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Synthesis of a Trisaccharide Analogue of Moenomycin A_{12} Implications of New Moenomycin Structure-Activity Relationships

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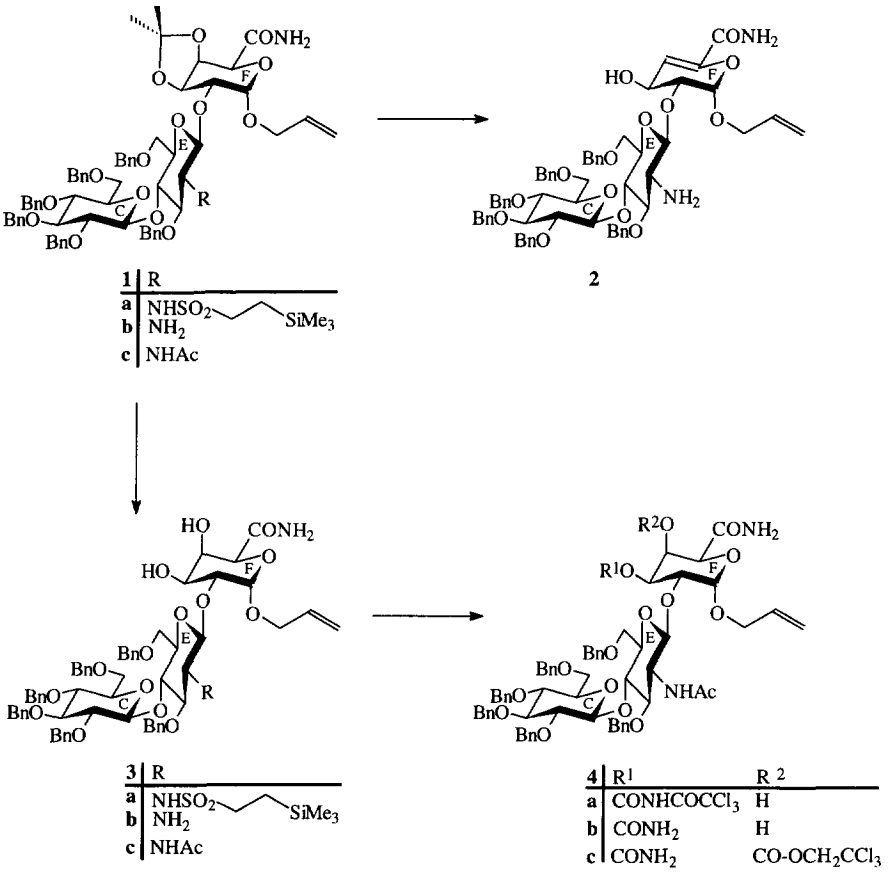
Abstract - A trisaccharide analogue of moenomycin A, **9a**, has been synthesized and has found to be antibiotically inactive. This compound differs from an active compound, **9b**, solely by the exchange NHAc→OH in unit C. A binding model for moenomycin-type transglycosylase inhibitors at the enzyme *penicillin binding protein* is proposed.

In the preceding publication[1] we described the synthesis of trisaccharide **1a** through a combination of Danishefsky's sulfonamidoglycosylation[2] approach and the Schmidt trichloroacetimidate procedure.[3] In the present paper we discuss (i) the conversion of **1a** into **9a**, (ii) the antibiotic properties of **9a**, and (iii) the implications of the biological results.

First **1a** had to be converted into **3c**. This was accomplished in two ways. One started with removal of the trimethylsilylethanesulfonyl group. Thus, **1a** (a 4:1 mixture of **1a** und the α-isomer at C-1^E which could not be separated[4]) was treated with caesium fluoride (30 equ.) in dry DMF.[5] Besides the desired amine **1b** (56%

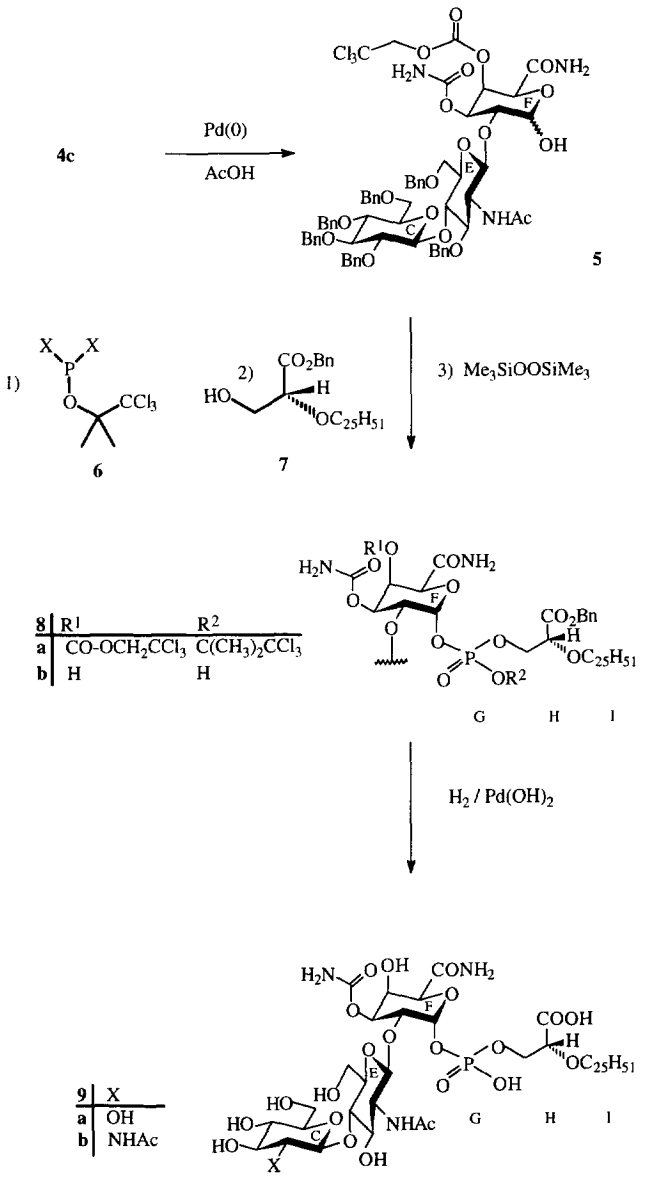
alongside with 14% of the α-isomer which was removed at this stage) in appreciable amounts the elimination product **2** (20%) was obtained. **2** was obviously formed by an E1cb type elimination with acetone as leaving group. **1b** was straightforwardly converted into **1c**, cleavage of the acetonide grouping (THF - 60 per cent acetic acid) to furnish **3c** turned out, however, to be a very sluggish process.



Scheme 1

Then the sequence of events was inverted. **1a** (4:1 mixture, vide supra) was hydrolyzed with aqueous trifluoroacetic acid to provide **3a** (100% of the 4:1 mixture), and the amine was then set free with caesium fluoride to give **3b** (4:1 mixture, 85%). The selective N-acetylation was reasonably well achieved on treatment of **3b** with acetyl chloride to give **3c** (63%) accompanied by 10% of the 1^E α-isomer. The conversion of **3c** into **4b** {(i) trichloroacetyl isocyanate: **3c→4a**, (ii) zinc in methanol: **4a→4b**} proceeded as desired.[6]

Formation of the dicarbamoylated product could, however, not completely be suppressed. Then the 4^F-OH group was protected by conversion of **4b** into the TROC derivative[7] **4c**. Removal of the allyl group was



Scheme 2

nicely achieved using the method of Nakayama et al.,[8] and **5** was obtained in 79% yield. The phosphoric acid diester grouping was installed making use of our version of the Ugi procedure.[6] Thus, **6** (X = Cl) was converted into the bistriazolide **6** (X = 1,2,4-triazolyl) and this was in turn treated with **5**. The reaction product was then allowed to react with the moenomycin derived glyceric acid derivative **7**[9,10] to furnish the corresponding phosphorous acid triester which was oxidized with bistrimethylsilylperoxide[11] to provide **8a** in 65% overall yield. A single signal in the ^{31}P NMR spectrum was observed at δ = -6.5 (pyridine-d_5) indicating that only one stereoisomer had been isolated. On reduction with zinc-copper couple (Imai conditions[12]) **8b** was formed. The ^{31}P NMR spectrum (pyridine-d_5) displayed two signals at δ = 0.4 and -4.7 in a 4:1 ratio. Unfortunately, we were unable to find any means to separate two compounds. Finally, hydrogenolytic removal of the benzyl protecting groups (Pearlman catalyst[13]) furnished **9a** (31%). FAB MS, HRMS, ^{13}C and ^{1}H NMR spectra (SDS micelles[14]) were in full accord with structure **9a**.

A second compound (compound **X**, 47%) was isolated from the debenzylation reaction. The FAB MS of this product exhibited a (presumed) molecular ion which was 17 mass units higher than that of **9a**. The exact mass as reported in the experimental part to our opinion is not in a simple relation to the mass of **9a**. The ^{13}C, ^{31}P, and ^{1}H chemical shifts differ only marginally from those of **9a**. Until now we have been unable to assign a structure to this compound.

Antibiotic and transglycosylase inhibiting properties of 9a and compound X

The biological activities of **9a** and compound **X** were studied in the Izaki, Matsuhashi, and Strominger[15] test (slightly modified version[16]) which measures the inhibition of the UDP-N-acetylmuramyl pentapeptide-dependent incorporation of [^{14}C]UDP-N-acetylglucosamine into cross-linked high-molecular weight peptidoglycan, and by the inhibitory effect of **9a** and compound **X** directly on the transglycosylation reaction (determined by the *in vitro* assay developed earlier in one of our laboratories[17] using a crude extract from an over-producer of polymerase PBP1b (*E.coli JA200 plc19-19)* and as substrate lipid II which is the immediate precursor of un-

Table 1: Effect of compounds **9a**, **9b**,[6] **X**, and of moenomycin A (for comparison) on the *in-vitro* UDP-N-acetylmuramyl pentapeptide-dependent incorporation of [^{14}C]UDP-N-acetyl-glucosamine into cross-linked high-molecular weight peptidoglycan.

	% inhibition			
concentration (mg/L)	moenomycin A	**9a**	**9b**	**X**
10	95	90	93	78
1	87	38	86	18
0.1	24	0	18	2

Table 2: Effect of **9a, 9b**,[6] **X,** and of moenomycin A (for comparison) on the *in-vitro* formation of uncross-linked peptidoglycan by transglycosylation.

	% inhibition			
final concentration (mg/L)	moenomycin A	**9a**	**9b**	**X**
10	100	-	100	-
1	100	0	93	0
0.1	78	-	43	-

Table 3: Minimum inhibitory concentrations (in mg/L) of compounds **9a**, **9b**,[6] **X**, and of moenomycin A (for comparison) against various test organisms.

test organism	moenomycin A	**9a**	**9b**	**X**
Staph.aureus SG 511	0.025	>64	12.5	>64
Staph.aureus 503	0.049	>64	12.5	>64
Strept. pyogenes A77	<0.002	2	0.781	8

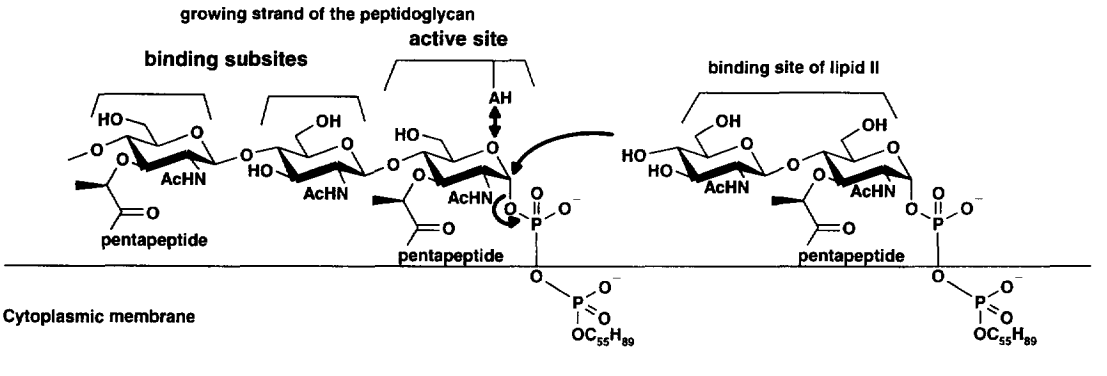
cross-linked peptidoglycan). Furthermore, the minimum inhibitory concentrations (MIC) against various microorganisms (serial two-fold agar dilution method, Müller Hinton Agar) have been determined. The results (see Tables 1, 2, and 3) demonstrate that both **9a** and compound **X** show greatly diminished activity in the *in-vivo* and the *in-vitro* test systems when compared with moenomycin A and are appreciably less active than the synthetic compound **9b**.

We conclude from these results that the hypothesis put forward in the preceding publication is invalid.

Discussion and molecular modelling of moenomycin and peptidoglycan

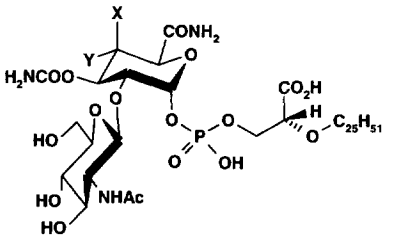
The two final steps of the biosynthesis of peptidoglycan from the lipid II precursor are the transglycosylation that extends the glycan chain and the transpeptidation that cross-links the glycan chains through peptidic bridges. These steps are catalysed by bifunctional enzymes named high-molecular weight *penicillin binding proteins* (PBPs) such as PBP 1A or PBP 1B of *E. coli*. The exact mechanism of the transglycosylation reaction is not known. However, it has been proposed that both the growing glycan strand and the disaccharide intermediate (lipid II) are anchored by their lipid chains into the cytoplasmic membrane (see Scheme 3). The reaction proceeds through the displacement of the pyrophosphate of the growing strand by the 4-hydroxyl group of the GlNAc unit of lipid II.[18] As indicated in Scheme 3 it is likely that both lipid II and a large piece of peptidoglycan interact with the PBPs. The binding site(s) of the growing peptidoglycan could be related to

the binding sites of the murein hydrolases (lysosyme or Slt 70) which have several subsites each for one sugar unit of the peptidoglycan (Scheme 3).[19]

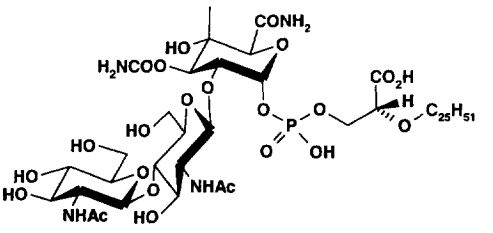


Scheme 3: Schematic representation of the transglycosylation reaction.

The transglycosylase activity of the high-molecular weight PBPs is inhibited by moenomycins and by moenomycin analogues such as disaccharide **10a** or trisaccharides **11** (moenomycin A series) and **9b** (moenomycin A_{12} series) whereas the disaccharide of the A_{12} series with D-*galacto* configuration in the uron-amide unit (**10b**) is inactive.[20]



10	X	Y
a	CH_3	OH
b	OH	H



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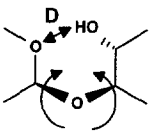
Scheme 4

Motivated by the experimental results discussed above, we wanted to look more closely at some possible structural analogies between a growing peptidoglycan strand and the moenomycins and their trisaccharide analogues, respectively.

We constructed a simplified model of peptidoglycan (2 disaccharide units without the pentapeptide chain) and of moenomycin, both with only one negatively charged anomeric phosphate. These structures were minimised using the CVFF force field (in vacuum) which has been shown to give good results for disaccharide conformations.[21]

The conformations found for the glycosidic linkages (MurNAc β 1-4 → Glc Nac in the peptidoglycan strand and B → C ; C → E in the moenomycin model) were close to the X-ray conformations N-acetyl-lactosamine, H_2O and N,N´-diacetyl chitobiose, 3 H_2O, respectively (Table 4).

Table 4: Comparison of calculated and experimental structural data of some disaccharides.



Φ_1 Φ_2

	Φ_1	Φ_2	D (O-O) A
N-acetyl-lactosamine, H_2O[22]	-88°	98°	2.8
β-N, N'-diacetyl-chitobiose, $3H_2O$[23]	-90°	77°	2.8
peptidoglycan MurNAc β→1-4 GlcNAc	-79°	108°	2.8
moenomycin			
B→C	- 72°	108°	2.7
C→E	- 75°	111°	2.8

The overall extended conformation of our peptidoglycan model (Figure 1) is also in agreement with the experimental and theoretical work on the 3D-structure of peptidoglycan.[24,25]

We have also found an extended conformation for moenomycin (Figure 2).

The moenomycin and peptidoglycan models were first superimposed on the basis of the phosphorylated sugar (see Figure 3). In this case, the glycan chains point into perpendicular directions.

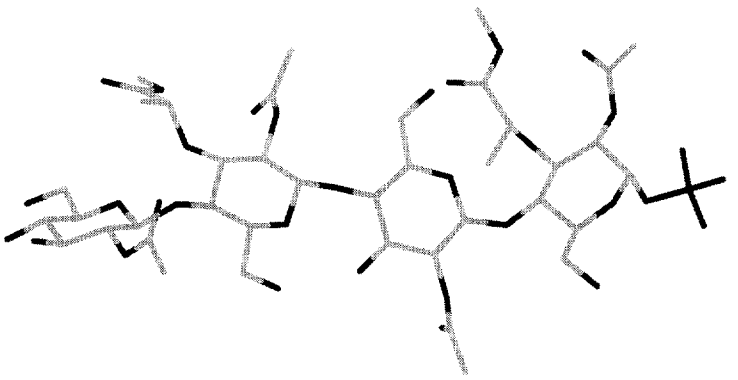


Figure 1: Optimized peptidoglycan model.

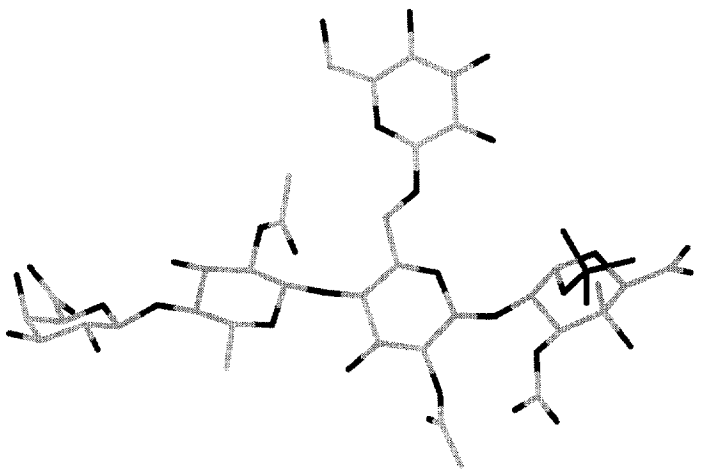


Figure 2: Optimized moenomycin model.

On the other hand, when the two molecules were superimposed on the basis of the sugar chains, a perfect overlapping of the four sugar rings (Figure 4) is observed. One striking feature of this docking is that the NHAc groups of units C and E of moenomycin are perfectly superimposed with those of the peptidoglycan model.

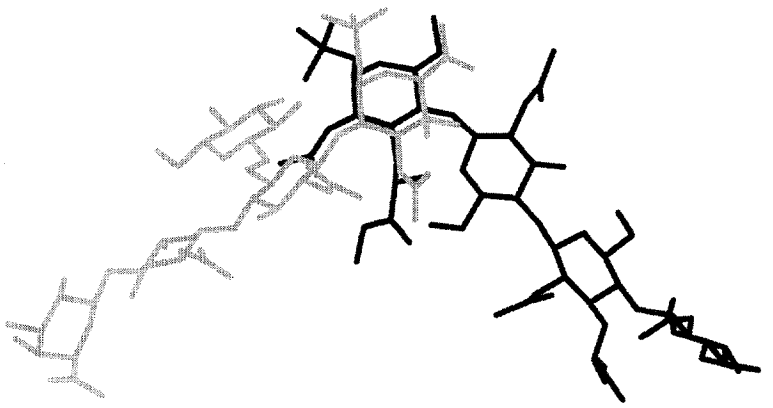


Figure 3: Superimposed peptidoglycan (black) and moenomycin (grey) models. For details see text.

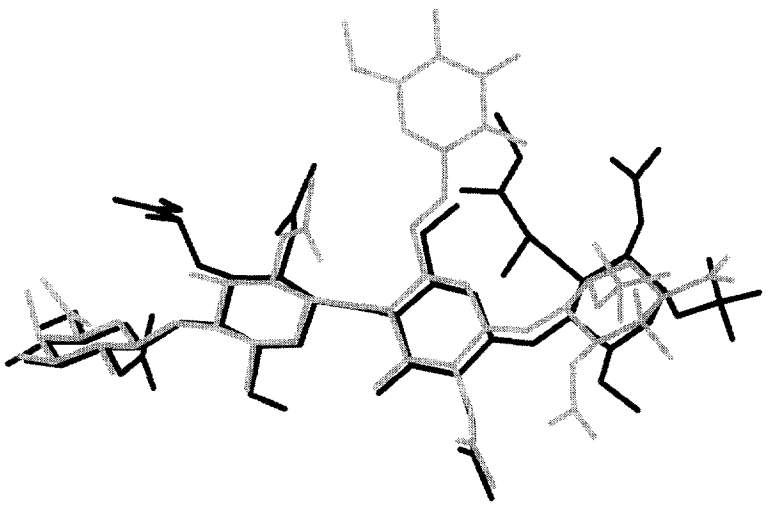


Figure 4: Superimposed peptidoglycan (black) and moenomycin (grey) models. For details see text.

If we now look more carefully at the phosphorylated sugars (Figure 5), we can make two important observations:

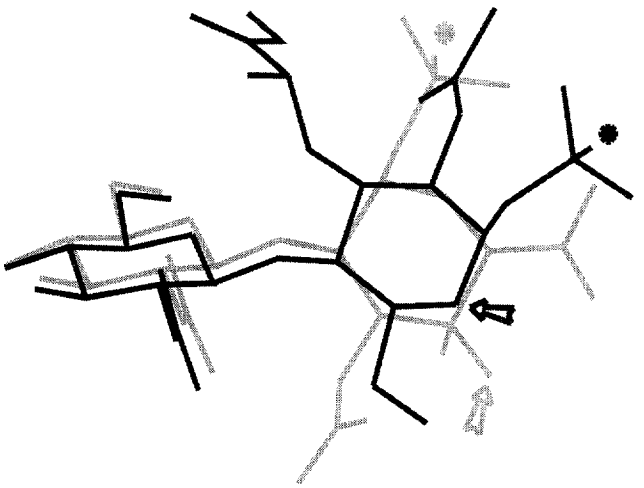
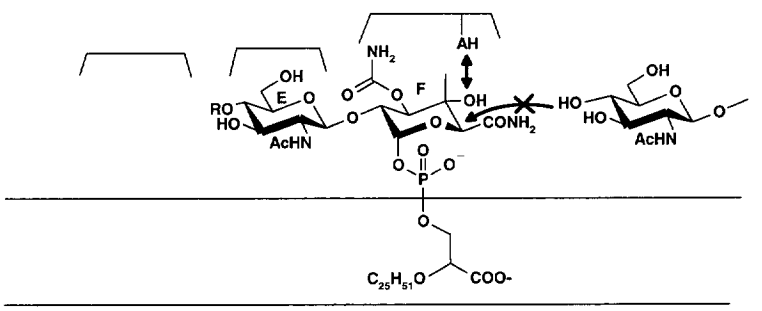


Figure 5: Superimposed peptidoglycan (black) and moenomycin (grey) models. For details see text.

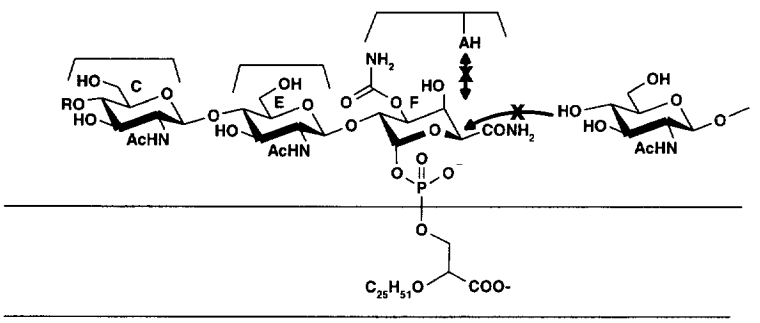
- the anomeric phosphate in moenomycin is shifted away from the peptidoglycan phosphate position but it still points into the same direction (see *)
- the 4-OH group in unit F of moenomycin is in the region of the ring oxygen of the phosphorylated peptidoglycan sugar unit (see ↓).

Our hypothesis is that if moenomycin competes with peptidoglycan for binding to the enzyme in the way shown in Figure 4, there is no anomeric phosphate at the position where the substitution should occur (Schemes 3 and 5).



Scheme 5: Binding model for the moenomycin A (D-*gluco*) series.

The comparison of the transglycosylation reaction as indicated in Scheme 3 with the inhibition model (Scheme 5) can also explain the differences in the structure-activity relationships in the moenomycin A and A_{12} series, respectively, i.e. why the equatorial 4-OH group in unit F is so important for the activity. In the moenomycin A series (Scheme 5), the binding interaction with the equatorial 4-OH group could replace the interaction with the ring oxygen of the phosphorylated unit of the growing peptidoglycan chain. This type of binding has already been suggested to explain the chemoselectivity of β-galactosidase.[26] In the moenomycin A_{12} series with an axial 4-OH group in unit F (Scheme 6) this interaction does not exist. The structure-activity relationships show that there has to be a compensation by the third sugar unit C. Based on the experimental and modelling results presented in this paper we think that it is the NHAc group of unit C, located at the same position as in peptidoglycan (Figure 4) which furnishes the extra contribution (as compared with **9a** and **10b**) to the binding of trisaccharide **9b** to the enzyme.



Scheme 6: Binding model for the moenomycin A_{12} (D-*galacto*) series.

The question whether the antibiotic activity of disaccharide analogues of moenomycin A (**10a**) should also be explained by the present model or is due to competition at the lipid II binding site is actively studied in our laboratories. The results will be reported in due course.

EXPERIMENTAL

For general methods and instrumentation, see the preceding paper.

Reaction of 1a with caesium fluoride

1a (393 mg, 0.302 mmol), CsF (1247 mg, 8.239 mmol), and DMF (50 mL) were stirred at 90°C for 3 d. After solvent evaporation (55°C) FC (petroleum ether-$CHCl_3$-methanol 80:80:3) provided **1b** (192 mg, 56%), the

1^E α-epimer (48 mg, 14%), elimination product **2** (55 mg, 17%), and its 1^E α-epimer (10 mg, 3%). TLC: petroleum ether-$CHCl_3$-methanol 8:8:1.

Allyl 2-*O*-[3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-2-amino-2-deoxy-β-D-glucopyranosyl]-3,4-*O*-isopropylidene-α-D-galactopyranosiduronamide (1b)

^1H NMR (400 MHz, $CDCl_3$): δ = 7.30 - 7.03 (m, Ar-H´s), 6.45 (broad s, $CONH_2$), 5.67 (broad s, $CONH_2$), 5.20 (CH^{benzyl}), 5.08 (d, $J_{1F, 2F}$ = 3.4 Hz, 1-H^F), 4.92 - 4.75 (m, 6H), 4.62 - 4.47 (m, 8H), 4.43 - 4.34 (m, 4H), 4.07 - 4.00 (m, 2H, including 1H of $OC\underline{H}_2CH=CH_2$), 3.85 (dd, partially hidden, J = 10.1 Hz, J = 4.3 Hz, 1H), 3.80 (dd, partially hidden, $J_{1F, 2F}$ = 3.4 Hz, $J_{2F, 3F}$ = 8.3 Hz, 2-H^F), 3.71 (dd, J = 10.9 Hz, J = 1.7 Hz, 1H), 3.67 - 3.52 (m, 5H, including 3-H^C, CH_2-6^C), 3.46 - 3.30 (m, 4H, including 3-H^E), 2.92 (dd, $J_{1E, 2E}$ = 8.0 Hz, $J_{2E, 3E}$ = 10.0 Hz, 2-H^E), 1.39 and 1.29 (2*s, O-$C(CH_3)_2$-O), allyl group signals at: 5.85 (m, $OCH_2C\underline{H}=CH_2$), 5.27 (dq, 1H of $CH_2CH=C\underline{H}_2$), 5.13 (dq, $OCH_2CH=C\underline{H}_2$), 4.12 (ddt, 1H of $OC\underline{H}_2CH=CH_2$).- ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 170.9 ($CONH_2$), 110.0 (O-$\underline{C}(CH_3)_2$-O), 105.2 (C-1^E), 103.2 (C-1^C), 98.6 (C-1^F), 85.4, 84.0, 83.2, 77.7, 77.1, 76.1, 75.9, 75.55, 75.50, 75.4, 75.1, 74.1, 73.8, 69.8, 69.5, 68.8, 56.9 (C-2^E), 28.8 and 26.9 (O-$C(\underline{C}H_3)_2$-O), 140.0 - 138.5 (6 Ar-C^i´s), 128.9 - 127.9 (Ar-C´s), allyl group signals at: 133.8 ($OCH_2\underline{C}H=CH_2$), 118.3 ($OCH_2CH=\underline{C}H_2$).- $C_{66}H_{76}N_2O_{15}$ (1137.33, 1136.52), FAB MS: m/z = 1137.2 ($[M+H]^+$), 1079.2 ($[f]^+$).

Allyl 2-*O*-[3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-2-amino-2-deoxy-α-D-glucopyranosyl]-3,4-*O*-isopropylidene-α-D-galactopyranosiduronamide (1^E α-epimer of 1a)

^1H NMR (200 MHz, $CDCl_3$, characteristic signals): δ = 7.31 - 7.24 (m, Ar-H´s), 6.45 (broad s, $CONH_2$), 6.05 - 5.80 (m, $OCH_2C\underline{H}=CH_2$), 5.45 (broad s, $CONH_2$), 5.04 (d, $J_{1F, 2F}$ = 3.7 Hz, 1-H^F), 4.89 (d, $J_{1E, 2E}$ = 3.7 Hz, 1-H^E), 2.80 (dd, $J_{2E, 3E}$ = 9.8 Hz, 2-H^E), 1.32 and 1.27 (2*s, O-$C(CH_3)_2$-O).- ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 170.8 ($CONH_2$), 110.0 (O-$\underline{C}(CH_3)_2$-O), 103.3 (C-1^C), 98.6 (C-1^F), 96.3 (C-1^E), 85.4, 83.2, 82.9, 78.5, 78.4, 77.9, 77.7, 77.1, 76.1, 75.6, 75.4, 75.3, 74.8, 74.4, 73.9, 73.8, 71.6, 70.1, 69.5, 69.0, 68.1, 55.8 (C-2^E), 28.5 and 26.8 (O-$C(CH_3)_2$-O), 140.0 - 138.3 (6 Ar-C^i´s), 128.9 - 127.8 (Ar-C´s), allyl group signals at: 133.7 ($OCH_2\underline{C}H=CH_2$), 118.8 ($OCH_2CH=\underline{C}H_2$).- $C_{66}H_{76}N_2O_{15}$ (1137.33, 1136.52), FAB MS: m/z = 1137.2 ($[M+H]^+$).

Allyl 2-*O*-[3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-2-amino-2-deoxy-β-D-glucopyranosyl]-4-deoxy-β-L-*threo*-hex-4-ene-pyranosiduronamide (2)

^1H NMR (400 MHz, H,H COSY, $CDCl_3$): δ = 7.30 - 7.07 (m, Ar-H´s), 6.20 (broad s, $CONH_2$), 6.00 (d, $J_{3F, 4F}$ = 2.4 Hz, 4-H^F), 5.68 (broad s, $CONH_2$), 5.23 (d, $J_{1F, 2F}$ = 2.7 Hz, 1-H^F), 5.12 - 5.02 (m, 1H of $OCH_2CH=C\underline{H}_2$, CH^{benzyl}), 4.84 (CH^{benzyl}), 4.78 - 4.68 (m, 4H), 4.57 - 4.45 (m, 4H, including $3CH^{benzyl}$, 3-H^F), 4.41 (d, partially hidden, $J_{1E, 2E}$ = 8.2 Hz, 1-H^E), 4.40 (d, $J_{1C, 2C}$ = 7.8 Hz, partially hidden, 1-H^C), 4.37 - 4.31 (m, $3CH^{benzyl}$), 3.96 (t, broad, $J_{3E, 4E} = J_{4E, 5E}$ = 9.2 Hz, 4-H^E), 3.80 - 3.75 (m, 5-H^E), 3.69 (dd, $J_{2F, 3F}$ = 8.7 Hz, 2-H^F), 3.65 (dd, J = 10.8 Hz, J = 1.5 Hz, 1H), 3.60 - 3.24 (m, 4H), 3.39 - 3.34 (m, 4H, including 2-H^C, 6-H^E, 3-H^E), 2.80 (dd, $J_{2E, 3E}$ = 9.9 Hz, 2-H^E), 2.22 (broad s, OH (exchangable with D_2O)), allyl group signals at: 5.79 (m, $OCH_2C\underline{H}=CH_2$), 5.22 - 5.15 (m, 1H of $OCH_2CH=C\underline{H}_2$), 4.11 - 4.08 (m, $OC\underline{H}_2CH=CH_2$).- ^{13}C NMR (100.6 MHz, APT, C,H COSY, $CDCl_3$): δ = 164.3 ($CONH_2$), 142.1 (C-5^F), 110.9 (C-4^F), 105.8 (C-1^E), 103.3 (C-1^C), 99.8 (C-1^F), 85.5 (CH), 83.2 (CH), 82.6 (CH), 80.7 (C-2^F), 78.5 (CH), 77.2 (C-4^E), 76.2 (CH_2), 75.9 (CH), 75.7 (CH_2), 75.5 (CH), 75.4 (CH_2), 75.0 (CH_2), 73.8 (2*CH_2), 70.6 (CH_2), 69.6 (CH_2), 68.7 (CH_2), 65.5 (CH), 56.6 (C-2^E), allyl group signals at: 134.3 ($OCH_2\underline{C}H=CH_2$), 118.0 ($OCH_2CH=\underline{C}H_2$),

70.9 ($O\underline{C}H_2CH=CH_2$), 139.4 - 138.6 (Ar-C^{i}'s), 128.9 - 128.0 (Ar-C's).- $C_{63}H_{70}N_2O_{14}$ (1079.26, 1078.49), FAB MS: m/z = 1101.4 ($[M+Na]^+$), 1079.4 ($[M+H]^+$).

Allyl 2-*O*-[3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-2-amino-2-deoxy-α-D-glucopyranosyl]-4-deoxy-β-L-*threo*-hex-4-ene-pyranosiduronamide (1^E α-epimer of 2)

^1H NMR (400 MHz, H,H COSY, $CDCl_3$): δ = 6.38 (broad s, $CONH_2$), 6.09 (d, $J_{3F,\,4F}$ = 2.7 Hz, 4-H^F), 5.81 (broad s, $CONH_2$), 5.23 - 5.18 (m, 3H, including $CH^{benzyl\,(a)}$, 1H of $OCH_2CH=C\underline{H}_2$ and d, $J_{1E,\,2E} \approx$ 2.7 Hz, 1-H^F), 5.04 (d, $J_{1E,\,2E}$ = 3.6 Hz, 1-H^E), 4.88 - 4.79 (m, 5H, including $3CH^{benzyl}$), 4.52 (dd, partially hidden, $J_{2F,\,3F}$ = 8.0 Hz, $J_{3F,\,4F}$ = 2.7 Hz, 3-H^F), 4.39 (d, $J_{1C,\,2C}$ = 7.9 Hz, 1-H^C), 4.13 - 4.08 (m, 5-H^E), 3.90 (t, $J_{3E,\,4E} = J_{4E,\,5E} \approx$ 9.1 Hz, 4-H^E), 3.82 - 3.74 (m, CH_2-6^E, 4-H^F and d, $J_{2F,\,3F} \approx$ 8.2 Hz, 2-H^F), 3.69 - 3.60 (m, 4H, including CH_2-6^C, 4-H^C), 3.56 (t, $J_{2C,\,3C} = J_{3C,\,4C}$ = 8.9 Hz, 3-H^C), 3.51 (t, broad, $J_{2,\,3} = J_{3,\,4} \approx$ 9.6 Hz, 3-H^E), 3.44 (dd, 2-H^C), 3.39 - 3.34 (m, 5-H^C), 2.80 (dd, 2-H^E), 2.22 (broad s, OH (exchangable with D_2O)), allyl group signals at: 5.87 (m, $OCH_2C\underline{H}=CH_2$), 5.34 (dq, 1H of $CH_2CH=C\underline{H}_2$), 4.23 (ddt, $OC\underline{H}_2CH=CH_2$), benzyl group signals at: 7.30 - 7.07 (m, Ar-H's), 5.21, 4.63 ($CH^{benzyl\,(a)}$, AB), 4.95, 4.58 ($CH_2^{benzyl\,(b)}$, AB), 4.52, 4.35 ($CH^{benzyl\,(d)}$, AB), 4.45 ($CH_2^{benzyl\,(e)}$, AB).- ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 164.0 ($CONH_2$), 142.4 (C-5^F), 110.2 (C-4^F), 103.4 (C-1^C), 100.1 (C-1^E), 98.5 (C-1^F), 85.4, 83.2, 82.3, 79.3, 78.5, 78.0, 76.1, 75.7, 75.5, 75.4, 75.3, 73.8 (2*), 72.2, 70.6, 69.5, 68.6, 65.7, 55.7 (C-2^E), allyl group signals at: 134.2 ($OCH_2\underline{C}H=CH_2$), 118.3 ($OCH_2CH=\underline{C}H_2$), 139.7 - 138.1 (Ar-C^{i}'s), 128.9 - 127.9 (Ar-C's).- $C_{63}H_{70}N_2O_{14}$ (1079.26, 1078.49), FAB MS: m/z = 1101.4 ($[M+Na]^+$), 1079.4 ($[M+H]^+$), 523.1 ($[c]^+$).

Allyl 2-*O*-[3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranosyl]-3,4-*O*-isopropyliden-α-D-galactopyranosiduronamide (1c)

To **1b** (66 mg, 0.058 mmol) pyridine (660 µL) and acetic anhydride (330 µL) were added at 0°C and the mixture was stirred at 25°C for 3d. After solvent evaporation pure **1c** was obtained in quantitative yield (68 mg, 100%).- ^1H NMR (400 MHz, H,H COSY, $CDCl_3$): δ = 6.30 (broad s, $CONH_2$), 5.54 (broad s, $CONH_2$), 5.54 (d, broad, $J_{NH,\,2E}$ = 7.6 Hz, N$\underline{H}$Ac), 5.09 (d, $J_{1E,\,2E}$ = 7.9 Hz, 1-H^E), 4.97 (d, $J_{1F,\,2F}$ = 3.3 Hz, 1-H^F), 4.80- 4.66 (m, 5H, including $2CH^{benzyl}$, 5-H^F), 4.50 - 4.30 (m, 9H, including $4CH^{benzyl}$, 4-H^F, 1-H^C), 4.22 (dd, $J_{2F,\,3F}$ = 8.0 Hz, $J_{3F,\,4F}$ = 5.3 Hz, 3-H^F), 4.17 - 4.11 (m, 3-H^E), 3.88 (t, $J_{2E,\,3E} = J_{3E,\,4E} \approx$ 8.7 Hz, 3-H^E), 3.74 (dd, J = 10.4 Hz, J = 4.4 Hz, 1H), 3.68 (dd, $J_{2F,\,3F}$ = 8.0 Hz, 2-H^F), 3.61 (dd, J ≈ 10.5 Hz, J ≈ 1.7 Hz, 1H), 3.60 - 3.42 (m, 5H, including 6^C-H', 4-H^C, 3-H^C, 4-H^E), 3.34 (t, $J_{1C,\,2C} = J_{2C,\,3C}$ = 8.0 Hz, 2-H^C), 3.25 - 3.21 (m, 5-H^C), 3.20 - 3.16 (m, 2-H^E), 1.72 (s, NHAc), 1.39 and 1.28 (2*s, O-C(CH_3)$_2$-O), allyl group signals at: 5.65 (m, $OCH_2C\underline{H}=CH_2$), 5.18 (dq, 1H of $CH_2CH=C\underline{H}_2$), 5.05 (dq, 1H of $OCH_2CH=C\underline{H}_2$), benzyl group signals at: 7.30 - 7.05 (m, Ar-H's); 4.91, 4.83, 4.55 (CH^{benzyl}).- ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 171.1 and 171.0 (NHAc and $CONH_2$), 110.0 (O-$\underline{C}$(CH_3)$_2$-O), 103.4 (C-1^C), 101.1 (C-1^E), 98.4 (C-1^F), 85.4, 83.3, 78.8, 78.4, 78.0, 76.1, 75.5, 75.5, 75.4, 75.3, 74.7, 74.1, 73.9, 73.7, 70.0, 69.5, 69.4, 69.0, 58.9 (C-2^E), 28.7 and 27.0 (O-C($\underline{C}H_3$)$_2$-O), 23.2 (NHAc), allyl group signals at: 133.9 ($OCH_2\underline{C}H=CH_2$), 118.3 ($OCH_2CH=\underline{C}H_2$), benzyl group signals at: 139.8 - 138.6 (6 Ar-C^{i}'s); 129.5 - 127.8 (Ar-C's).- $C_{68}H_{78}N_2O_{16}$ (1179.38, 1178.55), FAB MS: m/z = 1201.5 ($[M+Na]^+$), 1179.5 ($[M+H]^+$), 906.4 ($[e]^+$).

Allyl 2-*O*-[3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-2-acetamido-2-deoxy-α-D-glucopyranosyl]-3,4-*O*-isopropyliden-α-D-galactopyranosiduronamide (1^E α-epimer of 1c)

The 1^E α-epimer of **1b** was converted into the 1^E α-epimer of **1c** as described for **1b→1c**, yield: 100%.-^1H NMR (400 MHz, H,H COSY, $CDCl_3$): δ = 6.49 (broad s, $CONH_2$), 5.55 (broad s, $CONH_2$), 5.37 (d, broad,

$J_{NH, 2E}$ = 8.4 Hz, NHAc), 5.03 (d, partially hidden, $J_{1E, 2E}$ = 3.7 Hz, 1-H^E), 4.98 (d, $J_{1F, 2F}$ = 3.5 Hz, 1-H^F), 4.86 - 4.79 (m, 3H, including 5-H^F), 4.66 - 4.58 (m, 4H, including 4-H^F), 4.55 (d, $J_{1C, 2C}$ = 7.6 Hz, 1-H^C), 4.49 - 4.39 (m, 2H), 4.30 (dd, $J_{2F, 3F}$ = 7.9 Hz, $J_{3F, 4F}$ = 5.5 Hz, 3-H^F), 4.25 - 4.16 (m, 3H, including 1H of $OCH_2CH=CH_2$, 2-H^E), 4.07 - 3.93 (m, 3H, including 1H of $OCH_2CH=CH_2$), 3.82 (dd, $J_{2F, 3F}$ = 7.4 Hz, 2-H^F), 3.77 - 3.52 (m, 6H, including 4-H^C, CH_2-6^C, 3-H^C), 3.46 (dd, broad, $J_{2C, 3C}$ = 8.0 Hz, 2-H^C), 3.34 - 3.25 (m, 5-H^C), 1.80 (s, NHAc), 1.36 and 1.27 (O-C(CH_3)$_2$-O), allyl group signals at: 5.85 (m, $OCH_2CH=CH_2$), 5.33 - 5.20 (m, 1H of $OCH_2CH=CH_2$), 4.07 - 3.93 (m, 1H of $OCH_2CH=CH_2$), benzyl group signals at: 7.40 - 7.10 (m, Ar-H's); 5.06, 4.94, 4.92, 4.41 (CH^{benzyl}).- ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 170.7 and 170.2 (NHAc and $CONH_2$), 110.1 (O-C(CH_3)$_2$-O), 103.4 (C-1^C), 96.2 (C-1^F), 95.7 (C-1^E), 85.4, 83.1, 78.5, 78.4, 78.0, 77.7, 77.3, 76.1, 75.3, 74.6, 74.4, 73.8, 73.7, 71.5, 69.7, 69.1, 67.9, 52.7 (C-2^E), 28.3 and 26.6 (O-C(CH_3)$_2$-O), 23.8 (NHAc), allyl group signals at: 133.4 ($OCH_2CH=CH_2$), 118.7 ($OCH_2CH=CH_2$), 140.0 - 138.4 (Ar-C^i's), 129.5 - 127.7 (Ar-C's).- $C_{68}H_{78}N_2O_{16}$ (1179.38, 1178.55), FAB MS: m/z = 1201.6 ($[M+Na]^+$), 906.4 ($[e]^+$).

Preparation of 3c

a) To a solution of **1c** (177 mg, 0.150 mmol) in THF (4 mL) 60 per cent acetic acid (3 mL) was added and the mixture was stirred at 60°C for 4 d. Solvent evaporation (addition of toluene) followed by FC (petroleum ether-$CHCl_3$-methanol 12:12:0.6) yielded **3c** (138 mg, 81%).

b) A mixture of **3a** (4:1 mixture of **3a** and its 1^E α-epimer, 3.40 g, 2.70 mmol), caesium fluoride (11.00 g, 72.41 mmol) in DMF (200 mL) was stirred at 85°C for 3 d. Solvent evaporation and FC (petroleum ether-$CHCl_3$-methanol 9.2:9.2:1 → 9.2:9.2:2) provided **3b** (4:1 mixture of stereoisomers, 2.51 g, 85%), the ^{1}H NMR (400 MHz, $CDCl_3$) of which showed that the 2-(trimethylsilyl)ethyl grouping was not present anymore. TLC: petroleum ether-$CHCl_3$-methanol-acetic acid 10:10:2:1, R_f values of the stereoisomers: 0.21 and 0.10). 1.5121 g (1.379 mmol) of this material were dissolved in CH_2Cl_2 (40 mL). Triethylamine (300 µl) and acetyl chloride (128 µL, 1.793 mmol) were added at 0°C and the mixture was stirred at 0°C for 3 h. Excess acetyl chloride was destroyed with methanol (300 µL). Solvent evaporation and FC (petroleum ether-$CHCl_3$-methanol 12:12:1) provided **3c** (0.989 g, 63%) and its 1^E α-epimer (0.157 g, 10%).- TLC: petroleum ether-$CHCl_3$-methanol 10:10:3.

Allyl 2-*O*-[3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranosyl]-α-D-galactopyranosiduronamide (3c)

IR (KBr): 3690 - 3120 (OH, NH), 1748, 1670, 1650 cm^{-1}.- ^{1}H NMR (H,H COSY, 400 MHz, $CDCl_3$): δ = 6.61 (broad s, $CONH_2$), 6.51 (broad s, $CONH_2$), 6.42 (d, broad, $J_{NH, 2E}$ = 6.8 Hz, NHAc), 5.17 (d, $J_{1F, 2F}$ = 3.3 Hz, 1-H^F), 5.02 (d, $J_{1E, 2E}$ = 8.0 Hz, 1-H^E), 4.90 - 4.82 (m, 4H), 4.56 - 4.51 (m, 2H), 4.48 - 4.30 (m, 6H), 4.18 - 3.85 (m, 8H, including 3-H^F, 2-H^F, $OCH_2CH=CH_2$), 3.75 - 3.53 (m, 7H, including 3-H^C, 2-H^E), 3.46 (t, broad, $J_{1C, 2C} = J_{2C, 3C} \approx$ 8.3 Hz, 2-H^C), 3.39 - 3.34 (m, 5-H^C), 2.62 (broad s, OH), 1.82 (s, NHAc), allyl group signals at: 5.89 (m, $OCH_2CH=CH_2$), 5.31 - 5.24 (m, 1H of $OCH_2CH=CH_2$), 5.15 - 5.10 (m, 1H of $OCH_2CH=CH_2$), benzyl group signals at: 7.30 - 7.05 (m, Ar-H's); 4.96, 4.82, 4.68, 4.58, (CH^{benzyl} signals).- ^{13}C NMR (100.6 MHz, APT, C,H COSY, $CDCl_3$): δ = 172.8 and 172.1 ($CONH_2$ and NHAc), 103.4 and 102.8 (C-1^C and C-1^E), 98.8 (C-1^F), 85.4 (CH), 83.2 (CH), 79.7 (CH), 78.5 (CH), 78.4 (CH), 77.7 (CH), 76.1 (CH_2), 75.6 (CH), 75.6 (CH_2), 75.4 (CH_2), 74.3 (CH_2), 73.7 (2*CH_2), 71.7 (CH), 70.1 (CH), 69.8 (CH_2), 69.3 (CH_2), 69.0 (CH_2), 68.5 (CH), 57.0 (C-2^E), 24.0 (NHAc), allyl group signals at: 134.3 ($OCH_2CH=CH_2$), 118.0

($OCH_2CH=\underline{C}H_2$), 139.5 - 138.2 (Ar-C^{i}'s); 128.8 - 127.6 (Ar-C's).- $C_{65}H_{74}N_2O_{16}$ (1139.30, 1138.57), FAB MS: m/z = 1161.5 ([M+Na]$^+$), 1139.5 ([M+H]$^+$), 906.4 ([e]$^+$).

Allyl 2-*O*-[3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-2-acetamido-2-deoxy-α-D-glucopyranosyl]-α-D-galactopyranosiduronamide (1^E α-epimer of 3c)

IR (KBr): 3690 - 3102 (OH, NH), 1675 cm^{-1}.- ^{1}H-NMR (200 MHz, $CDCl_3$, characteristic signals): δ = 7.30 - 7.05 (m, Ar-H's), 6.52 (broad s, $CONH_2$), 5.92 - 5.71 (m, $OCH_2\underline{C}H=CH_2$) 5.63 (broad s, $CONH_2$), 5.63 - 5.12 (m, 3H, including $OCH_2CH=C\underline{H}_2$), 5.00 (d, $J_{1,2}$ = 4.4 Hz, 1-H^E), 1.78 (s, NHAc).- ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 171.8 ($CONH_2$), 170.2 (NHAc), 103.3 (C-1^C), 97.3 and 96.6 (C-1^F and C-1^E), 85.4, 83.1, 78.3, 77.7, 76.2, 76.1, 75.6, 75.3, 74.1, 74.0, 73.8, 71.8, 71.7, 70.1, 69.3, 68.6, 68.5, 52.7 (C-2^E), 23.7 (NHAc), allyl group signals at : 133.8 ($OCH_2\underline{C}H=CH_2$), 118.2 ($OCH_2CH=\underline{C}H_2$), 139.7 - 138.2 (Ar-C^{i}'s), 128.9 - 127.9 (Ar-C's).- $C_{65}H_{74}N_2O_{16}$ (1139.30, 1138.57), FAB MS: m/z = 1177.2 ([M+K]$^+$), 1161.1 ([M+Na]$^+$), 1139.1 ([M+H]$^+$), 906.2 ([e]$^+$).

Allyl 2-*O*-[3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-2-deoxy-2-(2-trimethylsilyl-ethanesulfonylamino)-β-D-glucopyranosyl]-α-D-galactopyranosiduronamide (≈4:1 mixture of 3a and its 1^E α-epimer)

A roughly 4:1 mixture of **1a** and its 1^E α-epimer (2.70 g, 2.10 mmol) was dissolved in trifluoroacetic acid (70 mL) and water (7 mL), and the mixture was stirred at 25°C for 80 min. At 0°C water (300 mL) was added. After lyophilization a mixture of **3a** and its 1^E α-epimer was obtained in quantitative yield (2.61 g). **3a** and the 1^E α-epimer had different R_F values (0.34 and 0.30, respectively, petroleum ether-$CHCl_3$-methanol-acetic acid 10:10:2:1) but the two compounds were not separated at this stage.- ^{13}C NMR (100.6 MHz, $CDCl_3$, characteristic signals): δ = 172.9 ($CONH_2^{\beta}$), 172.2 ($CONH_2^{\alpha}$), 104.3 (C-$1^{E\beta}$), 103.1 (broad, C-$1^{C\alpha,\beta}$), 99.0 (C-$1^{E\alpha}$), 96.9 (broad, C-$1^{F\alpha,\beta}$), 58.7 (C-$2^{E\beta}$), 57.6 (C-$2^{E\alpha}$), 51.2 ($CH_2(a)^{\alpha}$), 50.6 ($CH_2(a)^{\beta}$), 10.2 (CH_2(broad) $^{\alpha,\beta}$), -1.6 ($Si(C\underline{H}_3)_3^{\beta}$), -1.7 ($Si(C\underline{H}_3)_3^{\alpha}$), two further signals in this region indicated some impurities; allyl group signals at: 134.4 ($OCH_2\underline{C}H=CH_2^{\beta}$), 134.1 ($OCH_2\underline{C}H=CH_2^{\alpha}$), 118.3 ($OCH_2CH=\underline{C}H_2^{\alpha}$), 117.9 ($OCH_2CH=\underline{C}H_2$ $^{\beta}$), 139.3 - 138.2 (Ar-C^{i}'s), 128.9 - 127.9 (Ar-C's).- $C_{68}H_{84}N_2O_{17}SSi$ (1261.56, 1260.53), FAB MS: m/z = 1283.4 ([M+Na]$^+$).

Carbamoylation of 3c

To a solution of **3c** (117 mg, 0.102 mmol) in CH_2Cl_2 (4 mL) at 0°C trichloroacetyl isocyanate (14 µl, 0.188 mmol) was slowly added and the mixture was stirred at 0°C for 2 h and 45 min. Excess reagent was destroyed with methanol (0.5 mL). Solvents were removed with a stream of argon. The raw material was dried for 12 h at 10 Pa. The residue was dissolved in methanol (6 mL), zinc dust (64 mg) was added, and the mixture was stirred at 20°C for 12 h. After filtration over Celite® and washing with methanol the combined organic solutions were evaporated. FC (petroleum ether-$CHCl_3$-methanol-acetic acid 32:13:2.5:1.5) provided **4b** (77 mg, 63%) alongside with the 3,4-dicarbamoyl product (33 mg, 26%).- TLC: petroleum ether-$CHCl_3$-methanol-acetic acid 10:10:3:0.1.

Allyl 2-*O*-[3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranosyl]-3-*O*-carbamoyl-α-D-galactopyranosiduronamide (4b)

IR (KBr): 3680 - 3100 (NH, OH), 1700 cm^{-1}.- ^{1}H NMR (400 MHz, H,H COSY, pyridine-d_5): δ = 9.15 (d, broad, $J_{NH,\,2E}$ = 8.0 Hz, NHAc), 8.49 (broad s, $CONH_2$), 7.79 (broad s, $CONH_2$), 5.82 (dd, $J_{3F,\,4F}$ = 3.1 Hz,

$J_{2F,3F}$ =10.6 Hz, 3-H^F), 5.68 (d, $J_{1E,2E}$ = 8.2 Hz, 1-H^E), 5.57 (d, $J_{1F,2F}$ = 3.6 Hz, 1-H^F), 5.52 (m, $w_{½}$ = 6.5 Hz, 4-H^F), 5.04 - 4.91 (m, 9H, including 2-H^F, 1H of $OCH_2CH=C\underline{H}_2$), 4.86 (broad s, OH), 4.80 - 4.69 (m, 4H, including 3-H^E), 4.40 (t, $J_{3E,4E} = J_{4E,5E}$ = 9.4 Hz, 4-H^E), 4.15 (dd, J = 14.9 Hz, J = 5.0 Hz, 1H), 3.92 - 3.77 (m, 7H, including 2-H^E), 3.72 - 3.60 (m, 4H, including 5-H^E), 2.14 (s, NHAc), allyl group signals at: 5.92 - 5.84 (m, $OCH_2C\underline{H}=CH_2$), 5.35 - 5.28 (m, 1H of $OCH_2CH=C\underline{H}_2$), 4.21 (ddt, 1H of $OC\underline{H}_2CH=CH_2$), 4.08 (ddt, 1H of $OC\underline{H}_2CH=CH_2$), benzyl group signals at: 7.80 - 7.20 (m, Ar-H´s); 5.47, 5.15 - 5.09 (presumably A parts of 2 AB systems), 4.58, 4.52 (2*) (CH^{benzyl}).- ^{13}C NMR (100.6 MHz, pyridine-d_5): δ = 172.4 and 171.5 ($CONH_2$ and NHAc), 158.1 ($OCONH_2$), 103.6 and 102.4 (C-1^C and C-1^E), 99.6 (C-1^F), 85.6, 83.4, 80.0, 78.8, 78.2, 75.9 (2*), 75.7, 75.4, 75.2, 75.1, 74.7, 73.82, 73.76, 73.4, 69.8, 69.3, 69.2, 58.3 (C-2^E), 23.9 (NHAc), allyl group signals at: 134.9 ($OCH_2\underline{C}H=CH_2$), 117.2 ($OCH_2CH=\underline{C}H_2$), 140.9 - 139.3 (Ar-C^i´s), 129.1 - 127.8 (Ar-C´s).- $C_{66}H_{75}N_3O_{17}$ (1182.32, 1181.58), FAB MS: m/z = 1204.6 ([M+Na]$^+$), 928.3 ([e-H+Na]$^+$), 906.4 ([e]$^+$).

Allyl 2-*O*-[3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-2-acetamido-deoxy-β-D-glucopyranosyl]-3,4-di-*O*-carbamoyl-α-D-galactopyranosiduronamide (formula not shown)

^1H NMR (400 MHz, H,H COSY, pyridine-d_5): δ = 9.05 (d, broad, $J_{NH,2E}$ = 7.51 Hz, N$\underline{H}$Ac), 8.51 (broad s, $CONH_2$), 7.68 (broad s, $CONH_2$), 6.70 (m, $w_{½} \approx$ 6.3 Hz, 4-H^F), 6.00 (dd, $J_{2F,3F}$ = 10.8 Hz, $J_{3F,4F}$ = 3.4 Hz, 3-H^F), 5.58 (d, $J_{1F,2F}$ = 3.6 Hz, 1-H^F), 5.48 (d, partially hidden, $J_{1,2} \approx$ 7.5 Hz, 1-H^E), 5.11 - 4.91 (m, 14H, including 5-H^F), 4.79 - 4.67 (m, 4H), 4.58 - 4.54 (m, 3 H, including 2-H^F, CH^{benzyl}), 4.35 (t, $J_{3E,4E} = J_{4E,5E}$ = 9.4 Hz, 4-H^E), 4.15 - 4.07 (m, 2H, including 1H of $OC\underline{H}_2CH=CH_2$), 3.92 - 3.77 (m, 6H, including 2-H^E), 3.72 - 3.60 (m, 4H), 2.16 (s, NHAc), allyl group signals at: 5.86 - 5.80 (m, $OCH_2C\underline{H}=CH_2$), 5.32 - 5.27 (m, 1H of $OCH_2CH=C\underline{H}_2$), 4.21 (ddt, 1H of $OC\underline{H}_2CH=CH_2$), benzyl group signals at: 7.70 - 7.10 (m, Ar-H´s); 4.52 (2*) (CH^{benzyl}).- ^{13}C NMR (100.6 MHz, pyridine-d_5): δ = 170.7 and 170.2 ($CONH_2$ and NHAc), 157.3 and 157.0 (2*$OCONH_2$), 103.2 (C-1^C), 102.1 (C-1^E), 99.0 (C-1^F), 85.1, 83.0, 79.6, 78.4, 77.8, 75.5, 75.4, 75.2, 74.9, 74.7, 74.2, 73.3 (2*(?)), 71.0, 70.6, 70.4, 69.3, 69.1, 68.8, 57.6 (C-2^E), 23.4 (NHAc), allyl group signals at: 134.3 ($OCH_2\underline{C}H=CH_2$), 116.8 ($OCH_2CH=\underline{C}H_2$), 140.5 - 138.8 (Ar-C^i´s), 128.7 - 127.3 (Ar-C´s).- $C_{67}H_{76}N_4O_{18}$ (1225.36, 1224.52), FAB MS: m/z = 1247.7 ([M+Na]$^+$), 928.3 ([e-H+Na]$^+$), 906.4 ([e]$^+$).

Allyl 2-*O*-[3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranosyl]-4-*O*-(2,2,2-trichloroethoxycarbonyl)-3-*O*-carbamoyl-α-D-galactopyranosiduronamide (4c)

To a solution of **4b** (77 mg, 0.065 mmol) in pyridine (6 mL) trichloroethyl chloroformate (14 µl, 0.098 mmol) was added and the reaction mixture was stirred at 20°C for 6 h. Then a second portion of trichloroethyl chloroformate (14 µL, 0.098 mmol) was added. Stirring was continued for 2 h. Methanol (125 µL) was added, solvents were removed, and subsequent FC (petroleum ether-$CHCl_3$-methanol 24:24:1.5) provided **4c** (80 mg, 92%).- TLC: petroleum ether-$CHCl_3$-methanol-acetic acid 15:10:2:1.- IR (KBr): 3690 - 3130 (NH), 1775, 1745, 1700, 1600, 1550 cm^{-1}.- ^1H NMR (400 MHz, H,H COSY, pyridine-d_5): δ = 9.15 (d, broad, $J_{NH,2E}$ = 7.3 Hz, N$\underline{H}$Ac), 8.78 (broad s, $CONH_2$), 7.91 (broad s, $CONH_2$), 6.62 (d, broad, $J_{3F,4F}$ = 3.4 Hz, 4-H^F), 6.03 (dd, $J_{2F,3F}$ = 10.8 Hz, 3-H^F), 5.60 (d, $J_{1E,2E}$ = 8.4 Hz, 1-H^E), 5.57 (d, $J_{1F,2F}$ = 3.6 Hz, 1-H^F), 5.05 - 4.95 (m, 14H, including 5-H^F), 4.82 - 4.74 (m, 3H), 4.60 - 4.56 (m, 2H, including 2-H^F), 4.38 (t, J = 9.4 Hz, 1H), 4.21 - 4.07 (m, 3H, including 2H of $OC\underline{H}_2CH=CH_2$), 3.92 - 3.60 (m, 9H, including 2-H^E), 2.13 (s, NHAc), allyl group signals at: 5.91 - 5.82 (m, $OCH_2C\underline{H}=CH_2$), 5.32 - 5.25 (m, 1H of $OCH_2CH=C\underline{H}_2$), benzyl group signals at: 7.80 - 7.10 (m, Ar-H´s); 5.48, 5.16 - 5.09 (presumably A parts of 2 AB systems), 4.71, 4.52

(2*) (CH^{benzyl}).- ^{13}C NMR (100.6 MHz, pyridine-d_5): δ = 171.2 and 170.0 (NHAc and $CONH_2$), 157.4 ($OCONH_2$), 154.6 ($O\underline{C}OCH_2CCl_3$), 103.6 (C-1^C), 102.3 (C-1^E), 99.3 (C-1^F), 95.5 ($OCOCH_2\underline{C}Cl_3$), 85.5, 83.4, 79.8, 78.8, 78.2, 77.4, 76.9, 76.5, 75.8, 75.5, 75.4, 75.1, 74.8, 73.8, 73.7, 70.5, 70.0, 69.7 (2*), 69.3, 58.4 (C-2^E), 23.8 (NHAc), allyl group signals at: 134.6 ($OCH_2\underline{C}H=CH_2$), 117.5 ($OCH_2CH=\underline{C}H_2$), 140.9 - 139.3 (6 Ar-C^i's), 129.1 - 127.7 (Ar-C's).- $C_{69}H_{76}Cl_3N_3O_{18}$ (1341.73, 1339.48), FAB MS: m/z = 1378.2 ($[M+K]^+$), 906.2 ($[e]^+$).- HRMS: $[M+K]^+$: calc 1378.3826, found 1378.3800.

2-*O*-[3,6-Di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranosyl]-4-*O*-(2,2,2-trichloroethoxycarbonyl)-3-*O*-carbamoyl-α-D-galactopyranuronamide (5)

Under careful exclusion of air a mixture of **4c** (785 mg, 0.585 mmol) and commercial tetrakis(triphenylphosphine)palladium(0) (631 mg, 0.456 mmol) was suspended in oxygen-free acetic acid (40 mL). The reaction mixture was stirred at 20°C for 3.5 h and at 40°C for 2 h. Then water (700 mL) was added. After lyophilization the residue was taken up in ethyl acetate (300 mL). The mixture was washed with sat. aq. NaCl (2 x 50 mL). The organic solution was evaporated. FC (petroleum ether-ethyl acetate 1:2→1:10) furnished **5** (554 mg, 73%). After lyophilization and FC (same solvent), from the NaCl solution a second crop of **5** (42 mg, 6%) could be obtained.- IR (KBr): 3800 - 3100 (NH), 1767, 1751, 1722, 1679, 1559 cm^{-1}.- ^1H NMR (400 MHz, H,H COSY, pyridine-d_5): δ = 9.02 (d, broad, $J_{NH,\,2E}$ = 7.7 Hz, N<u>H</u>Ac), 8.67 (broad s, $CONH_2$), 7.88 (broad s, $CONH_2$), 6.72 (m, $w_{½} \approx$ 8.3 Hz, 4-H^F), 6.25 (dd, $J_{2F,\,3F}$ = 10.4 Hz, $J_{3F,\,4F}$ = 3.1 Hz, 3-H^F), 6.14 (d, $J_{1F,\,2F}$ = 3.3 Hz, 1-H^F), 5.60 (d, $J_{1E,\,2E}$ = 8.2 Hz, 1-H^E), 5.43 (m, $w_{½} \approx$ 3.4 Hz, 5-H^F), 5.05 - 4.91 (m, 7H, including 1-H^C), 4.82 - 4.70 (m, 4H, including 3-H^E), 4.68 (dd, 2-H^F), 4.39 (t, $J_{3E,\,4E} = J_{4E,\,5E}$ = 9.4 Hz, 4-H^E), 4.15 (dd, J = 11.2 Hz, J = 3.8 Hz, 1H), 3.93 - 3.60 (m, 11H, including 2-H^E, 5-H^E, 2-H^C), 2.17 (s, NHAc), benzyl group signals at: 7.70 - 7.20 (m, Ar-H's); 5.48, 5.15 - 5.09 (presumably A parts of 2 AB systems), 4.62, 4.55 (2*), 4.49 (CH^{benzyl}).- ^{13}C NMR (100.6 MHz, C,H COSY, APT, pyridine-d_5): δ = 171.3 and 170.8 (NHAc and $CONH_2$), 157.6 ($OCONH_2$), 154.7 ($O\underline{C}OCH_2CCl_3$), 103.5 (C-1^C), 102.6 (C-1^E), 95.5 ($OCOCH_2\underline{C}Cl_3$), 94.1 (C-1^F), 85.5 (CH), 83.4 (CH), 80.1 (C-2^F), 78.8 (CH), 78.0 (C-4^E), 77.4 (CH_2), 77.0 (CH), 76.4 (C-4^F), 75.8 (CH_2), 75.6 (CH), 75.4 (CH_2), 75.2 (CH_2), 74.7 (CH_2), 73.7 (2*CH_2), 70.4 (C-3^F), 70.2 (C-5^F), 69.7 (CH_2), 69.1 (CH_2), 58.1 (C-2^E), 23.8 (NHAc), 140.9 - 139.2 (Ar-C^i's); 129.4 - 127.8 (Ar-C's).- $C_{66}H_{72}Cl_3N_3O_{19}$ (1317.67, 1315.38), FAB MS: m/z = 1338.3 ($[M+Na]^+$), 1316.2 ($[M+H]^+$), 906.9 ($[e]^+$).- HRMS: $[M+Na]^+$: calc 1338.3723, found 1338.3717.

2-*O*-[3,6-Di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranosyl]-4-*O*-(2,2,2-trichloroethoxycarbonyl)-3-*O*-carbamoyl-1-*O*{[(R)-2-benzyloxycarbonyl-2-(3,8,8,11,14,18-hexamethylnonadecyloxy)-ethoxy]-(2-trichloromethyl-2-propyloxy)-phosphoryl}-α-D-galactopyranuronamide (8a)

To a suspension of 1H-1,2,4-triazole (66 mg, 0.924 mmol) in 1:4 pyridine-CH_2Cl_2 (2 mL) at 0°C 2,2,2-trichloro-1,1-dimethylethyl dichlorophosphite (48 µl, 0.240 mmol) was added. The mixture was stirred at 0°C for 20 min. To the then clear solution **5** (240 mg, 0.185 mmol) dissolved in 1:4 pyridine-CH_2Cl_2 (1.5 mL) was added and the reaction mixture was stirred at 0°C for 4 h. Within 2 h a solution of **7** (250 mg, 0.457 mmol) in 1:4 pyridine-CH_2Cl_2 (1.8 mL) was added. Stirring at 0°C was continued for 1 h. Bis(trimethylsilyl)peroxide (98 µL, 0.462 mmol) was added and the mixture was stirred at 0°C for 1 h and at 22°C for 17 h. Then toluene was added and the solvents were removed by evaporation at 22°C. FC (petroleum ether-ethyl acetate 3:2→0:1) provided **8a** (249 mg, 65%).- TLC: petroleum ether-$CHCl_3$-methanol-acetic acid 10:10:1.5:0.1.- ^1H NMR (400 MHz, H,H COSY, pyridine-d_5, characteristic signals): δ = 9.18 (broad s, NHAc), 8.88 (broad s, $CONH_2$),

8.05 (broad s, $CONH_2$), 6.69 - 6.63 (m, broad, 1-H^F), 6.61 (m, $w_{½} \approx 12$ Hz, 4-H^F), 6.04 (dd, broad, $J_{2F, 3F} \approx 10.7$ Hz, $J_{3F, 4F} \approx 3.3$ Hz, 3-H^F), 5.62 - 5.50 (m, CH^{benzyl}, d, partially hidden, $J_{1E, 2E} \approx 7.9$ Hz, 1-H^E), 5.34 (m, $w_{½} \approx 5.4$ Hz, 5-H^F), 2.13 (s, NHAc), 2.01 and 1.99 (2*s, $PO(C\underline{H}_3)_2CCl_3$), 1.87 - 0.82 (CH, CH_2 and CH_3, parts H and I).- ^{13}C NMR (100.6 MHz, APT, pyridine-d_5): δ = 170.6 and 169.7 and 168.5 (NHAc and $CONH_2$ and CO_2Bn), 156.6 ($OCONH_2$), 153.9 ($O\underline{C}OCH_2CCl_3$), 103.1 (C-1^C), 102.7 (C-1^E), 97.9 (d, $^3J_{C, P} = 6.0$ Hz, C-1^F), 95.0 ($OCOCH_2\underline{C}Cl_3$), 90.63 / 90.56 ($PO(CH_3)_2\underline{C}Cl_3$), 85.2, 83.1, 79.7 (C-$2^F$), 78.3, 78.2, 77.8, 77.0, 75.6, 75.5 (CH_2), 75.4, 75.3, 75.0, 74.9, 74.7, 73.6, 73.3, 71.6, 69.8, 69.3, 68.8, 68.5, 68.1 (C-3^H, broad), 68.0, 66.9, 61.6, 57.7 (C-2^E), 42.1 - 19.4 (NHAc, CH, CH_2 and CH_3, parts H and I), 140.4 - 139.0 (6 Ar-C^{i}'s), 136.3 (CO_2Bn, Ar-C^i), 129.1 - 127.4 (Ar-C's). The following signals could not be assigned: 106.3, 106.1, 54.8, 14.0, 11.0.- ^{31}P NMR (121.5 MHz, pyridine-d_5) δ = - 6.5.- $C_{105}H_{138}Cl_6N_3O_{25}P$ (2085.95, 2081.75), FAB MS: m/z = 2104.7 ([M+Na]$^+$), 906.9 ([e]$^+$).-HRMS: [M+Na]$^+$: calc 2104.7385, found 2104.7390.

2-*O*-[3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranosyl]-3-*O*-carbamoyl-1-*O*{[(R)-2-benzyloxycarbonyl-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy]-hydroxyphosphoryl}-α-D-galactopyranuronamide (8b)

A mixture of **8a** (230 mg, 0.111 mmol), freshly prepared zinc-copper couple (174 mg), pyridine (8 mL) and 2,4-pentanedione (180 µL) was stirred at 22°C for 4 h. Work-up was performed by extraction with CH_2Cl_2 (3 x 10 mL), ethanol (3x 10 mL), and methanol (3x 10 mL), combining the organic solutions, addition of toluene and solvent evaporation. The residue was dried at 10 Pa, then taken up in ethanol (40 mL). Water was added until a slight turbidity was produced. Dowex WX50/200 (H^+ form, 2 g) was added and this mixture was stirred for 1 h. After filtration the resin was washed with CH_2Cl_2, ethanol, and methanol. Solvent evaporation and FC (petroleum ether-$CHCl_3$-methanol-acetic acid 10:10:5:0.1→10:10:10:2.5) furnished a product which according to all spectra was **8b** (150 mg, 77%). Only a second signal in the ^{31}P NMR indicated an impurity (vide infra).- TLC: petroleum ether-$CHCl_3$-methanol-acetic acid 10:10:1.5:0.1. A number of other TLC systems were tried. In neither of them an impurity could be detected.- ^1H NMR (300 MHz, pyridine-d_5, only broad signals): δ = 9.15 (NHAc), 8.30 and 7.84 ($CONH_2$), 5.75 (1-H^F), 2.25 (NHAc).- ^1H NMR (400 MHz, H,H COSY, CD_3OD-$CDCl_3$ 2:1, all signals were broad), characteristic chemical shifts δ = 7.82 (s, N$\underline{H}$Ac), 5.75 (m, $w_{½} \approx 14.5$ Hz, 1-H^F), 5.02 - 4.95 (m, 3-H^F), 1.87 (s, NHAc).- ^{13}C NMR (100.6 MHz, CD_3OD-$CDCl_3$ 2:1): δ = 173.9 (broad) 172.4 (NHAc, $CONH_2$, and CO_2Bn), 158.6 ($OCONH_2$), 104.1 (C-1^C, C-1^E, partially hidden), 96.7 (C-1^F, broad), 86.0, 83.9, 81.6 (C-2^F, broad), 79.8 (broad), 79.0 (2*), 78.2 (C-2^H, broad), 76.9, 76.2, 76.1 (2*), 75.5, 74.5 (2*), 73.3 (broad), 72.8 (broad), 71.1 (2*), 70.5, 70.1 (?), 69.2 (C-1^I, broad), 68.4, 67.4 (C-3^H, broad), 65.6, 56.0 (C-2^E), 43.3 - 20.5 (NHAc, CH, CH_2, and CH_3, parts H and I), 140.6 - 139.2 (6 Ar-C^{i}'s), 136.9 (CO_2Bn, Ar-C^i), 129.8 - 128.2 (Ar-C's).- ^{31}P NMR (121.5 MHz, pyridine -d_5) δ = 0.4, - 4.7 (ratio roughly 4:1).- $C_{98}H_{132}N_3O_{23}P$ (1751.11, 1749.90), FAB MS: m/z = 1794.8 ([M+2Na-H]$^+$), 1146.4 ([f-H+Na]$^+$), 928.4 ([e-H+Na]$^+$).- HRMS: [M+2Na-H]$^+$: calc 1794.8706, found 1794.8710.

Hydrogenolysis of 8b

A mixture of **8b** (56 mg, 0.032 mmol), $Pd(OH)_2$ / C (135 mg), and 3:1:0.1 methanol-dioxane-acetic acid (4 mL) was stirred at 25°C for 6 h in an atmosphere of hydrogen. The product was isolated by the following procedure: (i) centrifugation, (ii) decantation, (iii) addition of petroleum ether (2 mL) to the residue and stirring, (iv) addition of 1:1 methanol-H_2O (10 mL) and further stirring for 20 min, (v), centrifugation, decantation. This procedure was repeated 5 times. The combined organic solutions were evaporated. By FC ($CHCl_3$-

methanol-H_2O 18:13:0.9) compound **X** and **9a** were separated. Subsequent purification by Sephadex G 10 chromatography (≈ 10 g, H_2O) provided **9a** (11 mg, 31%), compound **X** (17 mg, 47%).- TLC: $CHCl_3$-methanol-H_2O 18:13:2.7, $CHCl_3$-methanol-acetic acid 10:12:1, 2-propanol-NH_4OH (2 N) 7:2.

2-*O*-[2-Acetamido-2-deoxy-4-*O*-(β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranosyl]-3-*O*-carbamoyl-1-*O*{[(R)-2-carboxycarbonyl-2-(3,8,8,11,14,18-hexamethylnonadecyloxy)-ethoxy]-hydroxyphosphoryl}-α-D-galactopyranuronamide (9a)

^1H NMR (400 MHz, 5 mg of **9a** + 80 mg of SDS-d_{25}, in D_2O (0.6 mL), H,H COSY, TOCSY): 5.80 (m, $w_{½}$ ≈ 18 Hz, 1-H^F), 4.96 (d, broad, J = 10.3 Hz, 3-H^F), 4.58 (d, J = 7.9 Hz, 1-$H^{C\ or\ E}$), 4.48 (d, J = 8.2 Hz, 1-$H^{E\ or\ C}$), 4.38 (broad s, 4-H^F), 4.07 (broad s, 5-H^F (?)), 3.98 (t, partially hidden, J ≈ 6.4 Hz, 2-H^F), 3.54 (t, J ≈ 4.5 Hz), 3.23 (t, J ≈ 8.4 Hz), 1.96 (s, NHAc).- ^{13}C NMR (100.6 MHz, $CDCl_3$-CD_3OD-D_2O 18:14:2.7): δ = 175.0, 173.6, and 173.1 (NHAc, $CONH_2$, and CO_2H), 158.9 ($OCONH_2$), 104.3 and 103.9 (C-1^C and C-1^E), 96.5 (C-1^F, broad), 80.2 (C-2^F, broad), 79.83, 79.76, 77.8, 77.4, 76.8, 76.2, 74.8, 73.7, 72.9, 72.1, 71.3, 71.2, 69.7 (?), 69.4, 66.9 (broad), 65.0 (?), 62.4, 61.3, 56.7 (C-2^E), 43.4 - 20.5 (NHAc, CH, CH_2 and CH_3, parts H and I).- ^{31}P NMR (121.5 MHz, $CDCl_3$-CD_3OD-D_2O 18:14:2.7) δ = 2.3.- $C_{49}H_{90}N_3O_{23}P$ (1120.23, 1119.57), FAB MS (lactic acid): m/z = 1158.6 ($[M+K]^+$), 1142.6 ($[M+Na]^+$), 1120.6 ($[M+H]^+$).- HRMS: $[M+Na]^+$: calc 1142.5600, found 1142.5586.

Compound X

^1H NMR (400 MHz, 5 mg of compound **X** + 70 mg of SDS-d_{25} in D_2O (0.6 mL), H,H COSY, TOCSY): δ = 5.82 (m, $w_{½}$ = 16 Hz, 1-H^F), 4.95 (d, broad, J ≈ 10.9 Hz, 3-H^F), 4.57 (d, J = 7.7 Hz, 1-$H^{C\ or\ E}$), 4.46 (d, J = 11.0 Hz, 1-$H^{E\ or\ C}$), 4.38 (broad s, 4-H^F), 4.02 - 3.98 (dd, broad, partially hidden, J ≈ 8.8 Hz, 2-H^F), 3.55 (t, J ≈ 4.5 Hz.)), 3.25 (t, J ≈ 8.7 Hz), 1.96 (s, NHAc).- ^{13}C NMR (100.6 MHz, $CDCl_3$-CD_3OD-D_2O 18:14:2.7): δ = 174.9, 173.9, and 169.7[27] (NHAc, $CONH_2$, and CO_2H), 158.9 ($OCONH_2$), 104.2 and 104.0 (C-1^C and C-1^E), 96.6 (C-1^F, broad), 80.3 (C-2^F, broad), 77.8, 77.4, 76.8, 76.1, 74.8, 73.7, 72.9, 72.2, 71.3, 69.6 (?), 69.4, 67.7[27] (broad), 65.4[27], 65.1, 62.4, 62.0 (?), 61.3, 56.6 (C-2^E), 43.4 - 20.5 (NHAc, CH, CH_2 and CH_3, parts H and I). The following signals could not be assigned: 15.1, 12.1.- ^{31}P NMR (121.5 MHz, $CDCl_3$-CD_3OD-D_2O 18:14:2.7) δ: = - 2.1.- FAB MS (lactic acid): m/z = 1175.6 ($[M+K]^+$), 1159.6 ($[M+Na]^+$), 1137.7 ($[M+H]^+$).- HRMS: $[M+Na]^+$: found 1159.6061.

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Dedicated with great admiration to Professor Hans Paulsen on the occasion of his 75th birthday

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