Pergamon

Tetrahedron 57 (2001) 4139–4146

TETRAHEDRON

Moenomycin analogues with modified lipid side chains from indium-mediated Barbier-type reactions

Stefan Vogel,[a] Katherina Stembera,[a] Lothar Hennig,[a] Matthias Findeisen,[a] Sabine Giesa,[a] Peter Welzel[a,*] and Maxime Lampilas[b,*]

[a]*Fakultät für Chemie und Mineralogie der Universität Leipzig, Johannisallee 29, D-04103 Leipzig, Germany*
[b]*Aventis, F-93230 Romainville, France*

Dedicated to Professor Joachim Thiem on the occasion of his 60th birthday

Received 26 January 2001; accepted 6 March 2001

Abstract—From moenomycin A both the chromophore part and the lipid side chain were degraded by ozonolysis to give an analogue with a glycolaldehyde unit in 2-position of the glyceric acid moiety. The aldehyde was converted to a number of homoallylic alcohols by indium-mediated Barbier-type reactions with allylic and benzylic halides. With exception of the phytyl bromide-derived reaction product all compounds were antibiotically inactive.

1. Introduction

The transglycosylation reaction in peptidoglycan biosynthesis is a highly promising target for new antibiotics. The moenomycins,[1] see moenomycin A (**1**, Scheme 1) have been shown to interfere with this biosynthetic step interacting with the enzyme(s).[2] A mechanism for their mode of action has been proposed.[3–5] It is assumed that they are anchored to the cytoplasmic membrane via the lipid part and bind then highly selective to the active site of the enzyme via the C-E-F trisaccharide. Units A, B, and D have been shown to be of minor importance for the antibiotic activity.[6] Whereas the structural requirements for antibiotic activity in the carbohydrate part have been investigated in detail[6,7] much less is known about how the membrane anchoring orientates moenomycin in the correct way for the interaction with the enzyme. Hydrogenation of the lipid part gives a decahydro derivative that is antibiotically fully active.[8] However, it has been shown previously that converting the glyceric acid part into its methyl ester or introducing a single OH group to C-17 or C-18 of the lipid part abolishes the antibiotic activity completely. Similarly, cleavage of the bond between the glyceric acid unit and the lipid part leads to a compound devoid of any antibiotic activity.[9,10] In addition, membrane anchoring is of concern in the context of pharmacokinetics. First results along these lines have been obtained making use of fluorescence methods.[11]

2. Results and discussion

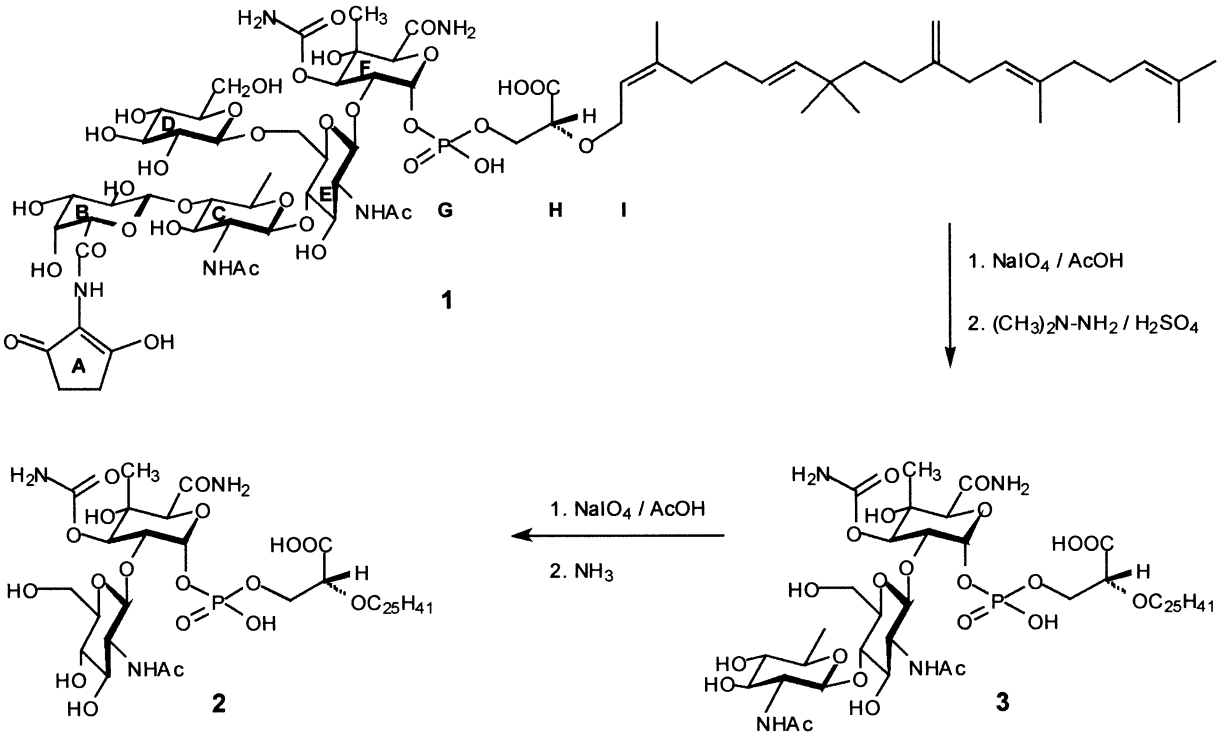
Here we wish to describe reactions that can be used very efficiently to modify the lipid part of moenomycin and truncated analogues derived therefrom.[12] We found that ozonising moenomycin A in methanolic solution leads to a precipitate that is only soluble in water and mixtures of polar solvents and water. After lyophilisation the structural analysis revealed this compound to be aldehyde **4** in which in addition to the removal of the chromophore part[13] the lipid chain was lost (Scheme 2). NMR spectra of **4** in aqueous solution displayed only the hydrate form (^{13}C: δ=89.4), but high resolution ESI MS (solution in 3:7 water-methanol) indicated the presence of the aldehyde, the hydrate and the hemiacetal. Obviously, the primary ozonide breaks down completely in one direction. The yield of the aldehyde in this astonishingly efficient degradation reaction was 98%.

In a similar way under somewhat modified experimental conditions the tri- and disaccharide analogues of moenomycin A (**3** and **2**) were degraded to furnish aldehydes **6** and **8**, respectively. Trisaccharide **3** was obtained from moenomycin A by periodate degradation followed by dimethylhydrazine treatment (Barry degradation). From **3** the disaccharide analogue **2** was obtained by diol cleavage with periodate followed by β-elimination with ammonia as described previously.[1,14] Hydride reduction of aldehydes **4** and **6** provided the corresponding primary alcohols **5** and **7** in high yields (Scheme 1).

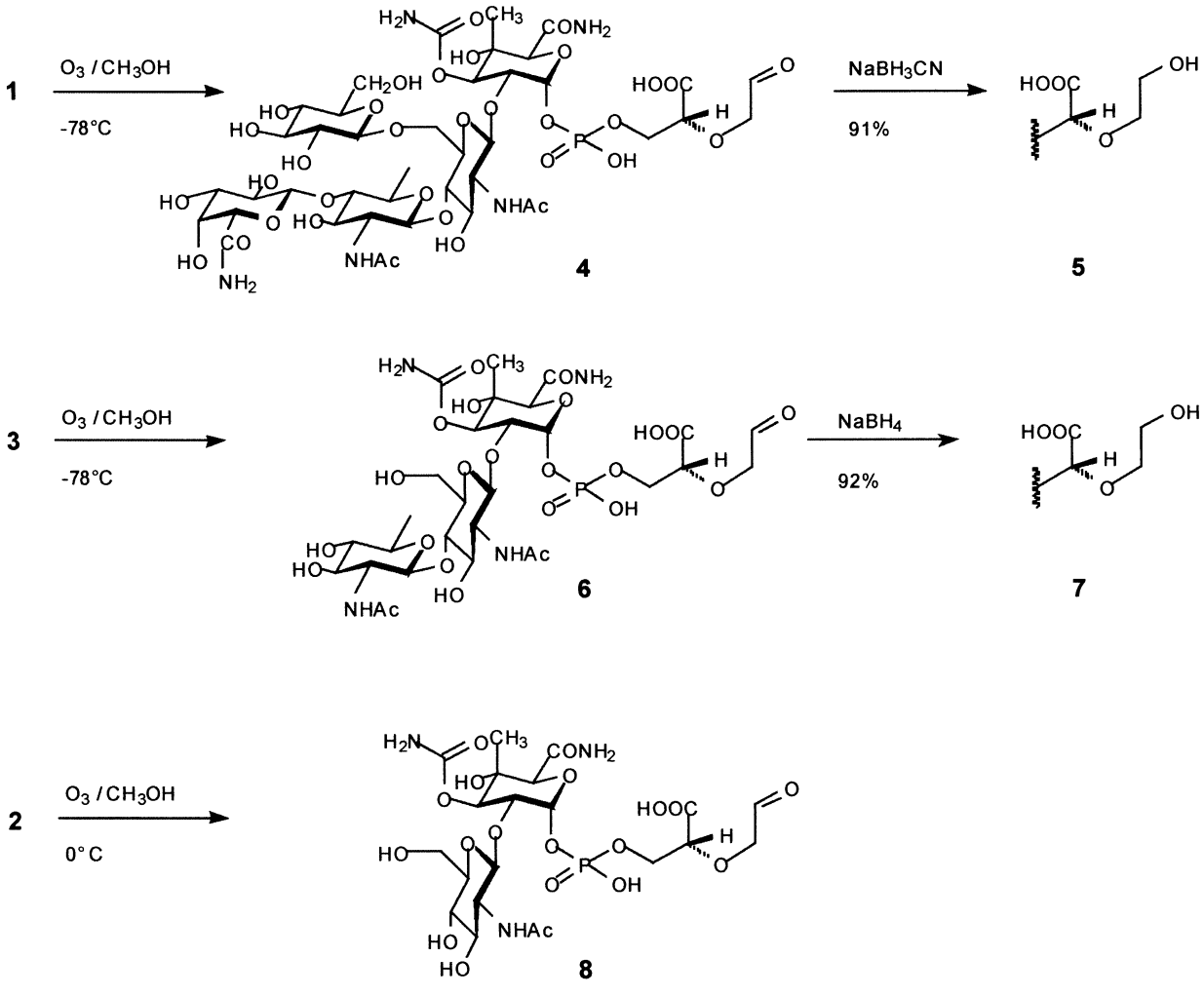
For the conversion of aldehyde **4** into derivatives with new lipid chains reactions are needed that tolerate both an

Keywords: antibiotics; Barbier reactions; phospholipids; carbohydrates.
* Corresponding authors. Tel: 49 341 97 36551; fax: +49 341 9736599; e-mail: welzel@organik.chemie.uni-leipzig.de; Fax: +33 1 49915087; e-mail: Maxime.Lampilas@aventis.com

0040–4020/01/$ - see front matter
PII: S0040-4020(01)00301-5

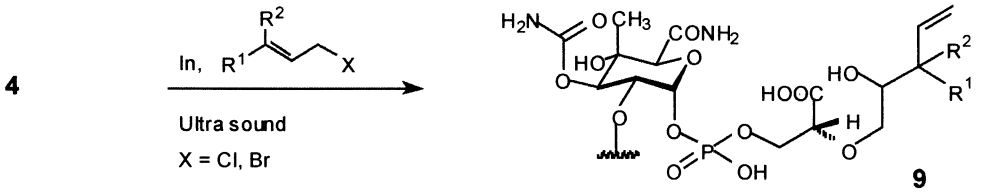


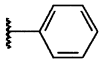
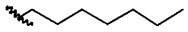
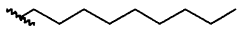
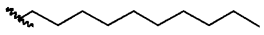
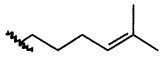
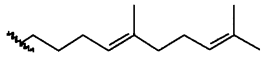
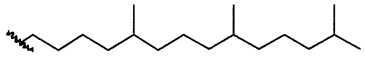
Scheme 1.



Scheme 2.

Table 1. Indium-mediated Barbier reactions of **4**



Product	R^1	R^2	Time [h]	Yield [%]
9a	H	H	12	80
9b	H		24	95
9c	H		24	21
9d	H		24	28
9e	H		24	28
9f	CH_3		24	30
9g	CH_3		24	14
9h	CH_3		24	5

aqueous medium and the many different functional groups of **4**. We expected that indium-mediated Barbier-type reactions in aqueous solution as pioneered by Li and Chan[15] would fulfill these requirements. In the event, reaction of **4** with allyl bromide provided homoallyl alcohol **9a** in 80% yield. The example with cinnamyl chloride demonstrated that chlorides of this type work equally well. The yield of **9b** was 95%. A number of different allyl bromides were submitted to the indium-mediated Barbier reaction with **4**. The yields varied quite considerably as a consequence of the low solubility of some of the bromides in water–methanol mixtures.

The structures of the products and the yields are collected in Table 1. The structures of all compounds were secured by ESI MS and NMR methods (see Experimental). The stereochemistry of the reactions has not been investigated nor have mixtures of stereoisomers been separated.

3. Antibiotic activity of compounds 9

The minimal inhibitory concentrations (MIC) against seven different *Staph. aureus* strains were determined by a micro dilution method on micro titer plates. With the exception of **9h** all compounds turned out to be inactive (Table 2). This result demonstrates once again the high importance of correct membrane anchoring of transglycosylase inhibitors of the moenomycin type and that polar groups in the side chain destroy the antibiotic activity. Example **9h** indicates, however, that by sufficiently elongating the lipid chain antibiotic activity may be recovered.

Table 2. MIC against various test organisms

	MIC [μg/mL]						
Strain	**1**	**9c**	**9d**	**9e**	**9f**	**9g**	**9h**
ATCC 25923	0.125	>32	>32	>32	>32	32	2.0
ATCC 29213	0.060	>32	>32	>32	>32	32	1.3
PEG 18	0.030	>32	>32	>32	>32	32	1.2
PEG 5	0.060	>32	>32	>32	>32	32	1.0
MRSA 1309	0.125	>32	>32	>32	>32	32	1.6
ATCC 6538P	0.038	>32	>32	>32	>32	16	0.6
SG 511	0.125	>32	>32	>32	>32	32	2.0

4. Experimental

General: NMR: GEMINI 200 (Varian), GEMINI 2000 (Varian), GEMINI 300 (Varian), DRX 400 (Bruker), DRX 600 (Bruker); chemical shifts are given in δ values, CH_3, CH_2, CH groups and quaternary carbons when identified by APT are indicated by (-) (CH_3, CH) and (+) (CH_2, C_q), respectively. ^{31}P shifts are referenced to H_3PO_4 as external standard.—Mass spectrometry: FAB MS: VG Autospec (Fisons, 3-nitrobenzylalcohol matrix), ESI MS: FT-ICR-MS Apex II (Bruker Daltonics, water –methanol, negative ion mode).

MIC values were determined by a serial two-fold micro dilution method (Iso-Sensitest medium, Oxoid). A series of decreasing concentrations of the compound under investigation was prepared in the medium. For inoculations $1x10^5$ cfu/mL were used. After 24 h at 37° C the MICs were determined (absence of visible turbidity).

4.1. Structure data

4.1.1. 2-*O*-{2-Acetamido-4-*O*-[2-acetamido-2,6-dideoxy-β-D-glucopyranosyl]-2-deoxy-β-D-glucopyranosyl}-3-*O*-carbamoyl-1-*O*-{[(R)-2-carboxy-2-((2*Z*,6*E*,13*E*)-3,8,8,14,18-pentamethyl-11-methylene-nonadeca-2,6,13,17-tetraen-1-yloxy)-ethoxy]-hydroxyphosphoryl}-4-C-methyl-α-D-glycopyranuronamide (3). To a solution of moenomycin A (800 mg, 505 μmol) in water (3 ml), an oxidation solution (9 ml, prepared from sodium metaperiodate (1.05 g, 5mmol) and sodium acetate trihydrate (1.38 g, 10 mmol) dissolved in 50% aqueous acetic acid (12 ml) was

heated to 80°C until a clear solution resulted) was added at 40°C. After stirring in the dark for 4.5 h and cooling to 20°C precipitated salts were removed by filtration. Soluble inorganic salts were removed by chromatography on HP-20 material (200 g, elution with H_2O, 600 ml and then methanol, 1000 ml). The combined methanolic fractions were concentrated to yield 825.3 mg of a brownish residue. The residue was dissolved in 3 ml of water and 3 ml of a *N,N*-dimethylhydrazine solution (*N,N*-dimethylhydrazine (0.94 ml, 12 mmol) was dissolved in 2-propanol (2.8 ml) and sulfuric acid (1 M was added until pH 4.5 was reached) was added at 60°C. After stirring for 5 h at 85°C and cooling to 20°C inorganic salts were removed by HP-20 chromatography (200g, elution with H_2O, 600 ml and methanol, 1000 ml). The crude product was purified by FC ($CHCl_3$-CH_3OH-H_2O 18:11:2.7) and freeze-dried to give pure **3** (167.9 mg, 29%).- ^{1}H NMR (H,H COSY, 400MHz, CD_3OD): characteristic signals at δ=0.85 (s, CH_3-23, CH_3-24^I), 1.13 (s, CH_3-4^F), 1.21 (d, CH_3-6^C), 1.20–1.30 (m, CH_2-9^I), 1.49, 1.50, 1.56, 1.65 (s, CH_3-19^I, s, CH_3-20^I, s, CH_3-21^I, s, CH_3-25^I), 1.76–1.81 (m, CH_2-10^I), 1.94 (s, $NHCOCH_3^E$, $NHCOCH_3^C$), 1.85–2.10 (m, CH_2-16^I, CH_2-15^I, CH_2-5^I, CH_2-4^I), 2.58 (d, CH_2-12^I), 4.97–5.03 (H-3^F, H-13^I, H-17^I), 5.15–5.35 (H-2^I, H-6^I, H-7^I), 5.82 (m, H-1^F), $J_{5C\text{-}6C}$=6.0 Hz, $J_{12I-13I}$=7.1 Hz.- ^{13}C NMR (APT, 150 MHz, CD_3OD): δ=15.42, 15.61 (CH_3-4^F, C-21^I), 17.07, 17.18 (C-6^C, C-20^I), 22.41, 22.66 ($NHCOCH_3^E$, $NHCOCH_3^C$), 23.34 (C-25^I), 25.17 (C-19^I), 26.81 (C-16^I), 27.09 (C-23^I, C-24^I), 31.46 (C-10^I), 31.81 (C-5^I), 32.55 (C-4^I), 35.07 (C-12^I), 35.55 (C-8^I), 39.93 (C-15^I), 41.97 (C-9^I), 55.20–56.33 (C-2^E, C-2^C), 60.31 (C-6^E), 65.93 (C-1^H, C-1^I), 70.73–79.49 (broad signals) (C-5^C, C-3^C, C-5^F, C-3^E, C-2^F, C-4^F, C-5^E, C-3^F), 80.80 (C-4^E), 94.98 (C-1^F), 102.38, 103.09 (C-1^C, C-1^E), 108.55 (C-22^I), 121.95 (b, C-2^I, (?)), 122.54 (C-13^I), 124.46 (C-17^I), 125.89 (C-6^I), 131.27 (C-18^I), 136.38 (C-14^I), 140.74 (C-3, 7^I), 150.01 (C-11^I), 158.58 ($OCONH_2^F$), 172.97–173.48 ($NHCOCH_3^E$, $CONH_2^F$), 176.91 (C-3^H).- ^{31}P NMR (81 MHz, D_2O): δ=-0.48.- $C_{52}H_{85}N_4O_{22}P$ (1149.24, 1148.54), ESI MS (negative mode): m/z=1147.53536 (1147.53203) $[M-H]^-$, 573.26232 (573.26380) $[M-2H]^{2-}$.

4.1.2. 2-*O*-{2-Acetamido-2-deoxy-β-D-glucopyranosyl)-3-*O*-carbamoyl-1-*O*-{[(R)-2-carboxy-2-((2*Z*,6*E*,13*E*)-3,8,8,14,18-pentamethyl-11-methylene-nonadeca-2,6,13,17-tetraen-1-yloxy)-ethoxy]-hydroxyphosphoryl}-4-*C*-methyl-α-D-glycopyranuronamide (2). To a solution of **3** (30 mg, 26 μmol) in water (50 μl) a $NaIO_4$ oxidation solution (vide supra, 300 μl) was added. After stirring for 5 h at 40°C ethanediol (15 μl) was added and the solution was stirred for 1 h. The mixture was cooled to 0°C and 25% aqueous NH_3 (1 ml) was added. After stirring for 12 h at 0°C the mixture was neutralised with acetic acid and purified by Sephadex LH-20® gel filtration (H_2O-CH_3OH 1:4). Ultrafiltration of the crude product and solvent evaporation yielded **2** (15.8 mg, 64%).- ^{1}H NMR (H,H COSY, 400MHz, CD_3OD): characteristic signals at δ=0.95 (bs, CH_3-23, CH_3-24^I), 1.23 (s, CH_3-4^F, signal doubling), 1.15–1.40 (m, CH_2-9^I), 1.59, 1.60, 1.66, 1.73 (s, CH_3-19^I, s, CH_3-20^I, s, CH_3-21^I, s, CH_3-25^I), 1.86–1.91 (m, CH_2-10^I), 2.01 (s, $NHCOCH_3^E$), 1.95–2.20 (m, CH_2-16^I, CH_2-15^I, CH_2-5^I, CH_2-4^I), 2.68 (d, CH_2-12^I), 5.08–5.15 (H-3^F, H-13^I, H-17^I), 5.25–5.45 (H-2^I, H-6^I, H-7^I), 5.94 (m, H-1^F), $J_{12I-13I}$=7.3 Hz.- ^{13}C NMR (50 MHz, CD_3OD): δ=16.25 (CH_3-4^F, C-21^I), 17.87 (C-20^I), 23.46 ($NHCOCH_3^E$), 23.97 (C-25^I), 27.83 (C-19^I, C-16^I, C-23^I, C-24^I), 32.09–33.29 (C-5^I, C-10^I, C-4^I), 35.93 (C-8^I), 36.36 (C-12^I), 40.69 (C-15^I), 42.71 (C-9^I), 57.13 (C-2^E), 62.20 (C-6^E), 67.08, 68.41 (C-1^I, C-1^H), 71.49–79.15 (C-5^F, C-3^E, C-2^F, C-4^F, C-5^E, C-3^F, C-4^E), 95.87 (C-1^F), 103.88 (C-1^E), 109.36 (C-22^I), 122.84 (C-2^I), 123.40 (C-13^I), 125.19 (C-17^I), 126.83 (C-6^I), 141.42 (C-3^I, C-7^I), 159.33 ($OCONH_2^F$), 174.29–174.49 ($NHCOCH_3^E$, $CONH_2^F$).– ^{31}P NMR (162 MHz, D_2O) δ=-1.66.- $C_{44}H_{72}N_3O_{18}P$ (962.04, 961.48), FAB MS: m/z=1000.2 $[M+K]^+$, 984.3 $[M+Na]^+$.

4.1.3. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryl-oxy)-2-(2-oxoethoxy)-propionic acid (4). A solution of moenomycin A (1061.7 mg, 671 μmol) in methanol (15 ml) was cooled to −78°C. The solution was saturated with O_3/O_2 (Fischer OZON 502, flow rate 50 l/h=2 g/h O_3) at -78°C until the a light-blue colour persisted (ca. 30 min). Then oxygen was bubbled through the solution and the mixture was then allowed to warm to 20°C. The precipitate was collected by filtration and rinsed with cold methanol. The product was dissolved in water and lyophilised to give **4** (98%, 786.4 mg).- ^{1}H NMR (H,H COSY, 400 MHz, D_2O): characteristic signals at δ=1.06 (s, CH_3-4^F), 1.22 (d, CH_3-6^C), 1.91, 1.94 (s, $NHCOCH_3^E$, s, $NHCOCH_3^C$), 3.14 (dd, H-2^D), 3.66-3.75 (H_X-6^D), 4.06 (s, H-4^B, H-5^B), 4.28 (H-5^F), 4.35 (d, H-1^D), 4.85 (d, H-3^F), 5.02 (dd, H-2^I), 5.63 (q, H-1^F), $J_{5C\text{-}6C}$=6.0 Hz, $J_{2D\text{-}3D}$=8.1 Hz, $J_{1D\text{-}2D}$=7.8 Hz, $J_{2F\text{-}3F}$=10.2 Hz, $J_{1F\text{-}2F}$=3.5 Hz, $^3J_{1F\text{-}P}$=6.0 Hz.- ^{13}C NMR (APT, HMBC, HMQC, 100 MHz, D_2O): δ=15.63 (CH_3-4^F), 17.55 (CH_3-6^C), 23.23, 23.33 ($NHCOCH_3^E$, $NHCOCH_3^C$), 56.01, 56.40 (C-2^E, C-2^C), 61.70 (C-6^D), 67.56 (d, C-3^H), 69.75 (+), 69.82, 70.62, 71.50, 72.03, 72.91, 73.10, 73.42, 73.72, 73.84 (+), 73.96 (+), 74.05, 74.38, 75.15, 75.78, 76.70, 76.91, 77.32, 77.41 (C-5^C, C-5^F, C-3^C, C-4^D, C-4^B, C-2^B, C-3^B, C-6^E, C-5^B, C-3^E, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D, C-1^I), 80.65 (C-4^E), 81.28 (m, C-2^H), 83.95 (C-4^C), 89.39 (m, C-2^I), 95.25 (d, C-1^F), 102.14, 103.01, 103.51, 104.11 (C-1^C, C-1^E, C-1^B, C-1^D), 159.07 ($OCONH_2^F$), 173.61 ($CONH_2^F$), 174.08 ($CONH_2^B$), 175.16, 175.48 ($NHCOCH_3^E$, $NHCOCH_3^C$), 176.43 (C-1^H).- ^{31}P NMR (81 MHz, D_2O): δ=-0.93.- $C_{41}H_{66}N_5O_{33}P$ (1187.96, 1187.33777, aldehyde), $C_{41}H_{67}N_5O_{34}P$ (1205.97, 1205.34833, aldehyde hydrate), $C_{42}H_{70}N_5O_{34}P$ (1220.00, 1219.36343, hemiacetal), ESI MS (negative mode): m/z=1186.33972 (1186.32983) $[M-H]^-$, 592.66422 (592.66161) $[M-2H]^{2-}$ (aldehyde), 601.67005 (601.66889) $[M-2H]^{2-}$ (aldehyde hydrate), 1218.37262 (1218.35561) $[M-H]^-$ (hemiacetale).

4.1.4. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryl-oxy)-2-(2-hydroxyethoxy)-propionic acid (5). To a solution of **4** (100 mg, 84 μmol) in water (500 μl) a solution of $NaBH_3CN$ (10.6 mg, 168 μmol) in water (200 μl) was

added and the solution was stirred for 12 h at 20°C. The reaction mixture was directly applied to a Sephadex LH-20® column (H_2O-CH_3OH 1:4). After evaporation of solvents and lyophilisation **5** was obtained (91%, 91.2 mg).- ^{1}H NMR (H,H COSY, 400MHz, D_2O): characteristic signals at δ=1.12 (s, CH_3-4^F), 1.29 (d, CH_3-6^C), 1.93, 2.00 (s, NHCOCH_3^E, s, NHCOCH_3^C), 3.29 (dd, H-2^D), 3.80-3.90 (H_X-6^D), 4.13 (s, H-4^B, H-5^B), 4.35 (s, H-5^F), 4.92 (d, H-3^F), 5.69 (q, H-1^F), $J_{5C\text{-}6C}$=5.9 Hz, $J_{2D\text{-}3D}$=9.3 Hz, $J_{2F\text{-}3F}$=10.7 Hz, $J_{1F\text{-}2F=}$3.5 Hz, $^3J_{1F\text{-}P}$= 5.7 Hz.- ^{13}C NMR (50 MHz, D_2O, APT): δ=14.89 (CH_3-4^F), 16.80 (CH_3-6^C), 22.49, 22.58 (NHCO*C*H_3^E, NHCO*C*H_3^C), 55.33 (C-2^E), 55.67 (C-2^C), 60.87, 60.97 (C-2^I, C-6^D), 66.99 (d, C-3^H), 69.07, 69.89, 70.75, 71.38, 71.44 (+), 72.19, 72.35, 72.66, 73.00, 73.23 (+), 73.30, 73.65, 74.41, 75.05, 75.96, 76.18, 76.52, 76.69 (C-5^C, C-3^C, C-5^F, C-4^D, C-4^B, C-2^B, C-3^B, C-6^E, C-5^B, C-3^E, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D, CH_2-1^I), 79.94 (C-4^E), 80.66 (d, C-2^H), 83.21 (C-4^C), 94.52 (d, C-1^F), 101.38, 102.24, 102.77, 103.38 (C-1^C, C-1^E, C-1^B, C-1^D), 158.33 (O*C*ONH_2^F), 172.86 (CONH_2^F), 173.34 (CONH_2^B), 174.39, 174.73 (NH*C*OCH$_3^E$, NH*C*OCH$_3^C$), 176.50 (C-1^H).- ^{31}P NMR (81 MHz, D_2O): δ=-1.49.- $C_{41}H_{68}N_5O_{33}P$ (1189.98, 1189.35341) ESI MS (negative mode): m/z= 1189.36641 (1189.35341) [M-H]$^-$, 593.67260 (593.66943) [M-2H]$^{2-}$.

4.1.5. (*R*)-3-{[2-Acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy]-hydroxyphosphoryloxy}-2-(2-oxoethoxy)-propionic acid (6). A solution of **3** (100 mg, 87 μmol) in methanol (1.5 ml) was cooled to -78°C. The solution was saturated with O_3/O_2 (Fischer OZON 502, flow rate 50 l/h=2 g/h O_3) at -78°C until a light-blue colour persisted (ca. 30 min) and 30 min at 0°C. Next oxygen was bubbled through the solution and the mixture was then allowed to warm to 20°C. The precipitate was collected by filtration and rinsed with cold methanol. The product was dissolved in water and lyophilised to give **6** (38 mg, 51%).- ^{1}H NMR (H,H COSY, DQF-COSY, 400 MHz, D_2O): δ=1.22 (s, CH_3-4^F), 1.30 (d, CH_3-6^C), 2.03, 2.07 (s, NHCOCH_3^E, s, NHCOCH_3^C), 3.22 (t, H-4^C), 4.45 (s, H-5^F), 4.53 (d, H-1^C), 4.63 (d, H-1^E), 5.02 (d, H-3^F), 5.22 (dd, H-2^I), 5.86 (q, H-1^F), $J_{5C\text{-}6C}$=6.0 Hz, $J_{3C\text{-}4C}$=9.0 Hz, $J_{1C\text{-}2C}$=10.5 Hz, $J_{1E\text{-}2E}$=8.0 Hz, $J_{2F\text{-}3F}$=10.5 Hz, $J_{1IA\text{-}2I}$=$J_{1IB\text{-}2I}$=4.5 Hz, $J_{1F\text{-}2F=}$3.5 Hz, $^3J_{1F\text{-}P}$=7.0 Hz.- ^{13}C NMR (APT, HETCOR, 100 MHz, D_2O): δ=14.18 (CH_3-4^F), 16.15 (CH_3-6^C), 21.65, 21.79 (NHCO*C*H_3^E, NHCO*C*H_3^C), 54.49, 55.23 (C-2^E, C-2^C), 59.70 (C-6^E), 65.48 (m, C-3^H), 71.48, 71.90 (+), 72.14, 72.31, 72.43 (+), 72.77, 73.78, 74.13, 74.43, 76.72, 76.80 (C-5^C, C-3^E, C-3^C, C-2^F, C-4^F, C-5^E, C-3^F, C-4^C, C-5^F, C-1^I), 79.31 (C-4^E), 79.80 (bs, C-2^H), 87.76 (C-2^I), 93.93 (d, C-1^F), 101.00, 101.54 (C-1^C, C-1^E), 157.76 (O*C*ONH_2^F), 172.12, 173.66, 174.09 (CONH_2^F, NH*C*OCH$_3^E$, NH*C*OCH$_3^C$).- ^{31}P NMR (81 MHz, D_2O) δ=-1.05.- $C_{29}H_{47}N_4O_{23}P$ (850.68, 850.23, aldehyde), $C_{29}H_{49}N_4O_{24}P$ (868.69, 868.25, aldehyde hydrate), ESI MS (negative mode): m/z=849.4 [M-H]$^-$, 423.3 [M-2H]$^{2-}$ (aldehyde), 867.4 [M-H]$^-$, 432.2 [M-2H]$^{2-}$ (aldehyde hydrate).

4.1.6. (*R*)-3-{[2-Acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(12)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy]hydroxyphosphoryloxy}-2-(2-hydroxyethoxy)-propionic acid (7). To a solution of **6** (14.8 mg, 17 μmol) in water (500 μl) a solution of $NaBH_4$ (1.3 mg, 34 μmol) in water (500 μl) was added and the solution was stirred for 12 h. The reaction mixture was directly applied to a Sephadex PD-10® column (H_2O). After solvent evaporation and lyophilisation **7** (92%, 13.6 mg) was obtained.- ^{1}H NMR (H,H COSY, 400 MHz, D_2O): characteristic signals at δ=1.10 (s, CH_3-4^F), 1.18 (d, CH_3-6^C), 1.91, 1.94 (s, NHCOCH_3^E, s, NHCOCH_3^C), 4.90 (d, H-3^F), 5.74 (m, H-1^F), $J_{5C\text{-}6C}$=4.6 Hz, $J_{2F\text{-}3F}$=10.2 Hz.- ^{13}C NMR (50 MHz, D_2O): δ=14.82 (CH_3-4^F), 16.81 (CH_3-6^C), 22.32, 22.47 (NHCO*C*H_3^E, NHCO*C*H_3^C), 55.37, 55.88 (C-2^E, C-2^C), 60.41, 60.88 (C-6^E, C-2^I), 67.02 (d, C-3^H), 71.37, 72.13, 72.71, 72.97, 73.09, 73.47, 74.47, 74.79, 75.11, 77.41 (C-5^C, C-3^E, C-3^C, C-2^F, C-4^F, C-5^E, C-3^F, C-4^C, C-5^F, C-1^I), 80.09 (C-4^E), 81.05 (d, C-2^H), 94.58 (C-1^F), 101.67, 102.11 (C-1^C, C-1^E), 158.43 (O*C*ONH_2^F), 172.73, 174.30, 174.77 (CONH_2^F, NH*C*OCH$_3^E$, NH*C*OCH$_3^C$), 176.96 (C-1^H).- ^{31}P NMR (81 MHz, D_2O) δ=-2.30.- $C_{29}H_{49}N_4O_{23}P$ (852.69, 852.25) ESI MS (negative mode): m/z=873.22296 (873.22664) [M-2H+Na]$^-$.

4.1.7. (*R*)-3-{[2-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyran-uronamidosyloxy]hydroxyphosphoryloxy}-2-(2-oxoethoxy)-propionic acid (8). A solution of **2** (20 mg, 17 μmol) in methanol (2 ml) was cooled to -78°C. The solution was saturated with O_3/O_2 (Fischer OZON 502, flow rate 50 l/h=2 g/h O_3) at -78°C until a light-blue colour persisted (ca. 60 min) and was then stirred at 0°C for 60 min. Oxygen was bubbled through the solution and the mixture was allowed to warm to 20°C. CH_2Cl_2 (0.5 ml) was added and the precipitate was collected by filtration and rinsed with cold methanol. The crude product was purified by FC (ethyl acetate-2-propanol-H_2O 4:5:4) and a Sephadex PD-10® column (H_2O). The product was dissolved in water and freeze-dried to give **8** (10.8 mg, 64%).- ^{1}H NMR (400 MHz, D_2O): characteristic signals at δ=1.14 (s, CH_3-4^F), 2.03 (s, NHCOCH_3^E), 4.37 (H-5^F), 4.95 (d, H-3^F), 5.78 (m, H-1^F), $J_{2F\text{-}3F}$=10.2 Hz.- ^{13}C NMR (100 MHz, D_2O, broad signals): δ=15.24 (CH_3-4^F), 22.93 (NHCO*C*H_3^E), 61.41 (C-6^E), 70.47, 72.87, 73.41, 73.54, 75.26, 76.33, 77.80, 77.94 (C-3^E, C-2^F, C-5^F, C-3^C, C-4^F, C-5^E, C-3^F, C-1^I, C-2^H), 95.02 (C-1^F), 102.85 (C-1^E), 158.89 (O*C*ONH_2^F), 173.18, 174.84 (CONH_2^F, NH*C*OCH$_3^E$).- ^{31}P NMR (162 MHz, D_2O) δ=-1.15.- $C_{21}H_{34}N_3O_{19}P$ (663.48, 663.15).

4.1.8. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-((*Ξ*)-2-hydroxy-4-pentenyloxy)propionic acid (9a). To a solution of **4** (21.3 mg, 18 μmol) in a mixture of water (600 μl) and methanol (400 μl) allyl bromide (4.3 mg, 36 μmol) and indium (4.1 mg, 36 μmol) were added and the mixture was sonicated for 10 min. The mixture then was stirred rapidly at 20°C for 12 h. After solvent evaporation the residue was redissolved in water and filtered (MILLEX LCR_{13}™). The filtrate was directly applied to a Sephadex PD-10™ column (water) and the eluent freeze-dried to give **9a** (18.2 mg,

80%).- ^{1}H NMR (400MHz, D_2O): characteristic signals at δ=1.13 (s, CH_3-4^F), 1.29 (d, CH_3-6^C), 1.94, 2.00 (s, $NHCOCH_3$^{E}, s, $NHCOCH_3$^{C}), 4.13 (s, H-4^B, H-5^B), 4.35 (s, H-5^F), 4.49 (d, H-1^D), 4.92 (d, H-3^F), 5.03 (d, CH=C*H*$_{trans}$-5^I), 5.03 (d, CH=C*H*$_{cis}$-5^I), 5.69 (q, H-1^F), 5.70–5.80 (m, C*H*=CH_2-4^I), $J_{4\text{-}5\text{-}cis}$=9.1 Hz, $J_{4\text{-}5\text{-}trans}$= 15.6 Hz.-^{13}C NMR (100 MHz, D_2O): δ=14.25 (CH_3-4^F), 16.21 (CH_3-6^C), 21.85 ($NHCOCH_3$^{E}, $NHCOCH_3$^{C}), 36.49 (C-3^I), 54.71, 55.06 (C-2^E, C-2^C), 60.37 (C-6^D), 68.41 (C-3^H), 69.28, 70.15, 70.69, 71.57, 71.76, 72.05, 72.63, 73.08, 73.42, 73.86, 74.51, 75.36, 75.57 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-3^C, C-5^F, C-6^E, C-5^B, C-3^E, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D, C-1^I, C-2^I), 79.34 (C-4^E), 79.90 (C-2^H), 82.60 (C-4^C), 93.96 (C-1^F), 100.84, 101.80, 102.19, 102.82 (C-1^C, C-1^E, C-1^B, C-1^D), 117.35 (C-5^I), 134.00 (C-4^I), 157.74 (O*C*ONH_2^{F}), 172.33, 172.80 ($CONH_2$^{B}, $CONH_2$^{F}), 174.20 (NH*C*OCH_3^{E}, NH*C*OCH_3^{C}).- ^{31}P NMR (81 MHz, D_2O): δ=-1.53.- $C_{44}H_{72}N_5O_{33}P$ (1230.05, 1229.38) g/mol, ESI MS (negative mode): m/z=1250.35304 (1250.35927) $[M\text{-}2H+Na]^-$, 1228.37638 (1228.37744) $[M\text{-}H]^-$, 613.68415 (613.68508) $[M\text{-}2H]^{2-}$, FAB MS: m/z=1252.3 $[M+Na]^+$, 1230.2 $[M+H]^+$.

4.1.9. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-((2Ξ,3Ξ)-2-hydroxy-3,7-dimethyl-3-vinyl-6-octenyloxy)-propionic acid (9f). To a solution of **4** (60 mg, 50 μmol) in a mixture of water (400 μl) and methanol (1.6 ml) geranyl bromide (43.8 mg, 200 μmol) and indium (23.2 mg, 200 μmol) were added. The mixture was sonicated for 60 min and then stirred at 20°C for 24 h. The crude reaction mixture was directly applied to a Sephadex™ LH-20 column (H_2O-CH_3OH 1:4). Product fractions were concentrated and freeze-dried. The crude product was purified by FC (ethyl acetate-2-propanol-H_2O 6:4:2) and subsequently by gel filtration (Sephadex LH-20®, H_2O-CH_3OH 1:4). The combined fractions were concentrated and freeze-dried to give **9f** (20.3 mg, 30%).- ^{1}H NMR (H,H COSY, 400MHz, D_2O): characteristic signals at δ=0.96 (s, CH_3-10^I), 1.18 (s, CH_3-4^F), 1.33 (d, CH_3-6^C), 1.35–1.40 (m, CH_2-4^I), 1.56, 1.64 (s, CH_3-8^I, CH_3-9^I), 1.85–1.93 (m, CH_2-5^I), 1.99, 2.05 (s, $NHCOCH_3$^{E}, s, $NHCOCH_3$^{C}), 3.26 (dd, H-2^D), 4.18 (s, H-4^B, H-5^B), 4.41 (s, H-5^F), 4.47 (d, H-1^D), 4.98 (d, H-3^F), 5.03 (m, CH=C*H*$_{trans}$-12^I), 5.12 (m, CH=C*H*$_{cis}$-12^I), 5.05 (m, C*H*=$C(CH_3)_2$-6^I), 5.75 (m, H-1^F), 5.75–5.85 (m, C*H*=CH_2-11^I), $J_{5C\text{-}6C}$=4.9 Hz, $J_{2D\text{-}3D}$=8.3 Hz, $J_{1D\text{-}2D}$=8.8 Hz, $J_{2F\text{-}3F}$=10.8 Hz, $J_{11I\text{-}12I\text{-}cis}$=11.3 Hz, $J_{11I\text{-}12I\text{-}trans}$=18.1 Hz.-^{13}C NMR (100 MHz, D_2O): δ=15.19 (CH_3-4^F), 16.21, 16.70, 16.79 (CH_3-6^C, C-9^I, C-5^I), 22.27–22.43 ($NHCOCH_3$^{E}, $NHCOCH_3$^{C}, C-8^I), 24.83 (C-10^I), 37.62 (C-4^I), 42.94 (C-3^I), 55.38–55.60 (C-2^E, C-2^C), 61.43 (C-6^D), 69.31–77.24 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-3^C, C-5^F, C-6^E, C-5^B, C-3^E, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D, C-1^I, C-2^I, C-2^H), 80.95 (C-4^E), 83.51 (C-4^C), 94.68 (C-1^F), 101.78, 102.42, 103.16, 103.43 (C-1^C, C-1^E, C-1^B, C-1^D), 113.49 (C-12^I), 124.86 (C-6^I), 131.22 (C-7^I), 143.48 (C-11^I), 158.07 (O*C*ONH_2^{F}), 172.89–173.54 ($CONH_2$^{B}, $CONH_2$^{F}, NH*C*OCH_3^{E}, NH*C*OCH_3^{C}).- ^{31}P NMR (81 MHz, D_2O): δ=-2.09.- $C_{51}H_{84}N_5O_{33}P$ (1326.23, 1325.47), ESI MS (negative mode): m/z=1324.49514 (1324.47134) $[M\text{-}H]^-$, 661.73478 (661.73203) $[M\text{-}2H]^{2-}$.

4.1.10. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-((2Ξ,3Ξ)-2-hydroxy-3-phenyl-4-pentenyl-oxy)-propionic acid (9b). 9b was prepared from **4** (60.3 mg, 50 μmol) and cinnamyl chloride (38.7 mg, 200 μmol) as described for **9f**. Yield: 63mg, 95%.- ^{1}H NMR (H,H COSY, D_2O, 400MHz): characteristic signals at δ=1.19 (s, CH_3-4^F), 1.32 (d, CH_3-6^C), 1.99, 2.04 (s, $NHCOCH_3$^{E}, s, $NHCOCH_3$^{C}), 3.27 (dd, H-2^D), 4.17 (s, H-4^B, H-5^B), 4.40 (s, H-5^F), 4.46 (d, H-1^D), 4.98 (d, H-3^F), 5.09 (d, CH=C*H*$_{cis}$-11^I), 5.15 (d, CH=C*H*$_{trans}$-11^I), 5.75 (m, H-1^F), 6.00–6.12 (m, C*H*=CH_2-10^I), 7.26–7.39 (m, H-5^I–H-9^I), $J_{5C\text{-}6C}$= 5.3 Hz, $J_{2D\text{-}3D}$=8.5 Hz, $J_{1D\text{-}2D}$=7.8 Hz, $J_{2F\text{-}3F}$=10.3 Hz, $J_{10I\text{-}11I\text{-}cis}$=10.2 Hz, $J_{10I\text{-}11I\text{-}trans}$=17.3 Hz.- ^{13}C NMR (100 MHz, D_2O): δ=15.03 (CH_3-4^F), 16.90 (CH_3-6^C), 22.56, 22.65 ($NHCOCH_3$^{E}, $NHCOCH_3$^{C}), 53.50 (C-3^I), 55.24, 55.76 (C-2^E, C-2^C), 61.05 (C-6^D), 66.97 (C-3^H), 68.90, 69.15, 69.94, 70.85, 71.37, 72.18, 72.46, 72.59, 72.73, 72.84, 73.17, 73.28, 73.35, 73.76, 74.50, 75.13, 76.07, 76.27, 76.37, 76.47 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-3^C, C-5^F, C-6^E, C-5^B, C-3^E, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D, C-1^I, C-2^I), 79.88 (C-4^E), 80.21 (C-2^H), 83.25 (C-4^C), 94.80 (C-1^F), 101.45, 102.41, 102.83, 103.42 (C-1^C, C-1^E, C-1^B, C-1^D), 117.28 (C-11^I), 127.29 (C-7^I), 128.69, 129.22 (C-5^I, C-9^I, C-6^I, C-8^I), 138.64 (C-10^I), 141.45 (C-4^I), 158.37 (O*C*ONH_2^{F}), 172.92–174.82 ($CONH_2$^{B}, $CONH_2$^{F}, NH*C*OCH_3^{E}, NH*C*OCH_3^{C}).- ^{31}P NMR (81 MHz, D_2O) δ=-1.43.- $C_{50}H_{76}N_5O_{33}P$ (1306.15, 1305.42), ESI MS (negative mode): m/z=1304.40652 (1304.40874) $[M\text{-}H]^-$, 651.70161 (651.70031) $[M\text{-}2H]^{2-}$, FAB MS: m/z=1306.2 $[M+H]^+$.

4.1.11. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-((2Ξ,3Ξ,6*E*)-2-hydroxy-3,7,11-trimethyl-3-vinyl-6,10-dodecadienyloxy)-propionic acid (9g). 9g was prepared from **4** (60 mg, 50 μmol) and (*E,E*)-farnesyl bromide (57.6 mg, 200 μmol) as described for **9f**. Yield: 9.8 mg, 14%.- ^{1}H NMR (H,H COSY, 400MHz, D_2O, assigments were made according to the geranyl derivative): characteristic signals at δ=0.95 (s, CH_3-15^I), 1.11, 1.12 (3H, ?), 1.17 (s, CH_3-4^F), 1.33 (d, CH_3-6^C), 1.36–1.40 (m, CH_2-4^I), 1.53 (CH_3-14^I), 1.57, 1.64 (s, CH_3-12^I, s, CH_3-13^I), 1.85–1.92 (m, CH_2-5^I), 1.95–2.00 (m, CH_2-8^I), 2.02–2.10 (m, CH_2-9^I), 1.98, 2.05 (s, $NHCOCH_3$^{E}, s, $NHCOCH_3$^{C}), 3.25 (dd, H-2^D), 4.17 (s, H-4^B, H-5^B), 4.40 (s, H-5^F), 4.46 (d, H-1^D), 4.96 (d, H-3^F), 5.03 (d, CH=C*H*$_{trans}$-17^I), 5.12 (d, CH=C*H*$_{cis}$-17^I), 5.13–5.20 (m, C*H*=$C(CH_3)_2$-10^I, C*H*=$C(CH_3)$-CH_2-6^I), 5.75 (m, H-1^F), 5.76–5.81 (m, C*H*=CH_2-11^I), $J_{5C\text{-}6C}$=5.8 Hz, $J_{2D\text{-}3D}$=7.8 Hz, $J_{1D\text{-}2D}$=7.8 Hz, $J_{2F\text{-}3F}$= 10.5 Hz, $J_{16I\text{-}17I\text{-}cis}$=11.5 Hz, $J_{16I\text{-}17I\text{-}trans}$=17.8 Hz.- ^{13}C NMR (100 MHz, D_2O): δ=16.01, 16.13 (CH_3-14^I, CH_3-4^F), 17.77 (CH_3-6^C, C-13^I), 23.13 (C-5^I), 23.37–23.43 ($NHCOCH_3$^{E}, $NHCOCH_3$^{C}), 25.86 (CH_3-12^I, CH_3-15^I), 27.34 (C-9^I), 38.53

(C-4^{I}), 40.41 (C-8^{I}), 56.44–56.54 (C-2^{E}, C-2^{C}), 62.28 (C-6^{D}), 68.61 (C-1^{I}), 69.70 (d, C-3^{H}), 70.22, 71.25, 71.91, 72.32, 73.42, 73.70, 74.17, 74.53, 74.64, 75.55, 75.94, 76.86, 77.34, 77.47 (C-5^{C}, C-4^{D}, C-4^{B}, C-2^{B}, C-3^{B}, C-3^{C}, C-5^{F}, C-6^{E}, C-5^{B}, C-3^{E}, C-2^{F}, C-4^{F}, C-5^{E}, C-2^{D}, C-3^{F}, C-5^{D}, C-3^{D}, C-2^{I}), 81.70 (C-4^{E}), 84.36 (C-4^{C}), 95.62 (C-1^{F}), 102.64, 103.24, 104.02, 104.36 (C-1^{C}, C-1^{E}, C-1^{B}, C-1^{D}), 114.63 (C-17^{I}), 125.15 (C-10^{I}), 125.97 (C-6^{I}), 132.70 (C-11^{I}), 135.83 (C-7^{I}), 144.36 (C-16^{I}), 159.04 (O*C*ONH$_2^{F}$), 173.95–174.74 (CONH$_2^{B}$, CONH$_2^{F}$, NH*C*OCH$_3^{E}$, NH*C*OCH$_3^{C}$).- ^{31}P NMR (81 MHz, D$_2$O): δ=0.37.- $C_{56}H_{92}N_5O_{33}P$ (1394.35, 1393.54), ESI MS (negative mode): m/z=1392.53420 (1392.53394) [M-H]$^-$, 695.76399 (695.76333) [M-2H]$^{2-}$.

4.1.12. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-[(2*Ξ*,3*Ξ*)-2-hydroxy-3-methyl-3-((4*Ξ*,8*Ξ*)-4,8,12-trimethyltridecyl)-4-pentenyloxy]-propionic acid (9h). 9h was prepared from **4** (75 mg, 63 μmol) and phytyl bromide (90.1 mg, 252 μmol, mixture of *E/Z*-isomers) as described for **9f**. Yield: 4.7 mg, 5%.- ^{1}H NMR (H,H COSY, 400MHz, D$_2$O): characteristic signals at δ=0.80–0.84 (CH$_3$-16^{I}, CH$_3$-17^{I}, CH$_3$-18^{I}, CH$_3$-19^{I}), 1.19 (s, CH$_3$-4^{F}), 1.24 (CH$_3$-20^{I}), 1.36 (d, CH$_3$-6^{C}), 0.90–1.45 (m, CH$_2$-4^{I}–CH$_2$-15^{I}), 1.97, 2.00 (s, NHCOCH$_3^{E}$, s, NHCOCH$_3^{C}$), 3.99 (s, H-5^{F}), 4.85–5.05 (m, CH=C*H*$_2$-22^{I}), 5.06 (d, H-3^{F}), 5.81 (m, H-1^{F}), 5.71–5.90 (m, C*H*=CH$_2$-21^{I}), $J_{5C\text{-}6C}$=5.3 Hz, $J_{2F\text{-}3F}$=10.2 Hz.- ^{31}P NMR (81 MHz, CD$_3$OD): δ=-0.48.- $C_{61}H_{106}N_5O_{33}P$ (1468.51, 1467.65), ESI MS (negative mode): m/z=1466.63970 (1466.64349) [M-H]$^-$, 732.81659 (732.81811) [M-2H]$^{2-}$, FAB MS: m/z=1512.55 [M+2Na-H]$^+$, 1490.56 [M+Na]$^+$.

4.1.13. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[α-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-((2*Ξ*,3*Ξ*)-3-heptyl-2-hydroxy-4-pentenyl-oxy)-propionic acid (9c). 9c was prepared from **4** (60 mg, 50 μmol) and 1-bromo-2-decene (44.3 mg, 200 μmol) as described for **9f**. Yield: 13.9 mg, 21%.- ^{1}H NMR (H,H COSY, 400MHz, D$_2$O): characteristic signals at δ=0.80 (t, CH$_3$-10^{I}), 1.17 (s, CH$_3$-4^{F}), 1.10–1.40 (m, CH$_2$-4^{I}–CH$_2$-9^{I}), 1.33 (d, CH$_3$-6^{C}), 1.98, 2.05 (s, NHCOCH$_3^{E}$, s, NHCOCH$_3^{C}$), 2.07–2.17 (m, H-3^{I}), 3.25 (dd, H-2^{D}), 4.17 (s, H-4^{B}, H-5^{B}), 4.40 (s, H-5^{F}), 4.46 (d, H-1^{D}), 4.97 (d, H-3^{F}), 5.05 (d, CH=C*H*$_{trans}$-12^{I}), 5.08 (d, CH=C*H*$_{cis}$-12^{I}), 5.55–5.70 (m, C*H*=CH$_2$-11^{I}), 5.73 (m, H-1^{F}), $J_{5C\text{-}6C}$=6.3 Hz, $J_{2D\text{-}3D}$=8.3 Hz, $J_{1D\text{-}2D}$=7.8 Hz, $J_{2F\text{-}3F}$=9.9 Hz, $J_{11I\text{-}12I\text{-}cis}$=10.5 Hz, $J_{11I\text{-}12I\text{-}trans}$=17.3 Hz.- ^{13}C NMR (CD$_3$OD-D$_2$O, 100 MHz): δ=14.38 (C-10^{I}), 16.12 (CH$_3$-4^{F}), 17.78 (CH$_3$-6^{C}), 23.36–23.41 (NHCO*C*H$_3^{E}$, NHCO*C*H$_3^{C}$, C-9^{I}), 26.85, 27.83, 28.00, 29.36, 29.99, 30.24, 30.91, 31.70, 32.58 (C-4^{I}–C-8^{I}, multiple signal sets were observed due to isomerism), 45.63 (C-3^{I}), 56.26, 56.54 (C-2^{E}, C-2^{C}), 62.25 (C-6^{D}), 68.33, 69.65 (C-3^{H}, C-1^{I}), 70.24, 71.23, 71.93, 72.34, 73.44, 73.74, 74.15, 74.59, 74.91, 75.57, 75.78, 77.42, 77.52, 78.34 (C-5^{C}, C-4^{D}, C-4^{B}, C-2^{B}, C-3^{B}, C-6^{E}, C-5^{B}, C-3^{E}, C-3^{C}, C-5^{F}, C-2^{F}, C-4^{F}, C-5^{E}, C-2^{D}, C-3^{F}, C-5^{D}, C-3^{D}, C-2^{I}), 81.66 (C-4^{E}), 81.99 (C-2^{H}), 84.39 (C-4^{C}), 95.67 (C-1^{F}), 102.68, 103.52, 104.06, 104.30 (C-1^{C}, C-1^{E}, C-1^{B}, C-1^{D}), 117.48 (C-12^{I}), 139.40, 139.93 (C-11^{I}, signal doubling due to isomerism), 159.07 (O*C*ONH$_2^{F}$), 173.95–174.71 (CONH$_2^{B}$, CONH$_2^{F}$, NH*C*OCH$_3^{E}$, NH*C*OCH$_3^{C}$), 176.86 (C-1^{H}).- ^{31}P NMR (81 MHz, CD$_3$OD-D$_2$O): δ=-0.76.- $C_{51}H_{86}N_5O_{33}P$ (1328.24, 1327.49), ESI MS (negative mode): m/z=1326.49918 (1326.48699) [M-H]$^-$, 662.73955 (662.73986) [M-2H]$^{2-}$.

4.1.14. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-((2*Ξ*,3*Ξ*)-3-nonyl-2-hydroxy-4-pentenyl-oxy)-propionic acid (9d). 9d was prepared from **4** (60 mg, 50 μmol) and 1-bromo-2-dodecene (50 mg, 200 μmol) as described for **9f**. Yield: 25.8 mg, 38%.- ^{1}H NMR (H,H COSY, 400MHz, D$_2$O): characteristic signals at δ=0.80 (t, CH$_3$-12^{I}), 1.17 (s, CH$_3$-4^{F}), 1.15-1.38 (m, CH$_2$-4^{I}–CH$_2$-11^{I}), 1.33 (d, CH$_3$-6^{C}), 1.98, 2.04 (s, NHCOCH$_3^{E}$, s, NHCOCH$_3^{C}$), 2.08–2.18 (m, H-3^{I}), 3.25 (dd, H-2^{D}), 4.17 (s, H-4^{B}, H-5^{B}), 4.40 (s, H-5^{F}), 4.46 (d, H-1^{D}), 4.97 (d, H-3^{F}), 5.04 (d, CH=C*H*$_{trans}$-14^{I}), 5.07 (d, CH=C*H*$_{cis}$-14^{I}), 5.55–5.70 (m, C*H*=CH$_2$-13^{I}), 5.73 (m, H-1^{F}), $J_{5C\text{-}6C}$=5.8 Hz, $J_{2D\text{-}3D}$=7.8 Hz, $J_{1D\text{-}2D}$=7.8 Hz, $J_{2F\text{-}3F}$=11.0 Hz, $J_{13I\text{-}14I\text{-}cis}$=7.8 Hz, $J_{13I\text{-}14I\text{-}trans}$=15.2 Hz.- ^{13}C NMR (100 MHz, CD$_3$OD-D$_2$O): δ=14.11 (C-12^{I}), 15.92 (CH$_3$-4^{F}), 17.51 (CH$_3$-6^{C}), 23.14 (NHCO*C*H$_3^{E}$, NHCO*C*H$_3^{C}$, C-11^{I}), 27.59, 27.77, 29.77, 30.03, 30.11, 30.73, 31.53, 32.42 (C-4^{I}–C-10^{I}, multiple signal sets were observed due to isomerism), 45.36 (C-3^{I}), 55.91, 56.33 (C-2^{E}, C-2^{C}), 62.05 (C-6^{D}), 67.37 (C-3^{H}), 69.39, 70.03, 70.72, 71.04, 71.44, 71.72, 72.13, 73.16, 73.38, 73.58, 73.93, 74.44, 75.36, 75.64, 77.24, 77.34, 78.03 (C-5^{C}, C-4^{D}, C-4^{B}, C-2^{B}, C-3^{B}, C-3^{C}, C-5^{F}, C-6^{E}, C-5^{B}, C-3^{E}, C-2^{F}, C-4^{F}, C-5^{E}, C-2^{D}, C-3^{F}, C-5^{D}, C-3^{D}, C-1^{I}, C-2^{I}), 81.47 (C-4^{E}), 84.19 (C-4^{C}), 95.43 (C-1^{F}), 102.46, 103.31, 103.85, 104.04 (C-1^{C}, C-1^{E}, C-1^{B}, C-1^{D}), 117.19 (C-14^{I}), 139.11, 139.74 (C-13^{I}, signal doubling due to isomerism), 159.79 (O*C*ONH$_2^{F}$), 173.62-174.31 (CONH$_2^{B}$, CONH$_2^{F}$, NH*C*OCH$_3^{E}$, NH*C*OCH$_3^{C}$).- ^{31}P NMR (81 MHz, CD$_3$OD-D$_2$O): δ=-2.04.- $C_{53}H_{90}N_5O_{33}P$ (1356.30, 1355.52), ESI MS (negative mode): m/z=1354.52390 (1354.51829) [M-H]$^-$, 676.75438 (676.75551) [M-2H]$^{2-}$.

4.1.15. (*R*)-3-({β-D-Galactopyranuronamidosyl-(1→4)-2-acetamido-2,6-dideoxy-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-carbamoyl-4-*C*-methyl-α-D-glucopyranuronamidosyloxy}hydroxyphosphoryloxy)-2-((2*Ξ*,3*Ξ*)-3-decyl-2-hydroxy-4-pentenyl-oxy)-propionic acid (9e). 9e was prepared from **4** (60 mg, 50 μmol) and 1-bromo-2-tridecene (52.8 mg, 200 μmol) as described for **9f**. Yield: 19.2 mg, 28%.- ^{1}H NMR (H,H COSY, 400MHz, D$_2$O): characteristic signals at δ=0.80 (t, CH$_3$-13^{I}), 1.17 (s, CH$_3$-4^{F}), 1.15-1.38 (m, CH$_2$-4^{I}–CH$_2$-12^{I}), 1.33 (d, CH$_3$-6^{C}), 1.98, 2.05 (s, NHCOCH$_3^{E}$, s, NHCOCH$_3^{C}$), 2.08–2.18 (m, H-3^{I}), 3.25 (dd, H-2^{D}), 4.17 (s, H-4^{B}, H-5^{B}), 4.40 (s, H-5^{F}), 4.46 (d, H-1^{D}), 4.98 (d, H-3^{F}), 5.04 (d, CH=C*H*$_{trans}$-15^{I}), 5.08 (d, CH=C*H*$_{cis}$-15^{I}),

5.55–5.70 (m, C*H*=CH_2-14^I), 5.73 (m, H-1^F), J_{5C-6C}=6.3 Hz, J_{2D-3D}=8.4 Hz, J_{1D-2D}=7.8 Hz, J_{2F-3F}= 10.5 Hz, $J_{14I-15I-cis}$=11.5 Hz, $J_{14I-15I-trans}$=16.7 Hz.- ^{13}C NMR (100 MHz, CD_3OD-D_2O): δ=14.70 (C-13^I), 16.41 (CH_3-4^F), 18.09 (CH_3-6^C), 23.67 (NHCO*C*H_3^E, NHCO*C*H_3^C, C-12^I), 30.29, 30.55, 32.92 (C-4^I–C-11^I), 45.95 (C-3^I), 55.57–56.90 (C-2^E, C-2^C), 62.57 (C-6^D), 70.59–78.48 (C-5^C, C-4^D, C-4^B, C-2^B, C-3^B, C-3^C, C-5^F, C-6^E, C-5^B, C-3^E, C-2^F, C-4^F, C-5^E, C-2^D, C-3^F, C-5^D, C-3^D, C-2^I, C-2^H, broad signals), 84.96 (C-4^C), 102.96–104.65 (C-1^C, C-1^E, C-1^B, C-1^D), 118.01 (C-15^I), 140.28 (C-14^I), 159.37 (O*C*ONH_2^F), 174.24–175.14 ($CONH_2^B$, $CONH_2^F$, NH*C*OCH_3^E, NH*C*OCH_3^C).- ^{31}P NMR (81 MHz, CD_3OD-D_2O): δ=-0.76.- $C_{54}H_{92}N_5O_{33}P$ (1370.32, 1369.54), ESI MS (negative mode): m/z=1368.53369 (1368.53394) $[M-H]^-$, 683.76234 (683.76333) $[M-2H]^{2-}$.

4.1.16. (*E*)-1-Bromo-2-decene. A solution of *E*-decen-2-ol (250 mg, 1.6 mmol), CBr_4 (730 mg, 2.2 mmol) and Ph_3P (525 mg, 2.0 mmol) in CH_2Cl_2 (5 ml) was stirred for 3 h at 0°C. Subsequently water (20 ml) was added and the solution was stirred for an additional 30 min. The aqueous phase was extracted with CH_2Cl_2 (4x30 ml) and the combined organic fractions dried over $MgSO_4$. The solvents were removed under reduced pressure. The crude product was dissolved in n-hexane (4x5 ml) and the combined n-hexane fractions evaporated to give product (*E*)-1-bromo-2-decene (320 mg, 91%, used without further purification).- ^{1}H NMR (400 MHz, $CDCl_3$, H,H COSY) δ=0.81 (t, CH_3-10), 1.15–1.25 (m, CH_2-6–CH_2-9), 1.30 (m, CH_2-5), 1.98 (m, CH_2-4), 3.87 (d, CH_2-1), 5.5–5.6 (m, H-2), 5.6–5.7 (m, H-3), J_{1-2}=7.1 Hz.- ^{13}C NMR (50 MHz, $CDCl_3$) δ=14.11 (CH_3-10), 22.67 (C-9). All other allyl bromides were prepared accordingly.

Acknowledgements

We wish to thank Dr Françis Maquin and Dr Christian Bonhomme for their support. The Leipzig group gratefully acknowledges financial support by the Deutsche Forschungsgemeinschaft (Innovationskolleg 'Chemisches Signal und biologische Antwort') and the Fonds der Chemischen Industrie. S. V. wishes to thank Hoechst Marion Roussel for a fellowship.

References

1. Donnerstag, A.; Marzian, S.; Müller, D.; Welzel, P.; Böttger, D.; Stärk, A.; Fehlhaber, H.-W.; Markus, A.; van Heijenoort, Y.; van Heijenoort, J. *Tetrahedron* **1995**, *51*, 1931–1940 and references therein.
2. van Heijenoort, J.; van Heijenoort, Y.; Welzel, P. In *Antibiotic Inhibition of Bacterial Cell Wall Surface Assembly and Function*, Actor, P., Daneo-Moore, L., Higgins, M. L., Salton, M. R. J., Shockman, G. D., Eds.; American Society for Microbiology: Washington, 1988; pp 549–557.
3. Ritzeler, O.; Hennig, L.; Findeisen, M.; Welzel, P.; Müller, D.; Markus, A.; Lemoine, G.; Lampilas, M.; van Heijenoort, J. *Tetrahedron* **1997**, *53*, 1675–1694. Kosmol, R.; Hennig, L.; Findeisen, M.; Müller, D.; Markus, A.; van Heijenoort, J. *J. Prakt. Chem.* **1997**, *339*, 340–358.
4. Terrak, M.; Ghosh, T. K.; van Heijenoort, J.; Van Beemen, J.; Lampilas, M.; Aszodi, J.; Ayala, J. A.; Ghuysen, J.-M.; Nguyen-Destèche, M. *Mol. Microbiol.* **1999**, *34*, 350–364.
5. For a recent review on transgylcosylase inhibition, see Goldman, R. C.; Gange, D. *Curr. Med. Chem.* **2000**, *7*, 801–820.
6. El-Abadla, N.; Lampilas, M.; Hennig, L.; Findeisen, M.; Welzel, P.; Müller, D.; Markus, A.; van Heijenoort, J. *Tetrahedron* **1999**, *55*, 699–722 and references therein.
7. For an NMR structure of moenomycin in aqueous solution, see: Kurz, M.; Guba, W.; Vertesy, L. *Eur. J. Biochem.* **1998**, *252*, 500–507.
8. Welzel, P. In *Antibiotics and Antiviral Compounds—Chemical Synthesis and Modifications*, Krohn, K., Kirst, H., Maas, H., Eds.; VCH: Weinheim, 1993; pp 373.
9. Marzian, S.; Happel, M.; Wagner, U.; Müller, D.; Welzel, P.; Fehlhaber, H.-W.; Stärk, A.; Schütz, H.-J.; Markus, A.; Limbert, M.; van Heijenoort, Y.; van Heijenoort, J. *Tetrahedron* **1994**, *50*, 5299–5308.
10. Vogel, S.; Buchynskyy, A.; Stembera, K.; Richter, K.; Hennig, L.; Müller, D.; Welzel, P.; Maquin, F.; Bonhomme, C.; Lampilas, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1963–1965.
11. Anikin, A.; Buchynskyy, A.; Kempin, U.; Stembera, K.; Welzel, P.; Lantzsch, G. *Angew. Chem.* **1999**, *111*, 3931–3935. Anikin, A.; Buchynskyy, A.; Kempin, U.; Stembera, K.; Welzel, P.; Lantzsch, G. *Angew. Chem. Int. Ed.* **1999**, *38*, 3703–3707.
12. Part of the results have been reported in preliminary form. See ref. 10.
13. Welzel, P.; Wietfeld, B.; Kunisch, F.; Schubert, Th.; Hobert, K.; Duddeck, H.; Müller, D.; Huber, G.; Maggio, J. E.; Williams, D. H. *Tetrahedron* **1983**, *39*, 1583–1591.
14. 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. Scherkenbeck, J.; Hiltmann, A.; Hobert, K.; Bankova, W.; Siegels, T.; Kaiser, M.; Müller, D.; Veith, H. J.; Fehlhaber, H.-W.; Seibert, G.; Markus, A.; Limbert, M.; Huber, G.; Böttger, D.; Stärk, A.; Takahashi, S.; van Heijenoort, Y.; van Heijenoort, J.; Welzel, P. *Tetrahedron* **1993**, *49*, 3091–3100.
15. For a recent review, see: Li, C.-J.; Chan, T.-H. *Tetrahedron* **1999**, *55*, 11149–11176.