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Moenomycin A: The Role of the Methyl Group in the Moenuronamide Unit and a General Discussion of Structure-Activity Relationships

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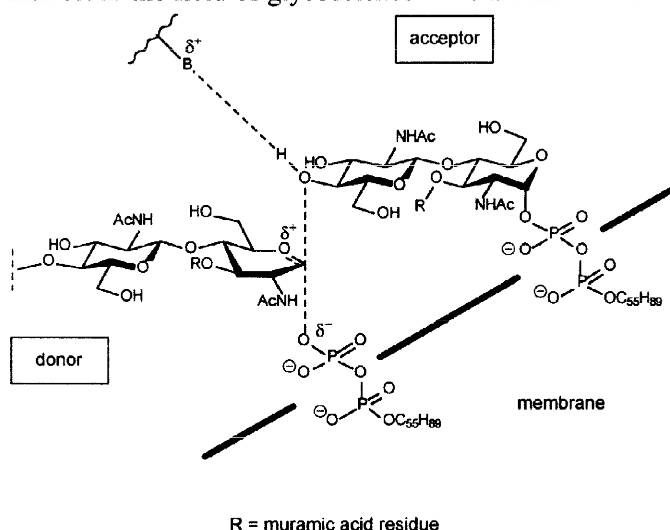
Abstract - Two disaccharide analogues **1b** and **17a** of moenomycin A have been synthesized and their antibiotic and transglycosylase-inhibiting properties have been determined. The results permit for the first time to arrive at a general view of the structural requirements in this class of compounds necessary to elicit antibiotic activity. © 1998 Elsevier Science Ltd. All rights reserved.

Key words: Antibiotics, carbohydrates, phospholipids, structure-activity

Introduction

Enzymatic glycosyl transfer and the development of glycosyltransferase inhibitors is an area of great current interest in the field of glycoscience.[2] We are interested in the so-called transglycosylation step in the biosynthesis of bacterial cell wall peptidoglycan and its inhibition by the moenomycin-type antibiotics.[3] The transglycosylation reaction is believed to proceed in such a way that the growing peptidoglycan chain linked to a C_{55} lipid (bacterioprenol) via a pyrophosphate bridge acts as the glycosyl donor whereas a disaccharide intermediate, the so-called lipid II, is the glycosyl acceptor (see Scheme 1). This mode of glycan chain elongation has been demonstrated for a poorly lytic mutant of *Bacillus licheniformis*.[4] In *E. coli* the transglycosylation reaction is catalyzed by the high-molecular weight penicillin-binding proteins such as the PBPs 1a and 1b. These are bifunctional enzymes which catalyze in addition to the transglycosylation the so-called transpepti-



Scheme 1: Transition state of the transglycosylation reaction (speculative)

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Moenomycin A

dation reaction, in which the sugar strands are cross-linked via short peptide chains.[5] The transglycosylase has been shown to be located in the N-terminal module (at the outer surface of the cytoplasmic membrane) but the active site is still unknown. The moenomycin-type antibiotics are most probably substrate analogous inhibitors of the transglycosylase.[3]

Studies on structure-activity relationships have revealed that moenomycin A degradation product **1a** contains presumably all the essential structural informations necessary to inhibit the transglycosylase,[6] at least in the *in-vitro* assays (vide infra). The role of the branching methyl group in unit F remained, however, elusive.

As discussed in the preceding publication, several attempts to synthesize **1b** failed.[7] It is the purpose of the present paper to uncover a successful route to **1b** and to the closely related compound **17a** in which the C_{25}

1	R
a	CH_3
b	H

lipid unit is replaced by a short alkyl chain. The transglycosylase inhibiting and antibiotic properties of **1b** and **17a** will be reported and there will be a general discussion of the structure-activity relationships of disaccharide and trisaccharide analogues of moenomycin A.

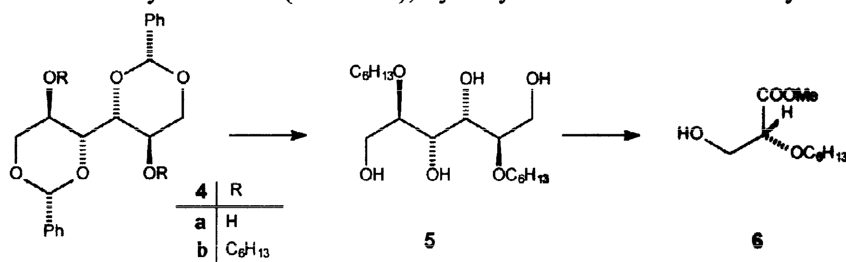
Synthetic planning

Moenomycin analogues can be assembled as indicated in formula **2**. The 2-*O*-alkylglyceric acid unit with

R = decahydromoenocinyl, $C_{25}H_{51}$ (see formula **1**) is available by degradation of moenomycin.[8] For the synthesis of other 2-*O*-alkylated glyceric acids a route was developed a number of years ago.[9,10] The phosphoric acid diester is constructed using the phosphite triester methodology.[11] For the synthesis of unit F we decided to use again, as in previous (unsuccessful) attempts, D-glucurono-6,3-lactone (**3**)[12] as starting material since this option promised to guarantee a minimum of protecting group manipulations. It was clear that the previously encountered and at that time unsurmountable solubility problems[13] had to be solved in the present work.

Synthesis of 6

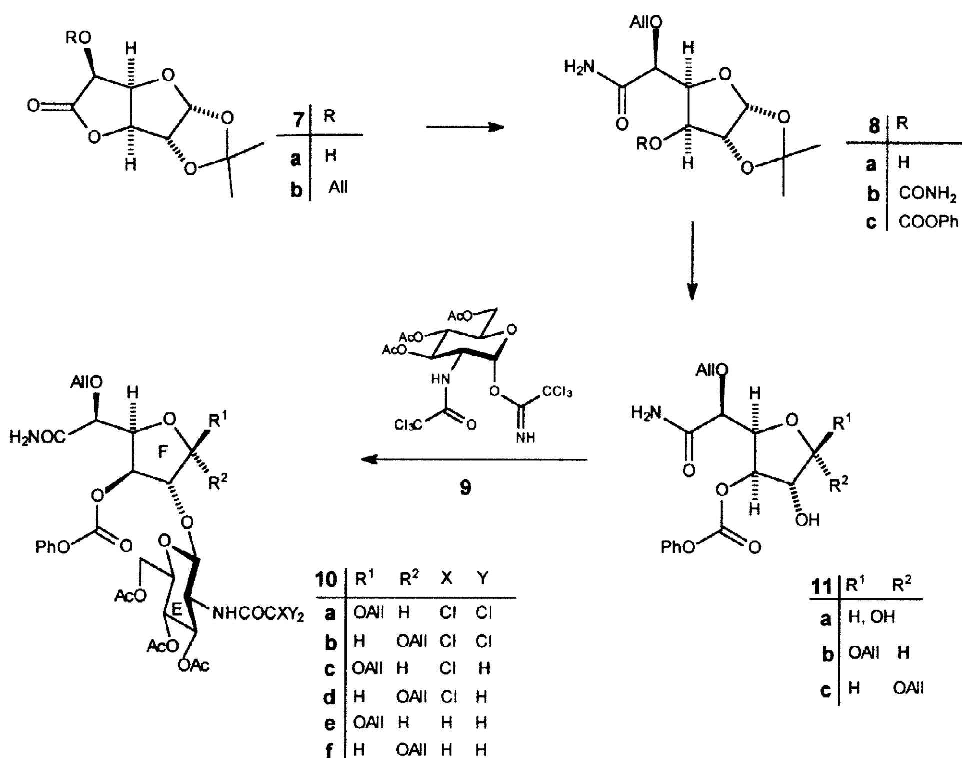
Protection of D-mannitol as its 1,3:4,6-bis(benzylidene)acetal **4a**, followed by alkylation of the remaining free alcohol groups with n-hexyl bromide (**4a** → **4b**), hydrolytic removal of the benzylidene acetals (**4b** →



5), Malaprade cleavage of the vicinal diol, oxidation of the intermediate aldehyde to the corresponding acid, and esterification of the acid provided **6**. As reported previously,[9] oxidation of the 2-*O*-hexyl glyceraldehyde with bromine gave the best results.

Synthesis of the glycosyl acceptor

Alkylation[14] of the 5-OH group of **7a** using allyl trichloroacetimidate[15,16] in a trifluoromethanesulfonic acid-catalyzed reaction gave allyl ether **7b** in 61% yield. The lactonic ring in **7b** was opend with NH_3 in methanolic solution[17] to furnish the known uronamide **8a**.[13] The next step was a crucial event in the synthesis.

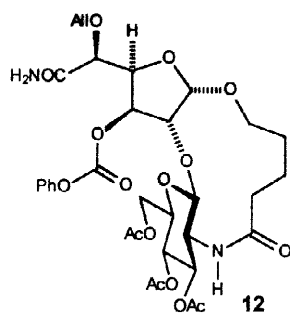


Previously,[13] we have introduced the carbamoyl group by reacting the free OH group with trichloroacetyl isocyanate and subsequent removal of the trichloroacetyl group from the resulting trichloroacetyl urethane[18] to give **8b**. Most probably the solubility problems referred to above were associated with the presence of several amide and urethane groups, respectively, in the intermediates on the way to **1b**.[13] And indeed, exchange of the urethane group by a latent equivalent solved practically all solubility problems and allowed to proceed with the synthesis as desired. Thus, **8a** on reaction with phenyl chloroformate[19] in dichloromethane in the presence of 1.0 eq of Steglich's base and triethylamine provided phenyl carbonate **8c** which was nicely soluble in organic solvents of medium polarity.

The acetonide protecting group was then removed with 90 per cent trifluoroacetic acid at 20°C to form **11a** in quantitative yield as a mixture of anomers which was in turn converted under Fischer conditions to a mixture of allyl glycosides **11b** (45%) and **11c** (25%).

Disaccharide formation

For the disaccharide formation we wanted to make use of the Blatter, Beau, Jacquinet method which was introduced some time ago[20] avoiding the notoriously low reactivity of the oxazoline derived from N-acetylglucosamine.[21] In the event, TMSOTf-promoted reaction of **11c** with the oxazoline prepared in-situ from **9** gave the desired disaccharide **10b** in 76% yield, 11% of **11c** were recovered. Glycosidation of the β-isomer **11b** under the same reaction conditions furnished **10a** in 60% yield, and 29% of **11b** were recovered. The structures of the two disaccharides, especially the ß-glycosidic linkage of unit E to unit F are in agreement with all spectroscopic data.



Dehalogenation of the trichloroacetyl group caused a number of problems, especially in the case of **10b**. First, we studied the reaction of **10a** with tributyltin hydride under the conditons of Blatter et al.[20] It turned out that the yields varied from 40-85%. Sometimes, the chloroacetamide **10c** was isolated. On the other hand, reduction with freshly prepared Zn-Cu couple in acetic acid provided the desired N-acetylamino compound **10e** reproducibly in 85% yield.

The α-isomer proved to be much more troublesome. Reaction of **10b** (0.024 mol/L in toluene / N,N-dimethylacetamide) using 4.5 eq of tributyltin hydride provided the desired product **10f** only in 38% yield alongside with macrocyclisation product **12** (30% yield).

As expected, an increase of the hydride concentration (10 eq of tributyltin hydride) at 0.24 M concentration furnished the desired product **10f** in a better yield (52%). **12** was formed only in 8% yield. However in this case, a product **X** was formed in which according to the FAB mass spectrum the NHAc group was present as desired but, in addition, tributyltin hydride addition to one of the allylic double bonds had occurred. Thus, the Zn-Cu method seems to be superior when compared with the Bu_3SnH dehalogenation, especially when allyl protecting groups are used. Another approach to convert the trichloroacetyl to an acetyl group based on work from Weygand's laboratory was reported recently.[22]

Urethane formation and allyl group removal

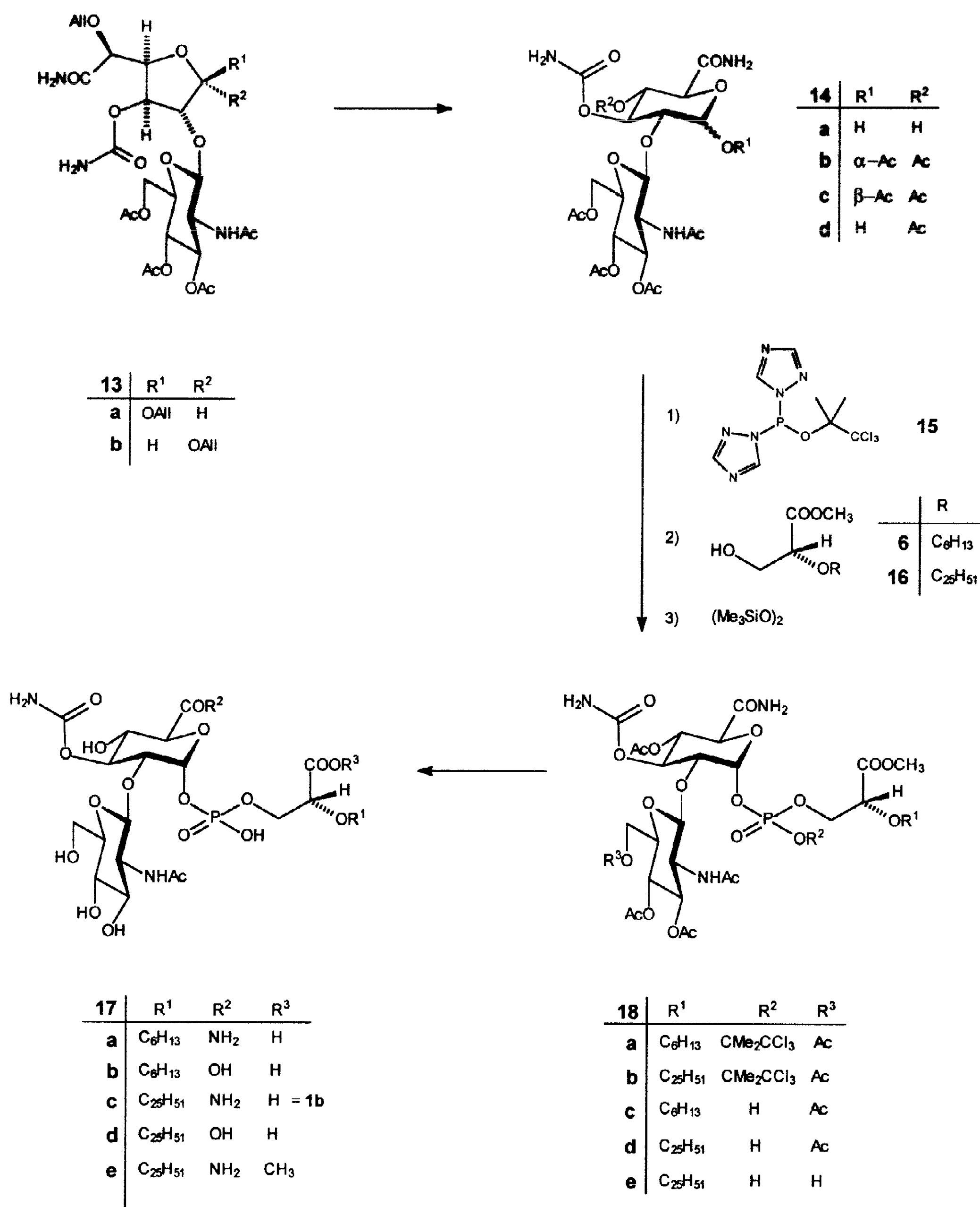
After extensive experimentation[23] the following order of functional group manipulations was found to be the most effective: (i) conversion of the phenyl carbonate into the urethane with ammonia-dioxane-pyridine (97% of **13a** and 78% of **13b**),[19] (ii) removal of the allyl protecting groups making use of the Nakayama method[24] (80% in the α- and 85% in the β-series), and (iii) acetylation of the free OH groups to give the α-acetate **14b** (87%, after purification). The presence of the urethane grouping was indicated by a signal at δ = 157.67 (urethane C) in the ^{13}C NMR and a broad singlet (2 H) at δ = 7.57 in the ^{1}H NMR spectra. Acetylation immediately after deallylation was performed for two reasons: (i) to protect the 4^F-OH group and (ii) to avoid solubility problems in the phosphorylation step. The acetyl group at the anomeric position was then removed with hydrazinium acetate in DMF (Excoffier method[25]). The reaction mixture was transferred directly onto the top of a FC column (Sephadex LH-20). This was found to be the best method for the purification and afforded **14d** in 95% yield.

Construction of the phosphoric triester moiety and completion of the synthesis

For the construction of the phosphoric acid diester grouping we used the Ugi variant[26] of the phosphite methodology[11] adapted to the synthesis of moenomycin analogues.[18,27]

Thus, bistriazolide **15** (formed *in-situ* from the corresponding dichlorophosphite) was treated with disaccharide **14d** (dissolved in 7:1 CH_2Cl_2-pyridine) and the reaction mixture was stirred at 0°C for 2 h. The reaction product was allowed to react with the C_6H_{13}-lipid compound **6** (added in three portions over a period of 2 h)

to furnish the corresponding phosphorous acid triester, which in turn was oxidized with bis(trimethylsilyl)peroxide [28,29,30] to provide after purification a pure diastereomer **18a** (55%) and a mixture of



two P-diastereomers (23%). Analogously, phosphoric acid triester **18b** was obtained making use of moenocinol derived H-I part **16**. A pure diastereomer was obtained in 47% and a mixture of two P-diastereomers in 34% yield.

Removal of the trichloroethyl group from **18a** was achieved with freshly prepared Zn-Cu couple in pyridine in the presence of 2,4-pentanedione (Imai conditions[31]). After stirring for 1 h at room temperature the reaction was complete to give a 8:1 mixture of two products (69%), one with five (**18c**) and the second with four acetyl groups. The ratio was determined by ^{1}H NMR in [D$_5$]pyridine. Similarly the phosphate protecting group was removed from **18b** (here the reaction was more sluggish). Again, a 1:1 mixture (^{1}H NMR in

CD_3OD) of pentaacetyl (**18d**) and a tetraacetyl compound (probaly **18e**) was obtained (68%). Both mixtures were used without further purification.
The ester protecting groups in the mixture containing **18c** were removed by hydrolysis with 0.3 mol/L aqueous lithium hydroxide in 2:1 methanol-water under carefully controlled reaction conditions (see Experimental). Even then a mixture of two products was formed, which could be separated by careful chromatography. The slightly less polar compound was uronamide **17a** (44%) and the more polar compound turned out to be the uronic acid **17b** (25%). Basic hydrolysis of the ester protecting groups in the mixture of **18d** and **18e** provided a mixture of three compounds as shown by TLC. The least polar compound was a minor reaction product (< 6%) and turned out to be methylester **17e**. The major products were (with decreasing R_f value) the desired uronamide **1b** (45%) and the uronic acid **17d** (21%).

Activities of 17a and 1b in various test systems
The minimum inhibitory concentration (MIC) against various micro-organisms have been determined by a

Table 1: Minimum inhibitory concentration (in µg/mL) of **17a, 1b, 17d** and of moenomycin A against various test organisms

Strain	MIC (µg/mL)			
	moenomycin A	**17a**	**1b (17c)**	**17d**
Staph. aureus SG 511	0.098	> 50	> 50	> 50
Staph. aureus 503	0.049	> 50	> 50	> 50
Staph. epidermidis ZH 2c	0.025	> 50	> 50	> 50
Ent. faecium Md8B	> 128	> 50	> 50	> 50
Strept. pyogenes VR3	>128	> 50	> 50	> 50
Strept. pyogenes 77A	< 0.002	> 50	1.563	3.125

serial two-fold agar dilution method (Müller Hinton Agar). The activity of these compounds and of moenomycin A (for comparison) are collected in Table 1.
The results demonstrate that the synthetic compounds are practically inactive against *gram*-positive bacteria. The *in-vitro* activity of the compounds was studied in the test of Izaki, Matsuhashi, and Strominger[32] (slightly modified version[33]) 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.
The results (Table 2) demonstrate that in this *in-vitro* system, synthetic compound **1b** is an as active inhibitor as moenomycin A itself, but the uronic acid **17d** is less active than **1b** and moenomycin A (**1**), compound **17a** is inactive.

Table 2: Effect of compounds **17a, 1b (17c), 17d** and 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.

final concentration	% inhibition			
(µg/mL)	moenomycin A	**17a**	**1b (17c)**	**17d**
100	98	33	95	91
10	97	21	91	86
1	97	11	87	67

In addition, the inhibitory effect of these compounds directly on the transglycosylation reaction was determined by the *in-vitro* assay of van Heijenoort[xxx,34] using a crude extract from an over-producer of polymerase PBP 1b (*E. coli JA200 plc19-19*) and as substrate the lipid II intermediate which is the immediate precursor of uncross-linked peptidoglycan.
The results demonstrate that in this *in-vitro* system, the synthetic compounds **17a**, **17b**, **17d** are inactive, whereas compound **1b** is as active as moenomycin A itself and degradation product **1a** (Table 3).

Table 3: Effect of **1b**, Moenomycin A and **1a** (for comparison) on the *in-vitro* formation of uncross-linked peptidoglycan by transglycosylation.

final concentration µg/mL	% inhibition		
	moenomycin A	1a	1b
10	100	100	100
1	95	85	92

Discussion of the test results.
Over the years, disaccharide and trisaccharide analogues of moenomycin A have been prepared for the evaluation of structure-activity relationships.

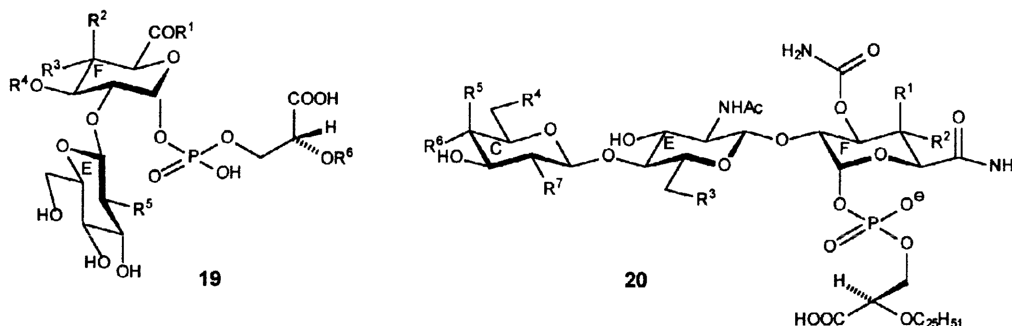


Table 4. Antibiotic and transglycosylase-inhibiting properties of disaccharide analogues of moenomycin A

19	R^1	R^2	R^3	R^4	R^5	R^6	MIC values against *Staph.aureus SG* 511 µg/mL	Izaki, Matsuhashi, Strominger test system[32, 33, a] % inhibition at 1 µg/mL	van Heijenoort test system,[34, a] % inhibition at 1 µg/mL	Ref.
a (1a)	NH_2	CH_3	OH	$CONH_2$	NHAc	$C_{25}H_{51}$	12.500	n.d.	100	35
							> 50			36
b (1b)	NH_2	H	OH	$CONH_2$	NHAc	$C_{25}H_{51}$	> 50	87	100	36
c (17a)	NH_2	H	OH	$CONH_2$	NHAc	C_6H_{13}	> 50	11	inactive	36
d	NH_2	OH	H	$CONH_2$	NHAc	$C_{25}H_{51}$	> 50	10	n.d.	18
e	OH	H	OH	$CONH_2$	NHAc	$C_{25}H_{51}$	n.d.	67	inactive	36
f	OCH_3	CH_3	OH	$CONH_2$	NHAc	$C_{25}H_{51}$	n.d.	n.d.	inactive	37
g	OH	CH_3	OH	$CONH_2$	NHAc	$C_{25}H_{51}$	n.d.	n.d.	26	37
h	NH_2	CH_3	OH	H	NHAc	$C_{25}H_{51}$	n.d.	n.d.	inactive	37
i	NH_2	H	H	$CONH_2$	NHAc	$C_{25}H_{51}$	n.d.	n.d.	inactive	38
j	NH_2	H	OH	$CONH_2$	OH	$C_{25}H_{51}$	> 80	n.d.	inactive	39

[a] For details, see text

In the disaccharide series compounds **1a** and **1b** have full activity (when compared with moenomycin A) as transglycosylase inhibitors. This results means that at least in the van Heijenoort test system the methyl

group at C-4 of unit F is not involved in eliciting activity. On the other hand, all other derivatives are either inactive or of significantly lower activity (see Table 4). Compound **1b** is thus the structurally simplest transglycosylase inhibitor known to-date.

With respect to the *in-vivo* activity against *Staph.aureus* SG 511 compound **1a** seems to be at a borderline (see Table 4). Previously, a weak activity has been determined whereas in a recent experiment the compound was inactive.

Obviously, there is a discrepancy between the *in-vivo* and the *in-vitro* test systems (vide infra). Of great significance is also the observation that compound **1b** is active in van Heijenoort's *in-vitro* inhibition test system whereas the analogous compound with the short lipid chain (**17a**) is completely inactive. This point will also be discussed below.

Table 5. Antibiotic and transglycosylase-inhibiting properties of trisaccharide analogues of moenomycin A

20	R^1	R^2	R^3	R^4	R^5	R^6	R^7	MIC values against *Staph. aureus SG 511* µg/mL	Izaki, Matsuhashi, Strominger test system[32, 33, a] % inhibition at 1 µg/mL	van Heijenoort test system,[34, a] % inhibition at 1 µg/mL	Ref
a	CH_3	OH	OH	H	H	OH	NHAc	3.13	n.d.	100	35
b	OH	H	OH	H	H	OH	NHAc	6.25	91	100	40
c	OH	H	H	H	H	OH	NHAc	6.25	n.d.	100	41
d	OH	H	OH	OH	H	OH	NHAc	8.25	86	93	42
e	OH	H	OH	OH	OH	H	OH	> 50	0	0	43
f	OH	H	OH	OH	H	OH	OH	>64	38	0	44

[a] For details, see text.

In the trisaccharide series compounds that are transglycosylase inhibitors show also antibiotic properties against *Staph.aureus SG 511*. Unit F may have D-*gluco* or D-*galacto* configuration. In this series it was found that the NHAc group of unit C is of prime importance since compounds **20e** and **20f** are inactive (see Table 5).

All the work that has been performed in the field of moenomycin-type compounds seems to justify the conclusion that these compounds are substrate analogous inhibitors of the transglycosylase.

It may be assumed that the transglycosylases like other glycosyltransferases have two binding sites, one for the glycosyl donor and one for the acceptor.[2] As Scheme 1 indicates, glycosyl donor and glycosyl acceptor in the transglycosylation reaction are structurally very similar. It is, therefore, impossible to conclude from a simple inspection of structures where the moenomycin antibiotics bind. The most obvious explanation of the structure-activity relationships summarized above is as follows:

The *in-vivo* and *in-vitro* activities have to be considered separately. The *in-vivo* activity of moenomycin A and its antibiotically active analogues originates from an unspecific binding to the membrane with the lipid part and then by a selective recognition of the sugar unit by the donor binding site of the enzyme. This view is indicated (i) by the fact that an intact lipid chain [45] and at least a trisaccharide sugar part are needed to elicit the activity and (ii) by the observation that the NHAc group in unit C is essential.[44] A mechanism for the inhibition of the transglycosylase has recently been proposed. The suggested conformation at the enzyme is indicated in formula **20**. Some recent NMR studies[46] may support the conclusions drawn from the work on structure-activity relationships and from force-field calculations.[44]

Most probably the acceptor binding site at the enzyme of the intact microorganism is not accessible to the antibiotics. The disaccharide analogues are thus antibiotically inactive. However, in the *in-vitro* test systems particulate fractions are used in which the enzyme is obviously more exposed. Under these conditions also the acceptor binding site can be reached. Therefore, in these test systems disaccharide analogues may be

active, provided that a specific binding interaction between the compound and the binding site of the enzyme exists. This is the case only for **1a** and **1b** whereas all the other disaccharide analogues prepared until now do not meet the essential structural requirements (see Table 4). Here, too, the unspecific binding to the membrane must be strong enough. The C_6H_{13} lipid side chain does not supply sufficient binding energy as indicated by synthetic compound **17a** which is inactive.

From these results one may conclude that affinity purification and affinity labeling studies of the transglycosylase domain of PBP 1b using moenomycin-type compounds have to be designed carefully.

Another point that has to be considered is the sensitivity of the *in-vitro* test systems. These tests may lead to a strong underestimation of the inhibitory activity of moenomycin. Under such circumstances compounds would appear to have activities in the range of moenomycin which in reality (*in-vivo* test systems) are orders of magnitude less active than moenomycin.

Why are the moenomycin-type compounds only active against *gram*-positive bacteria?

It is well-known that the moenomycin antibiotics are active only against *gram-positive* bacteria whereas they show strong inhibition against the transglycosylase isolated from *E.coli*. This holds even for the trisaccharide analogues. There may be two reasons for this:

Table 6. Minimum inhibitory concentrations of moenomycin A and of vancomycin ainst various test organisms and the effect of polymyxin B nonapeptide on the IC's

Antibiotic and permeabilizer	MIC (μg/mL)			
	E. coli 078	*E. coli* TEM	*Staph. aureus* SG 511	*Staph. aureus* 285
PMBNP	7.8	7.8	62.5	125
Moenomycin A	250.0	125.0	0.06	0.12
Moenomycin A + PMBNP (0.1 μg/mL)	31.2	15.6	0.03	0.12
Moenomycin A + PMBNP (1 μg/mL)	3.9	1.0	0.03	0.12
Moenomycin A + PMBNP (10 μg/mL)	-	-	0.12	0.12
Vancomycin	62.5	62.5	0.25	0.25
Vancomycin + PMBNP (0.1 μg/mL)	31.2	15.6	0.25	0.25
Vancomycin + PMBNP (1 μg/mL)	31.2	7.8	0.5	0.25
Vancomycin + PMBNP (10 μg/mL)	-	-	0.5	0.5

(i) *Gram*-positive and *gram*-negative bacteria differ in the lipid composition of their plasma membranes. Whereas, for example, it has been reported that the main fraction of the plasma membrane of *E.coli* is phosphatidylethanolamine (a zwitterionic lipid) the main constitutents of *gram*-positive bacteria such as *Staph. aureus* are anionic phospholipids such as phosphatidylglycerol.[47]

(ii) Many *gram*-negative bacteria have been shown to be resistant to antibiotics as a result of the permeability barrier of their outer membrane (OM).

The outer monolayer of OM is made up from a lipopolysaccharide (LPS). LPS is polyanionic because of a number of negative charges in its lipid A and inner-core parts. Adjacent polyanionic LPS molecules are linked electrostatically to each other by divalent cations.

Polycationic compounds and chelators such as EDTA have been shown to be able to disorganize the OM and permeabilize it to compounds which could normally not pass it.[48] In order to better understand the selectivity of the moenomycin antibiotics, the MIC's of moenomycin A and of vancomycin against a number of test organisms have been studied in the presence of polymyxin B nonapeptide (PMBNP) a compound well-known for its ability to increase the permeability of the outer membrane. The results (collected in Table 6)

show that PMBNP increases the sensitivity of *E.coli* to moenomycin (and vancomycin, for comparison) dramatically in a dose-dependent manner. The effect is more pronounced for moenomycin A than for vancomycin. As expected, no effect was found for *Staph. aureus*. The results may be taken as an indication that the low antibiotic activity of the moenomycin-type compounds against *gram*-negative bacteria results from the low permeability of the outer membrane to these compounds rather than from different constituents of the cytoplasmic membranes of *gram*-positive and *gram*-negative bacteria.

EXPERIMENTAL

General

NMR: Gemini 200 and Gemini 2000 (Varian, ^{1}H NMR 200 MHz, ^{13}C NMR 50.3 MHz), Gemini 300 (Varian, ^{1}H NMR 300 MHz, ^{13}C NMR 75.5 MHz, ^{31}P NMR 121.5 MHz), Unity 400 (Varian, ^{1}H NMR 400 MHz, ^{13}C NMR 100.6 MHz, ^{31}P NMR 161.9 MHz), chemical shifts are given in δ values, the ^{31}P NMR shifts are based on external phosphoric acid; FAB MS: VG AUTOSPEC (matrix: lactic acid or 3-nitrobenzyl alcohol), two molecular masses are always communicated, the first was calculated using the International Atomic Masses, the second refers to ^{12}C, ^{1}H, ^{16}O, ^{14}N, ^{31}P, ^{35}Cl, ^{120}Sn (mono-isotopic masses), carbon and proton numbering in the subunits (see NMR data) as well as naming of the MS fragments follows the moenomycin nomenclature[37]; melting points (corrected, determined in capillary tubes): Büchi (B-540); analytical TLC spots of phosphates were identified with the phosphate-specific spraying reagent of Dittmer and Lester;[49] optical rotations (sodium D-line, 0.5 dm cell): Perkin-Elmer Model 141.- Elemental analyses were performed at the laboratory Ilse Beetz, Kronach. For all other methods and instrumentation, see ref.[7]- The minimum inhibitory concentrations (MIC) were determined by a serial two-fold micro dilution method (Müller-Hinton medium). A series of increasing concentrations of the compound under investigation was prepared in the medium and in the medium + Polymyxin B nonapeptide (PMBN, concentrations as indicated in Table 6), respectively. For inoculations 1 x 10^5 cfu/mL were used. After 24 h at 37°C the MIC's were determined (absence of visible turbidity). All MIC values are the result of double experiments.

1,3:4,6-Di-*O*-benzylidene-2,5-di-*O*-hexyl-D-mannitol (4b)

Compound **4a** (1.0 g, 2.8 mmol) was dissolved in dry DMF (14 mL), sodium hydride (0.2 g, 8.4 mmol) was added in portions, followed by 1-bromohexane (1.0 g, 0.86 mL, 6.2 mmol) and the mixture was heated at 70 °C for 3 h. The reaction mixture was cooled, diluted with ether (30 mL) and filtered, the filtrate was washed with 1 N HCl (3x 20 mL), brine (1x 20 mL) and water. The organic solution was then dried over Na_2SO_4 and the solvent was evaporated under reduced pressure to give 1.2 g of a crude product which was purified by FC on silica gel (hexanes-ethyl acetate 25:1) to give **4b** (1.1 g, 75%) as an oil.-^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.55-7.52 (m, 4 H, Ar-Hs), 7.42-7.28 (m, 6 H, Ar-Hs), 5.51 (s, 2 H, Ph-C<u>H</u>), 4.46 (dd, 2 H, 1-H, 6-H), 4.02 (d, 2 H, 3-H, 4-H), 3.89 (ddd, 2 H, 2-H, 5-H), 3.66-3.60 (m, 4 H, 2x alkyl-1-H, 1'-H, 6'-H), 3.54-3.48 (ddd, 2 H, 2x alkyl-1'-H), 1.62-1.52 (m, 4 H, 2x alkyl-CH_2-2), 1.40-1.24 (m, 12 H, 2x alkyl-CH_2-3, 4, 5), 0.90 (t, 6 H, 2x alkyl-CH_3), $^{2}J_{1,1'}$ = 10.8 Hz, $J_{1,2}$ = 5.0 Hz, $J_{1',2}$ = 14.6 Hz, $J_{2,3}$ = 9.5 Hz.- ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 137.54 (Ar-C-1), 128.43 (Ar-C-4), 127.78 (Ar-C-3, Ar-C-3'), 125.80 (Ar-C-2, Ar-C-2'), 100.75 (Ph-<u>C</u>H), 77.29 (C-3, C-4), 70.48, 69.41 (C-1, C-6, alkyl-C-1), 66.92 (C-2, C-5), 31.18, 29.63, 25.34, 22.21 (alkyl-C-2,3,4,5), 13.63 (alkyl-C-6).- IR ($CHCl_3$): 2920, 2860, 1100 cm^{-1}.- $C_{32}H_{46}O_6$ (526.71, 526.33).

2,5-Di-*O*-hexyl-D-mannitol (5)

A mixture of **4b** (1.00 g, 1.9 mmol), ethanol (37 mL), water (11 mL), and 12 N HCl (1.5 mL) was heated at reflux for 65 h. The reaction mixture was cooled and neutralized with saturated $NaHCO_3$. After removal of ethanol under reduced pressure, the remaining solution was extracted with $CHCl_3$, the organic phase was dried over Na_2SO_4, and the solvent evaporated. The crude residue was purified by FC ($CHCl_3$-methanol 20:1) to yield **5** (0.46 g, 70%).-^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.93 (t, 2 H, J = 6.0 Hz), 3.85-3.75 (m, 4 H), 3.70-3.45 (m, 6 H, 1'-H, 6'-H, 2x alkyl-CH_2-1), 3.25 (d, 2 H, 2x OH, ^{3}J = 5.1 Hz), 2.85 (broad s, 2 H, 2x OH), 1.65-1.50 (m, 4 H, 2x alkyl-CH_2-2), 1.40-1.20 (m, 12 H, alkyl-CH_2-3,4,5), 0.90 (t, 6 H, 2x alkyl-CH_3).-^{1}H NMR (200 MHz, $CDCl_3$+D_2O): Similar to the previous data, but there is no signal at δ = 3.25 and 2.85.-

^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 81.06 (C-2, C-5), 71.48, 70.18 (C-1, C-6, alkyl-C-1), 61.52 (C-3, C-4), 32.09, 30.46, 26.22, 23.05 (alkyl-C-2, 3, 4, 5), 14.48 (alkyl-C-6).- IR (KBr): 3580-3200, 2920, 2850, 1120, 1040 cm^{-1}.- $C_{18}H_{38}O_6$ (350.50, 350.27)

(*R*)-Methyl-2-(hexyloxy)-3-hydroxy-propionate (6)
A solution of $NaIO_4$ (4.84 g, 22.8 mmol) in water (60 mL) was added to the solution of compound **5** (2.10 g, 6.0 mmol) in THF (120 mL) at 20°C. After 12 min the reaction mixture was cooled to 0°C and bromine (1.9 mL) was added dropwise. The reaction was stirred for 2 h at 20°C, then 1 per cent H_2SO_4 (25 mL) was added. Extraction with ethyl acetate (5x 50 mL), washing the organic layer several times with $NaHCO_3$ until the red-brown colour disappeared, then with water, drying over $MgSO_4$ and solvent evaporation yielded the crude carboxylic acid (2.50 g). The crude acid was dissolved in 1.5% methanolic HCl (120 mL) and the mixture was stirred at 20°C for 24 h. After solvent evaporation the residue was taken up in dichloromethane, washed with saturated aq $NaHCO_3$, water, dried over Na_2SO_4. Solvent evaporation and subsequent FC (hexanes-ethyl acetate 7:3) provided **6** (1.55 g, 63%) as a yellow oil.- B.p.: 265-268°C.- $[\alpha]_D^{25.5}$ = + 4.16 (c 0.308, $CHCl_3$).- ^1H NMR (200 MHz, homo decoupling, $CDCl_3$): δ = 3.99 (dd, 1 H, 2-H, J = 3.7, 6.1 Hz), 3.90-3.80 (m, 2 H, CH_2-3), 3.78 (s, 3 H, $COOC\underline{H}_3$), 3.71, 3.42 (2 ddd, 2x 1 H, alkyl-CH_2-1, J = 13.3, 9.0, 6.7 Hz), 2.22 (t, 1 H, OH, J = 6.8 Hz), 1.74-1.55 (m, 2 H, alkyl-CH_2-2), 1.42-1.22 (m, 6 H, alkyl-CH_2-3,4, 5), 0.90 (t, 3 H, alkyl-CH_3).- After addition of 0.1, 0.1, 0.2, 0.2 eq. of $Eu(TFC)_3$ the methyl ester signal was in all cases observed as a singlet at δ = 3.89, 3.99, 4.20, 4.31, respectively.- ^{13}C NMR (C,H COSY, APT, 50.3 MHz, $CDCl_3$): δ = 171.79 (CO), 80.06 (C-2), 71.89 (alkyl-C-1), 63.87 (C-3), 52.51 ($COO\underline{C}H_3$), 32.06, 30.07, 26.09, 23.04 (alkyl-C-2,3,4,5), 14.48 (alkyl-CH_3).- IR ($CHCl_3$): 3592, 3027, 2956, 2932, 2868, 1749, 1181, 1128, 1050 cm^{-1}.- $C_{10}H_{20}O_4$ (204.27, 204.14).- FAB MS: m/z 227.1 $[M+Na]^+$, 205.2 $[M+H]^+$.

1,2-*O*-Isopropyliden-5-*O*-allyl-α-D-glucofuranosidurono-6,3-lactone (7b)
7b was prepared as described previously.-^1H NMR (200 MHz, $CDCl_3$): δ = 6.02 (d, 1 H, 1-H), 6.07-5.87 (m, 1 H, allyl-2-H), 5.38 (dq, 1 H, allyl-3-H_a), 5.28 (dq, 1 H, allyl-3-H_b), 4.95 (dd, 1 H, 4-H), 4.80 (d, 1 H, 2-H), 4.76 (d, 1 H, 3-H), 4.38-4.31 (m, 2 H, allyl-CH_2-1), 4.33 (d, 1 H, 5-H), 1.52, 1.35 (2 s, 2x 3 H, 2x CH_3), $J_{1,2}$ = 4.0 Hz, $J_{2,3}$ < 1.0 Hz, $J_{3,4}$ = 3.0 Hz, $J_{4,5}$ = 4.4 Hz, allyl group: $^4J_{1,3a}$ = 1.5 Hz, $J_{2,3a}$ = 17.2 Hz, $J_{2,3b}$ = 10.5 Hz, $^2J_{3a,3b}$ = 2.9 Hz[50].-^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 172.35 (C-6), 133.69 (allyl-C-2), 119.98 (allyl-C-3), 113.67 [$\underline{C}(CH_3)_2$], 107.46 (C-1), 83.01 (C-4), 82.22 (C-2), 77.82 (C-5), 75.48 (C-3), 72.58 (allyl-C-1), 27.34, 26.94 (2x CH_3).- $C_{12}H_{16}O_6$ (256.26, 256.09).- FAB MS: m/z 279.0 $[M+Na]^+$, 257.0 $[M+H]^+$, 241.0 $[M+H-CH_4]^+$, 199.0 $[M+H-CH_3COCH_3]^+$.

1,2-*O*-Isopropyliden-5-*O*-allyl-α-D-glucofuranosiduronamide (8a)
8a was prepared as described previously.- ^1H NMR (H,H COSY, 200 MHz, $CDCl_3$): δ = 6.86, 6.25 (2 broad s, 2x 1 H, $CONH_2$), 6.01-5.80 (m, 1 H, allyl-2-H), 5.90 (d, 1 H, 1-H), 5.41 (d, 1 H, OH), 5.34 (dq, 1 H, allyl-3-H_a), 5.26 (dq, 1 H, allyl-3-H_b), 4.56-4.50 (4-H), 4.49 (d, 1 H, 2-H), 4.31 (d, 1 H, 5-H), 4.26 (dd, 1 H, 3-H), 4.22-3.60 (m, 2 H, allyl-CH_2-1), 1.49, 1.31 (2 s, 2x 3 H, 2x CH_3), $J_{1,2}$ = 3.9 Hz, $J_{2,3}$ < 1.0 Hz, $J_{3,4}$ = 3.3 Hz, $J_{4,5}$ = 2.1 Hz, $J_{3,OH}$ = 5.8 Hz.- ^1H NMR (200 MHz, $CDCl_3+D_2O$): Similar to the previous data, but there are no signals at δ = 6.86, 6.25 and 5.41.- ^{13}C NMR (APT, 50.3 MHz, $CDCl_3$): δ = 173.75 (C-6), 133.37 (allyl-C-2), 119.66 (allyl-C-3), 112.47 ($\underline{C}(CH_3)_2$), 105.37 (C-1), 86.14 (C-4), 81.30 (C-2), 78.44 (C-5), 75.89 (C-3), 73.30 (allyl-C-1), 27.47, 26.74 (CH_3).- $C_{12}H_{19}NO_6$ (273.29, 273.12).- FAB MS: m/z 296.0 $[M+Na]^+$, 274.0 $[M+H]^+$, 258.0 $[M+H-CH_4]^+$, 216.0 $[M+H-CH_3COCH_3]^+$.

1,2-*O*-Isopropyliden-5-*O*-allyl-3-*O*-phenoxycarbonyl-α-D-glucofuranosiduronamide (8c)
To a mixture of **8a** (250 mg, 0.9 mmol), DMAP (112 mg, 1.0 eq.), dry CH_2Cl_2 (4 mL), dry triethylamine (125 μL, 1.0 eq.) phenyl chloroformate (0.13 mL, 1.1 eq.) was added dropwise and the reaction mixture was stirred at 20°C for 1 h. The reaction mixture was diluted with CH_2Cl_2, washed with a 10 per cent aqueous solution of tartaric acid, twice with a saturated aq. $NaHCO_3$, water and dried over Na_2SO_4. After solvent evaporation FC ($CHCl_3$-ethyl acetate 9:1) provided **8c** (340 mg, 96 %).- M.p.: 195-196°C (ethyl acetate).- $[\alpha]_D^{26}$ = +0.66 (c 21.25, $CHCl_3$).- ^1H NMR (200 MHz, $CDCl_3$): δ = 7.40-7.36 (2 H, Ar-3-H, Ar-3'-H), 7.27-

7.26 (m, 1 H, Ar-4-H), 7.20-7.15 (2 H, Ar-2-H, Ar-2'-H), 6.40 (broad s, 1 H, $CONH_2$), 6.10 (d, 1 H, 1-H), 6.00-5.85 (m, 1 H, allyl-2-H), ~5.80 (1 H, $CONH_2$), 5.40-5.18 (allyl-CH_2-3), 5.30 (d, 1 H, 3-H), 4.70 (d, 1 H, 2-H), 4.55 (dd, 1 H, 4-H), 4.20 (d, 1 H, 5-H), 4.20-3.90 (m, 2 H, allyl-CH_2-1), 1.51, 1.33 (2 s, 2x 3 H, 2x CH_3), $J_{1,2}$ = 3.8 Hz, $J_{2,3}$ < 1.0 Hz, $J_{3,4}$ = 2.9 Hz, $J_{4,5}$ = 6.9 Hz.- ^{13}C NMR (APT, 50.3 MHz, $CDCl_3$): δ = 172.65 (C-6), 153.08, 151.41 (Ar-C-1, OCOO), 133.80 (allyl-C-2), 130.10 (Ar-C-3, Ar-C-3'), 126.78 (Ar-C-4), 121.25 (Ar-C-2, Ar-C-2'), 119.10 (allyl-C-3), 113.19 ($\underline{C}(CH_3)_2$), 105.33 (C-1), 83.35 (C-2), 80.27 (C-3), 79.28 (C-4), 76.93 (C-5), 73.09 (allyl-C-1), 27.16, 26.76 (2x CH_3).- IR (KBr): 3660-3140, 1765, 1675, 1258, 1214, 1090, 1028 cm^{-1}.- FAB MS: m/z 416.0 $[M+Na]^+$, 394.0 $[M+H]^+$, 378.0 $[M+H-CH_4]^+$, 336.0 $[M+H-CH_3COCH_3]^+$, 198.0 $[M+H-CH_3COCH_3-PhOCOOH]^+$.- $C_{19}H_{23}NO_8$ (393.40, 393.14), calcd. C 57.99, H 5.90, N 3.56, found C 57.94, H 5.82, N 3.52.

5-*O*-Allyl-3-*O*-phenoxycarbonyl-D-glucofuranuronamide (11a)

A mixture of **8c** (200 mg, 0.5 mmol) and 90 per cent trifluoroacetic acid (1.2 mL) was stirred at 20°C for 40 min. Water (250 mL) was added and the solvents were removed by lyophilization to give **11a** (171 mg, 95%) as a mixture of anomers (**11a**α / **11a**β = 4/6 as determined by ^1H NMR).- M.p.: 119-120°C ($CHCl_3$-hexanes).- ^1H NMR (H,H COSY, 400 MHz, $[D_6]DMSO$): characteristic signals: *α-anomer:* δ = 5.20 (d, 1 H, 1-H), 5.13 (dd, 1 H, 3-H), 4.36 (dd, 1 H, 4-H), 4.14 (t, 1 H, 2-H), 3.83 (d, 1 H, 5-H), $J_{1,2}$ = 4.2 Hz, $J_{2,3}$ = 4.1 Hz, $J_{3,4}$ = 4.8 Hz, $J_{4,5}$ = 8.0 Hz; *β-anomer:* δ = 5.05 (s, 1 H, 1-H), 4.99 (dd, 1 H, 3-H), 4.31 (dd, 1 H, 4-H), 3.98 (d, 1 H, 2-H), 3.96 (d, 1 H, 5-H), $J_{1,2}$ < 1.0 Hz, $J_{2,3}$ = 1.5 Hz, $J_{3,4}$ = 4.8 Hz, $J_{4,5}$ = 9.0 Hz; *α- and β-anomers:* δ = 7.56 (s), 7.50 (s), 6.60 (broad s) (4 H, $CONH_2$), 7.45-7.42 (m, 4 H, Ar-3-H, Ar-3'-H), 7.33-7.27 (m, 2 H, Ar-4-H), 7.26-7.18 (m, 4 H, Ar-2-H, Ar-2'-H), 5.92-5.81 (m, 2 H, 2x allyl-2-H), 5.27-5.11 (m, 4 H, 2x allyl-CH_2-3), 3.40 (broad s, 2 H, 2x OH), 4.08-3.85 (m, 4H, allyl-CH_2-1).- ^{13}C NMR (C,H COSY, 100.6 MHz, $[D_6]DMSO$): characteristic signals: *α-anomer:* δ = 95.94 (C-1), 81.84 (C-3), 77.41 (C-5), 75.00 (C-4), 73.66 (C-2); *β-anomer:* δ = 103.06 (C-1), 81.39 (C-3), 78.91 (C-2), 77.80 (C-4), 77.29 (C-5); *α- and β-anomers:* δ = 171.34, 171.21 (C-6), 152.55, 150.75, 150.74 (OCOO, Ar-C-1), 134.42 (allyl-C-2), 129.73, 129.68 (Ar-C-3, Ar-C-3'), 126.24 (Ar-C-4), 121.12, 121.05 (Ar-C-2, Ar-C-2'), 117.2, 116.97 (allyl-C-3), 70.50, 70.14 (allyl-C-1).- IR (KBr): 3620-3100, 2345, 1763, 1674, 1638, 1264, 1212, 1078, 1049 cm^{-1}.- $C_{16}H_{19}NO_8$ (353.33, 353.11).- FAB MS: m/z 376.0 $[M+Na]^+$, 354.0 $[M+H]^+$, 336.0 $[M+H-H_2O]^+$.

Allyl glycoside formation

A mixture of **11a** (1.00 g, 2.83 mmol), Dowex 50 W X2 (H^+ form, ~ 1.5 g), 3Å molecular sieves (1.0 g), and allyl alcohol (20 mL) was stirred at 20°C for 2.5 h. Filtration, washing the resin with ethyl acetate, solvent evaporation and FC ($CHCl_3$-ethyl acetate 7:3, then 1:1) furnished **11b** (0.50 g, 45%) and **11c** (0.28 g, 25%).

Allyl 5-*O*-allyl-3-*O*-phenoxycarbonyl-α-D-glucofuranosiduronamide (11c)

M.p.: 153-154°C ($CHCl_3$-hexanes).- $[\alpha]_D^{28.5}$ = +99.2 (c 6.29, $CHCl_3$).- ^1H NMR (H,H COSY, 300 MHz, homo decoupling, 200 MHz, $CDCl_3$): δ = 7.43-7.35 (m, 2 H, Ar-3-H, Ar-3'-H)), 7.28-7.24 (m, 1 H, Ar-4-H), 7.23-7.16 (m, 2 H, Ar-2-H, Ar-2'-H), 6.59 (broad s, 1 H, $CONH_2$), 6.00-5.82 (m, 2 H, 2x allyl-2-H), 5.60 (broad s, 1 H, $CONH_2$), 5.41 (t, 1 H, 3-H), 5.38-5.20 (m, 4 H, 2x allyl-CH_2-3), 5.17 (d, 1 H, 1-H), 4.74 (dd, 1 H, 4-H), 4.56-4.47 (ddd, 1 H, 2-H), 4.38-4.09 (m, 4 H, 2x allyl-CH_2-1), 4.12 (d, 1 H, 5-H), 2.80 (d, 1 H, OH), $J_{1,2}$ = 4.8 Hz, $J_{2,3}$ = 6.2 Hz, $J_{3,4}$ = 6.2 Hz, $J_{4,5}$ = 6.6 Hz, $J_{2,OH}$ = 8.4 Hz.- ^{13}C NMR (C,H COSY, 75.46, 50.3 MHz, $CDCl_3$): δ = 172.74 (C-6), 153.54, 151.51 (OCOO, Ar-C-1), 133.92, 133.84 (allyl-C-2), 130.00 (Ar-C-3, Ar-C-3'), 126.67 (Ar-C-4), 121.35 (Ar-C-2, Ar-C-2'), 119.01, 118.50 (allyl-C-3), 99.73 (C-1), 81.96 (C-3), 79.19 (allyl-C-1), 77.31 (C-4), 75.84 (C-2), 73.94 (C-5), 69.69 (allyl-C-1).- IR (KBr): 3627-3182,1760, 1668, 1313, 1261, 1124, 1056, 1028 cm^{-1}.- FAB MS: m/z 416.2 $[M+Na]^+$, 394.2 $[M+H]^+$, 336.1 $[M+H-AllOH]^+$.- $C_{19}H_{23}NO_8$ (393.39, 393.14), calcd. C 57.99, H 5.90, N 3.56, found C 58.68, H 5.92, N 3.64.

Allyl 5-*O*-allyl-3-*O*-phenoxycarbonyl-β-D-glucofuranosiduronamide (11b)

M.p.: 110-111°C ($CHCl_3$-hexanes).- $[\alpha]_D^{26}$ = -52.7 (c 6.0, $CHCl_3$).- ^1H NMR (H,H COSY, 200 MHz, $CDCl_3$): δ = 7.41-7.33 (m, 2 H, Ar-3-H, Ar-3'-H), 7.30-7.22 (m, 1 H, Ar-4-H), 7.20-7.10 (m, 2 H, Ar-2-H,

Ar-2'-H), 6.50 (d, 1 H, $CONH_2$, J = 2.9 Hz), 6.05-5.84 (m, 2 H, 2x allyl-2-H), 5.60 (broad s, 1 H, $CONH_2$), 5.40-5.19 (m, 5 H, 2x allyl-CH_2-3, 3-H), 5.05 (d, 1 H, 1-H), 4.71 (t, 1 H, 4-H), 4.51 (broad s, 1 H, 2-H), 4.39-4.00 (m, 4 H, 2x allyl-CH_2-1), 4.10 (d, 1 H, 5-H), 3.56 (d, 1 H, OH), $J_{1,2}$ = 2.3 Hz, $J_{3,4}$ = 6.4 Hz, $J_{4,5}$ = 6.5 Hz, $J_{2,OH}$ = 3.7 Hz.- ^{13}C NMR (C,H COSY, 50.3 MHz, $CDCl_3$): δ =173.56 (C-6), 153.70, 151.41 (OCOO, Ar-C-1), 134.48, 134.09 (allyl-C-2), 130.01 (Ar-C-3, Ar-C-3'), 126.74 (Ar-C-4), 121.41 (Ar-C-2, Ar-C-2'), 118.77, 117.84 (allyl-C-3), 107.76 (C-1), 81.79 (C-3), 79.47 (C-4), 79.27 (C-2), 78.98 (C-5), 73.22, 69.76 (allyl-C-1).- IR (KBr): 3546-3104, 1763, 1679, 1644, 1273, 1260, 1214, 1118, 1093, 1045 cm^{-1}.- FAB MS: m/z 416.0 $[M+Na]^+$, 394.1 $[M+H]^+$, 336.0 $[M+H-AllOH]^+$.- $C_{19}H_{23}NO_8$ (393.39, 393.14), calcd. C 57.99, H 5.90, N 3.56, found C 56.87, H 5.96, N 3.65.

Allyl 2-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-5-*O*-allyl-3-*O*-phenoxycarbonyl-α-D-glucofuranosiduronamide (10b)

A mixture of **11c** (275 mg, 0.7 mmol), **9** (500 mg, 0.8 mmol) and activated 3Å molecular sieves (~ 690 mg) in anhydrous 1,2-dichloroethane (8.5 mL) was stirred for 1 h at 20°C under dry argon, then cooled to 0°C. Trimethylsilyl triflate in dry toluene (1.0 M, 151 μL, 0.2 eq) was injected into the reaction flask and the mixture was stirred at 0°C for 5 h. Progress of the reaction was controlled by TLC ($CHCl_3$-ethyl acetate 1:1). Triethylamine (138 μL) was added, and the mixture was filtered, diluted with ethyl acetate, washed with sat. aq. $NaHCO_3$ then with a 10 per cent aqueous solution of tartaric acid and water. The organic phase after drying, solvent evaporation and FC ($CHCl_3$-ethyl acetate 1:1) furnished **10b** (440 mg, 76%). 30 mg of **11c** were recovered.- M.p.: 162-163°C ($CHCl_3$-hexanes).- $[\alpha]_D^{28}$ = +51.8 (c 10.0, $CHCl_3$).- ^1H NMR (400 MHz, $[D_5]$pyridine): δ = 10.95 (d, 1 H, N<u>H</u>$COCCl_3$), 8.60, 8.25 (2 s, 2x1 H, $CONH_2$), 7.50-7.11 (m, 5 H, Ar-Hs), 6.23 (t, 1 H, 3^F-H), 6.13 (dd, 1 H, 3^E-H), 6.09-5.93 (m, 2 H, 2x allyl-2-H), 5.60 (d, 1 H, 1^E-H), 5.50 (t, 1 H, 4^E-H), 5.48 (d, 1 H, 1^F-H), 5.40-5.28 (m, 3 H, 4^F-H, 2x allyl-3-H_a), 5.18-5.08 (m, 2 H, 2x allyl-3-H_b), 5.01 (dd, 1 H, 2^F-H), 4.60 (d, 1 H, 5^F-H), 4.53-4.29 (m, 6 H, CH_2-6^E, 2x allyl-CH_2-1), 4.27-4.19 (m, 1 H, 2^E-H), 3.95 (ddd, 1 H, 5^E-H), 2.09, 2.03, 2.02 (3 s, 3x 3 H, 3x $COCH_3$), $J_{1E,2E}$ = 8.4 Hz, $J_{2E,3E}$ = 10.0 Hz, $J_{3E,4E}$ = 10.0 Hz, $J_{4E,5E}$ = 10.0 Hz, $J_{5E,6E}$ = 2.4 Hz, $J_{5E,6'E}$ = 4.6 Hz, $^2J_{6E,6'E}$ = 10.0 Hz, $J_{1F,2F}$ = 4.3 Hz, $J_{2F,3F}$ = 8.2 Hz, $J_{3F,4F}$ = 8.0 Hz, $J_{4F,5F}$ = 5.0 Hz, $J_{NH,2E}$ = 8.2 Hz.- ^{13}C NMR (C,H COSY, 100.6 MHz, $[D_5]$pyridine): δ = 172.73, 170.97, 170.29 (CO), 163.72 (<u>C</u>$OCCl_3$), 154.16, 152.29 (OCOO, Ar-C-1), 135.56, 135.27 (allyl-C-2), 130.27 (Ar-C-3, Ar-C-3'), 126.85 (Ar-C-4), 122.14 (Ar-C-2, Ar-C-2'), 118.24, 117.33 (allyl-C-2), 101.45 (C-1^E), 100.31 (C-1^F), 94.42 (CCl_3), 82.62 (C-2^F), 80.56 (C-3^F), 79.93 (C-5^F), 76.34 (C-4^F), 73.50, 73.01 (C-5^E, allyl-C-1), 72.47 (C-3^E), 69.94, 69.61 (allyl-C-1, C-4^E), 62.90 (C-6^E), 57.28 (C-2^E), 21.08, 20.93 (CO<u>C</u>H_3).- ^1H NMR (H,H COSY, homo decoupling, 200 MHz, $CDCl_3$): δ = 7.44-7.15 (m, 5 H, Ar-Hs), 7.05 (d, 1 H, $NHCOCCl_3$), 6.57, 5.70 (2 broad s, 2x 1 H, $CONH_2$), 6.00-5.78 (m, 2 H, 2x allyl-2-H), 5.58 (t, 1 H, 3^F-H), 5.45 (dd, 1 H, 3^E-H), 5.38-5.12 (m, 4 H, 2x allyl-CH_2-3), 5.09 (t, 1 H, 4^E-H), 5.07 (d, 1 H, 1^F-H), 5.02 (d, 1 H, 1^E-H), 4.72 (dd, 1 H, 4^F-H), 4.48 (dd, 1 H, 2^F-H), 4.28-3.91 (m, 6 H, CH_2-6^E, 2x allyl-CH_2-1), 4.06 (d, 1 H, 5^F-H), 3.95-3.85 (m ,1 H, 2^E-H), 3.78-3.67 (m, 1 H, 5^E-H), 2.07, 2.03, 2.02 (3 s, 3x 3 H, 3x $COCH_3$), $J_{1E,2E}$ = 8.3 Hz, $J_{2E,3E}$ = 9.2 Hz, $J_{3E,4E}$ = 10.7 Hz, $J_{4E,5E}$ = 10.4 Hz, $J_{NH,2E}$ = 8.3 Hz, $J_{1F,2F}$ = 4.4 Hz, $J_{2F,3F}$ = 7.5 Hz, $J_{3F,4F}$ = 7.5 Hz, $J_{4F,5F}$ = 4.6 Hz.- ^{13}C NMR (C,H COSY, APT, 50.3 MHz, $CDCl_3$): δ = 172.52, 171.00, 169.90 (CO), 162.47 (<u>C</u>$OCCl_3$), 153.18, 151.38 (OCOO, Ar-C-1), 134.40, 133.78 (allyl-C-2), 130.02 (Ar-C-3, Ar-C-3'), 126.72 (Ar-C-4), 121.24 (Ar-C-2, Ar-C-2'), 119.13, 117.87 (allyl-C-3), 100.49 (C-1^E), 99.51 (C-1^F), 92.73 (CCl_3), 82.04 (C-2^F), 79.32 (C-3^F), 79.05 (C-5^F), 75.98 (C-4^F), 73.69 (allyl-C-1), 72.62 (C-5^E), 71.84 (C-3^E), 68.91 (C-4^E), 69.38 (allyl-C-1), 62.43 (C-6^E), 56.55 (C-2^E), 21.18, 21.09, 21.04 (CO<u>C</u>H_3).- IR (KBr): 3600-3200, 1754, 1687, 1247, 1074, 1042 cm^{-1}.- FAB MS: m/z 851.2, 849.2, 847.2 $[M+Na]^+$, 829.2, 827.2, 825.2 $[M+H]^+$, 771.2, 769.2, 767.2 $[M+H-AllOH]^+$, 436.0, 434.0, 432.0 $[e]^+$.- $C_{33}H_{39}Cl_3N_2O_{16}$ (826.04, 824.14), calcd. C 48.05, H 4.77, N 3.40, found C 48.14, H 4.88, N 3.52.

Allyl 2-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-5-*O*-allyl-3-*O*-phenoxycarbonyl-β-D-glucofuranosiduronamide (10a)

11b (400 mg, 1.0 mmol) was converted to **10a** as described for the preparation of **10b**. The crude reaction product was purified by FC ($CHCl_3$-ethyl acetate 7:3, 6:4, 1:1) to give **10a** (500 mg, 60%); 29% of **11b** were recovered.- M.p.: 158-159°C ($CHCl_3$-hexanes).- $[\alpha]_D^{29}$ = -25.7 (c 10.5, $CHCl_3$).- ^1H NMR (H,H COSY, 400

MHz, [D_5]pyridine): δ = 10.75 (d, 1 H, $NHCOCCl_3$), 8.47, 8.35 (2 s, 2x 1 H, $CONH_2$), 7.40-7.18 (m, 5 H, Ar-Hs), 6.11-5.95 (m, 2 H, 2x allyl-2-H), 6.08 (dd, 1 H, 3^E-H), 5.85 (dd, 1 H, 3^F-H), 5.69 (d, 1 H, 1^E-H), 5.52 (t, 1 H, 4^E-H), 5.51 (s, 1 H, 1^F-H), 5.38-5.28 (m, 2 H, 2x allyl-3-H_a), 5.24 (dd, 1 H, 4^F-H), 5.18-5.08 (m, 3 H, 2x allyl-3-H_b, 2^F-H), 4.67 (d, 1 H, 5^F-H), 4.58-4.50 (m, 2 H, allyl-1-H, 2^E-H), 4.47 (dd, 1 H, $6'^E$-H), 4.42-4.33 (m, 1 H, allyl-1-H), 4.31 (dd, 1 H, 6^E-H), 4.25-4.12 (m, 2-H, 2x allyl-1'-H), 3.97 (ddd, 1 H, 5^E-H), 2.08, 2.01, 1.97 (3 s, 3x 3 H, 3x $COCH_3$), $J_{1E,2E}$ = 8.6 Hz, $J_{2E,3E}$ = 10.4 Hz, $J_{3E,4E}$ = 9.5 Hz, $J_{4E,5E}$ = 10.0 Hz, $J_{5E,6E}$ = 2.2 Hz, $J_{5E,6'E}$ = 4.2 Hz, $^2J_{6E,6'E}$ = 12.4 Hz, $J_{NH,2E}$ = 8.4 Hz, $J_{1F,2F}$ < 1.0 Hz, $J_{2F,3F}$ = 1.8 Hz, $J_{3F,4F}$ = 5.2 Hz, $J_{4F,5F}$ = 8.2 Hz.- ^{13}C NMR (C,H COSY, APT, 100.6 MHz, [D_5]pyridine): δ = 173.02, 171.01, 170.93, 170.27 (CO), 163.98 ($\underline{C}OCCl_3$), 153.97, 152.13 (Ar-C-1, OCOO), 135.32, 135.25 (allyl-C-2), 130.41 (Ar-C-3, Ar-C-3'), 127.07 (Ar-C-4), 122.00 (Ar-C-2, Ar-C-2'), 117.97, 117.49 (allyl-C-3), 107.30 (C-1^F), 100.63 (C-1^E), 94.27 (CCl_3), 86.34 (C-2^F), 80.62 (C-4^F), 80.40 (C-3^F), 79.53, (C-5^F), 73.03 (C-5^E), 72.71 (C-3^E), 72.27 (allyl-C-1), 69.76 (C-4^E), 62.57 (C-6^E), 57.13 (C-2^E), 21.00, 20.92 ($CO\underline{C}H_3$).- IR (KBr): 3550-3200, 1757, 1724, 1692, 1530, 1371, 1255, 1164, 1071 cm^{-1}.- FAB MS: m/z 851.2, 849.2, 847.2 $[M+Na]^+$, 829.2, 827.2, 825.2 $[M+H]^+$, 771.2, 769.2, 667.2 $[M+H-AllOH]^+$, 436.0, 434.0, 432.0 $[e]^+$.- $C_{33}H_{39}Cl_3N_2O_{16}$ (826.04, 824.14), calcd. C 48.05, H 4.77, N 3.40, found C 48.01, H 4.82, N 3.45.

Allyl 2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-5-*O*-allyl-3-*O*-phenoxycarbonyl-β-D-glucofuranosiduronamide (10e)

a) A solution of **10a** (100 mg, 0.12 mmol), AIBN (7 mg, 43 µmol) and tributyltin hydride (126 µL, 0.48 mmol, 4.0 eq) in DME (6 mL) was stirred at 80°C until no starting material remained (20 min). The reaction mixture was cooled to 20°C and concentrated. The solid residue was washed with hexanes to remove Bu_3SnCl and the crude product was purified by FC on silica gel (11 g) using ethyl acetate to give **10e** (70 mg, 80%). There were problems in the reproducibility of the reaction.

b) Reduction of **10a** with with Zn-Cu couple in acetic acid: To a solution of **10a** (500 mg, 0.61 mmol) in acetic acid (8 mL), excess of activated Zn-Cu couple (800 mg) was added under argon. The reaction mixture was stirred at 20°C. After 2.5 h an additional amount of Zn-Cu (500 mg) was added under argon and the reaction mixture was stirred for 18 h at 20°C. Then the reaction mixture was filtered and the residue washed repeatedly with $CHCl_3$, ethyl acetate and toluene. The solvents were evaporated under reduced pressure. The crude product was purified by FC on silica gel (85 g), eluted with $CHCl_3$-ethyl acetate 50:50, 30:70 then 0:100 to provide pure **10e** (373 mg, 85%).

c) Reduction of **10a** with with Zn-Cu couple in THF - acetic acid: To a solution of **10a** (1.0 g, 1.21 mmol) in THF (20 mL) and acetic acid (4 mL), excess of activated Zn-Cu (1.0 g) was added under argon. The reaction mixture was stirred at 55°C. After 2.5 h additional amount of Zn-Cu (725 mg) was added under argon and the reaction mixture was stirred at 55°C for 20 h. Then work-up of the reaction was performed as described above. Yield: 83%.- M.p.: 189-190°C ($CHCl_3$-hexanes).- $[\alpha]_D^{26}$ = -27.6 (c 5.8, $CHCl_3$).- ^{1}H NMR (H,H COSY, 400 MHz, [D_5]pyridine): δ = 9.42 (d, 1 H, $N\underline{H}COCH_3$), 8.46, 8.38 (2 s, 2x 1 H, $CONH_2$), 7.40-7.20 (m, 5 H, Ar-Hs), 6.10-5.85 (m, 2 H, 2x allyl-2-H), 6.00 (dd, 1 H, 3^E-H), 5.83 (dd,1 H, 3^F-H), 5.69 (d, 1 H, 1^E-H), 5.48 (t, 1 H, 4^E-H), 5.46 (broad s, 1 H, 1^F-H), 5.36-5.28 (m, 2 H, 2x allyl-3-H_a), 5.18 (dd, 1 H, 4^F-H), 5.13-5.08 (m, 3 H, 2x allyl-3-H_b, 2^F-H), 4.68 (d, 1 H, 5^F-H), 4.52-4.43 (m, 2 H, allyl-1-H, $6'^E$-H), 4.43-4.34 (m, 2 H, allyl-1-H, 2^E-H), 4.29 (dd, 1 H, 6^E-H), 4.24 (dd, 1 H, allyl-1'-H), 4.11 (dd, 1 H, allyl-1'-H), 3.95 (ddd, 1 H, 5^E-H), 2.09, 2.03, 2.00, 1.96 (4 s, 4x 3 H, 4x $COCH_3$), $J_{1E,2E}$ = 8.5 Hz, $J_{2E,3E}$ = 10.4 Hz, $J_{3E,4E}$ = 9.5 Hz, $J_{4E,5E}$ = 9.5 Hz, $J_{5E,6E}$ = 2.3 Hz, $J_{5E,6'E}$ = 4.8 Hz, $^2J_{6E,6'E}$ = 12.4 Hz, $J_{NH,2E}$ = 8.5 Hz, $J_{1F,2F}$ < 1.0 Hz, $J_{2F,3F}$ = 1.5 Hz, $J_{3F,4F}$ = 5.2 Hz, $J_{4F,5F}$ = 8.3 Hz.- ^{13}C NMR (C,H COSY, APT, 100.6 MHz, [D_5]pyridine): δ = 172.23, 170.52, 170.26, 170.11, 169.49 (CO), 153.17, 151.34 (OCOO, Ar-C-1), 134.45 (allyl-C-2), 129.56 (Ar-C-3, Ar-C-3'), 126.20 (Ar-C-4), 121.22 (Ar-C-2, Ar-C-2'), 117.20, 116.63 (allyl-C-3), 106.41 (C-1^F), 100.31 (C-1^E), 85.29 (C-2^F), 79.94 (C-3^F), 79.50 (C-4^F), 78.51 (C-5^F), 72.72 (C-3^E), 72.07 (C-5^E), 71.47 (allyl-C-1), 69.13 (C-4^E), 68.89 (allyl-C-1), 61.91 (C-6^E), 54.99 (C-2^E), 22.70, 20.18, 20.11 ($CO\underline{C}H_3$).- IR (KBr): 3700-3000, 1751, 1651, 1641, 1373, 1273, 1254, 1073, 1046 cm^{-1}.- FAB MS: m/z 745.1 $[M+Na]^+$, 723.1 $[M+H]^+$, 665.1 $[M+H-AllOH]^+$, 330.0 $[e]^+$.- $C_{33}H_{42}N_2O_{16}$ (722.70, 722.25), calcd. C 54.83, H 5.86, N 3.88, found C 54.96, H 5.86, N 3.81.

Allyl 2-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-chloroacetamido-β-D-glucopyranosyl)-5-*O*-allyl-3-*O*-phenoxycarbonyl-β-D-glucofuranosiduronamide (10c)

To a solution of **10a** (80 mg, 0.10 mmol), AIBN (6 mg, 37 μmol) in DME (4 mL) tributyltin hydride (100 μL, 0.38 mmole, 3.9 eq) was added at 20°C. The reaction mixture was stirred at 20°C for 5 min, then at 85°C for 40 min. Work up of the reaction mixture was performed as described to provide **10e** (33 mg, 47%) and **10c** (28 mg, 40%).-^{1}H NMR (300 MHz, $CDCl_3$): The spectrum is very similar to the spectrum of **10e** in $CDCl_3$, except for an AB system at δ = 3.55 and 4.00 for $COCH_2Cl$ and the signal of N<u>H</u>$COCH_2Cl$ observed as doublet at δ = 6.78 whereas for **10e** as doublet at δ = 5.78.- ^{13}C NMR (APT, 75.5 MHz, $CDCl_3$): The spectrum is very similar to the spectrum of **10e**, except of a signal at δ = 42.47 for CO<u>C</u>H_2Cl (CO<u>C</u>H_3 of **10e** at δ = 23.64).- IR (KBr): 3580-3200, 1755, 1670, 1541, 1372, 1257, 1168, 1071, 1044 cm^{-1}.- $C_{33}H_{41}ClN_2O_{16}$ (757.14, 756.21).- FAB MS: m/z 781.2, 779.2 $[M+Na]^+$, 759.2, 757.2 $[M+H]^+$, 701.2, 699.2 $[M+H-AllOH]^+$, 366.1, 364.1 $[e]^+$.

Dehalogenation of 10b

a) A solution of **10b** (40 mg, 0.05 mmol), AIBN (2 mg), Bu_3SnH (127 mL,10 eq.) in DMAC (100 mL) and toluene (100 mL) was heated at 80°C under argon. After 10 min a white solid precipitated. The reaction mixture was diluted with toluene (300 mL) and heated 45 min at 80°C. After solvent evaporation hexane was added and the precipitated material was washed with hexane to remove Bu_3SnCl. The crude product (35 mg) was then purified by FC ($CHCl_3$-ethyl acetate 1:1 to 0:1) to give **10f** (18 mg, 52%), compound X (13 mg) and **12** (3 mg).

b) A mixture of **10b** (270 mg, 0.33 mmol) and freshly prepared Zn-Cu couple (~516 mg) in acetic acid (11 mL) was stirred at 20°C. After 2 h, 20 h, 30 h further ~400, ~180, ~500 mg portions of Zn-Cu were added and the reaction mixture was stirred for another 15 h (45 h as a whole). After filtration the residue was repeatedly washed with CH_2Cl_2, ethyl acetate and toluene. The solvents were evaporated under reduced pressure and the crude product was purified by FC ($CHCl_3$-ethyl acetate 1:1) to give **10f** (118 mg, 50%) and **10d** (94 mg, 40%), TLC: ethyl acetate.

c) A mixture of **10b** (230 mg, 0.28 mmol) and freshly prepared Zn-Cu couple (~560 mg) in acetic acid (1 mL) and THF (6 mL) was stirred at 55°C. After 2 h a further portion of Zn-Cu (~280 mg) was added and the mixture was stirred for another 18 h at 55°C. The reaction mixture was cooled to 20°C, filtered and the residue was washed repeatedly with ethyl acetate, CH_2Cl_2 and toluene. From the combined filtrates solvents were evaporated under reduced pressure and the crude product was purified by FC (ethyl acetate) to provide a quantitative amount of **10f**.

Allyl 2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-5-*O*-allyl-3-*O*-phenoxycarbonyl-α-D-glucofuranosiduronamide (10f)

M.p.: 217-219°C ($CHCl_3$-hexanes), decomposition.- $[\alpha]_D^{26}$ = +63.9 (c 6.2, $CHCl_3$).- ^{1}H NMR (H,H COSY, 400 MHz, [D_5]pyridine): δ = 9.47 (d, 1 H, N<u>H</u>$COCH_3$), 8.60, 8.24 (2 s, 2x 1 H, $CONH_2$), 7.53-7.15 (m, 5 H, Ar-Hs), 6.24 (t, 1 H, 3^F-H), 6.17 (dd, 1 H, 3^E-H), 6.10-6.00 (m, 1 H, allyl-2-H), 6.00-5.90 (m, 1 H, allyl-2-H), 5.75 (d, 1 H, 1^E-H), 5.45 (d, 1 H, 1^F-H), 5.44 (t, 1 H, 4^E-H), 5.41-5.31 (m, 2 H, 2x allyl-3-H_a), 5.30 (dd, 1 H, 4^F-H), 5.17, 5.09 (2 dd, 2x 1 H, 2x allyl-3H_b), 5.03 (dd, 1 H, 2^F-H), 4.59 (d, 1 H, 5^F-H), 4.51 (dd, 1 H, $6'^E$-H), 4.42-4.28 (m, 4 H, 6^E-H, 3x allyl-1-H), 4.22-4.15 (m, 1 H, allyl-1-H), 4.10 (dt, 1 H, 2^E-H), 3.93 (ddd, 1 H, 5^E-H), 2.07, 2.04, 2.02, 2.01 (4 s, 4x 3 H, 4x $COCH_3$), $J_{1E,2E} = J_{NH,2E}$ = 8.3 Hz, $J_{2E,3E}$ = 10.7 Hz, $J_{3E,4E} = J_{4E,5E}$ = 9.7 Hz, $J_{5E,6E}$ = 2.0 Hz, $J_{5E,6'E}$ = 4.7 Hz, $^2J_{6E,6'E}$ = 12.8 Hz, $J_{1F,2F}$ = 4.6 Hz, $J_{2F,3F}$ = 8.3 Hz, $J_{3F,4F}$ = 7.4 Hz, $J_{4F,5F}$ = 4.9 Hz.- ^{13}C NMR (C,H COSY, APT, 100.6 MHz, [D_5]pyridine): δ = 172.85, 171.43, 170.99, 170.37 (CO), 154.32, 152.43 (OCOO, Ar-C-1), 135.52, 135.35 (allyl-C-2), 130.24 (Ar-C-3, Ar-C-3'), 126.86 (Ar-C-4), 122.36 (Ar-C-2, Ar-C-2'), 118.15, 117.33 (allyl-C-3), 101.67 (C-1^E), 100.21 (C-1^F), 82.65 (C-2^F), 80.40 (C-3^F), 80.31 (C-5^F), 76.60 (C-4^F), 73.60 (allyl-C-1), 73.11 (C-3^E), 72.75 (C-5^E), 70.21 (C-4^E), 69.46 (allyl-C1), 63.04 (C-6^E), 56.57 (C-2^E), 23.57, 21.01 (CH_3).- IR (KBr): 3660-3100, 1749, 1670, 1640, 1373, 1251, 1074, 1043 cm^{-1}.- FAB MS: m/z 745.5 $[M+Na]^+$, 665.5 $[M+H-AllOH]^+$, 330.3 $[e]^+$.- $C_{33}H_{42}N_2O_{16}$ (722.70, 722.25), calcd. C 54.83, H 5.86, N 3.88, found C 54.84, H 5.98, N 3.87.

Allyl 2-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-chloroacetamido-β-D-glucopyranosyl)-5-*O*-allyl-3-*O*-phenoxycarbonyl-α-D-glucofuranosiduronamide (10d)

a) Vide supra.

b) Reduction of 10b with with Zn in acetic acid. To a solution of **10b** (20 mg, 0.024 mmol) in acetic acid (250 mL), excess of activated Zn (washed with 1N HCl) was added. After 15 min the reaction mixture was filtered through silica gel with ethyl acetate. Solvent evaporation and FC (ethyl acetate) gave **10d** (15 mg, 82%).- ^{1}H NMR (400, 200 MHz, $CDCl_3$): δ = 7.42-7.23 (m, 5 H, Ar-Hs), 6.70 (d, 1 H, $N\underline{H}COCH_2Cl$), 6.68, 5.95 (2 s, 2x 1 H, $CONH_2$), 6.01-5.85 (m, 2 H, 2x allyl-2-H), 5.63 (t, 1 H, 3^F-H), 5.56 (t, 1 H, 3^E-H), 5.40-5.15 (m, 5 H, 2x allyl-CH_2-3, 4^E-H), 5.09 (d, 1 H, 1^F-H), 5.04 (d, 1 H, 1^E-H), 4.75 (dd, 1 H, 4^F-H), 4.45 (dd,1 H, 2^F-H), 4.30-4.15 (m, 6 H, CH_2-6^E, 2x allyl-CH_2-1), 4.13 (d, 1 H, 5^F-H), 3.85-3.72 (m, 1 H, 2^E-H), 3.78 (s, 2 H, $COCH_2Cl$), 3.60 (dt, 1 H, 5^E-H), 2.08, 2.03, 2.02 (3 s, 3x 3 H, 3x $COCH_3$), $J_{1E,2E}$ = 9.4 Hz (unexpectedly large J), $J_{2E,3E}$ = 9.2 Hz, $J_{3E,4E}$ = 9.0 Hz, $J_{4E,5E}$ = 8.8 Hz, $J_{NH,2E}$ = 7.6 Hz, $J_{1F,2F}$ = 4.4 Hz, $J_{2F,3F}$ = 7.6 Hz, $J_{3F,4F}$ = 7.7 Hz, $J_{4F,5F}$ = 4.5 Hz.- ^{13}C NMR (100.6, 50.3 MHz, $CDCl_3$): δ = 173.43, 171.03, 170.79, 167.33 (CO), 153.34, 149.25 (OCOO, Ar-C-1), 134.39, 133.79 (allyl-C-2), 130.00 (Ar-C-3, Ar-C-3'), 126.79 (Ar-C-4), 121.54 (Ar-C-2, Ar-C-2'), 119.22, 117.89 (allyl-C-3), 100.39 (C-1^E), 99.36 (C-1^F), 82.73 (C-2^F), 79.31 (C-3^F), 79.01 (C-5^F), 75.93 (C-4^F), 72.44 (allyl-C-1), 71.47 (C-5^E), 69.26 (C-3^E), 69.22 (C-4^E), 62.58 (C-6^E), 56.52 (C-2^E), 42.78 (CH_2Cl), 21.21, 21.12, 21.09 ($CO\underline{C}H_3$), 148.41, 135.78 and 125.17 could not be assigned.- ^{1}H NMR (H,H COSY, 400 MHz, [D_5]pyridine): δ = 9.97 (d, 1 H, $N\underline{H}COCH_3$), 8.69, 8.20 (2 s, 2x 1 H, $CONH_2$), 7.53-7.08 (m, 5 H, Ar-Hs), 6.18 (t, 1 H, 3^F-H), 6.13 (dd, 1 H, 3^E-H), 6.11-5.81 (m, 2 H, 2x allyl-2-H), 5.70 (d, 1 H, 1^E-H), 5.42 (t, 1 H, 4^E-H), 5.41 (d, 1 H, 1^F-H), 5.39-5.25 (m, 2 H, 2x allyl-3-H_a), 5.25 (dd, 1 H, 4^F-H), 5.17-5.01 (m, 2x H, 2x allyl-3H_b), 4.99 (dd, 1 H, 2^F-H), 4.55 (d, 1 H, 5^F-H), 4.52-4.14 (m, 7 H, CH_2-6^E, 2x allyl-CH_2-1, 2^E-H), 4.21, 4.15 (AB system, 2x 1 H, $COCH_2Cl$), 3.96-3.84 (m, 1 H, 5^E-H), 1.99, 1.98, 1.97 (3 s, 3x 3 H, 3x $COCH_3$), $J_{1E,2E}$ = 8.4 Hz, $J_{NH,2E}$ = 7.9 Hz, $J_{2E,3E}$ = 9.3 Hz, $J_{3E,4E}$ = $J_{4E,5E}$ = 10.6 Hz, $J_{1F,2F}$ = 4.4 Hz, $J_{2F,3F}$ = 7.7 Hz, $J_{3F,4F}$ = 7.6 Hz, $J_{4F,5F}$ = 5.4 Hz.- IR (KBr): 3600-3200, 1753, 1677, 1250, 1042 cm^{-1}.- $C_{33}H_{41}ClN_2O_{16}$ (757.15, 756.21).- FAB MS: m/z 781.1, 779.1 $[M+Na]^+$, 759.2, 757.2 $[M+H]^+$, 701.1, 699.1 $[M+H-AllOH]^+$, 366.1, 364.1 $[e]^+$

Cyclization product 12

^{1}H NMR (200 MHz, $CDCl_3$): The spectrum demonstrated that **12** contained only one allyl group.- ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 174.27, 172.78, 171.27, 171.21, 169.77 (CO) , 152.92, 151.58 (OCOO, Ar-C-1), 133.98 (allyl-C-2), 129.97 (Ar-C-3, Ar-C-3'), 126.58 (Ar-C-4), 121.30 (Ar-C-2, Ar-C-2'), 118.77 (allyl-C-3), 100.88, 98.90 (C-1^E, C-1^F), 79.96, 78.67, 77.66, 76.55, 75.61, 73.69, 73.40, 69.79, 68.68, 62.51, 52.11, 37.08, 29.21, 23.25, 21.14, 21.06, 18.01 (3x $CO\underline{C}H_3$, $HNCOCH_2CH_2CH_2$).- $C_{33}H_{42}N_2O_{16}$ (722.70, 722.25).- FAB MS: m/z 745.3 $[M+Na]^+$, 723.3 $[M+H]^+$.

Compound X

^{1}H NMR (200 MHz, $CDCl_3$): Only signals for one allyl group were present and multiplets between 0.60 and 1.90 ppm corresponding to a Bu_3Sn group.- $C_{45}H_{70}N_2O_{16}Sn$ (1013.74, 1014.37).- FAB MS: m/z 1037.2 $[M+Na]^+$, 957.2 $[M+H-AllOH]^+$, 330.1 $[e]^+$.

Allyl 2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-5-*O*-allyl-3-*O*-carbamoyl-α-D-glucofuranosiduronamide (13b)

To a solution of **10f** (115 mg, 0.16 mmol) in pyridine (3.5 mL), a solution of ammonia in dioxane (0.5 mol/L 3.2 mL, 10.0 eq) was injected and the reaction mixture was stirred at 20°C for 65 h. The solvents were evaporated (codistillation with toluene) under reduced pressure. The crude product was purified by FC ($CHCl_3$-methanol 97:3, 95:5 and 92:8) to yield **13b** (80 mg, 78%).- M.p.: 204-205°C ($CHCl_3$-methanol-hexanes), decomposition.- ^{1}H NMR (H,H COSY, 400 MHz, [D_5]pyridine): δ = 9.32 (d, 1 H, $N\underline{H}COCH_3$), 8.72, 7.90 (2 s, 2x 1 H, $CONH_2$), 7.57 (broad s, 2 H, H_2NCOO), 6.31 (dd, 1 H, 3^F-H), 6.11 (t, 1 H, 3^E-H), 6.07-5.89 (m, 2 H, 2x allyl-2-H), 5.65 (d, 1 H, 1^E-H), 5.42 (t, 1 H, 4^E-H), 5.41 (d, 1 H, 1^F-H), 5.37-5.28 (m, 2 H, 2x allyl-3-H_a), 5.15 (t, 1 H, 4^F-H), 5.12-5.04 (m, 2 H, 2x allyl-3H_b), 4.92 (2^F-H), 4.52-4.46 (1 H, $6'^E$-H, d, 1 H, 5^F-H), 4.36-4.15 (m, 6 H, 6^E-H, 2x allyl-CH_2-1, 2^E-H), 3.92 (ddd, 1 H, 5^E-H), 2.08, 2.03, 2.01, 1.99 (4 s,

4x 3 H, 4x $COCH_3$), $J_{1E,2E}$ = 8.4 Hz, $J_{2E,3E}$ = 9.8 Hz, $J_{3E,4E}$ = 9.8 Hz, $J_{4E,5E}$ = 9.8 Hz, $J_{5E,6'E}$ = 3.8 Hz, $J_{5E,6E}$ = 2.2 Hz, $J_{NH,2E}$ = 8.2 Hz, $J_{1F,2F}$ = 4.6 Hz, $J_{2F,3F}$ = 7.5 Hz, $J_{3F,4F}$ = 7.5 Hz, $J_{4F,5F}$ = 5.5 Hz.- ^{13}C NMR (C,H COSY, APT, 100.6 MHz, [D5]pyridine): δ = 174.10, 172.18, 170.32, 169.95, 169.28 (CO), 156.67 (H_2NCOO), 135.18 (allyl-C-2, hidden by a solvent peak), 116.85, 115.98 (allyl-C-3), 100.84 (C-1^E), 99.61 (C-1^F), 82.58 (C-2^F), 79.60 (C-5^F), 76.40 (C-4^F), 74.44 (C-3^F), 72.36 (C-3^E), 72.12 (allyl-C-1), 71.58 (C-5^E), 69.21 (C-4^E), 68.34 (allyl-C-1), 61.99 (C-6^E), 55.22 (C-2^E), 22.50, 19.98, 19.88 (4x $CO\underline{C}H_3$).- IR (KBr): 3700-3120, 1744, 1663, 1373, 1235, 1074, 1043 cm^{-1}.- FAB MS: m/z 668.1 $[M+Na]^+$, 646.1 $[M+H]^+$, 588.0 $[M+H-AllOH]^+$, 330.0 $[e]^+$.- $C_{27}H_{39}N_3O_{15}$ (645.62, 645.24), calcd. C 50.23, H 6.09, found C 50.39, H 5.97.

Allyl 2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-5-*O*-allyl-3-*O*-carbamoyl-β-D-glucofuranosiduronamide (13a)

To a solution of **10e** (625 mg, 0.87 mmol) in pyridine (18 mL), an 0.5 M ammonia-dioxane solution (17.3 mL, 10.0 eq) was injected and the reaction mixture was stirred at 20°C for 65 h. The solvents were evaporated (codistillation with toluene) under reduced pressure. The crude product was dissolved in $CHCl_3$-methanol, mixed with kieselguhr, solvents were evaporated, the residue was dried at 10 Pa and the crude product was transferred to the top of a FC column eluting with $CHCl_3$-methanol: 95:5 and 90:10 to furnish **13a** (541 mg, 97%), TLC: $CHCl_3$-methanol 9:1.- M.p.: 223-224°C ($CHCl_3$-methanol-hexanes), decomposition.- ^1H NMR (H,H COSY, 400 MHz, [D5]pyridine): δ = 9.50 (d, 1 H, $N\underline{H}COCH_3$), 8.28, 8.00 (2 s, 2x 1 H, $CONH_2$), 7.83 (broad s, 2 H, H_2NCOO), 6.07-5.90 (m, 2 H, 2x allyl-2-H), 5.90 (t, 1 H, 3^E-H), 5.85 (d, 1 H, 3^F-H), 5.62 (d, 1 H, 1^E-H), 5.48 (t, 1H, 4^E-H), 5.45 (broad s, 1 H, 1^F-H), 5.36-5.22 (m, 2 H, 2x allyl-3-H_a), 5.09-5.04 (m, 2 H, 2x allyl-3-H_b), 5.03 (broad s, 1 H, 2^F-H), 5.01 (dd, 1 H, 4^F-H), 4.64-4.53 (m, 1 H, 2^E-H), 4.56 (d, 1 H, 5^F-H), 4.53-4.48, 4.32-2.24, 4.18-4.06 (m, 6 H, 2x allyl-CH_2-1, CH_2-6^E), 3.96 (ddd, 1 H, 5^E-H), 2.07, 2.04, 1.99, 1.97 (4 s, 4x 3 H, 4 $COCH_3$), $J_{1E,2E}$ = 8.5 Hz, $J_{2E,3E}$ = 11.4 Hz, $J_{3E,4E}$ = 9.3 Hz, $J_{4E,5E}$ = 10.5 Hz, $J_{NH,2E}$ = 9.9 Hz, $J_{1F,2F}$ < 1.0 Hz, $J_{2F,3F}$ < 1.0 Hz, $J_{3F,4F}$ = 5.3 Hz, $J_{4F,5F}$ = 8.8 Hz.- ^{13}C NMR (C,H COSY, APT, 100.6 MHz, [D5]pyridine): δ = 173.65, 171.16, 170.97, 170.29 (CO), 157.77 (H_2NCOO), 135.50, 135.45 (allyl-C-2), 117.93, 117.28 (allyl-C-3), 107.82 (C-1^F), 101.47 (C-1^E), 87.07 (C-2^F), 81.44 (C-4^F), 79.46 (C-5^F), 76.04 (C-3^F), 74.04 (C-3^E), 72.82 (C-5^E), 72.28 (allyl-C-1), 69.93 (C-4^E), 69.67 (allyl-C-1), 62.71 (C-6^E), 55.37 (C-2^E), 23.51, 21.04 (4x $CO\underline{C}H_3$).- IR (KBr): 3660-3140, 1744, 1707, 1657, 1373, 1256, 1235, 1050 cm^{-1}.- FAB MS: m/z 668.1 $[M+Na]^+$, 646.1 $[M+H]^+$, 588.1 $[M+H-AllOH]^+$, 330.0 $[e]^+$.- $C_{27}H_{39}N_3O_{15}$ (645.62, 645.24), calcd. C 50.21, H 6.09, found C 50.05, H 6.16.

2-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3-*O*-carbamoyl-α-D-glucopyranuronamide (14a)

a) A mixture of **13a** (85 mg, 0.13 mmol) and $Pd(PPh_3)_4$ (152 mg, 1.0 eq) in acetic acid (1.2 mL) was stirred under argon at 20°C for 3 h. The solvent was removed by azeotropic evaporation with toluene. The residue was taken up in water and washed repeatedly with $CHCl_3$, then water was removed with lyophilization. The product was further purified by washing it with toluene-$CHCl_3$-methanol 10:10:3 to yield **14a** (64 mg, 85%). In a second reaction, 240 mg of **13a** were used to yield **14a** (166 mg, 79%). TLC: $CHCl_3$-methanol 9:1 and 4:1.

b) A mixture of **13b** (100 mg, 0.16 mmol) and $Pd(PPh_3)_4$ (164 mg, 0.9 eq) in acetic acid (3 mL) was stirred under argon at 20°C for 3.5 h. Subsequent removal of the solvent and purification as described above yielded **14a** (70 mg, 80%).- ^1H NMR (H,H COSY, 400 MHz, homo decoupling, 200 MHz, [D6]DMSO): δ = 7.39 (s, 1 H, $CONH_2$), 7.35 (d, 1 H, $N\underline{H}COCH_3$), 7.13 (s, 1 H, $CONH_2$), 6.81 (d, 1 H, 1-OH), 6.32 (broad s, 2 H, H_2NCOO), 5.15 (dd, 1 H, 1^F-H), 5.12 (broad s, 1 H, 4^F-OH), 5.10 (t, 1 H, 3^E-H), 4.87 (t, 1 H, 3^F-H), 4.81 (t, 1 H, 4^E-H), 4.68 (d, 1 H, 1^E-H), 4.22 (dd, 1 H, $6'^E$-H), 4.10-3.98 (m, 1 H, 6^E-H), 4.01 (d, 1 H, 5^F-H), 3.82 (ddd, 1 H, 5^E-H), 3.81-3.63 (m, 1 H, 2^E-H), 3.54-3.40 (m, 2 H, 4^F-H, 2^F-H), 2.03, 1.98, 1.92, 1.81 (4 s, 4x 3 H, 4 $COCH_3$), $J_{1E,2E}$ = 8.5 Hz, $J_{2E,3E}$ = 10.1 Hz, $J_{3E,4E}$ = 10.1 Hz, $J_{4E,5E}$ = 10.1 Hz, $J_{5E,6'E}$ = 4.2 Hz, $J_{5E,6E}$ = 2.5 Hz, $^2J_{6E,6'E}$ = 12.2 Hz, $J_{NH,2E}$ = 9.6 Hz, $J_{1F,2F}$ ~ 4.0 Hz, $J_{2F,3F}$ = 9.8 Hz, $J_{3F,4F}$ = 9.7 Hz, $J_{4F,5F}$ = 9.6 Hz, $J_{1F,OH}$ = 4.1 Hz.- ^{13}C NMR (C,H COSY, APT, 50.3, 100.6 MHz, [D6]DMSO): δ = 171.48, 170.37, 170.01, 169.54, 169.47 (CO), 156.93 (H_2NCOO), 102.32 (C-1^E), 92.17 (C-1^F), 79.24 (C-2^F), 73.12 (C-3^F, C-3^E), 71.13 (C-5^F), 71.03 (C-4^F), 70.85 (C-5^E), 68.83 (C-4^E), 62.03 (C-6^E), 53.25 (C-2^E), 23.01 ($NHCO\underline{C}H_3$), 20.81, 20.68,

20.60 (CO$\underline{C}H_3$).- FAB MS: m/z 588.0 $[M+Na]^+$, 566.0 $[M+H]^+$, 548.0 $[M+H-H_2O]^+$, 330.0 $[e]^+$.- $C_{21}H_{31}N_3O_{15}$ (565.49, 565.18), calcd. C 44.59, H 5.53, found C 45.00, H 5.60.

2-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-1,4-di-*O*-acetyl-3-*O*-carbamoyl-α-D-glucopyranuronamide (14b)

Acetic anhydride (1.3 mL) was added to a solution of **14a** (obtained from **13a**, 60 mg, 0.11 mmol) in pyridine (2 mL), the reaction mixture was stirred at 20°C for 2 h. The solvents were removed by azeotropic evaporation with toluene under reduced pressure. The crude product was dried over night under vacuum and purified by FC ($CHCl_3$-methanol 100:0, 100:3, 100:5 and 100:10) to give **14b** (α-isomer, 60 mg, 87%) as a white solid product and a mixture of **14b** and β-isomer **14c** (5 mg, 7%), TLC: $CHCl_3$-methanol 4:1.

When the reaction was repeated with 165 mg of **14a** (obtained from **13b**) the yield was: **14b** (153 mg, 81%), a mixture of **14b** and **14c** (7 mg 1% α, 3% β) and a mixture of **14b** and an unknown side product (28 mg, 7% α and 8% unknown).- ^1H NMR (H,H COSY, 400 MHz, 200 MHz, [D5]pyridine): δ = 9.32 (d, 1 H, N$\underline{H}$COCH3), 8.50, 8.32 (2 s, 2x 1 H, $CONH_2$), 7.71 (broad s, 2 H, H_2NCOO), 6.90 (d, 1 H, 1^F-H), 6.11 (dd, 1 H, 3^E-H), 6.08 (t, 1 H, 3^F-H), 5.95 (t, 1 H, 4^F-H), 5.58 (d, 1 H, 1^E-H), 5.38 (t, 1 H, 4^E-H), 4.80 (d, 1 H, 5^F-H), 4.48 (dd, 1 H, $6'^E$-H), 4.37 (dd, 1 H, 6^E-H), 4.23 (dd, 1 H, 2^F-H), 4.15-3.95 (m, 2 H, 2^E, 5^E -H), 2.19, 2.11, 2.10, 2.02, 1.99, 1.81 (6 s, 6x 3 H, 6 CO$\underline{C}H_3$), $J_{1E,2E}$ = 8.4 Hz, $J_{2E,3E}$ = 9.9 Hz, $J_{3E,4E}$ = 9.8 Hz, $J_{4E,5E}$ = 9.7 Hz, $J_{5E,6'E}$ = 4.5 Hz, $J_{5E,6E}$ = 2.4 Hz, $^2J_{6E,6'E}$ = 12.1 Hz, $J_{NH,2E}$ = 8.2 Hz, $J_{1F,2F}$ = 3.9 Hz, $J_{2F,3F}$ = 9.8 Hz, $J_{3F,4F}$ = 9.8 Hz, $J_{4F,5F}$ = 9.9 Hz.- ^{13}C NMR (C,H COSY, 100.6 MHz, APT, 50.3 MHz, [D5]pyridine): δ = 169.69, 169.46, 169.28, 168.76, 168.61, 167.96 (CO), 156.08 (H_2NCOO), 100.64 (C-1^E), 89.83 (C-1^F), 76.76 (C-2^F) , 71.36 (C-3^E) , 70.60 (C-5^E), 70.04 (C-3^F), 69.95 (C-5^F), 69.34 (C-4^F), 68.27 (C-4^E), 61.13 (C-6^E), 54.64 (C-2^E), 21.94 (NHCO$\underline{C}H_3$), 19.53, 19.31, 19.20, 19.12, 19.05 (5x CO$\underline{C}H_3$).- IR (KBr): 3600-3140, 1748, 1683, 1373, 1236, 1050 cm^{-1}.- FAB MS: m/z 672.0 $[M+Na]^+$, 650.0 $[M+H]^+$, 590.0 $[M+H-AcOH]^+$, 330.0 $[e]^+$.- $C_{25}H_{35}N_3O_{17}$ (649.56, 649.20), calcd. C 46.21, H 5.43, N 6.47, found C 46.30, H 5.48, N 6.52.

2-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-1,4-di-*O*-acetyl-3-*O*-carbamoyl-β-D-glucopyranuronamide (14c)

^1H NMR (200 MHz, [D5]pyridine) of the mixture of **14b** and **14c**: The spectrum of **14c** is very similar to that of **14b** but there is difference for δ = 9.30 (d, 1 H, N$\underline{H}$COCH3, $^3J_{NH,2E}$ = 7.9 Hz), 6.88 (d, 1 H, 1^F-H, $^3J_{1F,2F}$ = 3.8 Hz) for **14b**, δ = 8.67 (d, 1 H, N$\underline{H}$COCH3, $^3J_{NH,2E}$ = 8.7 Hz), 6.40 (d, 1 H, 1^F-H, $^3J_{1F,2F}$ = 7.0 Hz) for **14c**.

2-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-4-*O*-acetyl-3-*O*-carbamoyl-α-D-glucopyranuronamide (14d)

A mixture of **14b** (80 mg, 0.12 mmol), hydrazinium acetate (14 mg, 0.15 mmol, 1.2 eq.) in DMF (2.5 mL, base free) was stirred for 40 min at 20°C under argon. The reaction mixture was purified directly by GPC on Sephadex LH-20 (10 g, $CHCl_3$-methanol 1:1) to give **14d** (71 mg, 95%), TLC: $CHCl_3$-methanol 5:1.- ^1H NMR (H,H COSY, 400 MHz, homo decoupling, 200 MHz, [D6]DMSO): δ = 7.45 (s, 1 H, $CONH_2$), 7.40 (d, 1 H, N$\underline{H}$COCH3), 7.15 (s, 1 H, $CONH_2$), 7.06 (d, 1 H, 1^F-OH), 6.37 (broad s, 2 H, H_2NCOO), 5.18 (dd, 1 H, 1^F-H), 5.12 (dd, 1 H, 3^E-H), 4.98 (t, 1 H, 3^F-H), 4.90 (t, 1 H, 4^F-H), 4.82 (t, 1 H, 4^E-H), 4.69 (d, 1 H, 1^E-H), 4.22 (dd, 1 H, $6'^E$-H), 4.16 (d, 1 H, 5^F-H), 4.07 (dd, 1 H, 6^E-H), 3.84 (ddd, 1 H, 5^E-H), 3.73 (dt, 1 H, 2^E-H), 3.58 (dd, 1 H, 2^F-H), 2.03, 1.97, 1.91, 1.90, 1.75 (5 s, 5x 3 H, 5 $COCH_3$), $J_{1E,2E}$ = 8.5 Hz, $J_{2E,3E}$ = 10.2 Hz, $J_{3E,4E}$ = 9.7 Hz, $J_{4E,5E}$ = 9.8 Hz, $J_{5E,6'E}$ = 4.7 Hz,$J_{5E,6E}$ = 2.6 Hz, $^2J_{6E,6'E}$ = 12.3 Hz, $J_{NH,2E}$ = 9.4 Hz, $J_{1F,2F}$ = 3.1 Hz, $J_{2F,3F}$ = 10.0 Hz, $J_{3F,4F}$ = 9.8 Hz, $J_{4F,5F}$ = 9.8 Hz, $J_{1F,OH}$ = 4.9 Hz.- ^{13}C NMR (C,H COSY, 100.6 MHz, APT, 50.3 MHz, [D6]DMSO): δ = 170.38, 170.03, 169.54, 169.41, 169.01 (CO), 156.22 (H_2NCOO), 102.39 (C-1^E), 92.03 (C-1^F), 78.80 (C-2^F), 73.08 (C-3^E), 70.96, 70.89 (C-4^F, C-5^E), 70.60 (C-3^F), 68.80 (C-4^E), 68.52 (C-5^F), 62.02 (C-6^E), 53.19 (C-2^E), 22.94 (NHCO$\underline{C}H_3$), 20.83 (2x CO$\underline{C}H_3$), 20.69, 20.60 (2x CO$\underline{C}H_3$).- IR (KBr): 3640-3060, 2920, 2365, 1742, 1710, 1677, 1623, 1374, 1241, 1048 cm^{-1}.- $C_{23}H_{33}N_3O_{16}$ (607.53, 607.19), FAB MS: m/z 630.2 $[M+Na]^+$, 608.3 $[M+H]^+$, 330.1 $[e]^+$.

2-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-4-*O*-acetyl-3-*O*-carbamoyl-1-*O*-{[(R)-2-methoxycarbonyl-2-(hexyloxy)-ethoxy]-(2,2,2,trichloro-1,1-dimethylethoxy)-phosphoryl}-α-D-glucopyranuronamide (18a)

To a solution of 1H-1,2,4-triazole (27 mg, 0.40 mmol, 4.8 eq.) in 4:1 CH_2Cl_2-pyridine (0.8 mL) 2,2,2,-trichloro-1,1-dimethylethyl dichlorophosphite (21 µL, 0.10 mmol, 1.25 eq.) was added at 0°C. This mixture was stirred at 0°C under argon for 25 min. **14d** (50 mg, 0.08 mmol), dissolved in 7:1 CH_2Cl_2-pyridine (2.3 mL) was added and the reaction mixture was stirred for 2 h at 0°C. After addition of **6** (47 mg, 0.23 mmol, 2.8 eq.) dissolved in 7:1 CH_2Cl_2-pyridine (0.6 mL) in three portions over a period of 2 h , the mixture was stirred for 1.5 h at 0°C. Bis(trimethylsilyl)peroxide (35 µL, 0.17 mmol, 2.0 eq.) was injected into the reaction flask and the stirred mixture was maintained at 0°C for 1 h, then 17 h at 20°C. The solvents were removed by azeotropic evaporation with toluene (25°C, argon stream). The residue was dissolved in CH_2Cl_2-methanol, mixed with kieselguhr (600 mg), solvents were evaporated, the residue was dried at 10 Pa and the crude product was transferred to the top of a FC column (20g, $CHCl_3$-toluene-methanol 10:10:1 and 10:10:2) and further purified by FC (CH_2Cl_2-methanol 100:0, 97:3 and 95:5) to give pure P-diastereomer **18a** (47 mg, 55%) and a mixture of two P-diastereomers (20 mg, 23%), TLC: $CHCl_3$-methanol 3:1. The ^{1}H NMR spectrum indicated that the sample of **18a** contained some triazole. This impurity was removed in the next step.- ^{1}H NMR (H,H COSY, homo decoupling, 200 MHz, [D$_5$]pyridine): δ = 9.25 (d, 1 H, N<u>H</u>COCH$_3$), 8.65 (signal of $CONH_2$ hidden by a triazole peak), 8.08 (s, $CONH_2$), 7.68 (broad s, 2 H, H_2NCOO), 6.54 (dd, 1 H, 1^F-H), 6.12 (t, 1 H, 3^E-H), 5.97 (t, 1 H, 3^F-H), 5.92 (t, 1 H, 4^F-H), 5.55 (d, 1 H, 1^E-H), 5.45 (t, 1 H, 4^E-H), 4.99 (d, 1 H, 5^F-H), 4.80-4.62 (m, 2 H, CH_2-3^H), 4.62-4.36 (m, 3 H, CH_2-6^E, 2^H-H), 4.22-4.10 (m, 2 H, 2^F-H, 2^E-H), 4.05 (ddd, 1 H, 5^E-H), 3.84-3.71 (m, 1 H, $1'^I$-H, and s at 3.76, 3 H, $COOCH_3$), 3.65-3.51 (m, 1 H, 1^I-H), 2.19 (3 H), 2.16 (3 H), 2.14 (6 H), 2.07 (6 H), 2.00 (3 H), (5 s, 7 CH_3 of units E, F and G), 1.65-1.11 (8 H, signals of 2^I, 3^I, 4^I, 5^I-H's), 0.80 (t, 3 H, 6^I-CH_3), $J_{1E,2E}$ = 8.4 Hz, $J_{2E,3E}$ = 9.8 Hz, $J_{3E,4E}$ = 9.8 Hz, $J_{4E,5E}$ = 9.8 Hz, $J_{5E,6'E}$ = 4.0 Hz, $J_{5E,6E}$ = 2.9 Hz, $J_{NH,2E}$ = 8.1 Hz, $J_{1F,2F}$ = 3.1 Hz, $J_{2F,3F}$ = 9.5 Hz, $J_{3F,4F}$ = 9.7 Hz, $J_{4F,5F}$ = 9.7 Hz, $J_{1F,P}$ = 5.3 Hz.- ^{13}C NMR (C,H COSY, DEPT, 50.3 MHz, [D$_5$]pyridine): δ = 171.13, 170.98, 170.72, 170.63, 170.16, 169.92, 169.69 (CO), 157.37 (H_2NCOO), 106.61 (d, <u>C</u>Cl$_3$), 102.88 (C-1^E), 97.97 (d, C-1^F), 91.08 (d, <u>C</u>(CH$_3$)$_2$), 78.90 (d, C-2^F), 78.54 (d, C-2^H), 72.99 (C-3^E), 72.28 (C-5^E), 71.89 (C-1^I), 71.42 (C-5^F), 71.12 (C-3^F), 70.53 (C-4^F), 69.94 (C-4^E), 68.73 (d, C-3^H), 62.76 (C-6^E), 55.99 (C-2^E), 52.52 (COO<u>C</u>H$_3$), 32.04, 30.17, 26.11 (C-2^I, C-4^I, C-3^I), 24.00, 23.94 (C(<u>C</u>H$_3$)$_2$), 23.56 (NHCO<u>C</u>H$_3$), 23.08 (C-5^I), 21.12, 21.05, 20.85, 20.77 (3x $COCH_3$ of units E and F), 14.45 (C-6^I), $^2J_{1F,P}$ = 6.4 Hz, $J_{2F,P}$ = 10.1 Hz, $^2J_{1G,P}$ = 4.6 Hz, $J_{2G,P}$ = 14.6 Hz, $J_{2H,P}$ = 8.2 Hz, $^2J_{3H,P}$ = 5.6 Hz.- ^{31}P NMR (81.0 MHz, [D$_5$]pyridine): δ = -5.92.- $C_{37}H_{57}Cl_3N_3O_{22}P$ (1033.20, 1031.22), FAB MS: m/z 1058.3, 1056.3, 1054.3 $[M+Na]^+$, 1036.3, 1034.3, 1032.3 $[M+H]^+$, 612.2 $[f+Na-H]^+$, 330.1 $[e]^+$.

The second stereoisomer could not be obtained pure. The following characteristic signals were obtained for the second isomer from the mixture of the two isomers: ^{1}H NMR (200 MHz, [D$_5$]pyridine): δ = 6.63 (dd, 1 H, 1^F-H, $J_{1F,2F}$ = 3.4 Hz, $J_{1F,P}$ = 6.8 Hz).- ^{31}P NMR (81.0 MHz, [D$_5$]pyridine): δ = -4.29.

2-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-4-*O*-acetyl-3-*O*-carbamoyl-1-*O*-{[(R)-2-methoxycarbonyl-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy]-(2,2,2,trichloro-1,1-dimethylethyloxy)-phosphoryl}-α-D-glucopyranuronamide (18b)

14d (80 mg, 0.13 mmol) and the lipid part **16** (180 mg, 0.38 mmol, 2.9 eq.) were converted into the phosphoric acid triester as described for **18a**. FC ($CHCl_3$-methanol 100:2, 100:3, 100:4, 100:5 and 100:10) furnished a single P-diastereomer **18b** (80 mg, 47%, ^{1}H NMR indicated the presence of some triazole in the product which was used for the next step without further purification) and a mixture of the P-diastereomers (55 mg, 34%). TLC: $CHCl_3$-methanol 10:1 then 5:1.- ^{1}H NMR (H,H COSY, 400 MHz, [D$_5$]pyridine): δ = 9.23 (d, 1 H, N<u>H</u>COCH$_3$), 8.60, 8.06 (2 s, 2x 1 H, $CONH_2$), 7.65 (broad s, 2 H, H_2NCOO), 6.52 (dd, 1 H, 1^F-H), 6.11 (dd, 1 H, 3^E-H), 5.99 (t, 1 H, 3^F-H), 5.93 (t, 1 H, 4^F-H), 5.53 (d, 1 H, 1^E-H), 5.46 (t, 1 H, 4^E-H), 4.99 (d, 1 H, 5^F-H), 4.85-7.73 (m, 1 H, $3'^H$-H), 4.72-4.64 (m, 1 H, 3^H-H), 4.54 (dd, 1 H, $6'^E$-H), 4.52-4.48 (m, 1 H, 2^H-H), 4.42 (dd, 6^E-H), 4.17-4.09 (dd, 2 H, 2^F-H, 2^E-H), 4.05 (ddd, 1 H, 5^E-H), 3.92-3.82 (m, 1 H, $1'^I$-H), 3.79 (s, 3 H, $COOCH_3$), 3.72-3.64 (m, 1 H, 1^I-H), 2.19, 2.17, 2.16, 2.15, 2.08, 2.07, 2.01 (7 s, 7x 3 H, 7 CH_3 of units E, F and G), 1.86-0.87 (signals of unit I), $J_{1E,2E}$ = 8.4 Hz, $J_{2E,3E}$ = 9.7 Hz, $J_{3E,4E}$ = 9.8 Hz, $J_{4E,5E}$ = 9.8 Hz, $J_{5E,6'E}$

= 4.5 Hz, $J_{5E,6E}$ = 2.4 Hz, $^2J_{6E,6'E}$ = 12.2 Hz, $J_{NH,2E}$ = 8.0 Hz, $J_{1F,2F}$ = 3.4 Hz, $J_{2F,3F}$ = 9.5 Hz, $J_{3F,4F}$ = 9.7 Hz, $J_{4F,5F}$ = 9.7 Hz, $J_{1F,P}$ = 5.4 Hz.- ^{13}C NMR (C,H COSY, 100.6 MHz, APT 50.3 MHz, [D$_5$]pyridine): δ = 171.12, 170.98, 170.71, 170.64, 170.14, 169.92, 169.70 (CO), 157.36 (H_2NCOO), 106.63 (d, $\underline{C}Cl_3$), 102.86 (C-1^E), 97.96 (d, C-1^F), 91.09 (d, $\underline{C}(CH_3)_2$), 78.84 (d, C-2^F), 78.66 (d, C-2^H), 72.98 (C-3^E), 72.29 (C-5^E), 71.42 (C-5^F), 71.13 (C-3^F), 70.55 (C-4^F), 70.27 (C-1^I), 69.93 (C-4^E), 68.73 (d, C-3^H), 62.76 (C-6^E), 56.02 (C-2^E), 52.53 (COO$\underline{C}H_3$), 42.56-19.87 (signals of unit I, CH_3 signals of units E, F and G) $^2J_{1F,P}$ = 6.1 Hz, $J_{2F,P}$ = 13.5 Hz, $^2J_{1G,P}$ = 5.1 Hz, $J_{2G,P}$ = 14.8 Hz, $J_{2H,P}$ = 12.4 Hz, $^2J_{3H,P}$ = 5.1 Hz.- ^{31}P NMR (81.0 MHz, [D$_5$]pyridine): δ = -3.67.- $C_{56}H_{95}Cl_3N_3O_{22}P$ (1299.71, 1297.52), FAB MS: m/z 1324.7, 1322.7, 1320.7 [M+Na]$^+$, 612.2 [f+Na-H]$^+$, 330.1 [e]$^+$.

2-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-4-*O*-acetyl-3-*O*-carbamoyl-1-*O*-{[(R)-2-methoxycarbonyl-2-(hexyloxy)-ethoxy]-hydroxyphosphoryl}-α-D-glucopyranuronamide (18c)

To a solution of the triester **18a** (60 mg, 0.06 mmol) in dry pyridine (2.5 mL) Zn-Cu couple (freshly prepared, 60 mg, 0.91 mmol) and 2,4-pentanedione (80 μL, 0.68 mmol) were added and the mixture was stirred under argon for 1 h at 20°C. Excess Zn-Cu was removed by filtration and the residue washed with CH_2Cl_2 (3x 5 mL), ethanol (3x 5 mL) and methanol (3x 5 mL). After solvent evaporation the residue was dried at 10 Pa for 1 h, then taken up in 8:1 water-ethanol (9 mL). Dowex 50 W X2 /50-100 (H$^+$ form, 1.0 g) was added and the mixture stirred for 1 h. After filtration the resin was washed with CH_2Cl_2 (3x 5 mL), ethanol (3x 5 mL), methanol (3x 5 mL) and methanol-water 1:1 (3x 5 mL). The organic solvents were removed by evaporation under reduced pressure and the remaining aqueous solution was lyophilized. The crude product was filtered through a column of Sephadex LH-20 (5.0 g, $CHCl_3$-methanol 1:1) to give **18c** as a mixture of two products, one with 5-acetyl groups and the second with 4-acetyl groups, ratio by ^{1}H NMR 8:1 (35 mg, 69%). TLC: $CHCl_3$-methanol 3:1.- ^{1}H NMR (H,H COSY, 400 MHz, D_2O): δ = 5.83 (dd, 1 H, 1^F-H), 5.12 (dd, 1 H, 3^E-H), 5.07-5.00 (2 H, 3^F-H, 4^F-H), 4.92 (t, 1 H, 4^E-H), 4.76 (d, 1 H, 1^E-H), 4.40-4.35 (2 H, 2^H-H, 5^F-H), 4.24-4.14, 4.04-3.96 (2 m, 2x 1 H, CH_2-3^H), 3.95-3.88 (m, 2 H, 2^F-H, 2^E-H), 3.80-3.50 (CH_2-6^E, CH_2-1^I, 5^E-H), 3.75 (s, 3 H, $COOCH_3$), 2.00, 1.98, 1.94, 1.83 (4 s, signals for 5 CH_3 of units E and F), 1.58-1.48, 1.34-1.25, 1.25-1.18 (3 m, 2x 2 H, 1x 4 H, CH_2-2^I, CH_2-3^I, CH_2-4^I, CH_2-5^I), 0.80 (3 H, CH_3 of unit I), $J_{1E,2E}$ = 8.5 Hz, $J_{2E,3E}$ = 10.4 Hz, $J_{3E,4E}$ = 9.6 Hz, $J_{4E,5E}$ = 9.6 Hz, $J_{1F,2F}$ = 3.8 Hz, $J_{1F,P}$ = 7.1 Hz; The presence of the minor component was evident from the 1^F-H signal at δ = 5.69 (dd, $J_{1F,2F}$ = 2.9 Hz, $J_{1F,P}$ = 6.3 Hz) and from signals in the acetyl CH_3 region (partially hidden).- ^{13}C NMR (C,H COSY, 100.6 MHz, $CDCl_3$-CD_3OD 2:1): δ = 170.82, 170.10, 169.35 (probably 1, 2, and 3 overlapping CO signals), 156.08 (H_2NCOO), 128.26 (?), 100.87 (C-1^E), 94.13 (C-1^F), 77.92 (C-2^F, C-2^H), 71.62, 71.07, 70.88, 69.1 (C-5^E, C-3^E, C-5^F, C-4^F), 68.32 (C-3^F, C-1^I, C-4^E), 65.47 (C-3^H), 63.08 (C-6^E), 61.61 (?), 53.49 (C-2^E), 51.53 (COO$\underline{C}H_3$), 42.29 (?), 30.95, 28.99, 28.78, 24.83, (NHCO$\underline{C}H_3$, C-2^I, C-4^I, C-3^I, C-5^I), 21.91, 21.74, 19.75 (CH_3 of units E and F), 13.13 (C-6^I).- ^{31}P NMR (81.0 MHz, $CDCl_3$-CD_3OD 2:1): δ = -3.53.- $C_{33}H_{52}N_3O_{22}P$ (873.76, 873.28), FAB MS: m/z 934.2 [M+Na+K-H]$^+$, 918.2 [M+2Na-H]$^+$, 896.2 [M+Na]$^+$, 876.2 [M+2Na-H-CH_2CO]$^+$, 854.2 [M+Na-CH_2CO]$^+$, 834.2 [M+2Na-H-2x CH_2CO]$^+$, 812.2 [M+Na-2x CH_2CO]$^+$, 612.1 [f+Na-H]$^+$.

Treatment of 18b with Zn-Cu couple

To a solution of the triester **18b** (75 mg, 0.06 mmol) in dry pyridine (3.5 mL) Zn-Cu couple (freshly prepared, 70 mg, 1.14 mmol) and 2,4-pentanedione (90 μL, 0.77 mmol) were added and the mixture was stirred under argon at 20°C for 4 h. TLC ($CHCl_3$-methanol 3:1) showed that most of **18b** was still present. An additional amount of 2,4-pentanedione (30 μL, 0.26 mmol) was added and the reaction mixture was stirred at 20°C overnight. Excess Zn-Cu was removed by filtration and the residue washed with CH_2Cl_2 (3x 10 mL), ethanol (3x 10 mL) and methanol (3x 10 mL). After solvent evaporation the residue was dried at 10 Pa for 1 h. The same procedure was used for another amount of **18b** (54 mg, 0.042 mmol) and the residue of both reactions were taken up in 8:1 water-ethanol (30 mL). Dowex 50 W X2 /50-100 (H$^+$ form, 3.0 g) was added and the mixture was stirred for 1 h. After filtration the resin was washed with CH_2Cl_2 (3x 10 mL), ethanol (3x 10 mL), methanol (3x 10 mL) and methanol-water 1:1 (3x 10 mL). The organic solvents were removed by evaporation under reduced pressure and the remaining aqueous solution was lyophilized. The crude

product was dissolved in CH_2Cl_2-methanol and mixed with kieselguhr, solvents were evaporated, the residue was dried at 10 Pa and the crude product was transferred to the top of a FC column eluting with $CHCl_3$-methanol 4:1 then $CHCl_3$-methanol-H_2O 20:10:0.5 to give a mixture of two products **18d** (R = CH_3CO) and **18e** (R probably = H), one with 5-acetyl groups and the second with 4-acetyl groups, ratio by ^{1}H NMR 1:1 (77 mg, 68%).

2-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-4-*O*-acetyl-3-*O*-carbamoyl-1-*O*-{[(R)-2-methoxycarbonyl-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy]-hydroxy-phosphoryl}-α-D-glucopyranuronamide (18d)

^{1}H NMR (H,H COSY, 400 MHz, CD_3OD): for the mixture of **18d** and **18e**; *characteristic signals of the first compound:* δ = 5.99 (dd, 1 H, 1^F-H), 5.35 (t, 1 H, 3^E-H), 5.23 (t, 1 H, 4^F-H), 5.17 (t, 1 H, 3^F-H), 5.02 (t, 1 H, 4^E-H), 4.88 (d, 1 H, 1^E-H), 4.46 (d, 1 H, 5^F-H), 4.37 (dd, 1 H, $6'^E$-H), 4.31-4.08 (m, 2^H-H, CH_2-3^H), 4.17 (dd, 1 H, 6^E-H), 3.96-3.78 (m, 2^E-H, 5^E-H), 3.79 (m, overlapping $COOCH_3$, 1 H, 2^F-H), 3.72-3.56 (m, CH_2-1^I), $J_{1E,2E}$ = 8.9 Hz, $J_{2E,3E}$ = 9.8 Hz, $J_{3E,4E}$ = 9.8 Hz, $J_{4E,5E}$ = 9.7 Hz, $J_{5E,6'E}$ = 4.6 Hz, $J_{5E,6E}$ = 2.2 Hz, $^2J_{6E,6'E}$ = 12.1 Hz, $J_{1F,2F}$ = 3.4 Hz, $J_{2F,3F}$ = 9.8 Hz, $J_{3F,4F}$ = 9.7 Hz, $J_{4F,5F}$ = 9.7 Hz, $J_{1F,P}$ = 7.2 Hz; *characteristic signals of the second compound:* δ = 5.77 (dd, 1 H, 1^F-H), 5.26 (dd, 1 H, 3^E-H), 5.25 (t, 1 H, 3^F-H), 5.16 (t, 1 H, 4^F-H), 4.99 (t, 1 H, 4^E-H), 4.84 (hidden by solvent peak, 1^E-H), 4.49 (d, 1 H, 5^F-H), 4.31-4.08 (m, 2 H, CH_2-3^H), 3.96-3.78 (m, 2^E-H, 2^F-H, 2^H-H), 3.72-3.56 (m, 5^E-H, CH_2-6^E, CH_2-1^I), $J_{2E,3E}$ = 10.4 Hz, $J_{3E,4E}$ = 9.4 Hz, $J_{4E,5E}$ = 9.5 Hz, $J_{1F,2F}$ = 3.3 Hz, $J_{2F,3F}$ = 9.7 Hz, $J_{3F,4F}$ = 9.8 Hz, $J_{4F,5F}$ = 9.9 Hz, $J_{1F,P}$ = 6.5 Hz; *signals of the two compounds:* δ = 3.85, 3.83 (2 s, 2x 3 H, 2x $COOCH_3$), 2.11-1.93 (CH_3 signals of units E and F), 1.70-0.86 (signals of unit I).- ^{13}C NMR (C,H COSY, 75.46 MHz, CD_3OD): *characteristic signals of the first compound:* δ = 103.44 (C-1^E), 96.02 (d, C-1^F), 80.48 (d, C-2^F), 79.87 (d, C-2^H), 75.62 (C-1^I), 74.25 (C-3^E), 72.95 (C-5^E), 72.78 (C-4^F), 71.25 (C-3^F), 70.77 (C-4^E), 70.33 (C-5^F), 67.09 (d, C-3^H), 63.41 (C-6^E), 55.55 (C-2^E), $^2J_{1F,P}$ = 5.3 Hz, $J_{2F,P}$ = 8.4 Hz, $J_{2H,P}$ = 7.4 Hz, $^2J_{3H,P}$ = 7.5 Hz; *characteristic signals of the second compound:* δ = 103.34 (C-1^E), 96.02 (d, C-1^F), 80.10 (d, C-2^F), 79.20 (d, C-2^H), 75.62 (C-1^I), 73.88 (C-3^E), 72.44 (C-3^F), 71.45 (C-4^F), 70.86 (C-5^E), 70.71 (C-4^E), 70.17 (C-5^F), 66.97 (d, C-3^H), 61.65 (C-6^E), 55.40 (C-2^E), $J_{1F,P}$ = 5.3 Hz, $J_{2F,P}$ = 8.8 Hz, $J_{2H,P}$ = 8.3 Hz; *characteristic signals of the two compounds:* δ = 173.57, 172.99, 172.95, 172.64, 172.57, 172.47, 171.91, 171.79, 171.35, 171.29, 171.21, 171.18 (CO of units E, F and H), 158.56, 158.44 (H_2NCOO), 52.80 ($COO\underline{C}H_3$), 43.07-19.99 (CH_3 of units E and F and signals of unit I).- ^{31}P NMR (81.0 MHz, CD_3OD): δ = -1.06.- $C_{52}H_{90}N_3O_{22}P$ (1140.27, 1139.58), FAB MS: m/z 1184.5 $[M+2Na-H]^+$, 1162.5 $[M+Na]^+$, 1142.5 $[M+2Na-H-CH_2CO]^+$, 612.1 $[f+Na-H]^+$.- MALDI TOF (α-cyano-4-hydroxy-cinnamic acid): 1184.43 $[M+2Na-H]^+$, compound **18d**; 1142.43 $[M+2Na-H]^+$, compound **18e**.

Hydrolytic cleavage of the ester protecting groups of 18c

2:1 methanol (p.a)-water (bidist.) was degassed (stream of argon, sonication) for 15 min. A solution of **18c** (10.0 mg, 11 μmol) in the above solvent (1.0 mL) was also degassed and cooled to 0°C. Lithium hydroxide (degassed, 0.3 mol/L, 267 μL, 7.0 eq) was added and the reaction mixture was stirred at 0°C for 30 min, then at 20°C for 8 h. Excess base was neutralized by addition of Dowex 50 W X2 (H^+ form). Stirring at 20°C was continued for 30 min. After filtration the resin was washed with 1:1 methanol - water. The combined filtrates were concentrated and the remaining aqueous solution was lyophilized. TLC: $CHCl_3$-methanol-H_2O 18:13:2.7, the pH of the reaction was controlled with pH paper.-The same procedure was used in a second reaction (10.0 mg of **18c**). The crude products of both reactions were combined and purified by repeated FC (CH_2Cl_2-methanol-H_2O 18:11:2.7) to yield **17a** (7.0 mg, 44%) and **17b** (4.0 mg, 25%). The two products were filtered separately through Sephadex G10, 1 cm column, eluting with methanol-H_2O 1:1.

2-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-3-*O*-carbamoyl-1-*O*-{[(R)-2-carboxy-2-(hexyloxy)-ethoxy]-hydroxy-phosphoryl}-α-D-glucopyranuronamide (17a)

^{1}H NMR (H,H COSY, 400 MHz, 200 MHz, D_2O): δ = 5.76 (dd, 1 H, 1^F-H), 4.91 (t, 1 H, 3^F-H), 4.58 (d, 1 H, 1^E-H), 4.25 (d, 1 H, 5^F-H), 4.16-4.08 (m, 2 H), 3.96 (dd, 1 H, J = 5.9, 10.3 Hz), 3.76 (dt, 1 H, 2^F-H), 3.66 (t, 1 H, 4^F-H), 3.82-3.77, 3.65-3.52, 3.51-3.42, 3.35-3.30, (4 m, CH_2-6^E, CH_2-3^H, CH_2-1^I, 2^E-H, 5^E-H), 1.94 (s, 3 H, $NHCOC\underline{H}_3$), 1.56-1.48 (m, 2 H, CH_2 of unit I), 1.31-1.15 (m, 6 H, 3x CH_2 of unit I), 0.77 (t, 3 H, CH_3

of unit I), $J_{1E,2E}$ = 8.2 Hz, $J_{1F,2F}$ = 3.2 Hz, $J_{2F,3F}$ = 9.9 Hz, $J_{3F,4F}$ = 9.8 Hz, $J_{4F,5F}$ = 10.0 Hz, $J_{1F,P}$ = 6.8 Hz.- ^{13}C NMR (50.3 MHz, D_2O): δ = 175.92 (CO), 161.28 (H_2NCOO), 105.21 (C-1^E), 97.62 (C-1^F), 81.09 (C-2^F), 78.64 (C-2^H), 76.79, 76.08, 74.08, 72.76, 68.93, 68.85 (C-1^I, C-3^E, C-5^E , C-4^E, C-3^F, C-4^F, C-5^F, C-3^H), 63.73 (C-6^E), 58.48 (C-2^E), 33.76, 31.51, 27.72, 25.19 (4x CH_2 of unit I), 24.85 (NHCO$\underline{C}H_3$), 16.26 (C-6^I).- ^{31}P NMR (81.0 MHz, $CDCl_3$:CH_3OH:H_2O 1:2:0.5): δ = 1.45.- $C_{24}H_{42}N_3O_{18}P$ (691.58, 691.22), FAB MS: m/z 736.2 $[M+2Na-H]^+$, 714.2 $[M+Na]^+$.

2-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-3-*O*-carbamoyl-1-*O*-{[(R)-2-carboxy-2-(hexyloxy)-ethoxy]-hydroxy-phosphoryl}-α-D-glucopyranuronic acid (17b)

^{1}H NMR (H,H COSY, 200 MHz, 400 MHz, D_2O): characteristic signals; δ = 5.82 (dd, 1 H, 1^F-H), 5.00 (t, 1 H, 3^F-H), 4.61 (d, 1 H, 1^E-H), 4.32 (d, 1 H, 5^F-H), 4.25-3.95 (m, 2 H), 3.93-3.76, 3.75-3.48, 3.42-3.36 (3 m, 2^F-H, 4^F-H, 4^E-H, 2^E-H, 3^E-H, CH_2-6^E, CH_2-3^H, CH_2-1^I, 5^E-H), 2.02 (s, 3 H, NHCOC$\underline{H}_3$), 1.68-1.50 (m, 2 H, 1x CH_2 of unit I) 1.38-1.22 (m, 6 H, 3x CH_2 of unit I), 0.84 (t, 3 H, CH_3 of unit I), $J_{1E,2E}$ = 8.1 Hz, $J_{1F,2F}$ = 3.3 Hz, $J_{2F,3F}$ = 9.6 Hz, $J_{3F,4F}$ = 9.5 Hz, $J_{4F,5F}$ = 10.0 Hz, $J_{1F,P}$ = 6.2 Hz.- ^{13}C NMR (50.3 MHz, D_2O): δ = 181.19, 174.72 (CO), 158.88 (H_2NCOO), 102.75 (C-1^E), 95.02 (C-1^F), 78.66 (C-2^F), 76.06 (C-2^H), 74.31, 73.69, 71.61, 70.53, 70.20, 68.95, 66.01, 63.87 (C-1^I, C-3^E, C-5^E , C-4^E, C-3^F, C-4^F, C-5^F, C-3^H), 61.15 (C-6^E), 55.87 (C-2^E), 31.17, 28.93, 25.12, 22.59, 22.26 (4x CH_2 of unit I, NHCO$\underline{C}H_3$), 13.65 (C-6^I).- ^{31}P NMR (81.0 MHz, $CDCl_3$:CH_3OH:H_2O 1:2:0.5): δ = 1.45.- $C_{24}H_{41}N_3O_{19}P$ (692.57, 692.20), FAB MS: m/z 737.2 $[M+2Na-H]^+$, 715.2 $[M+Na]^+$.

Hydrolytic cleavage of the ester protecting groups of 18d and 18e

To a degassed solution (stream of argon, sonication) of **18d** and **18e** (15.0 mg, 13.0 μmol) in 2:1 p.a. methanol - bidist. water (1.8 mL) at 0°C degassed aqueous lithium hydroxide (0.3 mol/L, 307 μL, 7.0 eq.) was added. The reaction mixture was stirred at 0°C for 30 min, then at 20°C for 5 h. The reaction was stopped by addition of Dowex 50 W X2 (H^+ form, 0.5 g). Stirring at 20°C was continued for 30 min. After filtration the resin was washed with 2:1 methanol-water then with water. The combined filtrates were concentrated and the remaining aqueous solution was lyophilized. TLC: $CHCl_3$-methanol-H_2O 18:13:2.7, the pH of the reaction was controlled with pH paper.- The same procedure was used in a second experiment (10.0 mg of **18d** and **18e**) and the crude products of both reactions were combined and purified by repeated FC ($CHCl_3$-methanol-H_2O 18:11:1 then 18:11:2.7) to provide **1b** (15.0 mg, 45%), **17d** (7.0 mg, 21%), a mixture of **1b** and **17d** (3.0 mg, 9%) and a mixture of **1b** with a less polar product, probably methyl ester **17e**, (2.0 mg, 6%). The two pure specimen of **1b** and **17d** were filtered separately through Sephadex G10, 1 cm column, eluting with methanol-H_2O 1:1.

2-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-3-*O*-carbamoyl-1-*O*-{[(R)-2-carboxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy]-hydroxyphosphoryl}-α-D-glucopyranuronamide (1b)

^{1}H NMR (H,H COSY, 300 MHz, water suppression, 0.4 mg / 0.7 mL): signals that could be assigned: δ = 5.90 (dd, 1 H, 1^F-H, $J_{1F,2F}$ = 4.3 Hz, $J_{1F,P}$ = 7.4 Hz), 5.00 (t, 1 H, 3^F-H, $J_{2F,3F}$ = $J_{3F,4F}$ = 9.4 Hz), 4.80 (a signal hidden by the solvent, 1^E-H), 4.42 (d, 1 H, 5^F-H, $J_{4F,5F}$ = 9.9 Hz), 2.09 (s, 3H, NHCOC$\underline{H}_3$), 1.60-0.87 (signals of unit I).- ^{1}H NMR (200 MHz, [D_6]DMSO): signals that could be assigned: δ = 7.48 (s, 1 H, $CONH_2$), 7.46 (d, 1 H, N$\underline{H}$COCH$_3$), 7.18 (s, 1 H, $CONH_2$), 6.25 (broad s, 2 H, H_2NCOO), 5.79 (dd, 1H, 1^F-H, $J_{1F,2F}$ = 2.9 Hz, $J_{1F,P}$ = 6.5 Hz), 4.85 (t, 1H, 3^F-H, $J_{2F,3F}$ = $J_{3F,4F}$ = 9.5 Hz), 4.45 (d, 1H, 1^E-H, $J_{1E,2E}$ = 7.3 Hz), 4.05 (d, 1H, 5^F-H), 1.82 (s, 3 H, NHCOC$\underline{H}_3$), 1.59-0.81 (signals of unit I).- ^{1}H NMR (H,H COSY, 400 MHz, [D_6]DMSO: D_2O 4:1, at 70°C, better resolution than at 26°C): signals that could be assigned: δ = 5.69 (dd, 1H, 1^F-H, $J_{1F,2F}$ = 3.3 Hz, $J_{1F,P}$ = 5.6 Hz), 4.83 (t, 1H, 3^F-H, $J_{2F,3F}$ = $J_{3F,4F}$ = 9.52 Hz), 4.50 (d, 1H, 1^E-H, $J_{1E,2E}$ = 7.9 Hz), 4.10 (d, 1H, 5^F-H, $J_{4F,5F}$ = 9.9 Hz), 1.84 (s, 3H, NHCOC$\underline{H}_3$), 1.60-0.75 (signals of unit I).- ^{13}C NMR (50.3 MHz, D_2O:CD_3OD 1:1): only signals from unit I were observed, δ = 40.44-20.73 and a signal at δ = 68.80.- ^{13}C NMR (100.6 MHz, D_2O): δ = 173.84, 172.24 (CO), 157.91 (H_2NCOO), 102.07 (C-1^E), 94.48 (C-1^F), 78.00, 75.30 (C-2^F, C-2^H), 73.39, 72.68, 70.79, 69.15 (2x C), 68.01, 65.64 (C-1^I, C-3^E, C-5^E, C-4^E, C-3^F, C-4^F C-5^F, C-3^H), 60.01 (C-6^E), 55.02 (C-2^E), 42.86-19.40 (NHCO$\underline{C}H_3$, signals from unit I).- ^{31}P NMR (81.0

MHz, D_2O:CD_3OD 1:1): δ = -2.17.- $C_{43}H_{80}N_3O_{18}P$ (958.09, 957.52), FAB MS: **m/z 996.8** $[M+K]^+$, **980.8** $[M+Na]^+$, **958.8** $[M+H]^+$.

2-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-3-*O*-carbamoyl-1-*O*-{[(R)-2-carboxy-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy]-hydroxyphosphoryl}-α-D-glucopyranuronic acid (17d)
The NMR spectra were uninformative.- ^{31}P NMR (81.0 MHz, DMSO:D2O 4:1): δ = -2.81.- $C_{43}H_{79}N_2O_{19}P$ (959.08, 958.50), FAB MS: m/z 1003.8 $[M+2Na-H]^+$, 997.8 $[M+K]^+$, 981.8 $[M+Na]^+$, 959.8 $[M+H]^+$.

2-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-3-*O*-carbamoyl-1-*O*-{[(R)-2-methoxycarbonyl-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy]-hydroxyphosphoryl}-α-D-glucopyranuronamide (17e)
The NMR spectra were uninformative.- $C_{44}H_{82}N_3O_{18}P$ (972.12, 971.53), FAB MS: m/z 1010.3 $[M+K-H]^+$, 994.3 $[M+Na]^+$.

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