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The First Enzymatic Degradation Products of the Antibiotic Moenomycin A.

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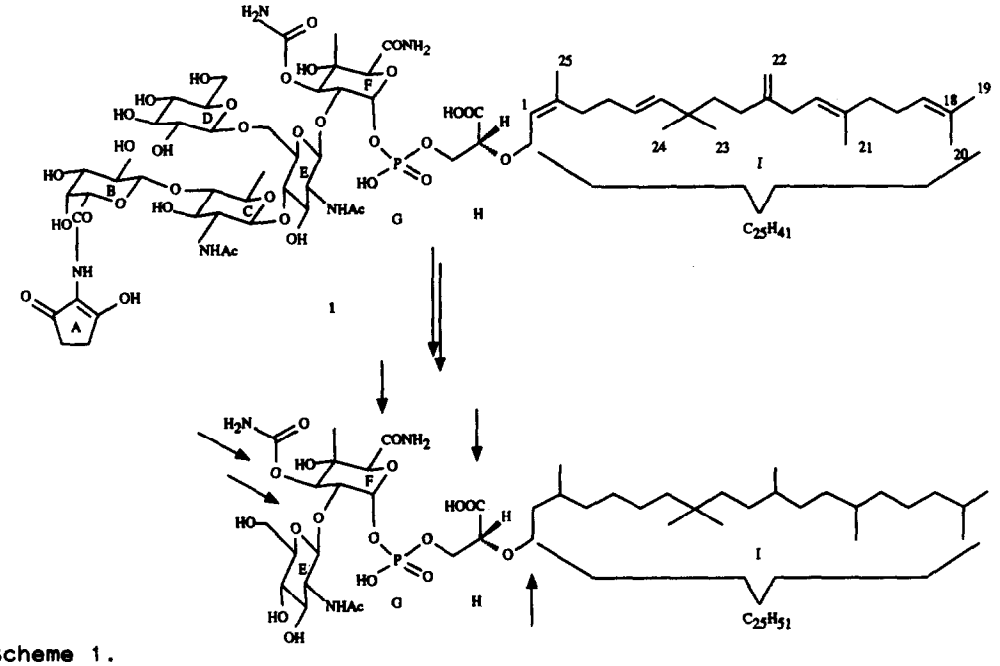
Abstract - Moenomycin A was degraded by enzymatic cleavage of the bond between the moenuronic acid moiety and the phosphate group. The phosphoric acid monoester **4a** could be dephosphorylated further to yield **3a**. Compound **3a** was used to confirm the previous configurational assignment at C-2 of the glycerate part of moenomycin A and to prepare *ent*-**4a**. **4a** and *ent*-**4a** are antibiotically inactive.

Introduction

The two successive final reactions in the biosynthesis of cross-linked peptidoglycan (a major constituent of the bacterial cell-wall) from the membrane precursor N-acetylglucosaminyl-N-acetylmuramyl-(pentapeptide)-pyrophosphoryl-undecaprenol are transglycosylation* which extends the glycan chain and transpeptidation which cross-links the glycan chains through two peptide units. A number of bifunctional enzymes (penicillin-binding proteins, PBP's) have been identified that catalyze both transglycosylation and transpeptidation. With cell-free systems from *E.coli* it was demonstrated that moenomycin A (**1**)[1] selectively inhibits the transglycosylation step through its action on the penicillin-binding protein 1b (PBP 1b)[2] and thus represents one of the rare examples of a transglycosylation inhibitor. A complicated stepwise degradation sequence[3] has demonstrated compound **2** to be the smallest moenomycin degradation product

with full (in-vitro) antibiotic activity (the arrows in **2**, Scheme 1, indicate cleavage reactions that lead to less active or inactive derivatives). Further progress in the context of structure - activity relations is slow because the chemical synthesis of structural analogues is complicated and



Scheme 1.

time-consuming.[4] Enzymatic degradation of moenomycin A which might constitute a useful alternative has hitherto not been reported. Here, we wish to describe as the first example of an enzymatic cleavage of moenomycin A formation of compound **4a**. This degradation reaction is expected to be very useful, aiding the structural elucidation of new moenomycin antibiotics, as well as making available important building blocks for partial syntheses. Both these aspects are substantiated below by an example.

Enzymatic degradation products of moenomycin A

From a contaminated culture broth of a Flavomycin^R production fermentation a Bacillus strain (*Bacillus* spec. DSM 4675) was isolated that was found to cleave moenomycin A into three degradation products, which were named M_A, M_B, and M_C. Initially, only M_B and M_C are formed. Then M_B is degraded further to give M_A. The first step, cleavage of the phosphoglycosidic linkage, is catalyzed by a cell wall associated enzyme which is named *moeno-*

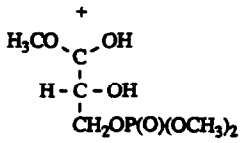
mycinase. This enzyme requires cobalt ions for its maximum activity. The dephosphorylation of M_B is catalyzed by a soluble phosphatase called *MBase*. This Bacillus enzyme can be replaced by commercially available phosphatases from sweet potato or bovine intestinal mucosa.[5]

M_A has structure **3a** (Scheme 2) which is closely related to moenocinol (the alcohol corresponding to unit I of **1**, see Scheme 1). A reference sample of moenocinol was prepared by acid degradation of moenomycin. Besides the products already reported by Tschesche et al.[6] we isolated an isomer of moenocinol which has probably the (2E) configuration. Some spectral data of this compound are provided in the Experimental. Also reported are the results of a systematic assignment of the ^1H and ^{13}C NMR spectra of moenocinol (see Table 3, Experimental). Comparison of the ^{13}C chemical shifts of moenocinol with the carbon resonances of **3a** (see Table 1) clearly indicates the presence of the moenocinol unit in **3a**. The remaining signals are due to the glyceric acid part. Also, the expected down-field shift of the C-1' and the C-2 signals (β effect) is observed. Methylation of **3a** yielded the methyl ester **3b**.

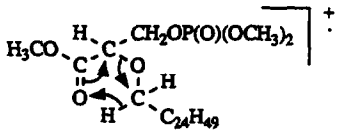
M_B has structure **4a**, again fully in accord with the ^{13}C NMR spectrum (Table 1). When **4a** was treated with methanol in the presence of an ion exchange resin (Dowex 50, H^+ form) an exceedingly unstable compound resulted which according to its ^1H and ^{13}C NMR spectra was probably methyl ester **4b**. Based on previous observations[7] we assume that a β-elimination process is responsible for the instability.

Hydrogenation of **4a** provided decahydro derivative **5a** esterification of which with diazomethane furnished **5b**. On the basis of NMR and MS data (see for example MS ions **a** and **b**) **5b** proved to be identical with a specimen obtained previously from chemical degradation of moenomycin A.[8]

M_C contains probably units A through F of moenomycin A (**1**) as far as can be judged from its ^{13}C NMR spectrum.[9] Further structural work has not been performed.



a



b

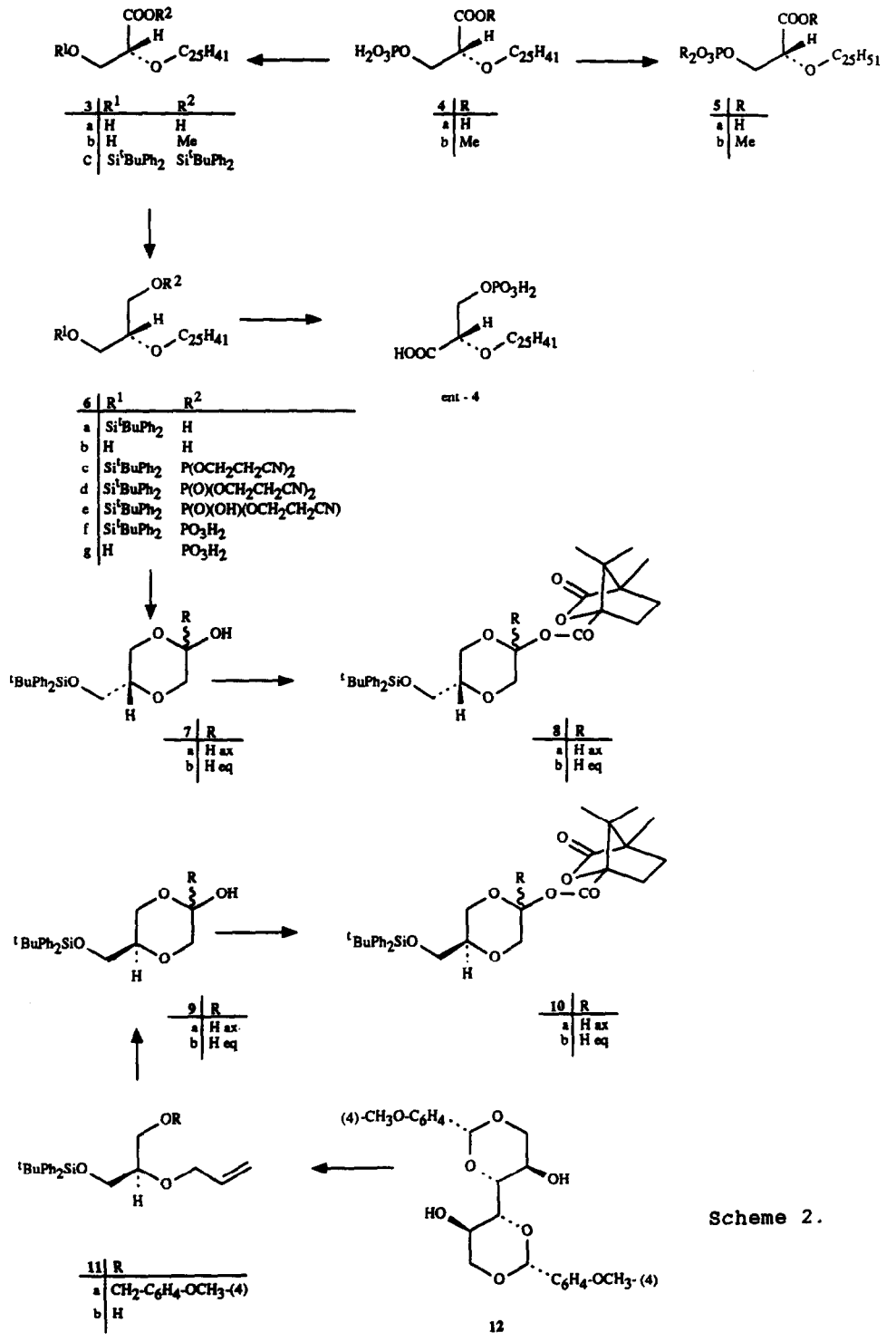
Table 1. ^{13}C NMR spectra of moenocinol[a], M_A (3a)[b], and M_B (4a)[c]

C[d]	moenocinol	3a	4a
1'	59.0	67.5	68.6
2'	124.3	123.5	124.5
3'	139.7	141.6	142.9
4'	32.2	32.3	34.6
5'	31.3	32.5	33.9
6'	125.2	126.7	128.0
7'	140.7	141.8	143.8
8'	35.5	35.9	37.7
9'	41.3	40.9	44.0
10'	31.3	30.7	33.9
11'	150.0	151.1	151.4
12'	34.9	33.4	37.1
13'	121.9	122.7	123.4
14'	136.4	137.3	138.3
15'	39.7	36.4	42.1
16'	26.6	27.7	29.4
17'	124.3	125.3	126.8
18'	131.4	132.2	133.1
19'	25.7	25.9	27.9
20'	17.7	17.8	19.9
21'	15.9	16.1	18.2
22'	108.3	109.2	111.1
23'/24'	27.3	27.3	29.6
25'	23.4	23.8	25.9
1		175.9	179.1
2		80.6	81.8
3		64.1	68.6

[a] in $CDCl_3$ solution, [b] in CD_3OD solution, [c] in D_2O solution, [d] for position numbers, see formula 1. The primed carbon numbers correspond to unit I ($C_{25}H_{41}$), the non-primed to the glyceric acid part, see unit H in 1.

Reinvestigation of the glyceric acid configuration in 1.

Many years ago, the configuration at C-2 of the glyceric acid unit present in 1 was determined by Huber.[10] After acid cleavage of moenomycin a mixture of 2- and 3-phosphoglycerate was isolated. Subsequent dephosphorylation with calf intestinal alkaline phosphatase furnished glyceric acid. On the basis of the very small negative optical rotation of the specimen thus obtained ($[\alpha]_D$ = -1.8) the (R) configuration was assigned. Our ready access to **3a** offered the opportunity to verify the earlier assignment. A crude specimen of **3a** on treatment with tert-butyldiphenylsilyl chloride under the conditions reported by Chaudhary and Hernandez[11] yielded fully protected **3c**. An attempt to reduce this compound with lithium aluminium hydride was unsuccessful since only the diol **6b** was formed by ester reduction and silyl ether cleavage.[12,13,14] However, careful reduction with diisobutylaluminium hydride (DIBAH)[15] led to the desired **6a**. In this case,



Scheme 2.

too, under non-optimized conditions the formation of **6b** was observed.[12] **6a** was then submitted to ozonolysis whereupon a mixture of the diastereomeric hemiacetals **7a** and **7b** was formed. Two signals in the ^{1}H NMR spectrum at δ = 4.80 (dd, $J_{2,3}$=8.5 Hz, $J_{2,3'}$=3.0 Hz) and δ = 4.95 ($W_{1/2}$=1.5 Hz) were indicative of the hemiacetal structure with the OH group in the equatorial and the axial position, respectively. The corresponding ^{13}C NMR signals were found at δ = 92.1 and 89.1, respectively. The **7a/7b** mixture was then esterified with (-)-camphanoyl chloride.[16] The resulting diastereomeric esters **8a** and **8b** were readily separated by silica gel chromatography. In the ^{1}H NMR spectra of **8a** and **8b** the three three-spin multiplets overlap and could only partly be analyzed. The 2-H signals appeared at low field and were characteristic of an axial proton in **8a** and an equatorial proton in **8b**. In **8a** also the 3-H_{ax} and 3-H_{eq} signals could be assigned. Because of the uncertainties in the analysis of the ^{1}H NMR spectra no definite assignment of the ring conformation was possible, and, therefore, the configuration at C-2 remained elusive (a question without relevance in the present context). Reference compounds **10a** and **10b** with known configuration at C-5 were prepared employing the route to optically active glycerol derivatives alkylated in the 2-position that we developed some time ago.[6,17,18] 1,3(R):4,6(R)-Bis-O-(4-methoxy-benzylidene)-D-mannitol (**12**) was converted to **11a** by (i) allylation of the free OH groups, (ii) reductive acetal opening, (iii) periodate cleavage of the 3,4 diol grouping, followed by sodium borohydride reduction, and (iv) silyl ether formation as described previously.[17,18] From **11a** the p-methoxybenzyl group was removed by oxidation with ceric ammonium nitrate in acetonitrile-water[19] to provide **11b**. Ozonolytic degradation of **11b** yielded the two hemiacetals **9a** and **9b** which on subsequent esterification with (-)-camphanoyl chloride furnished **10a** and **10b**, again readily separable by chromatography. The ^{1}H and ^{13}C NMR spectra of **8a** and **10a** are practically superimposable. Similarly, the ^{1}H NMR spectra of **8b** and **10b** are practically identical. However, on TLC (toluene - tert-butyl methyl ether 15:1, 3 times developed) **8a** and **10a** have distinctly different R_f values, and also those of **8b** and **10b** differ slightly. These results demonstrate unambiguously, that **8a** and **10a**, as well as **8b** and **10b**, have opposite configuration at C-5, in agreement with Huber's configurational assignment.[10]

Synthesis of *ent*-M$_8$ (*ent*-**4a**)

Nothing is known on how the antibiotic activity of moenomycin A (**1**) is related to the configuration of the glyceric acid moiety. As a first step directed towards an investigation of this problem, we synthesized *ent*-**4a**.

Our method for the synthesis of optically active glycerol derivatives mentioned above has already demonstrated its merits in the synthesis of optically active glycerate and 3-phosphoglycerate derivatives, O-alkylated in the 2-position, i.e. simple structural analogues of moenomycin A.[20] In the present case, however, this approach appeared to be of little value. The insufficient supply of the moenocinol-derived reagent needed for the 2- and 5-alkylation of **12** forms a serious obstacle. Neither degradation of moenomycin[6] nor total synthesis[21] make moenocinol available in substantial amounts.

On the other hand, M_A (**3a**) is now readily obtained from moenomycin A by the enzymatic degradations described above. It appeared, therefore, reasonable to prepare *ent*-**4a** from **3a**. This approach demanded (i) inversion of the absolute configuration by exchanging the oxidation levels at the terminal glycerol carbons and (ii) phosphorylation. **3a** was converted to **6a** as described above. For the introduction of the phosphate group the phosphite methodology was employed.[22,16] A number of usual phosphite and phosphate protecting groups such as benzyl, tert-butyl, allyl, or differently substituted phenyl groups seemed not compatible with the other functional groups present in **6a**. From suitable protecting groups such as the p-nitrophenylethyl,[23] the ß,ß,ß-trichloroethyl,[24] and the ß-cyanoethyl group, we selected the latter, which can be removed under mildly basic conditions by ß-elimination.[25,26,27,28,29] The desired phosphate **6f** was obtained only after much experimentation and optimization. The best yields were achieved by coupling of **6a** with bis(ß-cyanoethyl)-N,N-diisopropylaminophosphoramidite[30] in the presence of 1H-tetrazole, oxidation of the intermediate phosphite **6c** with bis(trimethylsilyl)peroxide[31,4] to give phosphoric acid triester **6d**, and deblocking with 0.5 mol/l aqueous lithium hydroxide at 20°C. TLC control showed the fast formation of diester **6e** (5 min) whereas the second ß-cyanoethyl group was split off much more reluctantly (20-40 min), an observation not unexpected in view of the large difference in the pK_a values of H_3PO_4 (2.12) and $H_2PO_4^-$ (7.21).[32] On treatment of triester **6d** with triethylamine[27b] only diester **6e** was obtained (see Experimental), similar observations were made using ammonia in methanol,[24b] aqueous ammonia,[25b] DBU or diisopropylamine in pyridine.[28] For the oxidation step (**6c** --> **6d**) both bis(trimethylsilyl)peroxide and tert-butyl hydroperoxide can be used. Finally, because of the great difference in the polarity of **6d**, **6e**, and **6f** the selection of the solvent was found to be critical (see Experimental).

Cleavage of the silylether grouping in **6f** to provide **6g** was achieved in quantitative yield with potassium fluoride in 3.4:1 methanol-water (6 d at 20°C).

Oxidation of **6g** to furnish the desired *ent*-**4a** turned out to be the most critical step of the synthesis. We had in mind to oxidize **6g** either directly to *ent*-**4a** with pyridinium dichromate[33] or first with a non-acidic Cr(VI) reagent followed by oxidation of the intermediate aldehyde with sodium chlorite under mild conditions.[34] These experiments met with no success. In all oxidations with the chromium reagents only decomposition was observed. We assume that the aldehyde obtained from **6g** is very labile and decomposes readily by a β-elimination process (vide supra for the instability of ester **4b**). A method avoiding this complication was needed and recourse was made to catalytic oxidation, a method that is known to convert primary alcohols to carboxylic acids quite selectively if the reaction process is carefully monitored. The pH of the medium has to be kept in the range of 7 to 8.[35] In a test experiment M_B (**4a**) was shown to be stable under the conditions of the catalytic oxidation. **6d** was then oxidized using this method and provided *ent*-**4a** in 50% yield. *Ent*-**4a** thus obtained gave the correct FAB MS spectrum. Especially useful for the identification was the ^{13}C NMR spectrum that was identical in every respect with that of **4a**.

Antibiotic activities of the moenomycin degradation products

The inhibitory effect of **4a** and *ent*-**4a** on the transglycosylation reaction was tested as described previously.[36] Tests were carried out twice with 1 and 10 mg/l of both **4a** and *ent*-**4a**. No inhibition was observed,[5] whereas it was verified that moenomycin was effective in this case. Further, the inhibition of the polymerization of the peptidoglycan sugar chains was studied with a slightly modified version of the assay described by Izaki[37] using UDP-N-acetylmuramyl pentapeptide isolated from *Bacillus cereus T* and cell membranes of *E.coli* JE5684. At a concentration of 1 mg/l **4a** and *ent*-**4a** were inactive. Antibacterial activity was finally determined by agar dilution tests with Mueller-Hinton agar.[38] The results in Table 2 show a slight activity only against *Streptococcus pyogenes* A77. These findings are in agreement with previous observations on structure-activity relations in the moenomycin area. Great care was taken in these experiments to obtain M_B completely free of moenomycin A by reversed-phase chromatography.

Table 2. *In vitro* activity of moenomycin A (1) and compounds M_A (3a), M_B (4a), and *ent*-M_B (*ent*-4a)

test organism	MIC (mg/l)			
	1	3a	4a	*ent*-4a
S.aureus SG 511	0.025	100.0	50.0	100.0
S.aureus 285			50.0	>100.0
S.aureus 503			50.0	>100.0
Strept.pyog. A 77	<0.002	12.5	3.125	50.0
Strept.pyog. 308			50.0	100.0

EXPERIMENTAL

For instrumentation and general methods, see ref. [10].

Acid cleavage of moenomycin.
Moenomycin complex (Flavomycin®, 20 g) was degraded as described by Tschesche et al.[8] Two successive separations (MPLC, silica gel, hexanes-ethyl acetate 10:1) yielded moenocinol (148 mg) and the (2E)-isomer (51 mg).

(2Z,6E,13E)-3,8,8,14,18-Pentamethyl-11-methylene-nonadeca-2,6,13,17-tetraen-1-ol (moenocinol).
The following NMR spectra were recorded: ^1H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100.6 MHz, $CDCl_3$), ^1H, ^1H COSY, ^1H, ^{13}C COSY, COLOC,[39] DEPT. The assignments of C-20/C-21, C-5/C-16 and the quaternary carbons are based on the COLOC experiment ($J_{C,H}$ = 5 Hz). Assignment of the C-5/C-10 and C-2/C-17 chemical shifts is based on a ^1H, ^{13}C COSY spectrum with a digital resolution of 0.25 Hz/point. The results are collected in Table 3.

(2E,6E,13E)-3,8,8,14,18-Pentamethyl-11-methylene-nonadeca-2,6,13,17-tetraen-1-ol.
IR (CCl_4): 3600, 3080, 1665, 1640 cm^{-1}.- ^1H NMR (400 MHz, $CDCl_3$): δ = CH_2-1 (4.12, dd, J=7 and 1 Hz); 2-H (5.38, tq, J_t=7 Hz, J_q=1 Hz); CH_2-4 and CH_2-5 (2.02-2.13); 6-H (2.24, dt, J_d=15 Hz, J_t=6 Hz); 7-H (5.32, d, ^{1rc} J_d=15 Hz); CH_2-9 (1.31-1.37); CH_2-10 (1.82-1.90); CH_2-12 (2.67, d, J_d=7.5 Hz); 13-H (5.13, t (^{1rc42}), J_t=7.5 Hz); CH_2-15 (1.96-2.02); CH_2-16 (2.02-2.13); 17-H (5.05, t (^{1rc42}), J_t=7 Hz); CH_3-19 (1.66, s); CH_3-20 (1.58, s), CH_3-21 (1.58, s); CH_3-22 (4.63, s (^{1rc42})), CH_3-23/CH_3-24 (0.96, s); CH_3-25 (1.64, s).- ^{13}C NMR (100.6 MHz, $CDCl_3$, DEPT): δ = C-1 (59.3, CH_2); C-2 (123.5, CH); C-3 (139.4, C); C-4 (31.3, CH_2); C-5 (31.0, CH_2); C-6 (125.6, CH); C-7 (140.2, CH); C-8 (35.5, C); C-9 (41.4, CH_2); C-10 (31.3, CH_2); C-11 (150.1, C); C-12 (34.9, CH_2); C-13 (121.9, CH); C-14 (136.4, C); C-15 (39,7, CH_2); C-16 (26.6, CH_2); C-17 (124.2, CH); C-18 (131.3, C); C-19 (25.6, CH_3); C-20 (17.6, CH_3); C-21 (15.9, CH_3); C-22 (108.3, CH_2); C-23/C-24 (27.3, CH_3); C-25 (16.2, CH_3).- $C_{25}H_{42}O$ (358.6), MS: m/z (%) = 358 (5), 340 (5), 271 (39), 69 (94), 41 (100).

Degradation of moenomycin to give M_A and M_B.[5]
The preparative conversions were carried out in a fermentor containing Flavomycin® (562 g), freeze-dried cells of Bacillus spec. DSM 4675 (658 g), glycine-NaOH buffer (0.1 mol/l, pH 8.5; 37.3 l), $CoCl_2$ ($1x10^{-4}$ mol/l), NaN_3 (0.02%) for 6-48 h at 37°C and 100 rpm. The progress of the reaction was monitored by TLC ($CHCl_3$-CH_3OH-CH_3COOH 80:10:1). The suspension was

then centrifuged, the solid material washed with acetone, and the aqueous solution extracted with ethyl acetate. Combining the two organic fractions and solvent evaporation gave crude M_A (123 g). Repeated extraction of the aqueous phase with 1-butanol, combining the extracts and solvent evaporation furnished crude M_B (96 g). M_A (20 g) was purified by LC (silica gel, 2.1 kg). The crude material was dissolved in as little as possible 1:1 hexanes-acetone and transferred onto the top of the column. Elution was first performed with hexanes-acetone 60:40 (10 l) and then with methanol (5 l). The methanol eluate (collected in 0.1 l fractions) contained M_A (13 g).- MPLC (500 g silica gel, chloroform-ethanol-water 4:7:1.5) of the M_B fraction (3.1 g) furnished pure M_B (1.3 g). The antibiotic activitiy was determined using a sample further purified by reverse-phase LC (RP 18, methanol - water 3:2). 500.0 mg yielded 388.2 mg of pure **4a**.

(R)-3-Hydroxy-2-((2Z,6E,13E)-3,8,8,14,18-pentamethyl-11-methylene-nonadeca-2,6,13,17-tetraenyloxy)-propanoic acid, M_A (**3a**).
^{13}C NMR (100.6 MHz, CD_3OD): see Table 1.- $C_{28}H_{46}O_4$ (446.7[40a], 446.3[40b]), MS: m/z (%) = 446 (1), 377 (1), 340 (2), 271 (20), 69 (100).

Methyl (R)-3-hydroxy-2-((2Z,6E,13E)-3,8,8,14,18-pentamethyl-11-methylene-nonadeca-2,6,13,17-tetraenyloxy)-propanoate, (**3b**).
An aqueous solution of **3a** was treated with an ion exchange resin (Dowex 50, H^+ form). After filtration the aqueous phase was freeze-dried. The free acid form of **3a** (18.5 mg, 0.04 mmol), dissolved in methanol (3 ml) was treated at 0°C with an excess of an ethereal diazomethane solution, and the mixture was left 2 h at 0°C and 12 h at 20°C. Solvent evaporation followed by LC of the residue (silica gel, hexanes-ethyl acetate 2:1) provided **3b** (3 mg, 16%).- ^1H NMR (80 MHz, $CDCl_3$): δ = 0.96 (s, 6H, CH_3-23' and CH_3-24'); 1.61 (s, 6H), 1.68 (s, 3H), 1.73 (s, 3H) (CH_3 signals); 1.90-2.12 (allyl. H's), 2.62 (br d, J=7 Hz, CH_2-12'), 3.78 (s, OCH_3), 3.60-4.30 (OCH_2 and OCH signals), 4.62 (br s, CH_2-22'), 4.87-5.47 (olefin. H's).- $C_{29}H_{48}O_4$ (460.7[40a], 460.4[40b]), MS: *m/z* (%) = 460 (0.03), 271 (10), 230 (19), 199 (68), 43 (100).-

(R)-2-((2Z,6E,13E)-3,8,8,14,18-Pentamethyl-11-methylene-nonadeca-2,6,13,17-tetraenyloxy)-3-phosphonooxy-propanoic acid, M_B (**4a**).
$[\alpha]^{20}_{436}$ = +5.3 ± 0.8 (c 1.07, water).- ^{13}C NMR (100.6 MHz, D_2O): see Table 1.- $C_{28}H_{47}O_7P$ (526.7[40a], 526.3[40b]), FAB MS (DMSO-glycerol): *m/z* = 615 $[M-3H+4Na]^+$, 593 $[M-2H+3Na]^+$, 571 $[M-H+2Na]^+$, 492, 267, 231, 185, 165, 143, 115.

Methyl (R)-2-((2Z,6E,13E)-3,8,8,14,18-pentamethyl-11-methylene-nonadeca-2,6,13,17-tetraenyloxy)-3-phosphonooxy-propanoate (**4b**).
To a solution of **4a** (54.6 mg, 0.104 mmol) in methanol (15 ml) ion exchange resin (Dowex 50 W, H^+ form, freshly regenerated and washed with methanol, 5g) was added, and the mixture was stirred for 14 d at 20°C. Filtration, solvent evaporation (30°C), addition of water, and lyophilization gave TLC pure **4b** (49.8 mg, 88%) which decomposed very quickly. In other experiments **4b** had to be purified by LC ($CHCl_3$-CH_3OH-H_2O 18:11:2.7).- ^1H NMR (400 MHz, $CDCl_3$, freshly filtered through Al_2O_3, otherwise **4b** decomposed rapidly): δ = 3.72 (OCH_3).- ^{13}C NMR (100.6 MHz, solvent as described of ^1H NMR): δ = 52.8 (OCH_3).

(R)-3-Hydroxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-propanoic acid (**5a**).
3a (14.9 mg, 28.3 µmol) and PtO_2 (4 mg) were stirred in methanol (3ml) and acetic acid (50 µl) under an atmosphere of hydrogen at 20°C for 3 d. Filtration of the reaction mixture and concentration in vacuo gave **5a** (13.5 mg, 89%).- $C_{28}H_{57}O_7P$ (536.7[40a], 536.4[40b]), FAB MS (DMSO-glycerol): *m/z* =

625 [M-3H+4Na]$^+$, 603 [M-2H+3Na]$^+$, 581 [M-H+2Na]$^+$, 558 [M-H+Na]$^+$, 514, 500, 498, 432, 404, 362, 340, 298, 288, 266, 186, 164, 142, 115, 93.

Table 3. NMR spectral data of moenocinol

position number	^{1}H NMR δ	^{1}H NMR signal structure	^{1}H,^{1}H COSY	^{13}C NMR COLOC	^{13}C NMR δ	^{13}C NMR DEPT
1	4.08	dd, J=7, J=1	2-H, 25-H	C-3	59.0	CH_2
2	5.40	td, J_t=7, J_d=1	1-H, 25-H		124.26	CH
3					139.7	C
4	2.02-2.14	m	5-H	C-3, C-2/17 C-5/10, C-25	32.2	CH_2
5	2.02-2.14	m	4-H, 6-H, 7-H, 17-H 19-H,20/21-H		31.32	CH_2
6	5.25	dt, J_d=15, J_t=6	7-H, 5/16-H		125.2	CH
7	5.34	d, J_d=15	5/16-H, 6-H	C-6	140.7	CH
8					35.5	C
9	1.30-1.38	m	10-H	C-8, C-23/24	41.3	CH_2
10	1.84-1.90	m	9-H, 22-H	C-8, C-11	31.28	CH_2
11					150.0	C
12	2.67	d, J_d=7.5	13-H, 15-H, 20/21-H	C-11, C-14 C-22	34.9	CH_2
13	5.13	t, J_t=7.5 lrc[a)]	12-H, 15-H 20/21-H		121.9	CH
14					136.4	C
15	1.95-2.02	m	12-H, 13-H, 20/21-H	C-14	39.7	CH_2
16	2.02-2.14	m	6-H, 7-H, 17-H, 19-H 20/21-H	C-14	26.7	CH_2
17	5.08	t, J_t=7	5/16-H, 19-H, 20/21-H		124.33	CH
18					131.4	C
19	1.66	s	5/16-H, 17-H	C-18, C-20	25.7	CH_3
20	1.58		5/16-H, 12-H 13-H, 17-H	C-13, C-14, C-19	17.7	CH_3
21	1.58		5/16-H, 12-H, 13-H, 17-H	C-13, C-14 C-15, C-18, C-19	15.9	CH_3
22	4.56	s, lrc[a)]	10-H, 12-H	C-5/10,C-22	108.3	CH_2
23/24	0.95	s		C-9, C-8	27.3	CH_3
25	1.72	s	1-H, 2-H	C-3, C-2/17 C-4	23.4	CH_3

[a)] long range coupling

The assignment of the carbon resonances are in full agreement with a previous assignment based on comparison with model compounds[41] and completes and modifies in two positions the assignments reported by Coates and Johnson.[21d]

Methyl (R)-3-hydroxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-propanoate (5b).
An aqueous solution of **5a** was treated with ion exchange resin (Dowex 50, H^+ form) to generate the free acid form of **5a**. After filtration and lyophilization 8.5 mg (15.9 µmol) of the sample were dissolved in methanol (2 ml), and an excess of an ethereal diazomethane solution was added at 0°C. The reaction mixture was kept at 0°C for 2 h and at 20°C for 12 h. Solvent evaporation and LC (silica gel, hexanes-ethyl acetate 1:1) furnished **5b** (4.0 mg, 44%).- ^{1}H NMR (80 MHz, $CDCl_3$): δ = 3.75 (s, OCH_3 and 2d, $^3J_{H,P}$=10 and 12 Hz, $P(OCH_3)_2$), 3.20 - 4.40 (OCH_2 and OCH multiplets).- $C_{31}H_{63}O_7P$ (578.8[40a], 578.4[40b]), MS: *m/z* (%) = 563 (0.1, M-CH_3), 519 (1, M-$COOCH_3$), 452 (1, M-$(CH_3O)_2P(O)OH$), 381 (3, M-$C_{14}H_{29}$, bond fission between C-8' and C-9'), 229 (8, **a**), 212 (6, **b**), 127 (20), 57 (100).

tert-Butyldiphenylsilanyl (R)-3-(tert-butyldiphenylsilanyloxy)-2-((2Z,6E,13E)-3,8,8,14,18-pentamethyl-11-methylene-nonadeca-2,6,13,17-tetraenyloxy)-propanoate (3c).
To a solution of crude M_A (1.76 g, dried by repeated codistillation with 10 ml portions of dry toluene) in CH_2Cl_2 (25.0 ml), triethylamine (1.5 ml, 10.60 mmol), 4-dimethylaminopyridine (79.2 mg, 0.62 mmol) dissolved in CH_2Cl_2 (2.0 ml), and tert-butyldiphenylsilyl chloride (4.0 ml, 15.36 mmol) were added. After stirring for 21 h at 20°C the reaction mixture was diluted with CH_2Cl_2 (200 ml) and then washed once with water (50 ml). The organic phase was briefly dried over Na_2SO_4, filtered and concentrated in vacuo to yield crude **3c** (5.57 g). For characterization a small scale reaction (52.6 mg of crude M_A) was carried out. After work-up and LC (hexanes-ethyl acetate 20:1) the NMR spectrum of the very sensitive product **3c** (28.4 mg, 0.030 mmol) was immediately taken.- ^{1}H NMR (60 MHz, $CDCl_3$): δ = 0.79-2.18 (48H, 12xCH_3, 6xCH_2), 2.57-2.77 (m, 2H, CH_2-12'), 3.65-4.20 (5H, 2-H, CH_2-3, CH_2-1'), 4.65 (2H, CH_2-22'), 4.90-5.50 (5H, 2'-H, 6'-H, 7'-H, 13'-H, 17'-H), 7.20-7.80 (20H, Ar-H's).

(S)-3-(tert-Butyldiphenylsilanyloxy)-2-((2Z,6E,13E)-3,8,8,14,18-pentamethyl-11-methylene-nonadeca-2,6,13,17-tetraenyloxy)-propanol (6a).
To a solution of crude **3c** (2.765 g) in CH_2Cl_2 (50 ml) a DIBAH solution (1.2 mol/l in toluene, 5.0 ml, 6.0 mmol) was added at 0°C within 30 min. After stirring at 0°C for 1 h and at 20°C for 1 h an additional amount of the DIBAH solution (1.0 ml, 1.2 mmol) was added dropwise to the reaction mixture at 20°C. Stirring was continued for 1 h. The reaction was stopped by adding first 7:3 toluene-methanol (5 ml) and then 5:3 methanol-water (5 ml) at 0°C. After dilution with CH_2Cl_2 (50 ml), the organic layer was washed with a 10% aqueous $NaHCO_3$ solution (50 ml) and with water (50 ml). Drying of the organic phase over Na_2SO_4, filtration, solvent evaporation and LC (hexanes-ethyl acetate 17:1) provided **6a** (432.1 mg).- $[\alpha]_D$ = -18.8 (c 0.99, $CHCl_3$).- ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.93 (s, 6H, CH_3-23' and CH_3-24'), 1.03 (s, 9H, $C(CH_3)_3$), 1.33-1.41 (m, 2H, CH_2-9'), 1.60 (s, 6H, 2xCH_3), 1.67 (s,[42] 3H, CH_3), 1.71 (s[42]), 3H, CH_3), 1.84-1.91 (m, 2H), 1.99-2.12 (8H), 2.68 (br d, J=7.1 Hz, 2H, CH_2-12'), 3.48-3.55 (m, 1H), 3.61-3.80 (m, 4H), 3.98 (dd[42], $J_{1'a,1'b}$=12.0 Hz, $J_{1'a,2'}$=7.0 Hz, 1H, 1'$_a$-H), 4.08 (dd (1rc[42]), 1'$_b$-H, $J_{1'a,1'b}$=12.0 Hz, $J_{1'b,2'}$=6.5 Hz, 1H), 4.68 (s (1rc[42]), 2H, CH_2-22', $W_{1/2}$=6.1 Hz), 5.06-5.37 (5H, 2'-H, 6'-H, 7'-H, 13'-H, 17'-H), 7.30-7.75 (10H, Ar-H's).- ^{13}C NMR (100.6 MHz, $CDCl_3$, DEPT): Cq signals 150.3 (C-11'), 140.5 (C-3'), 136.6, 135.0, 133.5, 133.4, 131.6, 35.8 (C-8'), 19.4; CH signals 140.8 (C-7'), 135.8, 130.0, 128.0, 125.5, 124,6, 122.2, 122.2, 79.5 (C-2); CH_2 signals 108.6 (C-22'), 66.7, 63.8, 63.1, 41.7, 40.0, 35.2, 32.8, 31.7, 27.0; CH_3 signals 27.6, 27.1, 26.0, 23.8, 17.9, 16.2.- IR ($CHCl_3$) 3585 cm^{-1} (OH).- MS: *m/z* (%) = 670 (0.2, M^+), 433 (1.2), 69 (100).- Anal. Calcd for $C_{44}H_{66}O_3Si$ (671.1[40a], 670.5[40b]: C, 78.75; H, 9.91. Found: C, 78.64; H, 9.83.

(R)-2-Allyloxy-3-tert-butyldiphenylsilanyloxy-propan-1-ol (**11b**).
(S)-2-Allyloxy-3-(4-methoxy-benzyloxy)-propan-1-ol[17] (200.0 mg, 0.79 mmol) was converted to (R)-2-allyloxy-3-tert-butyldiphenylsilanyloxy-3-(4-methoxy-benzyloxy)-propane (**11a**) as described in ref.[18]). The sample of **11a** thus obtained (250.0 mg) was not completely pure (containing silanol impurities) and was used without further purification. To a solution of this material in 9:1 acetonitrile-water (10.85 ml) pyridine (166 µl, 2.04 mmol) and cerium(IV) ammonium nitrate (1398.0 mg, 2.55 mmol) were added. The mixture was stirred at 20°C for 4 h. Usual work-up (CH_2Cl_2, one washing with sat. Na_2SO_3 and with sat. $NaHCO_3$ solution) followed by LC (CH_2Cl_2 - ethyl acetate 60:1) provided **11b** (103.4 mg, 62 %) based on (S)-2-allyloxy-3-(4-methoxy-benzyloxy)-propan-1-ol).- ^1H NMR (80 MHz, $CDCl_3$): δ = 1.05 (s, 9H, tert-butyl); 3.40-3.85 (5H, CH_2-1, CH_2-3, 2-H); 3.90-4.10 (m, 2H, $CH_2CH=CH_2$); 5.00-5.35 (2H, $=CH_2$); 5.60-6.10 (1H, $CH_2CH=CH_2$); 7.25-7.75 (10H, Ar-H's).- $C_{22}H_{30}O_3Si$ (370.6[40a], 370.2[40b]), MS: m/z (%) = 340 (0.5), 313.1261 (5, Calc for $C_{18}H_{21}O_3Si$ 313.1260, [M-tert-butyl]$^+$), 255 (5), 235 (50), 225 (30), 197 (55), 195 (58), 179 (90), 165 (85), 117 (100).

(5S)-5-(tert-Butyldiphenylsilanyloxy-methyl)-1,4-dioxan-2≡-ol (**7a/7b**).
At -78°C ozone (1.66 mmol O_3 per h) was passed through a solution of **6a** (77.6 mg, 0.12 mmol) and methanol (40.0 mg, 1.25 mmol) in CH_2Cl_2 (10 ml) for 10 min. Excess O_3 was removed by passing O_2 through the solution. At -78°C a solution of $NaBH_4$ (23.0 mg, 0.61 mmol) in methanol (5 ml) was added. The mixture was stirred and allowed to warm to 20°C within 2 h. Excess $NaBH_4$ was destroyed by addition of dilute acetic acid. Dowex 50 (H^+ form) was added to remove Na^+ ions. Filtration and repeated addition of methanol and solvent evaporation to remove trimethyl borate, followed by LC (hexanes - ethyl acetate 3:1) furnished a roughly 1:1 mixture of **7a/7b** (25.4 mg, 59 %).- ^1H NMR (400 MHz, $CDCl_3$): δ = 1.05 (s, tert-butyl); 3.20-4.05 (CH_2-3, CH_2-6, CH_2-OSi, 5-H); 4.80 (dd, $J_{2,3}$=8.5 Hz, $J_{2,3'}$=3.0 Hz, 2-H of **7a**); 4.95 (m, $W_{1/2}$=1.5 Hz, 2-H of **7b**); 7.35-7.65 (Ar-H's).- ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 19.5, 27.0, 61.5, 63.6, 64.0, 67.8, 69.5, 70.2, 74.4, 75.7, 89.1 (C-2 of **7b**), 92.1 (C-2 of **7a**), 128.0, 130.1, 133.4, 135.8.

Acylation of **7a/7b** with (-)-camphanoyl chloride.
To a solution of the **7a/7b** mixture (23.5 mg, 0.06 mmol) in pyridine (500 µl) at 0°C a solution of (-)-camphanoyl chloride (31.3 mg, 0.15 mmol) in pyridine (500 µl) was added. The reaction mixture was stirred at 20°C for 2 h. Usual work-up (CH_2Cl_2) and LC (CH_2Cl_2 - ethyl acetate 40:1) gave **8a** (18.4 mg) and **8b** (16.3 mg). The combined yield was 100%.

(5S)-5-(tert-Butyldiphenylsilanyloxy-methyl)-1,4-dioxan-2eq-yl (1S)-camphanoate (**8a**).[43]
^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR spectra (100.6 MHz, $CDCl_3$) were indistinguishable from those of **10a**.- $C_{31}H_{40}O_7Si$ (552.7[40a], 552.3[40b]), MS: m/z = 495.1850 (10, Calc for $C_{27}H_{31}O_7Si$: 495.1839, [M-tert-butyl]$^+$), 297 (100), 255 (60), 225 (40), 183 (55), 163 (75).

(5S)-5-(tert-Butyldiphenylsilanyloxy-methyl)-1,4-dioxan-2ax-yl (1S)-camphanoate (**8b**).[43]
^1H NMR (400 MHz, $CDCl_3$) was indistinguishable from that of **10b**.- $C_{31}H_{40}O_7Si$ (552.7[40a], 552.3[40b]), MS: m/z = 495.1830 (6, Calc for $C_{27}H_{31}O_7Si$: 495.1839, [M-tert-butyl]$^+$), 297 (100), 255 (15), 225 (15), 183 (25), 163 (35).

(5R)-5-(tert-Butyldiphenylsilanyloxy-methyl)-1,4-dioxan-2≡-ol (**9a/9b**).
Ozonolytic degradation of **11b** was performed as described for **6a**. Yield: 44.4 %. R_f value (hexanes - ethyl acetate 1:1) and ^1H NMR spectrum (80 MHz) were identical with those of **7a/7b**.

Acylation of **9a/9b** with (-)-Camphanoyl chloride.
Acylation of the **9a/9b** mixture with (-)-camphanoyl chloride and separation of **10a** and **10b** was performed as described for **8a** and **8b**. Combined yield: 100%.

(5R)-5-(tert-Butyldiphenylsilanyloxy-methyl)-1,4-dioxan-2eq-yl (1S)-camphanoate (**10a**).[43]
^{1}H NMR (400 MHz, $CDCl_3$, H,H COSY, NOE): δ = 0.95 (s, CH_3); 1.05 (s, tert-butyl); 1.08 (s, CH_3); 1.10 (s, CH_3); 1.65-1.75 (ddd, $|J_{5,5'}|$=14 Hz, $J_{5,6}$=9.5 Hz, $J_{5,6'}$=4.5 Hz, 5-H$_{camph}$); 1.85-1.95 (ddd, $J_{6,6'}$=15.0 Hz, $J_{6,5'}$=11 Hz, 6-H$_{camph}$; 2.00-2.10 (ddd, 5'-H $_{camph}$); 2.35-2.45 (ddd, 6'-H $_{camph}$); 3.48 (1H, dd, $|^2J|$=11.5 Hz, ^3J=7.0 Hz, 3-H$_{ax}$); 3.83 (dd, 1H, $|^2J|$=11.5 Hz, ^3J=3.0 Hz, 3-H$_{eq}$); 3.65-3.80 (4H) und 4.05-4.15 (1H) (CH_2-6, CH_2-O-Si, 5-H); 5.85 (dd, $J_{2,3}$=7.5 Hz, $J_{2,3'}$=3.0 Hz, 2-H); 7.35-7.65 (Ar-H's).- ^{13}C NMR (100.6 MHz, $CDCl_3$, DEPT): C_q signals 19.2 ($(CH_3)_3\underline{C}$), 54.5, 54.9, 90.7 (C-1$_{camph}$), 134.8 (Ar-C), 166.1, 177.9; CH signals 73.5 (C-5), 90.4 (C-2), 127.7, 129.8, 135.5 (Ar-C's); CH_2 signals 28.9, 30.6, 62.5, 65.9, 66.3; CH_3 signals 9.7, 16.7, 26.7 ($(\underline{C}H_3)_3C$).- $C_{31}H_{40}O_7Si$ (552.7[40a], 552.3[40b]), MS: m/z = 495.1830 (12, Calc for $C_{27}H_{31}O_7Si$: 495.1839, [M-tert-butyl]$^+$), 297 (100), 255 (75), 225 (45), 163 (65).

(5R)-5-(tert-Butyldiphenylsilanyloxy-methyl)-1,4-dioxan-2ax-yl (1S)-camphanoate (**10b**).[43]
^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.95-1.10 (4 CH_3 signals); 1.65-1.75 (ddd, $|J_{5,5'}|$=13.5 Hz, $J_{5,6}$=9.5 Hz, $J_{5,6'}$=4.5 Hz, 5-H$_{camph}$); 1.85-1.95 (ddd, $|J_{6,6'}|$=15.0 Hz, $J_{6,5'}$=10.5 Hz, 6-H$_{camph}$; 2.00-2.10 (ddd, 5-H'$_{camph}$); 2.35-2.45 (ddd, H-6'$_{camph}$); 3.63 (1H, dd, $|^2J|$=10.5 Hz, ^3J=5.5 Hz) and 3.70-3.90 (6H) (CH_2-3, CH_2-6, CH_2-OSi, 5-H); 6.00 (m, $W_{1/2}$=1.5 Hz, 2-H); 7.35-7.65 (Ar-H's).- ^{13}C NMR (100.6 MHz, $CDCl_3$, DEPT): C_q signals 19.5 ($(CH_3)_3\underline{C}$), 54.4, 55.0, 91.2 (C-1$_{camph}$), 133.2 (Ar-C), 166.7, 178.2; CH signals 75.1 (C-5), 90.6 (C-2), 128.0, 130.1, 135.8 (Ar-C's); CH_2 signals 29.2, 30.8, 63.7, 64.1, 67.7; CH_3 signals 10.0, 17.1, 27.0 ($(\underline{C}H_3)_3C$).- $C_{31}H_{40}O_7Si$ (552.7[40a], 552.3[40b]), MS: m/z = 495.1875 (6, Calc for $C_{27}H_{31}O_7Si$: 495.1839, [M-tert-butyl]$^+$), 297 (100), 255 (15), 225 (15), 183 (20), 163 (30).

2-Cyanoethyl (R)-3-(tert-butyldiphenylsilanyloxy)-2-((2Z,6E,13E)-3,8,8,14,18-pentamethyl-11-methylene-nonadeca-2,6,13,17-tetraenyloxy)-propyl phosphate (**6e**).
Bis(ß-cyanoethyl)-N,N-diisopropylaminophosphoramidite (35.4 mg, 0.130 mmol), dissolved in CH_3CN (1.5 ml), was added to a solution of **6a** (21.6 mg, 0.032 mmol) and 1H-tetrazole (15.8 mg, 0.226 mmol) in CH_3CN (1.5 ml). The reaction mixture was stirred for 45 min at 20°C. The intermediate phosphite triester **6c** was oxidized with tert-butyl hydroperoxide (80%, 38 µl, 0.304 mmol) within 2 h at 20°C. After addition of triethylamine (0.3 ml), stirring for further 5.25 h, concentration of the reaction mixture in vacuo, and LC ($CHCl_3$-methanol 5:1) **6e** was obtained (25.7 mg, 100%).- ^{1}H NMR (400 MHz, pyridine-d_5): δ = 1.03 (s, 6H, CH_3-23' and CH_3-24'), 1.14 (s, 9H, C(CH_3)$_3$), 1.49-1.56 (m, 2H), 1.58 (s, 3H), 1.66 (s, 3H, CH_3), 1.67 (s, 3H, CH_3), 1.75 (s[42], 3H, CH_3), 2.03-2.22 (10H), 2.83 (br d, J=7.2 Hz, 2H, CH_2-12'), 2.85-2.91 (br t, J=6.0 Hz, 2H, $OCH_2\underline{C}H_2CN$), 3.93-4.10 and 4.26-4.48 (9H, CH_2-1, 2-H, CH_2-3, CH_2-1', $O\underline{C}H_2CH_2CN$), 4.88-4.93 (br d,[42] J=7.5 Hz, 2H, CH_2-22'), 5.20 (m, 1H), 5.33-5.51 (m, 3H), 5.55-5.62 (m, 1H), 7.43-7.95 (10H, Ar-H's).- ^{13}C NMR (100.6 MHz, pyridine-d_5, DEPT) Cq signals 144.2, 138.4, 136.3, 133.7, 133.7, 118.8 ($OCH_2CH_2\underline{C}N$), 35.4 (C-8'), 19.1; CH signals 140.3 (C-7'), 135.7, 129.9, 128.0, 125.7, 124.5, 123.4-122.9 (one CH signal hidden by the pyridine signals), 122.2, 79.2 (d, C-2); CH_2 signals 108.7 (C-22'), 66.7, 65.2, 64.2, 60.6, 41.5, 39.7, 35.0, 32.5, 31.4, 26.7, 19.8 (d, $OCH_2\underline{C}H_2CN$, $J_{C,P}$=7.0 Hz); CH_3 signals 27.2, 26.7, 25.5, 23.3, 18.8, 17.4, 15.8.- ^{31}P NMR (62 MHz, pyridine-d_5, 85 per

cent H_3PO_4 as external standard): δ = -6.5 - -5.3 (broad signal).- $C_{47}H_{70}O_8SiPN$ (804.1[40a], 803.5[40b]), no FAB MS spectrum could be obtained from this compound.

(R)-3-(tert-Butyldiphenylsilanyloxy)-2-((2Z,6E,13E)-3,8,8,14,18-pentamethyl-11-methylene-nonadeca-2,6,13,17-tetraenyloxy)-propyl phosphate (**6f**).

Bis(β-cyanoethyl)-N,N-diisopropylaminophosphoramidite (54.7 mg, 0.202 mmol), dissolved in CH_3CN (2.0 ml), was added to a solution of **6a** (23.5 mg, 0.035 mmol) and 1H-tetrazole (13.6 mg, 0.195 mmol) in CH_3CN (2.0 ml) at 20°C. Bis(trimethylsilyl)peroxide (ca. 90 per cent as judged from the ^1H NMR spectrum, δ = 0.15, impurity signal at δ = 0.03) was added after 1 h, 3 h, and 23 h (three portions of 10 µl, 0.047 mmol). 1 h after the last addition the reaction mixture was diluted with pyridine (2.0 ml). Deblocking of the phosporic acid triester **6d** was accomplished with 0.5 mol/l aqueous lithium hydroxide (2.0 ml) at 20°C. After stirring for 30 min chromatographic separation of the reaction mixture on (HP-20, elution with water (1 l), then methanol (0.5 l) furnished (from the methanolic fraction) crude **6f** (37.3 mg). LC ($CHCl_3$-methanol 3:1) then yielded pure **6f** (21.5 mg, 82%).- After larger-scale experiments the reaction mixture was neutralized with gaseous CO_2, and the solvents were removed by lyophilization. The HP-20 and SiO_2 chromatographic separations were then performed as described above.- $[\alpha]_D$ = -2.02 (c 1.10, methanol).- ^1H NMR (400 MHz, pyridine-d_5): δ = 1.03-1.27 (15H, CH_3-23' and CH_3-24', $C(CH_3)_3$), 1.47-1.73 (14H), 1.74-1.80 (2H), 1.98-2.30 (8H), 2.80-2.93 (m, 2H, CH_2-12'), 3.40-4.53 (7H, CH_2-1, 2-H, CH_2-3, CH_2-1'), 4.85-5.02 (m, 2H, CH_2-22', $W_{1/2}$=21 Hz), 5.18-5.29 (m, 1H), 5.30-5.70 (4H), 7.45-8.05 (10H, Ar-H's).- ^{13}C NMR (100.6 MHz, pyridine-d_5, DEPT) Cq signals 136.6, 134.9, 134.2, 131.3, 35.8 (C-8'), 19.5; CH signals 140.5 (C-7'), 136.1, 130.1, 128.2, 126.1, 124.9, 124.0, 122.6, 79.6 (br, C-2); CH_2 signals 109.1 (C-22'), 66.9, 64.6 (broad signal, which corresponds presumably to two carbons), 41.8 (C-9'), 40.1 (C-15'), 35.3 (C-12'), 32.5, 31.8, 27.0 (C-16'); CH_3 signals 27.5, 27.1, 25.9, 23.6, 17.8 (C-20'), 16.1 (C-21').- ^{31}P-NMR (162 MHz, pyridine-d_5, 85 per cent H_3PO_4 as external standard): δ = -0.34.- $C_{44}H_{67}O_8SiP$ (751.1[40a], 750.4[40b]), FAB MS did not give a M^+ peak.

(R)-2-((2Z,6E,13E)-3,8,8,14,18-Pentamethyl-11-methylene-nonadeca-2,6,13,17-tetraenyloxy)-propyl phosphate (**6g**).

A solution of **6f** (54.1 mg, 0.072 mmol) and KF (161.6 mg, 2.781 mmol) in 3.4:1 methanol-water (12.3 ml) was stirred at 20°C for 6 d. After addition of water (5 ml) methanol was evaporated in vacuo. Chromatographic separation of the aqueous solution (HP-20, elution with water (1 l), methanol (1 l), evaporation of the methanolic fraction and subsequent LC (silica gel, $CHCl_3$-methanol-water 15:10:1) gave **6g** (36.8 mg, 100%).- In other experiments after methanol evaporation water was removed by lyophilization. After HP-20 separation as described above pure **6g** was obtained by RP-18 LC (methanol-water 4:1).- $[\alpha]_D$ = +2.28 (c 1.00, pyridine).- ^1H NMR (400 MHz, pyridine-d_5): δ = 1.08 (s, 6H, 2xCH_3), 1.50-1.60 (m, 2H), 1.63 (s, 3H, CH_3), 1.70 (s, 6H, 2xCH_3), 1.75-1.88 (3H), 2.00-2.28 (10 H), 2.82-2.97 (br d[42], J=7 Hz, 2H, CH_2-12'), 3.80 (br s, $W_{1/2}$=25 Hz, 1H), 4.16 (br s, $W_{1/2}$=29 Hz, 2H), 4.28 (br s, $W_{1/2}$=21 Hz, 2H), 4.54 (br s, $W_{1/2}$=41 Hz, 2H), 4.85-4.99 (br d, J=8 Hz, 2H, CH_2-22'), 5.17-5.28 (m, 1H), 5.33-5.58 (4H).- ^{13}C NMR (100.6 MHz, pyridine-d_5): δ = 149.3 (C-11'), 140.7 (C-7'), 136.7 (C-3'), 134.9 (C-14'), 131.4 (C-18'), 126.2 (C-6'), 124.9 (C-17'), 123.0 (C-2'), 122.6 (C-13'), 109.1 (C-22'), 79.3 (br, C-2), 71.3, 66.5, 64.0 (br), 61.0 (br), 41.9 (C-9'), 40.2 (C-15'), 35.9 (C-8'), 35.4 (C-12'), 32.8, 31.9, 27.6 (C-23' and C-24'), 27.1 (C-16'), 25.9 (C-19'), 23.5 (C-25'), 17.8 (C-20'), 16.2 (C-21').- $C_{28}H_{49}O_6P$ (512.7[40a], 512.3[40b]), FAB MS (glycerol): *m/z* (%) = 535 (4.0, $[M+Na]^+$), 557 (14.5, $[M+2xNa-H]^+$).

(S)-2-((2Z,6E,13E)-3,8,8,14,18-Pentamethyl-11-methylene-nonadeca-2,6,13,17-tetraenyloxy)-3-phosphonooxy-propanoic acid (*ent*-**4a**).
A mixture of **6g** (48.6 mg, 0.094 mmol), Adams' catalyst (207 mg, freshly prepared), $NaHCO_3$ (57.2 mg, 0.681 mmol), and water (2.5 ml, bidistilled) was stirred vigorously at 45°C while oxygen was bubbled through the reaction mixture. After 43 h a further specimen of Adams' catalyst (100 mg) was added. After 73 h the catalyst was removed by centrifugation. The catalyst had to be washed and centrifugated several times since the product was strongly adsorbed. Lyophilization, and LC (RP-18, methanol-water 3:2) gave *ent*-**4a** (24.5 mg, 50%).- $[\alpha]^{20}_{436}$ = -7.5 ± 0.8 (c 1.07, water).- ^{1}H NMR (400 MHz, D_2O): δ = 0.80 (s, 6H, 2xCH_3), 1.03-1.22 (m, 2H), 1.37 (s, 6H, 2xCH_3), 1.47 (s, 3H, CH_3), 1.58 (s, 3H, CH_3), 1.65-1.75 (m, 2H), 1.76-2.05 (8H), 2.46 (br d, J=5.8 Hz, 2H, CH_2-12'), 3.69-4.07 (5H), 4.48 (br s[42], 2H), 4.83-5.37 (5H).- ^{13}C NMR (100.6 MHz, D_2O): δ = 179.7 (C-1), 151.9 (C-11'), 144.2, 143.2, 138.7, 133.5, 128.3, 127.1, 124.8, 123.6, 111.4 (C-22'), 82.2 (C-2), 68.9, 68.6, 44.3 (C-9'), 42.4 (C-15'), 38.0 (C-8'), 37.4 (C-12'), 34.8, 34.2, 29.8 (C-23', C-24'), 29.3 (C-16'), 28.2 (C-19'), 26.1 (C-25'), 20.2 (C-20'), 18.4 (C-21').- $C_{28}H_{47}O_7P$ (526.7[40a], 526.3[40b]), FAB-MS (glycerol): *m/z* (%) = 615 (6.5, $[M+4xNa-3H]^+$), 593 (16.5, $[M+3xNa-2H]^+$), 571 (10.4, $[M+2xNa-H]^+$), 289 (17.0), 187 (21.7), 165 (41.7), 143 (55.6), 125 (25.2), 115 (60).

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References and Notes

1 Structure: Fehlhaber, H.-W.; Girg, M.; Seibert, G.; Hobert, K.; Welzel, P.; van Heijenoort, Y.; van Heijenoort, J. *Tetrahedron* **1990**, *46*, 1557-1568, and references therein.

2 van Heijenoort, J.; van Heijenoort, Y; Welzel, P. in Actor P.; Daneo-Moore, L.; Higgins, M.L.; Salton, M.J.R.; Shockman, G.D. (eds.) "Antibiotic Inhibition of Bacterial Cell Wall Surface Assembly and Function" American Society of Microbiology, Washington 1988, p. 549.

3 Welzel, P.; Kunisch, F.; Kruggel, F.; Stein, H.; Scherkenbeck, J.; Hiltmann, A.; Duddeck, H.; Müller, D.; Maggio, J.E.; Fehlhaber, H.-W.; Seibert, G.; van Heijenoort, Y.; van Heijenoort, J. *Tetrahedron* **1987**, *43*, 585-598, see also ref.[1]

4 Hohgardt, H.; Dietrich, W.; Kühne, H.; Müller, D.; Grzelak, D.; Welzel, P. *Tetrahedron* **1988**, *44*, 5771-5790.

5 Aretz, W.; Böttger, D.; Seibert, G.; Tumulka, A.; Welzel, P.; Hobert, K. (Hoechst AG), Eur.Pat.Appl. EP 355,679 (Cl. C07H13/12), 28.2.1990, DE Appl. 3,828,337, 20.8.1988; C.A. **1990**, *113*, 57469r. Experimental details will be published in a separate paper. The degradation product M_8 was originally believed to be antibiotically active. This observation could not be confirmed in the present studies.

6 Tschesche, R.; Brock, F.-X.; Duphorn, I. *Liebigs Ann.Chem.* **1968**, *720*, 58-70.

7 Schubert, Th.; Kunisch, F.; Welzel, P. *Tetrahedron* **1983**, *39*, 2211-2217.

8 Welzel, P.; Witteler, F.-J.; Müller, D. *Tetrahedron Lett.* **1976**, 1665-

1668.

9 Unpublished results.

10 Huber, G. J.Antibiotics **25**, 226-229 (1972).

11 Chaudhary, S.K.; Hernandez, O. *Tetrahedron Lett.* **1979**, 99-102.

12 Metten, K.-H., Dissertation, Ruhr-Universität Bochum, 1991.

13 Shekhani, M.S.; Khan, K.M.; Mahmood, K. *Tetrahedron Lett.* **1988**, *29*, 6161-6162.

14 Brussee, J.; Dofferhoff, F.; Kruse, C.G.; van der Gen, A. *Tetrahedron* **1990**, *46*, 1653-1658.

15 Winterfeldt, E. *Synthesis* **1975**, 617-630.

16 Kappes, D.; Gerlach, H. *Synth.Commun.* **1990**, *20*, 581-587.

17 Peters, U.; Bankova, W.; Welzel, P. *Tetrahedron* **1987**, *43*, 3803-3816.

18 Metten, K.-H.; Welzel, P. *Tetrahedron* **1990**, *46*, 5145-5154.

19 Classon, B.; Garegg, P.J.; Samuelsson, B. *Acta Chem.Scan., Ser B.* **1984**, *38*, 419-422; Masaki, Y.; Iwata, I.; Mukai, I; Oda, H.; Nagashima, H. *Chem.Lett.* **1989**, 659-662.

20 Schubert, Th.; Hobert, K.; Welzel, P. *Tetrahedron* **1983**, *39*, 2219-2221.

21 a) Tschesche, R.; Reden, J. *Liebigs Ann.Chem.* **1974**, 853-863; b) Grieco, P.A.; Masaki, Y.; Boxler, D. *J.Am.Chem.Soc.* **1975**, *97*, 1597-1599; c) Kocienski, P.J. *J.Org.Chem.* **1980**, *45*, 2037-2039; d) Coates, R.M.; Johnson, M.W. *J.Org.Chem.* **1980**, *45*, 2685-2697; d) Kocienski, P.; Todd, M. *J.Chem.Soc.,Perkin Trans.1* **1983**, 1777-1781; e) Böttger, D.; Welzel, P. *Tetrahedron Lett.* **1983**, *24*, 5201-5204; d) Stumpp, M.C.; Schmidt, R.R. *Tetrahedron* **1986**, *42*, 5941-5948.

22 For new references, see Perich, J.W.; Johns, R.B. *Synthesis* **1988**, 142-144; Perich, J.W.; Johns, R.B. *Tetrahedron Lett.* **1987**, *28*, 101-102; de Bont, H.B.A.; Veeneman, G.H. ; van Boom, J.H.; Liskamp, R.M.J. *Recl.Trav.Chim.Pays-Bas* **1987**, *106*, 641-642; Dreef, C.E.; Elie, C.J.J.; Hoogerhout, P.; van der Marel, G.A.; van Boom, J.H. *Tetrahedron Lett.* **1988**, *29*, 6513-6516; Bannwarth, W.; Trzeciak, A. *Helv.Chim.Acta* **1987**, *70*, 175-186, and references therein; Bannwarth, W.; Küng, E.; *Tetrahedron Lett.* **1989**, *30*, 4219-4222.

23 Cleaved with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN): Uhlmann, E.; Pfleiderer, W. *Tetrahedron Lett.* **1980**, *21*, 1181-1184; Pfleiderer, W.; Uhlmann, E. *Nucleic Acids Res. Special Pub.* **1978**, *4*, 525.

24 Cleaved with zinc dust: Eckstein, F.; Rizk, I. *Chem.Ber.* **1969**, *102*, 2362-2377; van Boom, J.H.; Burgers, P.M.J. *Recl.Trav.Chim.Pays-Bas* **1978**, *97*, 73-80; Adamiak, R.W.; Biala, E; Grzeskowiak, K.; Kierzek, R.; Kraszewski, A.; Markiewicz, W.T.; Stawinski, J.; Wiewiorowski, M. *Nucleic Acids Res.* **1977**, *4*, 2321-2329; cleaved with zinc-copper couple: Catlin, J.C.; Cramer, F. *J.Org.Chem.* **1973**, *38*, 245-250; Imai, J.; Torrence, P.F. *J.Org.Chem.* **1981**, *46*, 4015-4021; for other conditions see Adamiak, R.W.; Biala, E.; Grzeskowiak, K.; Kierzek, R.; Kraszewski, A.; Markiewicz, W.T.; Okupniak, J.; Stawinski, J.; Wiewiorowski, M. *Nucleic Acids Res.* **1978**, *5*, 1889-1905; Ogilvie, K.K.; Beaucage, S.L. *Nucleic Acids Res.* **1979**, *7*, 805-823.

25 a) Ogilvie, K.K.; Theriault, N.Y.; Seifert, J.-M.; Pon, R.T.; Nemer, M.J. *Can.J.Chem.* **1980**, *58*, 2686-2693; b) Westerduin, P.; Veeneman, G.H.; Marugg, J.E.; van der Marel, G.A.; van Boom, J.H. *Tetrahedron Lett.* **1986**, 27, 1211-1214.

[26] a) Tener, G.M. *J.Am.Chem.Soc.* **1961**, *83*, 159-168; b) Sinha, N.D.; Biernat, J.; McManus, J.; Köster, H. *Nucleic Acids Res.* **1984**, *12*, 4539-4557.

[27] Westerduin, P.; Veeneman, G.H.; van Boom, J.H. *Recl.Trav.Chim.Pays-Bas* **1987**, *106*, 601-606.

[28] a) Sood, A.K.; Narang, S.A. *Nucleic Acids Res.* **1977**, *4*, 2757-2765; b) Yau, E.K.; Ma, Y.-X.; Caruthers, M.H. *Tetrahedron Lett.* **1990**, *31*, 1953-1956.

[29] Sung, W.L.; Narang, S.A. *Can.J.Chem.* **1982**, *60*, 111-120.

[30] Uhlmann, E.; Engels, J. *Tetrahedron Lett.* **1986**, *27*, 1023-1026.

[31] a) Wozniak, L.; Kowalski, J.; Chojnowski, J. *Tetrahedron Lett.* **1985**, *26*, 4965-4968; b) Hayakawa, Y.; Uchiyma, M.; Noyori, R. *Tetrahedron Lett.* **1986**, *27*, 4191-4194, 4195-4196.

[32] Handbook of Chemistry and Physics, 56th edition, p. D 147, CRC press, Cleveland 1975-1976.

[33] Corey, E.J.; Schmidt, G. *Tetrahedron Lett.* **1979**, 399-402.

[34] Bal, B.S.; Childers, Jr., W.E.; Pinnick, H.W. *Tetrahedron* **1981**, *37*, 2091-2096; Schwab, J.M.; Klassen, J.B. *J.Am.Chem.Soc.* **1984**, *106*, 7217-7227; Mann, J.; Thomas, A. *Tetrahedron Lett.* **1986**, *27*, 3533-3534; Greck, C.; Grice, P.; Jones, A.B.; Ley, S.V. *Tetrahedron Lett.* **1987**, *28*, 5759-5762.

[35] Review: Heyns, K.; Paulsen, H., Rüdiger, G.; Weyer, J. *Fortschr.Chem.Forsch.* **1968/69**, *11*, 285-374. For some newer examples, see Welzel, P.; Bulian, H.-P.; Maulshagen, A.; Müller, D.; Snatzke, G. *Tetrahedron* **1984**, *40*, 3657-3666; Marino, J.P.; Fernández de la Pradilla, R.; Laborde, E. *J.Org.Chem.* **1987**, *52*, 4898-4913.

[36] van Heijenoort, Y.; Derrien, M.; van Heijenoort, J. *J.FEBS Lett.* **1979**, *89*, 141-144.

[37] Izaki, K.; Matsuhashi, M.; Strominger, J.L. *J.Biol.Chem.* **1968**, *243*, 3180-3192.

[38] Antibiotics in Laboratory Medicine, Lorian, V. (Ed), Baltimore 1986, pages 1-10.

[39] Kessler, H.; Griesinger, C.; Zarbock, J.; Looslie, H.R. *J.Magn.Reson.* **1984**, *57*, 331-336.

[40] Calculated a) using the International Atomic Masses, b) for ^{12}C, ^{1}H, ^{16}O, ^{14}N, ^{28}Si, ^{31}P (mono-isotopic mass)

[41] Böttger, D., Dissertation, Ruhr-Universität Bochum, 1983.

[42] Long range coupling.

[43] 2eq and 2ax, respectively, refers to the spatial disposition (axial or equatorial) of the substituent at C-2 as determined by ^{1}H NMR. For details, see text.