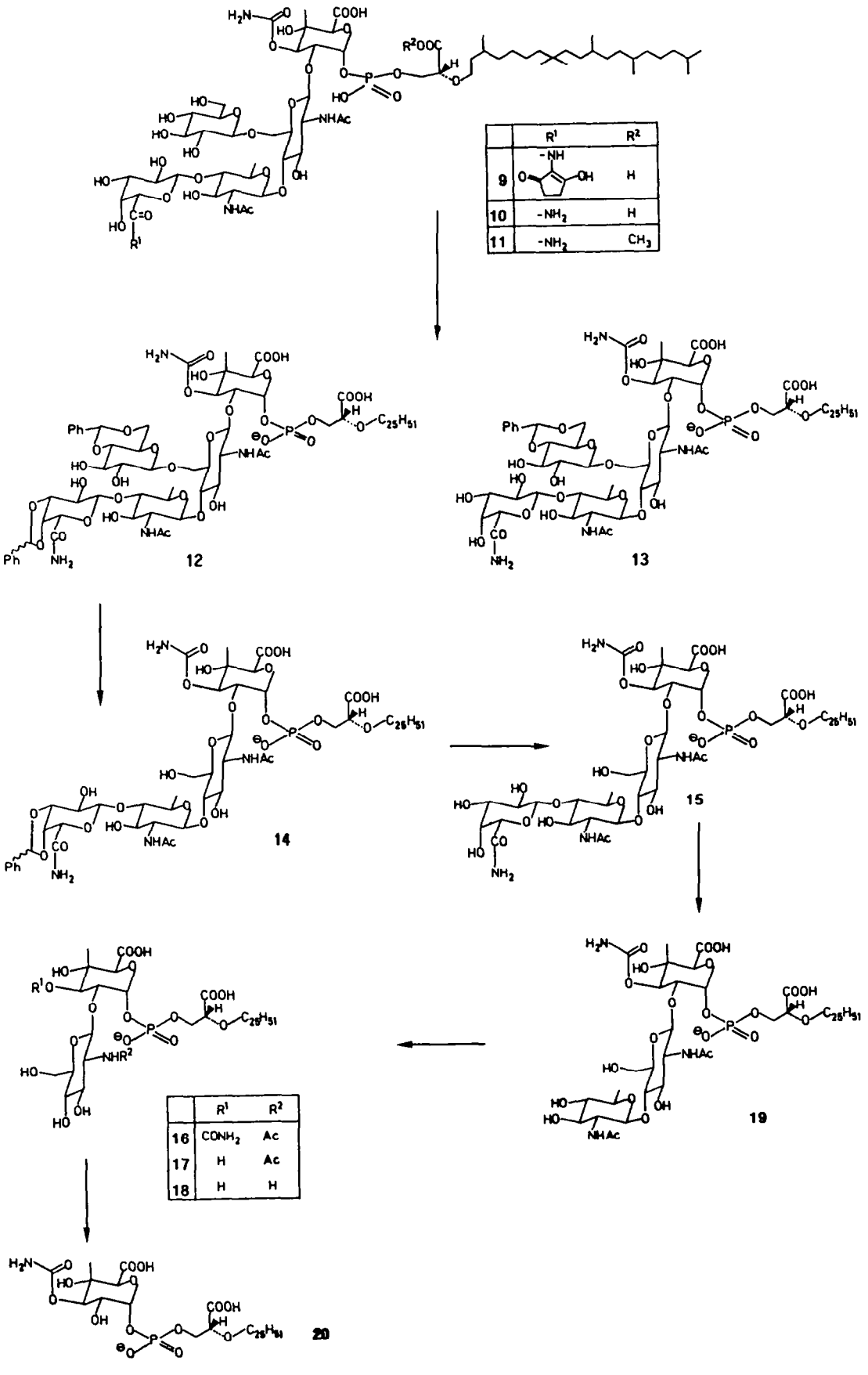
Stepwise degradation of moenomycin A [21]

In order to avoid complications by the acid-sensitive allyl ether bond between units G and F, **2** was hydrogenated. We have already described the hydrogenation of **2** in methanolic solution over Adam's catalyst to give the decahydro derivative **9**. [9] Later, we encountered occasionally difficulties with this procedure. This problem is, however, avoided, when pure **2** is used and when a small amount of acetic acid is added to the solvent. The reaction should be followed by HPLC and stopped after completion to avoid hydrogenation of unit A which occurs under acidic conditions (vide supra).

Next, unit A was removed from **9** to give **10**. This was previously accomplished [9] using ozonolysis as key reaction (see **5** → **6**). Compound **10** has now been obtained much more efficiently (88% yield) from **9** by oxidation with $K_3Fe(CN)_6$ in 0.37M K_2CO_3 solution and subsequent reversed-phase chromatography. We believe that an intermediate of type **7** is involved in this reaction.

For the first Barry degradation **10** was converted into **12** which has only one free diol grouping (in unit D). Formation of sugar benzylidene acetals is, in principle, a straightforward task. [22] For example, methyl α-D-glucopyranoside has been converted into its 4,6-benzylidene derivative with benzaldehyde-zinc chloride (80% [23]), or with $PhCHBr_2$ in pyridine (70% [24]), or with $PhCH(OCH_3)_2$ and a trace of p-toluenesulfonic acid in DMF (80% [25]). In the case of **9** all these methods gave poor results. The benzaldehyde-$ZnCl_2$ method worked only when DMSO was added as solvent since the solubility of **9** in benzaldehyde is very low. Even under carefully controlled conditions we were able to prepare **12** only in 14% yield along with **13** (15%). The $PhCHBr_2$-pyridine method provided **12** and **13** in yields of 7% and 22%, respectively. Reaction of **9** with $PhCH(OCH_3)_2$ / p-TsOH (for solubility reasons in DMSO) led to extensive decomposition. - The proposed structures for **12** and **13** rest on ^{13}C NMR (see Table 1) and FAB-MS results. The ^{13}C NMR spectrum of **12** shows two new acetal carbon signals, and very intense quasimolecular ions as well as a number of fragment ions containing units B and D are fully in accord with the presence of two benzylidene units. Fragment ion m/z 1248 ($DEFGHINa_2$+H) confirmed that the glucose unit D contained only one benzylidene grouping. The same signal appeared in the mass spectrum of **13**, pointing to the presence of the benzylidene group in unit D. This conclusion is supported by the fact that the C-6 signal of the glucose unit is shifted downfield (δ=68) from its normal position at δ=61 (c.f. **9** in Table 1) by the β-alkyl. Whether **12** is a single stereoisomer or a mixture of two diastereoisomers isomeric at the acetal carbon of the benzylidene group of unit B [24] is unclear.

Reaction of **12** with $NaIO_4$ in 50% acetic acid containing sodium acetate (5h at 40°),


R¹ R²
9 H
10 -NH₂ H
11 -NH₂ CH₃
12
13
14
15
16 CONH₂ Ac
17 H Ac
18 H H
19
20

followed by a) reversed-phase chromatography and lyophylization, b) reaction with a 100-fold excess of N,N-dimethylhydrazine in 2-propanol - 1M H_2SO_4 at pH 4 (2.5h at 20°C and 1h at 90°C), and d) lyophilization and chromatographic separation gave **14** in 37% overall yield.

Liberation of another diol grouping was achieved by removing the benzylidene group from **14** by hydrogenolysis (in 1:8 methanol-acetic acid, Pd/C catalyst, 47h) to give **15** quantitatively. Degradation under the conditions described above converted **15** into **19** (32%).

The trisaccharide **19** could be obtained from **10** directly by removing units B and D in one step. Thus, a) oxidation of **10** with an excess of $NaIO_4$ in 50% acetic acid containing sodium acetate (2h at 40°C), b) reversed-phase chromatography and lyophilization, c) treatment of the oxidation product with a 20-fold excess of N,N-dimethylhydrazine in 2-propanol - 1M H_2SO_4 at pH 4.5 (3h at 85°C), and d) chromatographic separation furnished **19** in 47% overall yield. **19** was submitted to another degradation cycle in which now ammonia was used instead of N,N-dimethylhydrazine. **16** was obtained in 44% yield. Under the same conditions, **16** was degraded to **20** in 46% yield.

Guided by the biochemical results which will be described below we also removed the urethane and the N-acetyl group from **16**. Treatment of **16** with 17% butylamine in methanol [26] (70h at reflux temp.) gave in a very clean reaction **17** which was isolated after chromatographic separation in 67% yield. Reaction with hydrazine hydrate (10h at 70°C) converted **16** into **18** which was isolated after rather difficult chromatographic separations in 43% yield.

^{13}C NMR spectra of compounds 10, 12, 13-20

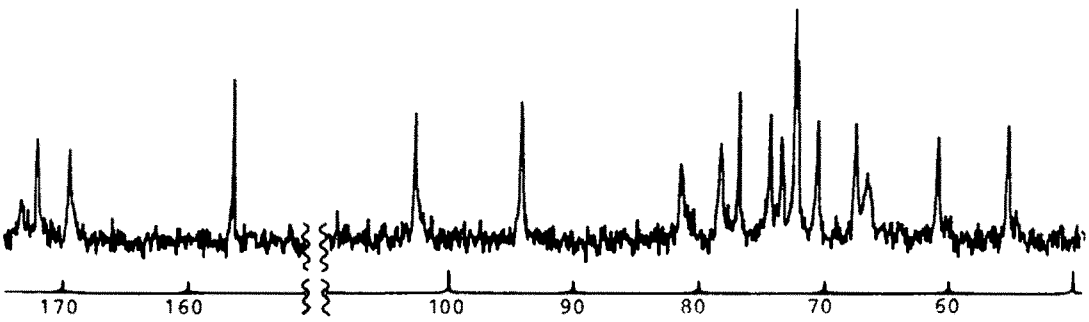


Fig. 1. The 62.9 MHz ^{13}C spectrum of **16** (in DMSO-d_6)

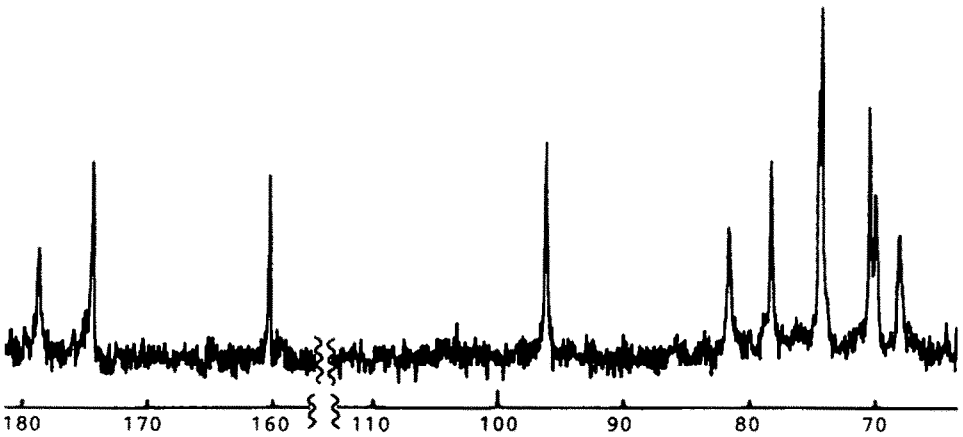


Fig. 2. The 62.9 MHz ^{13}C spectrum of **20** (in CH_2Cl_2-CD_3OD-H_2O=1:6:1)

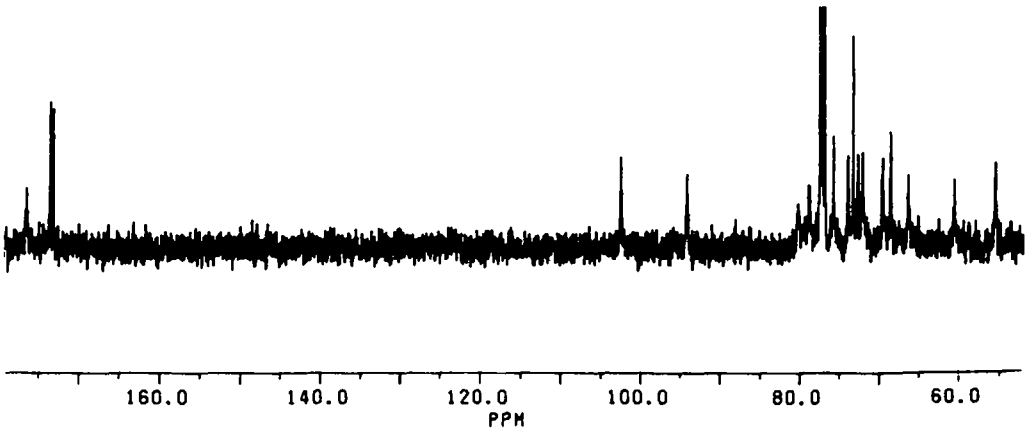


Fig. 3. The 100.6 MHz ^{13}C spectrum of **17** (in $CDCl_3$-CD_3OD-D_2O=18:11:2.7)

Table 1. ^{13}C-NMR spectral data of compounds **10, 12, 13 - 20** (δ-values)

10[c]	**12**[c]	**13**[c]	**14**[c]	**15**[c]	**19**[c]	**16**[c]	**20**[d]	**17**[e]	**18**[e]	Assignment	Unit
173.1	171.6	172.5	172.5	a	173.3	173.3	a	176.7	a	C-1	H
171.7	171.3	171.6	171.8	171.6	171.7	172.1	172.6	173.5	170.0	C-6	F
170.6	170.6	170.8	169.6	170.7	-	-	-	-	-	C-6	B
169.7	169.7	169.8	169.2	169.3	169.4	169.4	-	173.1	-	$NHCOCH_3$	C,E
		169.4									
156.5	156.7	157.0	156.6	156.7	156.6	156.3	158.0	-	-	$OCONH_2$	F
103.6	104.2	104.2	-	-	-	-	-	-	-	C-1	D
102.9	103.7	103.8	102.8	103.8	-	-	-	-	-	C-1	B
101.9	102.9	102.5	102.3	102.5	102.7	102.9	-	102.4	101.4	C-1	E
101.2	102.5	101.4	101.7	101.5	101.9	-	-	-	-	C-1	C
-	101.5	100.8	101.3	-	-	-	-	-	-	C-7[b]	B
-	101.0	-	-	-	-	-	-	-	-	C-7[b]	D
93.7	94.3	93.8	93.8	94.1	94.2	94.3	94.5	94.1	95.2	C-1	F
84.4	84.8	83.8	84.6	84.4	75.4	-	-	-	-	C-4	C
80.7	80.7	80.8	80.7	80.8	80.4	70.5	-	69.7	69.7	C-4	E
71.9	70.8	70	70.8	59.6	59.2	60.7	-	60.4	61.5	C-6	E
70.3	80.7	70	80.7	70.5	-	-	-	-	-	C-4	B
69.0	80.7	80.8	-	-	-	-	-	-	-	C-4	D
61.1	68.5	68.4	-	-	-	-	-	-	-	C-6	D
55.5	55.7	55.7	55.5	55.5	55.5	55.1	-	55.3	57.0	C-2	E
55.0	54.6	54.7	54.6	54.3	54.3	-	-	-	-	C-2	C
23.1	23.4	22.7	a	23.4	23.2	23.3	-	22.1	-	$NHCOCH_3$	C,E
		22.3									
17.3	17.3	17.3	17.4	17.4	18.0	-	-	-	-	C-6	C
16.2	16.2	16.2	16.3	16.4	16.4	16.9	16.7	a	a	CH_3-4	F

[a] Not observable. [b] Acetal carbon. [c] in DMSO-d_6. [d] in CH_2Cl_2-CD_3OD-H_2O=1:6:1
[e] in $CDCl_3$-CD_3OD-D_2O=18:11:2.7

The structural assignments of the moenomycin A degradation products described above rest mainly on the very informative ^{13}C NMR spectra. We have recently discussed the spectrum of **10** in some detail. For almost all units of **10** at least one signal could be assigned [28] with certainty, and the same is true for all compounds described in the present paper. The most characteristic signals are collected in Table 1. The ^{13}C NMR spectra of the final products of the degradation sequence are shown in Figures 1 to 3.

Mass spectra of compounds 9 - 20

Excellent positive ion FAB spectra of compounds **9** - **20** have been obtained with molecular ion clusters fully in accord with the proposed structures. In addition, we have previously shown for compounds **2**, **9**, **10**, and **11** that a number of typical fragments in the positive FAB mass spectra provide important structural informations about the various moieties of the molecules. The same type of fragments are found in the mass spectra of compounds **12** - **20**. Structural assignments to the mass peaks as given in the experimental part are tentative only since they have not been confirmed by appropriate methods but it should be stressed that these ions have been observed running the spectra in different matrices and on three different instruments. From the FAB mass spectra the position of the benzylidene groups in **12** and **13** has been determined as discussed above. - Another interesting point deserves being mentioned. Many of the compounds show abundant odd-electron molecular ions (see Experimental), for example **12**: m/z = 1672 ($M^{\cdot+}$), 1673 $(M+H)^+$; **13**: m/z = 1584 ($M^{\cdot+}$), 1585 $(M+H)^+$; **14**: m/z = 1444 $(M-H+Na)^{\cdot+}$; **15**: m/z = 1356 $(M-H+Na)^{\cdot+}$; **16**: m/z = 954 $(M-H+Na)^{\cdot+}$. Similarly, in the negative ion mode **9** showed m/z = 1590 $(M-2H)^{\cdot-}$ and **15**: m/z = 1332 $(M-2H)^{\cdot-}$, 1333 $(M-H)^-$. The reason for this unexpected behaviour is not yet understood.

Biological activity of compounds 2, 9, 10, 11, and 15 - 20

The inhibitory effect of **2** and the various degradation products on the transglycosylation reaction was determined using the convenient *in vitro* assay for the transglycosylation step which was recently developed in one of our laboratories. [3] The data in Table 2 show that the moenomycin derivatives **9**, **10**, **15**, **16**, and **19** are as efficient as moenomycin A (**2**) (and the trade product flavomycin®). This means that hydrogenation of the moenocinol part I of **2** and subsequent successive removal of units A, D, B, and C has no effect on the inhibitory efficiency. However, degradation of unit E from **16** (**16** → **20**) or methylation of the carboxyl group of the glyceric acid unit H in **10** (to give **11** [9]) leads to less efficient (10 and 100-fold, respectively) inhibitors. When the carbamoyl group is removed from **16** (**16** → **17**) a 100-fold decrease in inhibitory efficiency is observed. Further removal of the acetyl group leads to derivative (**18**) practically devoid of any inhibitory effect. The minimum inhibitory concentrations (MIC) of moenomycin A (**2**) and its degradation products against various microorganisms were determined by a serial two-fold agar dilution method. The results are presented in Table 3. For **2** the expected behaviour is observed: Strong activity against gram-positive bacteria and a weak one against *E.coli*. [1] The high MIC values against *E.coli* have been attributed to an inability of the antibiotic to penetrate to the site of peptidoglycan biosynthesis since the *E.coli* cell-free system is as sensitive to **2** as is for example *Staph. aureus in vivo*. [3] It should be pointed out that the values in Table 3 are based on one growth-medium (Müller Hinton Agar) only. Recently, it has been reported for *E.coli* that the MIC of moenomycin is highly dependent on the growth-medium. [29] Similar observations have been published for pholipomycin. [30]

Table 2. Effect of flavomycin®, moenomycin A (2), and degradation products 9 - 11 and 15 - 20 on the *in vitro* formation of peptidoglycan by transglycosylation [a]

Product	Final concentration	% inhibition
flavomycin®	10 µg/ml	100
	1 "	100
	0.1 "	23
(2)	10 µg/ml	100
	1 "	100
	0.1 "	54
(9)	10 µg/ml	100
	1 "	100
	0.1 "	0
(10)	10 µg/ml	100
	1 "	100
	0.1 "	8
(11)	100 µg/ml	100
	10 "	64
	1 "	0
(15)	10 µg/ml	100
	1 "	100
	0.1 "	75
(19)	10 µg/ml	100
	1 "	100
	0.1 "	30
(16)	10 µg/ml	100
	1 "	100
	0.1 "	45
(20)	100 µg/ml	100
	10 "	100
	1 "	53
(17)	100 µg/ml	100
	10 "	56
	1 "	0
(18)	100 µg/ml	16
	10 "	0
	1 "	0

[a] Assays were carried out as described previously with a particulate fraction from E.coli K 12. [3]

Table 3. Minimal inhibitory concentrations (in µg/ml) of moenomycin A (2) and degradation products 9, 10, 15 - 20 against various test organisms.

	Staph. aureus 503	Strept. pyogenes 308	Strept. pyogenes 77	Pseud. aeroginosa 1771m	E.coli DC2
(2)	0.19	3.13		25.00	25.00
(9)	< 0.19	6.25		25.00	50.00
(10)	1.56	6.25		> 100.00	> 100.00
(15)	3.13	12.50	0.78	> 50.00	> 50.00
(19)	3.13	12.50	0.39	25.00	50.00
(16)	12.50	12.50	1.56	12.50	50.00
(20)	> 50.00	6.25	3.13	> 50.00	> 50.00
(17)	> 50.00	> 50.00	12.50	> 50.00	> 50.00
(18)	> 50.00	> 50.00	> 50.00	> 50.00	> 50.00

In principle, the results obtained for Staph. aureus (see Table 3) parallel those for the E.coli cell-free system (vide supra): Compounds **2**, **9**, **10**, **15**, **16**, and **19** are active, whereas **17**, **18**, and **20** are not. **11** has already been reported to be without any antibiotic activity. [9] The increase in the MIC values against Staph. aureus in going from **2** to **16** may reflect increasing difficulties to pass the cell wall, by decreasing polarity of the compounds.

Conclusions

The results obtained in these studies have defined the structural features in the moenomycin-type antibiotics that are required for full biological (in vitro) activity:

(i) Units E, F, G, H, and I are essential,

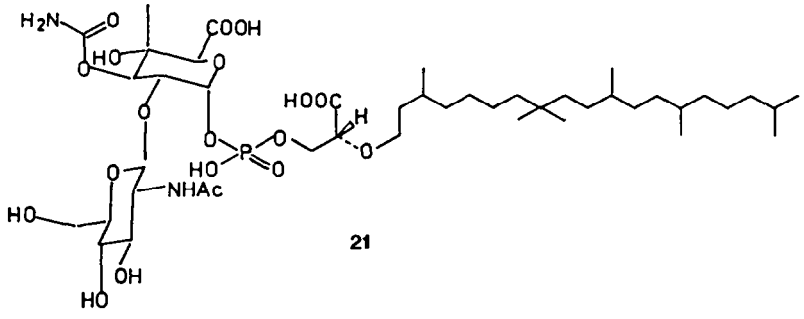
(ii) the moenocinol part I can be saturated,

(iii) the carboxyl group in unit H must be free, and

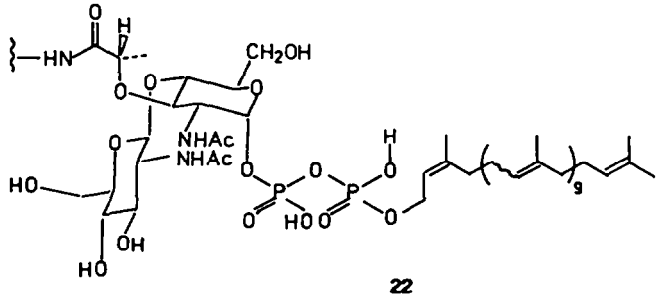
(iv) the carbamoyl group in the moenuronic acid moiety F must be present.

From these results we conclude that inhibition of the transglycosylation reaction by moenomycin A and the active degradation products is based on the structural analogy between these and the membrane substrate, e.g. **1**. [31] The structural analogy is especially apparent if **1** and **16** are compared in the conformations depicted in formulae **21** and **22**.

Recently, van Heijenoort et al. have demonstrated the absence of irreversible binding of **9** to E.coli membranes and membrane proteins. [29] The results taken together strongly suggest that moenomycin A is a competetive inhibitor of the peptidoglycan polymerase.



21



22

EXPERIMENTAL

General

The ^{13}C-NMR spectra were recorded on the Bruker WH 250 and AM 400 instruments. The FAB mass spectra were obtained using (i) a Finnigan MAT 731 instrument with a modified Saddle Field Ion Source, (ii) a Kratos MS-50 instrument with a Kratos FAB Source, (iii) an AEI MS 902 instrument with an M-Scan Atom Gun.

For crude reversed-phase separations polystyrene resin HP-20 (Mitsubishi) was used. Medium pressure liquid chromatography was performed using 25.0 cm x 1.0 cm glass tubes (column A), or 25.0 cm x 2.5 cm glass tubes (column B), or 40.0 cm x 4.5 cm glass tubes (column C), silica gel Grace (50 µm), the Duramat pump (Cfg), and (if possible) the UV detector Chromatochord III (Serva).

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% 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) at 0°C. 2N H_2SO_4 was added until a pH of 4.5 was reached (about 6.40 ml). Only freshly prepared solutions were used.

Decahydromoenomycin A (**9**).

Pure **2** (10.2 g, 6.45 mmol) and PtO_2 (3.0 g) were stirred in 20:1 methanol-acetic acid (630 ml) for 5 d at 20°C under hydrogen (atmospheric pressure). Progress of the reaction was followed by HPLC (5 µm RP-18, methanol-water-acetonitrile 6:3:1). Work-up and purification were performed as described previously [9] to give pure **9** (9.2 g, 89%).- $C_{69}H_{117}N_4O_{35}P$ (1592.72), FAB-MS [8] (matrix: glycerol): 1659 $(M-2H+3Na)^+$, 1637 $(M-H+2Na)^+$, 1615 $(M+Na)^+$, 1155 (DEFGHI+K), 1139 (DEFGHI+Na), 1078 (ABCDEF+Na), 862 (ABCDE+K), 846 (ABCDE+Na); after ion exchange (2x) for NH_4^+ (negative ion mode): 1590 $(M-2H)^-$, 1494 (M-A-H), 1319 (M-A-B-H), 1301, 1160, 1152 (ABCDEFG-H), 1114, 795, 767, 535 (GHI-H).

2-O-{ 2-Acetylamino-4-O-{ 2-acetylamino-4-O-((5S)-5-carbamoyl-β-L-arabinopyranosyl)-2,6-dideoxy-β-D-glucopyranosyl} -2-deoxy-6-O-β-D-glucopyranosyl-β-D-glucopyranosyl} -3-O-carbamoyl-1-O-{{ (S)-2-carboxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy -} hydroxyphosphoryl} -4-C-methyl-α-D-glucopyranuronic acid (**10**).

To a stirred solution of **9** (11.67 g, 7.4 mmol) in water (300 ml) were added at 0°C solutions of K_2CO_3 (16.72 g, 121 mmol) in water (30 ml) and of $K_3(Fe(CN_6))$ (26.60 g, 80.8 mmol) in water (30 ml). After 30 min, the reaction mixture was allowed to warm to 20°C and was stirred at this temp. for 1.5 h. Inorganic salts were removed by reversed-phase chromatography (20 g HP-20, elution with water (1.5 l) and then with methanol (2.2 l)). Evaporation of the methanol fraction followed by lyophilization gave pure **10** (9.7 g, 88%) identical with an authentic sample. [9] - $C_{64}H_{113}N_4O_{33}P$ (1496.70), FAB-MS (matrix: glycerol): 1573 $(M-H+2K)^+$, 1557 $(M-H+NaK)^+$, 1541 $(M-H+2Na)^+$, 1535 $(M+K)^+$, 1519 $(M+Na)^+$, 998 (BCDEF+K), 982 (BCDEF+Na), 873, 857, 835, 766 (BCDE+K), 750 (BCDE+Na), 613 (GHI-H+2K), 597 (GHI-H+Na+K), 575 (GHI+K), 559 (GHI+Na), 547.

Reaction of **10** with benzaldehyde/$ZnCl_2$.

To a solution of dry $ZnCl_2$ (1.34 g, 9.9 mmol) in benzaldehyde (3 ml, 28 mmol) was added at 90°C

under argon a solution of **10** (1.06 g, 0.72 mmol) in dry DMSO (3 ml). The mixture was then stirred under argon for 52h at 90-95°C. After cooling to 20°C the reaction mixture was directly separated by reversed-phase chromatography (70 g HP-20, elution with water (200 ml), methanol-water 1:1 (150 ml), 6:4 (200 ml), 7:3 (100 ml), and methanol (1000 ml). MPLC of the methanol fraction (after solvent evaporation and lyophilization, column C, chloroform-methanol-water 10:6:1) gave **12** (149.8 mg, 14%), pure **13** (165 mg, 15%), and further fractions containing **13** (582 mg).

Reaction of **10** with $C_6H_5CHBr_2$ - pyridine.

A solution of **10** (410 mg, 0.27 mmol) and $C_6H_5CHBr_2$ (205 mg, 0.82 mmol) in dry pyridine (3.5 ml) was stirred under argon at 110°C for 4h. Work-up procedure: a) Addition of water (35 ml) and acetic acid (3 ml) at 20°C, b) continous (10h) extraction with ether, c) concentration of the ether phase and lyophilization. The first fractions (500 ml) of a crude separation at 15 g SiO_2 (chloroform-methanol-water 18:11:2.7) were combined and (after solvent evaporation and lyophilization) separated by MPLC (2xcolumn B, chloroform-methanol-water 10:6:1) to give **12** (26 mg, 7%), **13** (85 mg, 22%), and fractions containing **10** and **13** (66%).

2-O-{2-Acetylamino-4-O-{2-acetylamino-4-O-((5S)-3,4-O-benzylidene-5-carbamoyl-β-L-arabinopyranosyl)-2,6-dideoxy-β-D-glucopyranosyl}-2-deoxy-6-O-(4,6-O-benzylidene-β-D-glucopyranosyl)-β-D-glucopyranosyl}-3-O-carbamoyl-1-O-{{(S)-2-carboxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy}-hydroxyphosphoryl}-4-C-methyl-α-D-glucopyranuronic acid (**12**).

$C_{78}H_{121}N_4O_{33}P$ (1672.77), FAB-MS (matrix: 3-amino-1,2-propanediol): 1738 $(M-3H+3Na)^+$, 1717 $(M-H+2Na)^+$, 1716 $(M-2H+2Na)^+$, 1695 $(M+Na)^+$, 1694 $(M-H+Na)^+$, 1673 $(M+H)^+$, 1672 (M^+), 1453 (CDEFGHI+2Na), 1431 (CDEFGHI+Na), 1294, 1278, 1248 (DEFGHI-H+2Na), 1226 (DEFGHI-H+Na), 1158 (BCDEF-H+Na), 942 (BCDE+K), 926 (BCDE+Na), 603 (GHI-2H+3Na), 581 (GHI-H+2Na), 559 (GHI+Na).

2-O-{2-Acetylamino-4-O-{2-acetylamino-4-O-((5S)-5-carbamoyl-β-L-arabinopyranosyl)-2,6-dideoxy-β-D-glucopyranosyl}-2-deoxy-6-O-(4,6-O-benzylidene-β-D-glucopyranosyl)-β-D-glucopyranosyl}-3-O-carbamoyl-1-O-{{(S)-2-carboxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy}-hydroxyphosphoryl}-4-C-methyl-α-D-glucopyranuronic acid (**13**).

$C_{71}H_{117}N_4O_{33}P$ (1584.73), FAB-MS (matrix: 1-thioglycerol-diglycerol): 1650 $(M-3H+3Na)^+$, 1644 $(M-2H+Na+K)^+$, 1628, 1607 $(M+Na)^+$, 1606 $(M-H+Na)^+$, 1585 $(M+H)^+$, 1584 (M^+), 1435, 1294, 1248 (DEFGHI-H+2Na), 1190 (BCDEFG+2Na), 1072, 1070 (BCDEF+Na), 1054, 838 (BCDE+Na), 603 (GHI-2H+3Na), 597 (GHI-H+Na+K), 581 (GHI-H+2Na), 559 (GHI+Na).

2-O-{2-Acetylamino-4-O-{2-acetylamino-4-O-((5S)-3,4-O-benzylidene-5-carbamoyl-β-L-arabinopyranosyl)-2,6-dideoxy-β-D-glucopyranosyl}-2-deoxy-β-D-glucopyranosyl}-3-O-carbamoyl-1-O-{{(S)-2-carboxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy}-hydroxyphosphoryl}-4-C-methyl-α-D-glucopyranuronic acid (**14**).

To **11** (200 mg, 0.12 mmol) was added the $NaIO_4$ solution (2.4 ml) and the mixture was stirred in the dark for 5 h at 40°C. Inorganic salts were removed by reversed-phase chromatography (20 g HP-20, elution with water (70 ml) and methanol (400 ml)). Upon evaporation of the methanol fraction and lyophilization 170 mg of reaction products were obtained. 95 mg of which were added to the dimetylhydrazine solution (240 µl), and the mixture was stirred at 85°C for 2.5 h. Addition of water (5 ml) at 20°C, lyophilization and MPLC (column B, chloroform-methanol-water 10:6:1) gave amorphous **14** (35 mg, 37% based on **11**).- $C_{65}H_{107}N_4O_{28}P$ (1422.68), FAB-MS (matrix: glycerol): 1482 $(M-2H+Na+K)^+$, 1460 $(M-H+K)^+$, 1444 $(M-H+Na)^+$, 1422 (M^+), 1225, 1181, 1163, 998, 559 (GHI+Na), 537 (GHI+H), 451.

2-O-{2-Acetylamino-4-O-{2-acetylamino-4-O-((5S)-5-carbamoyl-β-L-arabinopyranosyl)-2,6-dideoxy-β-D-glucopyranosyl}-2-deoxy-β-D-glucopyranosyl}-3-O-carbamoyl-1-O-{{(S)-2-carboxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy}-hydroxyphosphoryl}-4-C-methyl-α-D-glucopyranuronic acid (15).

14 (180 mg, 0.126 mmol) and 10% Pd/C (200 mg) were stirred in 1:8 methanol-acetic acid (4.5 ml) for 44 h at 20°C under hydrogen (atmospheric pressure). The mixture was then filtered and the catalyst washed several times with methanol. The combined filtrates were evaporated and lyophilized to give pure amorphous **15** (186.4 mg, 100%).- $C_{58}H_{103}N_4O_{28}P$ (1334.65), FAB-MS (matrix: 1-thioglycerol + NaCl): 1400 $(M-3H+3Na)^+$, 1394 $(M-2H+Na+K)^+$, 1378 $(M-2H+2Na)^+$; (Matrix: Glycerol): 1378 $(M-2H+2Na)^+$, 1356 $(M-H+Na)^+$, 1181, 1044, 994, 940, 820, 581 (GHI-H+2Na), 559 (GHI+Na); in the negative ion mode: 1439, 1412, 1370 $(M-3H+K)^-$, 1354 $(M-3H+Na)^-$, 1333 $(M-H)^-$, 1332 $(M-2H)^-$, 1311, 1179, 949, 894, 789, 557, 313, 239.

2-O-{2-Acetylamino-4-O-{2-acetylamino-2,6-dideoxy-β-D-glucopyranosyl}-2-deoxy-β-D-glucopyranosyl}-3-O-carbamoyl-1-O-{{(S)-2-carboxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy}-hydroxyphosphoryl}-4-C-methyl-α-D-glucopyranuronicacid (19).

a) **15** (165 mg, 0,12 mmol) was degraded by a) reaction with $NaIO_4$, b) reaction with dimethylhydrazine, as described for the degradation of **11**, to give **19** (66 mg, 32%).
b) To **18** (1.0 g, 0.7 mmol) was added the $NaIO_4$ solution (12 ml) and the mixture was stirred in the dark for 2 h at 40°C. Without further work-up the mixture was separated by reversed-phase chromatography (40 g HP-20, 600 ml water, then 600 ml methanol). Evaporation of the methanol fraction followed by lyophilization gave 830 mg oxidation products. 687 mg of this mixture were added in portions to the N,N-dimethylhydrazine solution (3 ml) and the mixture was stirred at 85°C for 3 h. After cooling to 20°C the mixture was separated by reversed-phase chromatography (40 g HP-20, 600 ml water, then 600 ml methanol). MPLC (2x) of the methanol fraction (after solvent evaporation and lyophilization) at SiO_2 (column B, chloroform-methanol-water 10:6:1) gave pure amorphous **19** (311 mg, 47%).- $C_{52}H_{94}N_3O_{23}P$ (1159.60), FAB-MS (matrix: glycerol): 1219 $(M-2H+Na+K)^+$, 1203 $(M-2H+2Na)^+$, 1197 $(M-H+K)^+$, 1181 $(M-H+Na)^+$, 1010, 994, 559 (GHI+Na).

2-O-(2-Acetylamino-2-deoxy-β-D-glucopyranosyl)-3-O-carbamoyl-1-O-{{(S)-2-carboxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy}-hydroxyphosphoryl}-4-C-methyl-α-D-glucopyranuronic acid (16).

To **19** (6.44 g, 5.56 mmol) was added the $NaIO_4$ solution (55.2 ml) and the mixture was stirred in the dark at 20°C for 4 h. Excess $NaIO_4$ was destroyed with ethylene glycol (1.4 ml, 1h at 20°C). 25% NH_3 solution (166 ml) was added at 0°C and the mixture was then stirred at 20°C for 16 h. Concentration of the solution at a rotatory evaporator followed by adjustment to pH 5.5 with 50 per cent acetic acid and chromatographic separation (a: 250 g HP-20, gradient water (2.5 l) methanol (3.2 l); b: MPLC, column C, chloroform-methanol-water 18:11:2.7) gave **16** (2.37 g, 44%).- $C_{44}H_{81}N_2O_{19}P$ (972.50), FAB-MS (matrix: glycerol + NaCl): 1038 $(M-3H+3Na)^+$, 1016 $(M-2H+2Na)^+$, 994 $(M-H+Na)^+$.

3-O-Carbamoyl-1-O-{{(S)-2-carboxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy}-hydroxyphosphoryl}-4-C-methyl-α-D-glucopyranuronic acid (20).

16 (212 mg, 0.21 mmol) was degraded exactly as described for the conversion of **19** to **16**. Work-up as described, reversed phase separation (15 g HP-20, water (200 ml), then methanol (800 ml)), MPLC of the methanol fraction (after solvent evaporation and lyophilization) at SiO_2 (column B, chloroform-methanol-water 17.5:7.5:1) gave pure amorphous **20** (75 mg, 46%).- $C_{36}H_{68}NO_{14}P$ (769.44), FAB-MS (matrix: thioglycerol): 835 $(M-3H+3Na)^+$, 813 $(M-2H+2Na)^+$, 791 $(M-H+Na)^+$.

2-O-(2-Acetylamino-2-deoxy-β-D-glucopyranosyl)-1-O-{{(S)-2-carboxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy}-hydroxyphosphoryl}-4-C-methyl-α-D-glucopyranuronic acid (17).

A solution of **16** (100.0 mg, 103 µmol) in 17% methanolic butylamine (20 ml) was stirred in a sealed tube at 70°C for 30 h. Butylamine (3.32 ml) was added and stirring at 70°C was continued for another 40 h. Working-up procedure: a) solvent evaporation, b) addition of ethanol (2x) and evaporation to dryness, c) dissolving the residue in water and lyophilization. Repeated (2x) MPLC (column B, chloroform-methanol-water 10:6:1) gave pure **17** (64.3 mg, 67%).- $C_{43}H_{80}NO_{18}P$ (929.51), FAB-MS (matrix: triethanolamine): 999, 967 $(M-H+K)^+$, 952 $(M+Na)^+$, 667, 649, 611, 559 (GHI+Na), 537 (GHI+H).

2-O-(2-Amino-2-deoxy-β-D-glucopyranosyl)-1-O-{{(S)-2-carboxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy}-hydroxyphosphoryl}-4-C-methyl-α-D-glucopyranuronic acid (18).

A mixture of **16** (76 mg, 78 µmol) and hydrazine hydrate (38 µl, 8.2 mmol) were heated in a sealed reaction flask for 7 h at 70°C. Excess hydrazine hydrate was removed by repeated (2x) water addition and lyophilization. Repeated MPLC (column A, ethyl acetate-methanol-water-acetic acid 14:11:2.7:0.04 and chloroform-methanol-water 9:9:1) provided pure **18** (15.2 mg, 43%).- $C_{41}H_{78}NO_{17}P$ (887.50), FAB-MS (matrix: triethanolamine): 986 $(M-2H+2K+1Na)^+$, 910 $(M+Na)^+$, 746 (FGHI-H+Na), 724 (FGHI), 613, 603 (GHI-2H+3Na), 597 (GHI-H+Na+K), 581 (GHI-H+2Na), 559 (GHI+Na).

Acknowledgments

The Bochum group thanks the Hoechst AG and the Fonds der Chemischen Industrie for generous financial support. The work from the Orsay group was supported by grants from the Centre National de la Recherche Scientifique.

References and Notes

1) Review: G.Huber, in "Antibiotics", ed. F.E.Hahn, Springer, Berlin, 1979, vol. V/1, p. 135.
2) Review: J.B.Ward, Pharmac.Ther. **25**, 327 (1984).
3) Y.van Heijenoort, M.Derrien, and J.van Heijenoort, FEBS Lett. **89**, 141 (1978); Y.van Heijenoort and J.van Heijenoort, Ibid. **110**, 241 (1980).
4) H.Suzuki, Y.van Heijenoort, T.Tamura, J.Mizoguchi, Y.Hirota, and J.van Heijenoort, FEBS Lett. **110**, 245 (1980).
5) J.Nakagawa, S.Tamaki, S.Tomioka, and M.Matsuhashi, J.Biol.Chem. **259**, 13937 (1984).
6) F.Ishino and M.Matsuhashi, Biochem.Biophys.Res.Commun. **101**, 905 (1981); M.Matsuhashi, F.Ishino, J.Nakagawa, K.Mitsui, S.Nakajima-Iijima, and S.Tamaki, in "β-Lactam Antibiotics", ed. M.Salton and G.D.Shockman, Academic Press, New York, 1981, p. 169.
7) M.Leduc, R.Kasra, and J.van Heijenoort, J.Bacteriol. **152**, 26 (1982); M.Leduc, C.Frehel, and J.van Heijenoort, Ibid. **161**, 627 (1985).
8) P.Welzel, F.-J.Witteler, D.Müller, and W.Riemer, Angew.Chem. **93**, 130 (1981); Angew.Chem.Int.Ed.Engl. **20**, 121 (1981).
9) P.Welzel, B.Wietfeld, F.Kunisch, Th.Schubert, K.Hobert, H.Duddeck, D.Müller, G.Huber, J.E.Maggio, and D.H.Williams, Tetrahedron **39**, 1583 (1983).
10) For the structure of the related antibiotic pholipomycine, see Sh.Takahashi, K.Serita, M.Arai, H.Seto, K.Furihata, and N.Otake, Tetrahedron Lett. **24**, 499 (1983).
11) U.Schacht and G.Huber, J.Antibiot. **22**, 597 (1969).
12) D.Lenoir, R.Tschesche, W.Wucherpfennig, G.Huber, and H.L.Weidenmüller, Antimicrob. Agents Chemother. 144 (1969).
13) P.Welzel, F.-J.Witteler, L.Hermsdorf, R.Tschesche, H.Buhlke, P.Michalke, J.Simons, H.-W.Fehlhaber, J.Blumbach, and G.Huber, Tetrahedron **37**, 105 (1981).

14) N.Langenfeld and P.Welzel, Tetrahedron Lett. 1833 (1978).
15) P.Welzel, G.Knupp, F.-J. Witteler, Th.Schubert, H.Duddeck, D.Müller, and G.Höfle, Tetrahedron **37**, 97 (1981).
16) F.-J.Witteler, P.Welzel, H.Duddeck, G.Höfle, W.Riemer, and H.Budzikiewicz, Tetrahedron Lett. 3493 (1979).
17) B.Lindberg, J.Lönngren, and S.Svensson, Adv.Carbohydr.Chem.Biochem. **31**, 184 (1975).
18) A.von Wartburg, M.Kuhn, and K.Huber, Helv.Chim.Acta **51**, 1317 (1968).
19) P.S.O'Colla, Methods Carbohydr.Chem., Academic Press, New York, London, 1965, vol. V, p. 382.
20) H.Paulsen, R.Jansen, and P.Stadler, Chem.Ber. **114**, 837 (1981).
21) Preliminary Communication: P.Welzel, F.Kunisch, F.Kruggel, H.Stein, A.Ponty, and H.Duddeck, Carbohydr.Res. **126**, C1 (1984).
22) A.N. de Belder, Adv.Carbohydr.Chem.Biochem. **34**, 179 (1977).
23) D.M.Hall, Carbohydr.Res. **86**, 158 (1980), and references therein.
24) P.J.Garegg and C.-G.Swahn, Acta Chem.Scand. **26**, 3895 (1972); P.J.Garegg and C.-G.Swahn, Methods Carbohydr.Chem., Academic Press, New York, London, 1980, vol. VIII, p. 317.
25) M.E.Evans, Methods Carbohydr. Chem., Academic Press, New York, London, 1980, vol. VIII, p. 313.
26) P.C.Durette, E.P.Meitzner, and T.Y.Shen, Tetrahedron Lett. 4013 (1979), see also T.Ogawa, H.Yamamoto, T.Kitajima, and T.Nukada, Carbohydr.Res. **123**, C8 (1983).
27) B.A.Dmitriev, Yu.A.Knirel, and N.K.Kochetkov, Carbohydr.Res. **29**, 451 (1973).
28) For leading references to methods for the assignment of ^{13}C NMR spectra of carbohydrates, see R.E.Hoffman, J.C.Christofides, D.B.Davies, and Ch.J.Lawson, Carbohydr.Res. **153**, 1 (1986).
29) Y.van Heijenoort, M.Leduc, H.Singer, and J.van Heijenoort, J.Gen.Microbiol., in press.
30) A.Torikata, H.Yoshikawa, T.Katayama, M.Arai, M.Nakahara, and N.Kitano, J.Antibiotics 30, 1060 (1977).
31) For early speculations on this point, see E.J.J.Lugtenberg, A.van Schijndel -van Dam, and T.H.M.van Bellegem, J.Bacteriol. **108**, 20 (1971); P.E.Linnett and J.L.Strominger, Antimicrob.Ag.Chemother. 4, 231 (1973).