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STRUCTURAL ANALOGUES OF THE ANTIBIOTIC MOENOMYCIN A WITH A D-GLUCOSE-DERIVED UNIT F

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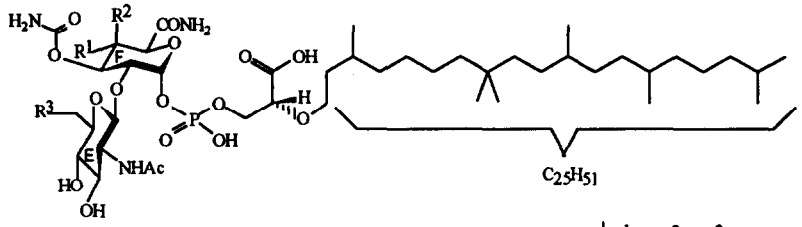
Abstract - Disaccharide derivative **1a** is the smallest transglycosylase inhibiting compound known up to now. Structurally closely related compounds **11b** and **19c** have been synthesized. They do not inhibit the transglycosylase demonstrating the high specificity of the interaction between inhibitor **1a** and the binding-site at the enzyme.

Introduction

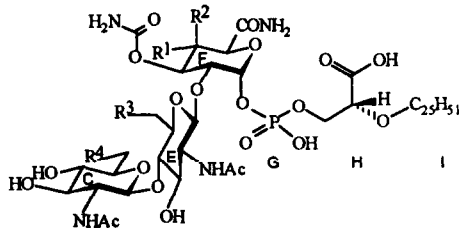
Among the different constituents of the bacterial cell wall, the most important for the survival and integrity of the cell is peptidoglycan, a ß-1,4-linked glycan consisting of a repeating unit N-acetylglucosaminyl-N-acetylmuramyl-L-Ala-D-isoGlu-L-Lys (or DAP)-D-Ala. The peptide chains are at least partially cross-linked, either directly or through short peptide chains.[1]

The two successive final reactions in the biosynthesis of cross-linked peptidoglycan from the membrane precursor N-acetylmuramyl-(pentapeptide)-pyrophosphoryl-undecaprenol are (i) the transglycosylation that extends the glycan chain and (ii) the transpeptidation that cross-links the glycan chains through two peptide units. A number of bifunctional enzymes (penicillin-binding proteins, PBP's) have been identified that catalyze both transglycosylation and transpeptidation.[2] Moenomycin A and related antibiotics belong to the rare compounds that have been found to inhibit efficiently the transglycosylase activity of the PBP's.[3] Extensive moenomycin degradation studies as well as syntheses of structural analogues have been performed in order to establish the structural basis of the transglycosylase inhibition.[4] At present, disaccharide derivative **1a** is the smallest structural analogue of the moenomycins that elicits full transglycosylase inhibiting activity.[5] On the contrary, both **1b**[6] and **1c**,[7] which differ from **1a** configurationally (D-

galacto vs. D-gluco configuration in unit F) and by the lack of the methyl group at C-4^F, are completely inactive. In the trisaccharide series, compounds both with a moenuronic acid- and a D-galacturonic acid-derived unit F (**2a**, **2b**, and **2c**) are fully active inhibitors of the transglycosylase.[7,8] Still unresolved is the question whether in the disaccharide series transglycosylase inhibiting activity is linked to D-gluco configuration in unit F and/or the presence of the C-4^F-methyl group. Compound **1d** would possibly answer at least some of these questions. The present paper describes work that was set out to establish whether D-gluco configuration of unit F is a prerequisite of transglycosylase inhibiting activity in the disaccharides.



1	R^1	R^2	R^3
a	OH	CH_3	OH
b	H	OH	OH
c	H	OH	H
d	OH	H	OH



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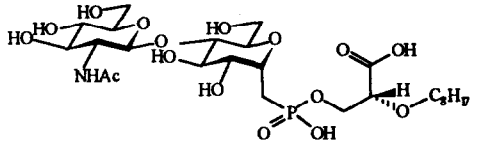


3

2	R^1	R^2	R^3	R^4
a	OH	CH_3	OH	H
b	H	OH	H	H
c	H	OH	OH	OH

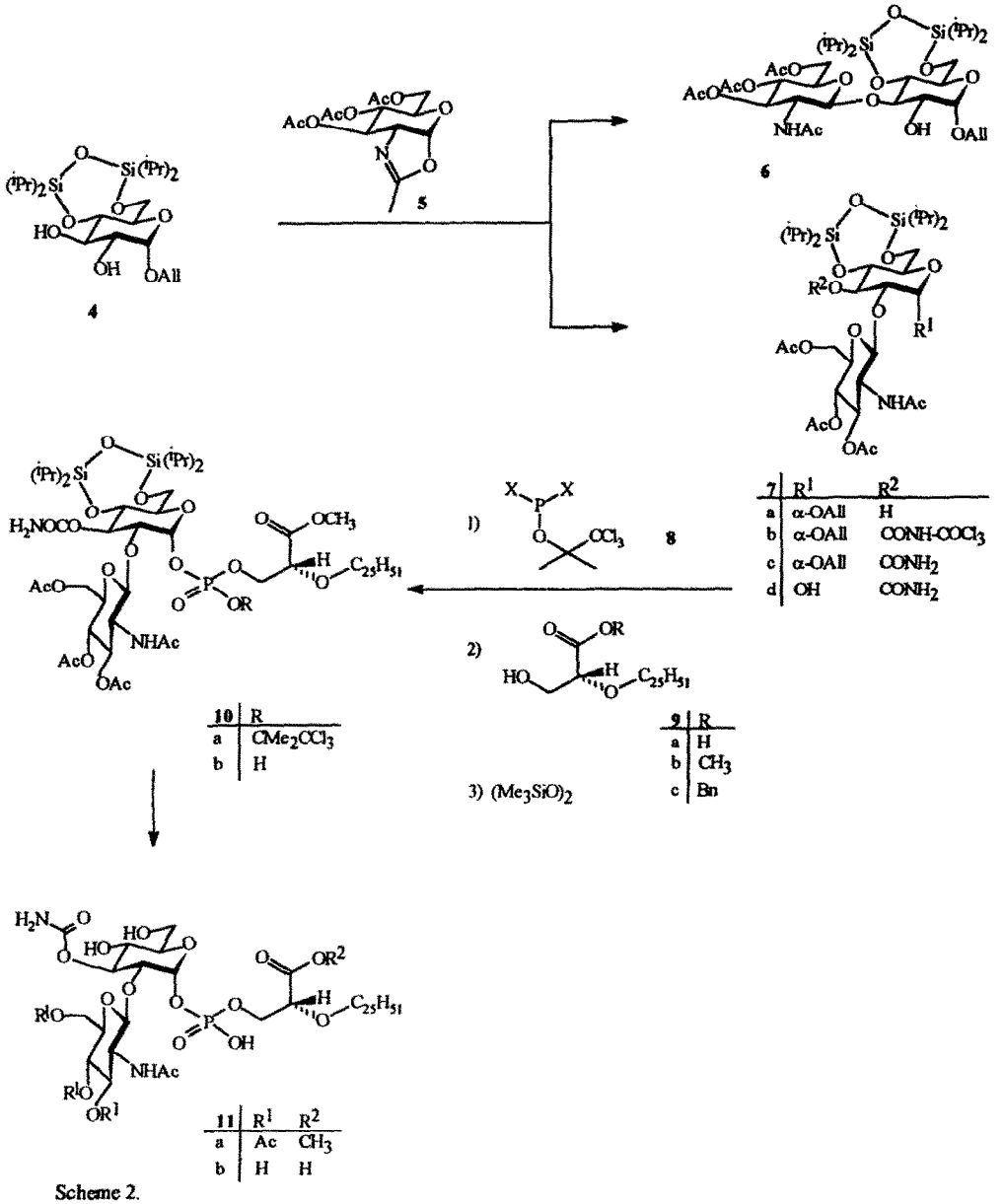
Scheme 1.

Synthesis of a Structural Analogue 11b of Moenomycin A with a D-Glucose-derived Unit F

Guided by results from van Boom's laboratory[9] we expected TIPS-protected **4**[10] to react regioselectively at the 2-position with glycosyl donor **5**.[11,12] In the event, the p-toluenesulfonic acid-catalyzed reaction between **4** and **5** (in 1:1 toluene-nitromethane solution) furnished only a 1.8:1 ratio of **7a** and **6** (total yield 71%). The structural assignments rest mainly on one- and two-dimensional NMR spectra (summarized in the Experimental). Most specifically, in the glucose unit the C-2 signal in **7a** and the C-3 signal in **6**, respectively, are downfield shifted by approximately 10 ppm when compared with **4** (ß effect). In the COSY spectrum of **6** a cross-peak for the coupling of the OH proton with 2-H was observed, and in the COSY spectrum of **7a** for the coupling between OH and 3-H. $J_{1,2}$ = 8.5 Hz in the N-acetyl-D-glucosamine unit was visible, indicative of the ß1→2 and ß1→3 linkage in **7a** and **6**, respectively.

For the introduction of the carbamoyl group, trichloroacetyl isocyanate (TAI) was used.[13] **7c** was obtained

in 79% overall yield by a one-pot procedure consisting of a) reaction of **7a** with TAI in CH_2Cl_2 (**7a**→**7b**), b) CH_3OH quench, and c) hydrolysis with 5% aq. K_2CO_3. Next, the allyl protecting group was removed by treatment with $PdCl_2$ in 0.1 mol/L sodium acetate in 20:1 acetic acid-water solution (21 h at 20°C) to give **7d** in 71% yield.[14]



7	R^1	R^2
a	α-OAll	H
b	α-OAll	CONH-$COCl_3$
c	α-OAll	$CONH_2$
d	OH	$CONH_2$

10	R
a	CMe_2CCl_3
b	H

9	R
a	H
b	CH_3
c	Bn

11	R^1	R^2
a	Ac	CH_3
b	H	H

Scheme 2.

For the construction of the phosphoric acid diester grouping we used the phosphite methodology as adapted to the synthesis of moenomycin analogues.[15] Thus, the sequence (i) treatment of 2,2,2-trichloro-1,1-dimethylethyl dichlorophosphite **8** (X=Cl) with two equivalents of 1H-1,2,4-triazole,[16] (ii) reaction of the thus prepared reagent **8** (X=triazolyl) with **7d**, (iii) subsequent addition of **9b**[17] and (iv) oxidation of the intermediate phosphite triester with bis(trimethylsilyl)peroxide[18] furnished the phosphate triester **10a** (probably a mixture of stereoisomers isomeric at the P centre). Removal of the phosphate protecting group was achieved under the Imai conditions[19] with freshly prepared Zn-Cu couple[6] to provide **10b.**

From **10b** the silyl protecting group was cleaved off with tetra-^{n}butylammonium fluoride (TBAF) in THF. **11a** was obtained in 72% yield. According to the ^{13}C NMR spectrum, it contained 20% of an impurity which we were unable to remove by chromatographic separation. From this impure sample the target compound **11b** was then obtained by base-catalyzed ester cleavage followed by careful chromatographic purification. Structural assignment rests on the ^{13}C NMR and mass spectral data. According to these spectra, **11b** was free of impurities.

The inhibitory effect of **11b** directly on the transglycosylation reaction was determined by the *in vitro* assay described previously[20] using as substrate the lipid intermediate which is the immediate precursor of uncross-linked peptidoglycan. In these experiments **11b** turned out to have no inhibitory effect at a final concentration of 10 μg/mL.

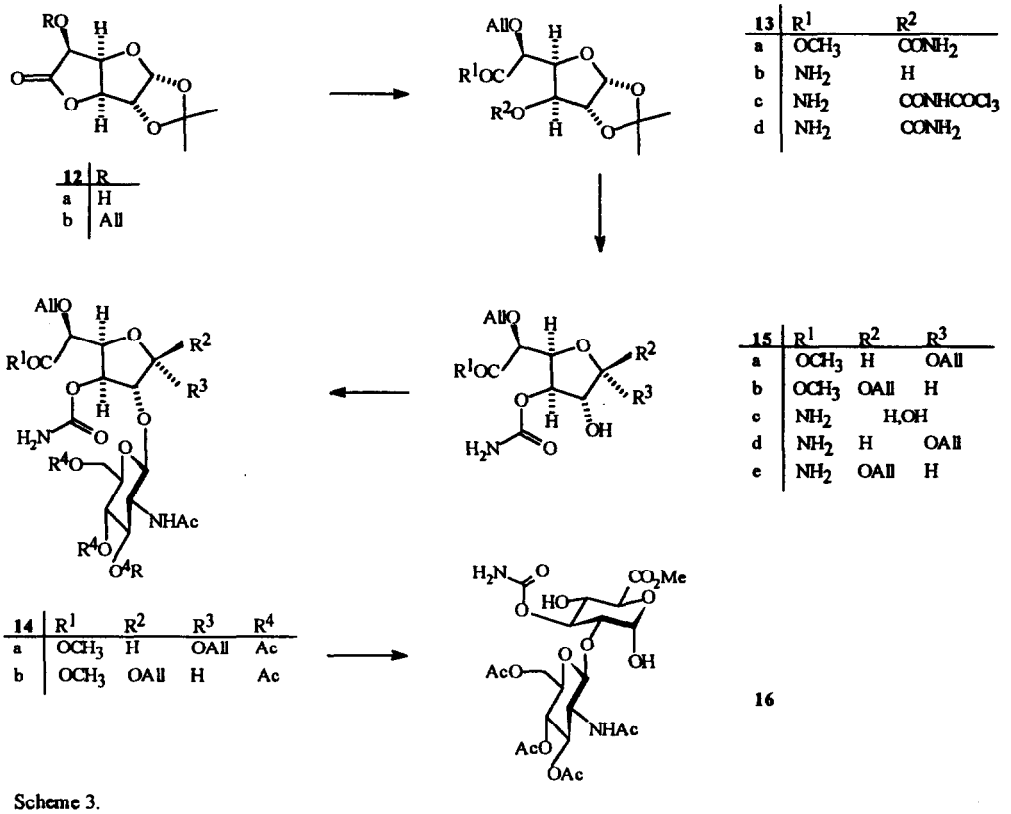
Studies on the Synthesis of 1d

D-Glucuronolactone derivative **12a** was expected to be a promising starting material for the synthesis of **1d** by a route invoking only a limited number of protecting group manipulations. **12a** was converted into **12b** either by trifluoromethanesulfonic acid-catalyzed reaction with allyl trichloroacetimidate[21, 22] or by treatment with allyl bromide in the presence of freshly prepared[23] silver oxide.[24] The latter procedure provided **12b** in better yields. In a model series, we wished to open the lactone by methanolysis. From precedent, the corresponding ester **14a** could be expected to be very sensitive and revert easily to the lactone.[25] Therefore, **12b** was treated with methanol in the presence of a weakly basic ion exchange resin,[26] and the reaction product was (after filtration and solvent evaporation) immediately converted into the urethane **13a** making use of the Kocovský procedure.[27]

With NH_3 in methanolic solution the lactonic ring of **12b** was opened to furnish uronamide **13b** in almost quantitative yield.[28] **13b** was then converted into **13d** by (i) treatment with trichloroacetyl isocyanate (**13b** →**13c**, 100% yield), (ii) removal of the trichloroacetyl group with Amberlite IRA 93 (OH^- form, 96% yield). The presence of the carbamoyl function in **13a** and **13d** was evident from the chemical shift of 3-H (≈5.2) and, in the case of **13d**, from the characteristic ^{13}C NMR signal at δ = 155.4.[29]

Simple reaction of **13a** with allylalcohol in the presence of a cation exchange resin (H^+ form) at elevated temperature[30] effected both acetonide cleavage and allyl glycoside formation. **15a** and **15b** were obtained in a 1:1.6 ratio (total yield: 67%). In the next step, **15a** turned out to be less reactive (vide infra) than **15b**. Therefore, from **15a** a further portion of **15b** was obtained by treatment with allyl alcohol in the presence of a cation exchange resin to establish the pseudo-equilibrium (34:66)[31] between both isomers. For the same type of transformation in the uronamide series a two-step procedure consisting of (i) acetonide cleavage with 90 per cent trifluoroacetic acid[32] (**13d**→**15c**, quantitative yield) and (ii) camphorsulfonic acid - catalyzed allyl glycoside formation (**15c**→ **15d** + **15e**, 1:1, 76%) was found to give better results.[33]

The stage was now set for joining these acceptors with **5** as N-acetylglucosamine donor. For the reaction of **15a** and **15b** with **5** the conditions had to be carefully optimized.[33] Nevertheless, the yields were at best modest, 47% of **14b** and 25% of **14a**. **15a** was the less reactive acceptor in these experiments, probably a result of the cis-disposition of the substituents at C-1 and C-2. Removal of the allyl protecting group both from **14a** and **14b** with $PdCl_2$ in an acetate buffer provided the desired **16**. The NMR spectra indicated that in solution only the α anomer was present. In the model series, the arrival at **16** demonstrated that, in principle, the synthesis of compounds of type **1d**, commencing from **12a** should be possible in the desired sense, i.e. without too much protecting group chemistry. However, as in many other instances, the results obtained for the model compounds could not be translated into the real series. All attempts, to achieve disaccharide formation between **15e** and **5** were completely fruitless. Very polar **15e** was only sparingly soluble in solvents such as acetonitrile or nitromethane. We believe that the low concentration of the glycosyl acceptor in the reaction mixtures is the main reason for the failure of these experiments. In any case, the glucuronolactone approach to moenomycin analogues such as **1d** had to be given up at this stage.



Scheme 3.

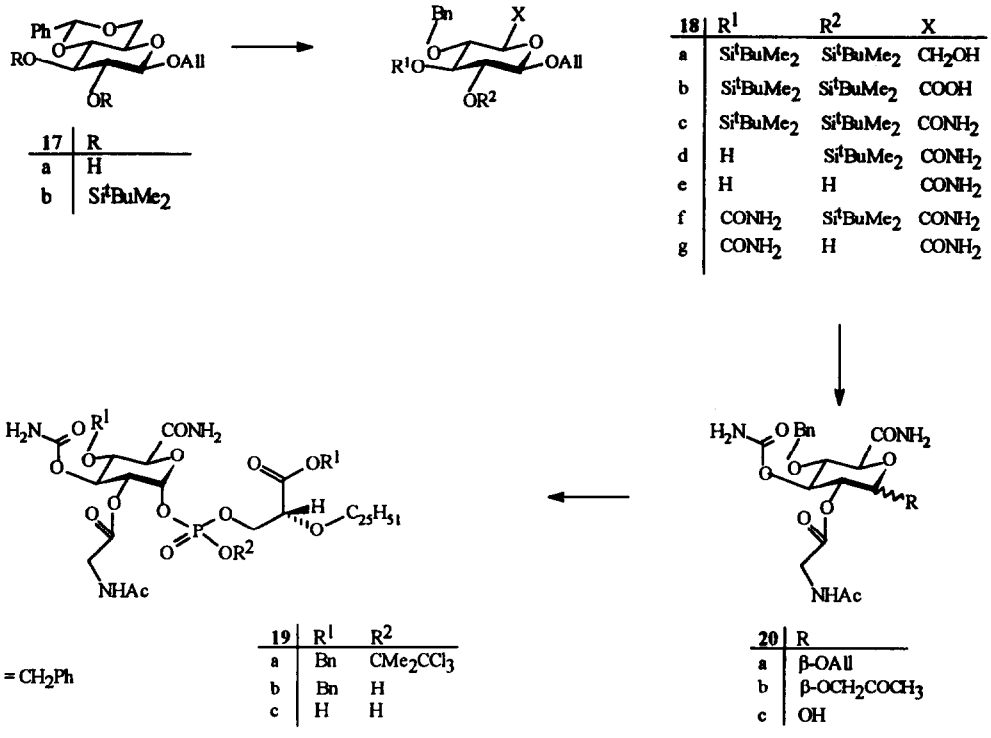
Synthesis of 19c

It was also tried to prepare compound **1d** via **18g**, but unfortunately, disaccharide formation using both the oxazolin method and the α-trichloroacetimidate of 2-azido-3,4,6-tri-O-benzyl-2-deoxy-D-glucopyranose as glycosyl donor[12] was completely unsuccessful, again for solubility reasons.[34] This will be discussed in detail

elsewhere. Here we describe the synthesis of **19c** where the aminosugar residue is mimicked by the N-acetylglycyl residue. It was speculated that the N-acetylated amino acid might replace C-2 with its substituent and the ring oxygen of the aminosugar unit E of **1d**. Starting material was **17a**,[35] a 4,6-benzylidene derivative of D-glucose with an allyl protecting group in the anomeric position. **17a** was converted into **17b** using Corey's procedure.[36] The benzylidene acetal was then to be opened reductively to furnish **18a**. For this type of operation a number of reagents have been recommended ($LiAlH_4$-$AlCl_3$,[37] borane-trimethylamine complex-$AlCl_3$,[38] $NaBH_3CN$-trimethylsilyl chloride[39]). In the case of **17b**, the $LiAlH_4$-$AlCl_3$ method was used, and **18a** was obtained in 50% yield. The position of the benzyl group was evident from the ^1H NMR spectrum (coupling between the OH proton and the CH_2-6 protons). For the oxidation of the primary OH group a two-step procedure was used, consisting of (i) Swern[40] and (ii) sodium chlorite oxidation.[41] Usually, uronic acid **18b** was immediately converted into amide **18c** making use of an improved version[42] of Staab's imidazolide procedure.

When **18c** under carefully selected conditions was treated in 95:5 THF-water solution with TBAF,[43] only the silyl ether grouping at C-3 was cleaved to furnish **18d** (70% yield). The carbamoyl grouping was established by treatment with trichloroacetyl isocyanate followed by reductive (Zn dust in methanol[44]) removal of the trichloroacetyl group (**18d**→**18f**, 90% yield). Getting rid of the remaining silyl protecting group of **18f**, though chemically simple, turned out to be experimentally demanding: (i) The reaction conditions had to be chosen cautiously (tetrabutylammonium fluoride in 95:5 THF-water), otherwise the carbamoyl group was also lost (formation of **18e**), (ii) extraction could be accomplished only with a very polar solvent (3:1 ethyl acetate-1-butanol), and (iii) tetrabutylammonium salts had to be removed by ion exchange chromatography. Highly polar **18g** was esterified with N-acetyl glycine in DMF solution by treatment with dicyclohexylcarbodiimide and Steglich's base[45] (**18g**→**20a**, 84% yield). At this stage we met another example of the difficulties associated with the removal of the allyl protecting group from the anomeric position.[46] Many of the established procedures could not be used for solubility reasons, and with Rh(I) no isomerization could be achieved. The $PdCl_2$ procedure gave unsatisfactory results (32% yield). We then applied our recently introduced two-step sequence which consists of (i) Wacker oxidation (**20a**→**20b**) and (ii) cleavage of the C-O bond α to the carbonyl group by electron transfer in the photoexcited state.[14] Lack of material excluded optimization of this step, but still, this method gave the best results (56% yield). For the construction of the phosphoric acid diester grouping we used the version described above (see Scheme 2). The ester grouping in **20c** demanded, however, a slight modification. In all previous cases methyl ester **9b** has been used as 2-O-alkyl glyceric acid equivalent. In the present case, methyl protecting was unacceptable in view of the ester linkage to the glycine unit which had to be retained in the final deprotection step. Thus, benzyl ester **9c** was used instead, prepared either from **9a** by acid-catalyzed esterification with benzyl alcohol, or from methyl ester **9b** by Ti(IV) isopropoxide-mediated transesterification.[47] For the final deprotection hydrogenolytic cleavage of the benzyl ester could be used to provide the desired target **19c**.

Inhibition of the UDP-N-acetylmuramyl pentapeptide-dependent incorporation of [^{14}C]UDP-N-acetylglucosamine into cross-linked high-molecular weight peptidoglycan was studied with a slightly modified[48] version of the assay described by Izaki, Matsuhashi, and Strominger.[49] Under these conditions **19c** turned out to be completely inactive.



17	R
a	H
b	Si^tBuMe_2

18	R^1	R^2	X
a	Si^tBuMe_2	Si^tBuMe_2	CH_2OH
b	Si^tBuMe_2	Si^tBuMe_2	COOH
c	Si^tBuMe_2	Si^tBuMe_2	$CONH_2$
d	H	Si^tBuMe_2	$CONH_2$
e	H	H	$CONH_2$
f	$CONH_2$	Si^tBuMe_2	$CONH_2$
g	$CONH_2$	H	$CONH_2$

19	R^1	R^2
a	Bn	CMe_2CCl_3
b	Bn	H
c	H	H

20	R
a	β-OAll
b	β-OCH_2COCH_3
c	OH

Scheme 4.

Conclusions

From the results summarized in the introductory part it is clear, that the structure-activity relations for transglycosylase inhibition are very strict and that monosaccharide analogues[50] and even disaccharides such as **3**, which was prepared recently,[51] can possibly not be expected to be active.[52] In keeping with this, structural analogues **11b** and **19c** are also inactive. It seems quite important now to test the transglycosylase-inhibiting properties of **1d**, and we hope that the difficulties associated with the synthesis of **1d** as discussed above can be overcome.

EXPERIMENTAL

O_2- or moisture-sensitive reactions were performed in oven-dried glassware under a positive pressure of argon. Liquids and solutions were transferred by syringe. Small-scale reactions were performed in Wheaton serum bottles sealed with aluminium caps with open top and Teflon-faced septum (Aldrich). Usual work-up means partitioning the reaction mixture between water and an organic solvent (given in parenthesis), drying the combined organic solutions over Na_2SO_4 and removal of solvent either by distillation in vacuo at 40°C using a rotatory evaporator or by lyophilization (using the Leybold-Heraeus GT2 apparature). Solvents were purified by standard techniques.- The instrumentation used was: ^1H NMR: AM 400 (Bruker, at 400 MHz); ^{13}C NMR: AM 400 (Bruker, at 100.6 MHz); FAB MS: MAT 731 (Varian) with a modified Saddle Field Ion Source (Ion Tech Ltd.); Liquid SIMS: MAT-CH-5

instrument (Varian) with a Cs ion gun or VG AUTOSPEC; LC (preparative gravitational liquid chromatography): silica gel (ICN Biomedicals Silica 63-100); MPLC (medium-pressure liquid chromatography): 30.0 cm x 2.5 cm or 40.0 cm x 1.5 cm glass tubes, 50 µm silica gel (Amicon), Duramat pump (CfG); analytical TLC: Merck precoated silica gel 60 F_{254} plates (0.2 mm), spots were identified by spraying with a 2.22 mol/L H_2SO_4 solution which contained $Ce(SO_4)_2x4H_2O$ (10 g/L) and $H_3[PO_4(Mo_3O_9)_4]xH_2O$ (25 g/L)[53] and heating at 140°C, or with the phosphate-specific spraying reagent of Dittmer and Lester.[54] Carbon and proton numbering in the subunits (see NMR data) follows the moenomycin nomenclature (see formula 1). Where appropriate, two molecular masses are 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 (mono-isotopic masses). Straightforward protecting group NMR signals are not reported.

Coupling of 4 and 5

Allyl 4,6-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-glucopyranoside (4) (132.6 mg, 0.287 mmol) was dissolved in a 0.117 mol/L solution of 2-methyl-(3,4,6-tri-O-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-d]-2-oxazoline (5) in 1:1 toluene-nitromethane (2.5 mL, 0.293 mmol). After addition of a 0.031 mol/L solution of anhydrous p-toluenesulfonic acid in toluene (0.5 mL, 15.5 µmol), the mixture was stirred at 70°C. After 14.5 h again 2.5 mL of the 0.117 mol/L solution of 5 (0.293 mmol) and 0.5 mL of the 0.031 mol/L solution of p-toluenesulfonic acid (15.5 µmol) were added. After 24 h at 70°C further 1.25 mL of the 0.231 mol/L solution of 5 (0.289 mmol) were introduced into the reaction flask. After a total of 40 h the reaction was stopped by addition of pyridine (1 mL). Evaporation at 40°C and MPLC (petrol-ethyl acetate-ethanol 6:1:0.6) gave **7a** (103.9 mg, 46%) and **6** (56.0 mg, 25%); 20.3 mg (16%) of 4 were recovered.

Allyl 3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-4,6-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-glucopyranoside (6)

M.p. 145-148°C (petrol-ethyl acetate).- ^{1}H NMR (400 MHz, $CDCl_3$, H,C COSY): unit E: δ = 4.84 (1-H), 3.97 (2-H), 5.04 (3-H), 5.10 (4-H), 3.61 (5-H), 4.01 (6-H), 4.21 (6-H'), 6.31 (NH), 1.90 ($NHCOCH_3$), 1.96, 1.99, 2.00 ($OCOCH_3$), $J_{1,2}$ = 8.5 Hz, $J_{5,6'}$ = 4.0 Hz, $J_{6,6'}$ = 12.2 Hz, $J_{2,NH}$ = 8.3 Hz; unit F: 4.90 (1-H), 3.51 (2-H), 3.72 (3-H), 3.71 (4-H), 3.51 (5-H), 3.83 (6-H), 4.08 (6-H'), 2.45 (OH), 0.82-1.15 (^{i}Pr signals), $J_{1,2}$ = 4.1 Hz, $J_{5,6}$ = 1.1 Hz, $J_{5,6'}$ = 1.9 Hz, $J_{2,OH}$ = 9.8 Hz, $J_{6,6'}$ = 12.7 Hz.- ^{13}C NMR (100.6 MHz, $CDCl_3$, DEPT): δ = 101.92 ($C\text{-}1^E$), 97.29 ($C\text{-}1^F$), 83.33 ($C\text{-}3^F$), 74.01 ($C\text{-}3^E$), 73.60 ($C\text{-}2^F$), 72.71 ($C\text{-}5^E$), 71.70 ($C\text{-}5^F$), 68.66 ($C\text{-}1^{allyl}$), 68.17 ($C\text{-}4^E$), 66.86 ($C\text{-}4^F$), 62.05 ($C\text{-}6^E$), 60.57 ($C\text{-}6^F$), 54.54 ($C\text{-}2^E$).- IR ($CHCl_3$): 3600-3160 (OH, NH), 1740 (C=O), 1680 (amide I), 1525 cm^{-1} (amide II).- FAB MS (matrix: DMSO-glycerol): m/z= 792 ($[M+H]^+$), 734, 330 ($[e]^+$).- $C_{35}H_{61}NO_{15}Si_2$ (792.04, 791.36), calc (for $C_{35}H_{61}NO_{15}Si_2$ x ethyl acetate) C 53.22, H 7.90, found C 53.46, H 7.80.

Allyl 2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-4,6-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-glucopyranoside (7a)

M.p. 246-249°C (petrol-ethyl acetate).- ^{1}H NMR (400 MHz, $CDCl_3$, H,C COSY): unit E: δ = 4.86 (1-H), 3.92 (2-H), 5.17 (3-H), 5.02 (4-H), 3.67 (5-H), 4.12 (CH_2-6), 5.69 (broad, NHAc), 1.93 ($NHCOCH_3$), 2.02-2.07 ($OCOCH_3$ signals), $J_{1,2}$ = 8.5 Hz, $J_{2,3}$ = 11 Hz, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz; unit F: 4.98 (1-H), 3.46 (2-H), 3.94 (3-H), 3.70 (4-H), 3.54 (5-H), 3.80 (6-H), 4.06 (6-H'), 2.67 (OH), 0.94-1.16 (^{i}Pr signals), $J_{1,2}$ = 4.0 Hz, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 1.1 Hz, $J_{5,6'}$ = 1.8 Hz, $J_{6,6'}$ = 12.7 Hz.- ^{13}C NMR (100.6 MHz, $CDCl_3$, DEPT): δ = 101.98 ($C\text{-}1^E$), 97.40 ($C\text{-}1^F$), 81.59 ($C\text{-}2^F$), 72.63 ($C\text{-}3^E$), 72.19 ($C\text{-}3^F$), 71.72 ($C\text{-}5^F$), 71.89 ($C\text{-}5^E$), 69.48 ($C\text{-}4^F$), 68.54 ($C\text{-}4^E$), 68.62 ($C\text{-}1^{allyl}$), 62.30 ($C\text{-}6^E$), 60.67 ($C\text{-}6^F$), 54.86 ($C\text{-}2^E$).- IR (nujol): 3580, 3260 (broad, OH, NH), 1740 (C=O), 1650 (amide I), 1570 cm^{-1} (amide II).- FAB MS (matrix: DMSO/glycerol): m/z = 792 ($[M+H]^+$), 734, 330 ($[e]^+$).- $C_{35}H_{61}NO_{15}Si_2$ (792.04, 791.36), calc C 53.08, H 7.76, found C 52.86, H 7.70.

Allyl 2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-4,6-O-(1,1,3,3-tetraisopropyl-disiloxane-1,3-diyl)-3-O-trichloroacetylcarbamoyl-α-D-glucopyranoside (7b)

To a solution of **7a** (31.1 mg, 39.3 µmol) in CH_2Cl_2 (0.9 mL) slowly trichloroacetyl isocyanate (6 µL, 50.6 µmol) was added. The reaction mixture was left at 0°C for 160 min and was then quenched with dry CH_3OH. Solvent evaporation and MPLC (petrol-$CHCl_3$-ethanol 7:1:0.7) gave **7b** (15.7 mg, 41 %) and a fraction of **7b** (22.0 mg) slightly contaminated with two degradation products.- IR ($CHCl_3$): 3580-3260 (NH), 1805 (CCl_3C=O), 1740 (C=O),

1675 cm^{-1} (amide I).- $C_{38}H_{61}Cl_3N_2O_{17}Si_2$ (980.4), calc C 46.55, H 6.27, found C 46.80, H 6.40.

Allyl 2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-carbamoyl-4,6-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-glucopyranoside (7c)

From **7a** (147.0 mg, 185.6 μmol) and trichloroacetyl isocyanate (27 μL, 0.228 mmol) **7b** was prepared as described above. After quenching with dry CH_3OH (0.5 mL) and addition of 5% aq. K_2CO_3 (3.0 mL) the mixture was stirred at 20°C for 10 d. Work-up (CH_2Cl_2) and MPLC (petrol-ethyl acetate-ethanol 6:1:0.6) gave **7c** (122.6 mg, 79 %).- IR ($CHCl_3$): 3560-3130 (NH), 1730 (C=O), 1675 (amide I), 1590 cm^{-1} (amide II).- LSI MS (matrix: DMSO-glycerol): m/z = 835 ($[M+H]^+$), 330 ($[e]^+$).- $C_{36}H_{62}N_2O_{16}Si_2$ (835.06, 834.36), calc C 51.78, H 7.48, found C 51.81, H 7.52.

2-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-carbamoyl-4,6-O-(1,1,3,3-tetra-isopropyldisiloxane-1,3-diyl)-D-glucopyranose (7d)

A mixture of **7c** (58.3 mg, 69.8 μmol) and $PdCl_2$ (30.8 mg, 173.8 μmol) in 0.1 mol/L aq NaOAc in 20:1 acetic acid-water (3.5 mL) was stirred at 20°C for 21 h. Usual work-up (ethyl acetate) and MPLC (petrol-$CHCl_3$-ethanol 6:1:0.8) gave **7d** (39.4 mg, 71 %); 5.5 mg (10 %) of **7c** were recovered.- ^1H NMR (80 MHz, $CDCl_3$) proved the removal of the allyl group.- IR ($CHCl_3$): 3580-3140 (NH), 1735 (C=O), 1670 (amide I), 1580 cm^{-1} (amide II).

2-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-carbamoyl-4,6-O-(1,1,3,3-tetra-isopropyldisiloxane-1,3-diyl)-1-O-{[(R)-2-methoxycarbonyl-2-(3,8,8,11,14,18-hexamethylnonadecyloxy)-ethoxy]-(2-trichloromethyl-2-propyloxy)-phosphoryl}-α-D-glucopyranose (10a)

To a solution of 1H-1,2,4-triazole (17.5 mg, 253.4 μmol) in 4:1 CH_2Cl_2-pyridine (0.2 mL) 1,1,1-trichloro-2-methyl-prop-2-yl dichlorophosphite **8** (X=Cl, 12.0 μL, 59.9 μmol) was added at 0°C, and the mixture was stirred at 0°C for 10 min. A solution of **7d** (39.1 mg, 49.2 μmol) in CH_2Cl_2 (0.4 mL) was added and stirring was continued at 0°C for 5.5 h. A solution of **9b** (27.3 mg, 58.0 μmol) in CH_2Cl_2 (0.3 mL) was added. After being stirred at 0°C for 2 h, the solution was treated at 0°C with bis(trimethylsilyl)peroxide (15.0 μL, 70.6 μmol) and stirred at 20°C for 18.5 h. MPLC (petrol-ethyl acetate-ethanol-triethylamine 7:1:0.5:0.08) gave **10a** (21.7 mg, 30 %).- ^1H NMR (80 MHz, $CDCl_3$): Characteristic signals at δ = 5.93 (dd, 1H, 1-H^F), 6.54 (d, broad, 1H, NHAc), $^3J_{P,H}$ = 6.5 Hz, $J_{1,2}{}^F$ = 3.4 Hz, $J_{NH,2}$ = 7.6 Hz.- Two $COOCH_3$ signals at δ = 3.80 and 3.82 indicated the presence of two diastereomeric phosphates.

2-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-carbamoyl-1-O-{[(R)-2-methoxy-carbonyl-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy]-hydroxy-phosphoryl}-4,6-O-(1,1,3,3-tetra-isopropyldisiloxane-1,3-diyl)-α-D-glucopyranose (10b), triethylammonium salt

Zn-Cu couple (5.9 mg) and 2,4-pentanedione (12.0 μL) were added to a solution of **10a** (7.9 mg, 5.3 μmol) in pyridine (0.3 mL), and the mixture was stirred under argon for 6 h at 20°C. Solids were filtered off and the filtrate was evaporated after addition of toluene. LC (ethyl acetate-methanol-triethylamine 8:1:0.3) furnished the triethylammonium salt of **10b** (5.7 mg, 76 %).- ^1H NMR (400 MHz, pyridine-d_5, H,C COSY): unit E: δ = 5.54 (1-H), 4.28 (2-H), 5.95 (3-H), 5.36 (4-H), 3.88 (5-H), 4.49 (6-H), 4.36 (6-H'), 8.98 (NHAc), 2.26 ($NHCOCH_3$), 1.94, 2.02, 2.09 ($OCOCH_3$ signals), $J_{1,2}$ = 8.3 Hz, $J_{2,3}$ = $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, $J_{2,NH}$ = 9.5 Hz; unit F: 7.46 (broad, $OCONH_2$), 6.40 (1-H), 4.22 (2-H), 5.87 (3-H), 4.34 (4-H), 4.42 (5-H), 4.33 (CH_2-6), $J_{1,2}$ = 3.2 Hz, $J_{1,P}$ = 6.8 Hz, $J_{2,P}$ = 2.8 Hz, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz; unit H: 4.52 (2-H), 4.72 (3-H), 4.63 (3-H'), 3.74 ($OCOCH_3$); unit I: 3.80 (1-H), 3.66 (1-H'), 1.74 (2-H), 1.51 (2-H').- ^{13}C NMR (100.6 MHz, pyridine-d_5, DEPT): δ = 157.56 ($OCONH_2$), 101.79 (C-1^E), 95.71 (C-1^F, $^2J_{C,P}$= 4.2 Hz), 79.81 (C-2^H, $^2J_{C,P}$= 6.7 Hz), 79.57 (C-2^F, $^3J_{C,P}$= 9.1 Hz), 73.75 (C-3^F), 73.39 (C-3^E), 73.09 (C-5^F), 71.97 (C-5^E), 69.82 (C-4^E), 69.68 (C-1^I), 69.16 (C-4^F), 66.31 (C-3^H, $^3J_{C,P}$= 8.3 Hz), 62.54 (C-6^E), 61.55 (C-6^F), 55.33 (C-2^E), 51.81 ($COOCH_3$).- IR ($CHCl_3$): 3550-3100 (NH), 2470 ($H\text{-}N^+Et_3$), 1740 (C=O), 1665 (amide I), 1595 (amide II), 1550 cm^{-1}.- $C_{62}H_{115}N_2O_{22}PSi_2xC_6H_{15}N$ (1428.93, 1427.84), FAB MS (matrix: DMSO-glycerol): m/z = 1429 ($[M+H]^+$), 330 ($[e]^+$).

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

To a solution of **10b** (41.0 mg, 28.7 μmol) in THF (1.0 mL) a 1 mol/L solution of TBAF in THF (80.0 μL) was added. The mixture was stirred at 20°C for 4 h. Addition of water (0.1 mL), lyophilization and MPLC ($CHCl_3$-

methanol-water 20:5:0.5) gave **11a** (24.3 mg, 78 %).- ^{13}C NMR (100.6 MHz, CD_3OD, DEPT): δ = 159.50 ($OCONH_2$), 103.11 (C-1^E), 96.23 (C-1^F), 80.21 ($^2J_{C,P}$= 8.6 Hz) and 79.74 ($^2J_{C,P}$ = 8.7 Hz, C-2^H and C-2^F), 75.63, 74.26, 73.85, 72.86, 70.58, 70.28, 70.25 (C-3^E, C-3^F, C-5^E, C-5^F, C-4^E, C-4^F, C-1^I), 66.92 (C-3^H), 63.42 (C-6^E), 62.18 (C-6^F), 55.70 (C-2^E).- According to the ^{13}C NMR spectrum **11a** contained ≈ 20% of an impurity).- $C_{50}H_{89}N_2O_{21}P$ (1085.23, 1084.57), FAB MS (matrix: DMSO-glycerol): m/z = 1129 ($[M+2Na-H]^+$), 1107 ($[M+Na]^+$), 655 ($[g+H+Na]^+$), 595, 573 ($[M-f+2Na]^+$).

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-glucopyranose (11b)

To a solution of **11a** (11.7 mg, 10.8 μmol) in anhydrous THF (2.0 mL) at 0°C a 0.29 mol/L aq. LiOH (0.5 mL) was added, and the mixture was stirred at 0°C for 8 min. Excess base was neutralized by addition of Dowex 50WX2-200, (H^+ form). Filtration, lyophilization and LC (SiO_2 (5.5 g), $CHCl_3$-methanol-water 18:7:0.9) provided a mixture of products containing **11b** (9.7 mg). 29.8 mg of such a mixture obtained in several runs was separated by reversed-phase LC (HP-20 (6.0 g), elution with water-methanol 10:0 to 0:10), followed by reversed-phase MPLC (column A, long, 19 g of RP 18, methanol-water-acetonitrile-triethylamine 8:1.1:3:0.1) to give pure **11b** (10.6 mg).- ^{13}C NMR (100.6 MHz, $CDCl_3$-methanol-d_4-D_2O 18:11:2.7): δ = 159.1 ($OCONH_2^F$), 103.0 (C-1^E), 95.9 (C-1^F), 80.0 (C-2^H), 78.2 (C-2^F), 76.9 (C-5^E), 74.8 (C-3^F), 74.7 (C-3^E), 73.2 (C-5^F), 70.8 (C-4^E), 69.1 (C-4^F, C-1^I), 66.9 (C-3^H), 61.5 (C-6^F, C-6^E), 56.9 (C-2^E), 23.1 ($NHCOCH_3^E$).- $C_{43}H_{81}N_2O_{18}P$ (945.09, 944.52), FAB MS (matrix: triethanol amine): m/z = 1011.5 ($[M+3Na-2H]^+$), 1005.5 ($[M+K+Na-H]^+$), 989.5 ($[M+2Na-H]^+$), 983.5 ($[M+K]^+$), 967.5 ($[M+Na]^+$), 613 ($[M-f+2K]^+$), 597 ($[M-f+Na+K]^+$), 581 ($[M-f+2Na]^+$), 575 ($[M-f+K+H]^+$), 559 ($[M-f+Na+H]^+$), 431 ($[f+Na-H]^+$), 409 ($[f]^+$).

1,2-O-Isopropyliden-5-O-allyl-α-D-glucofuranosidurono-6,3-lactone (12b)

(i) To a solution of **12a** (4.94 g, 22.8 mmol) and allyl trichloroacetimidate (4.68 g, 23.1 mmol) in CH_2Cl_2 (80 mL) trifluoromethanesulfonic acid (0.1 mL, 1.1 mmol) was added. The mixture was stirred at 20°C for 4.5 h. Then additional portions of allyl trichloroacetimidate (1.20 g, 5.9 mmol) und trifluoromethanesulfonic acid (0.1 mL, 1.1 mmol) were added and stirring was continued for 4.5h at 20°C. Work-up (CH_2Cl_2) and MPLC (petrol-ethyl acetate 9:2) furnished **12b** (3.28 g, 56%), 0.85 g (17%) of **12a** were recovered.

(ii) In the dark, to **12a** (66.4 mg, 307 μmol) and freshly prepared Ag_2O (142.3 mg, 615 μmol) allyl bromid (67 μL, 770 μmol) and CH_2Cl_2 (1.5 mL) were added and the mixture was stirred at 20°C for 3.5 h. Filtration, solvent evaporation, and LC (petrol-ethyl acetate 3:1) yielded **12b** (68.9 mg, 88%).- ^1H NMR (80 MHz, $CDCl_3$): δ = 6.01 (d, 1-H), 4.92 (dd, 4-H), 4.79 (d, 2-H), 4.74 (d, 3-H), 4.29 (d, 5-H), $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ < 1 Hz, $J_{3,4}$ = 2.6 Hz, $J_{4,5}$ = 4.2 Hz.- IR ($CHCl_3$): 1800 (C=O), 1597 cm^{-1} (C=C).- EI MS: m/z (%) = 256 ($M^{+\bullet}$, 3), 241 (22), 215 (8), 200 (5), 157 (39), 114 (26), 59 (57), 41 (100).- $C_{12}H_{16}O_6$ (256.3), calc C 56.25, H 6.29, found C 56.34, H 6.23.

Methyl (1,2-O-isopropyliden-5-O-allyl-3-O-carbamoyl-α-D-glucofuranosid)uronate (13a)

To **12b** (3.17 g, 12.4 mmol) dry methanol (70 mL) and Amberlite IRA-93® (OH^- form, ≈ 3 g,) were added, and the mixture was stirred at 20°C for 1h. After filtration, washing the resin with methanol, solvent evaporation and careful drying, the residue was redissolved in CH_2Cl_2 (40 mL), trichloroacetylisocyanate (1.33 mL, 11.2 mmol) was added and the mixture was stirred at 20°C for 20 min. Excess reagent was destroyed with methanol (0.5 mL). After concentration the remaining solution was transferred to the top of a column charged with alumina (neutral, grade 2, 60 g). Elution was performed with ethyl acetate. Fractions containing **12b** were resubmitted to the same treatment. The combined filtrates after solvent evaporation and LC (petrol-ethyl acetate 1:1) yielded **13a** (2.06 g, 50%), 0.44 g (14%) of **12b** were recovered.- M.p. 144.5 - 145°C (ethyl acetate-petrol).- ^1H NMR (80 MHz, $CDCl_3$): δ = 5.90 (d, 1-H), 5.24 (d, 3-H), 4.99 (broad, NH_2), 4.56 (d, 2-H), 4.45 (dd, 4-H), 4.08 (d, 5-H), 3.80 (s, OCH_3), $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ < 1Hz, $J_{3,4}$ = 3.0 Hz, $J_{4,5}$ = 8.6 Hz.- IR ($CHCl_3$): 3510, 3400, 3370 - 3100 (NH), 1760 (C=O), 1578 cm^{-1} (amide II).- EI MS: m/z (%) = 316 (7), 272 (10), 202 (20), 144 (30), 59 (42), 41 (100).- $C_{14}H_{21}O_8N$ (331.3), calc C 50.75, H 6.39, found C 50.74, H 6.39.

Acid-catalyzed reaction of 13a with allyl alcohol

A mixture containing **13a** (603.7 mg, 1.82 mmol), Dowex 50WX8® (H^+ form, 1.1 g), and allyl alcohol (10 mL) was

stirred at 80°C for 3.3 h. After filtration, washing the resin with ethyl acetate, solvent evaporation, and MPLC (petrol-ethyl acetate-ethanol-triethylamine 7:4:0.5:0.01) **13a** (47.8 mg, 8%), **15a** (158.8 mg, 26%), and **15b** (248.9 mg, 41%) were obtained.

Methyl (allyl-5-O-allyl-3-O-carbamoyl-α-D-glucofuranosid)uronate (15a)

M.p. 89 - 90°C (CH_2Cl_2-petrol).- ^{1}H NMR (400 MHz, CD_3OD): δ = 5.28 (t, 3-H), 5.01 (d, 1-H), 4.48 (t, 4-H), 4.24 (dd, 2-H), 4.11 (d, 5-H), 3.75 (s, OCH_3), $J_{1,2}$ = 4.3 Hz, $J_{2,3}$ = 6.3 Hz, $J_{3,4}$ = $J_{4,5}$ = 6.8 Hz.- ^{13}C NMR (100.6 MHz, CD_3OD, H,C COSY): δ = 172.69 (C-6), 158.51 ($CONH_2$), 101.47 (C-1), 78.49 (C-5), 77.98 (C-3), 77.54 (C-4), 76.20 (C-2), 70.10, 73.70 (C-1^{allyl}), 52.56 (OCH_3)-. IR ($CHCl_3$): 3545 - 3180 (NH, OH), 1745 (C=O), 1585 cm^{-1} (amide II). FAB MS (matrix: glycerol): m/z = 332 ([M+H]$^+$), 314 ([M+H-H_2O]$^+$), 274 ([M-OAll]$^+$).- $C_{14}H_{21}O_8N$ (331.3), calc C 50.75, H 6.39, found C 50.80, H 6.42.

Methyl (allyl-5-O-allyl-3-O-carbamoyl-ß-D-glucofuranosid)uronate (15b)

^{1}H NMR (400 MHz, aceton-d_6): δ = 5.05 (dd, 3-H), 4.89 (s, 1-H), 4.83 (d, OH), 4.42 (dd, 4-H), 4.13 (d, 5-H), 3.97 (dd, 2-H), 3.73 (s, OCH_3), $J_{1,2}$ < 1 Hz, $J_{2,3}$ = 1.2 Hz, $J_{2,OH}$ = 4.0 Hz, $J_{3,4}$ = 5.3 Hz, $J_{4,5}$ = 9.2 Hz.- ^{13}C NMR (100.6 MHz, aceton-d_6, H,C,COSY): δ = 171.99 (C-6), 156.57 ($CONH_2$), 108.86 (C-1), 80.45 (C-4), 80.08 (C-2), 77.97 (C-5), 77.45 (C-3), 68.94, 72.08 (C-1^{allyl}), 51.91 (OCH_3).- IR ($CHCl_3$): 3540 - 3150 (NH, OH), 1740 (C=O), 1585 cm^{-1} (amide II). FAB MS (matrix: glycerol): m/z = 274 ([M-OAll]$^+$).- $C_{14}H_{21}O_8N$ (331.3), calc C 50.75, H 6.39, found C 50.78, H 6.44.

Isomerization of 15a

A mixture of **15a** (38.5 mg, 116 µmol), Dowex 50WX8 (H^+ form, 200 mg), and allyl alcohol (1.5 mL) was stirred at 20°C for 3d. Filtration, washing of the resin with ethyl acetate, solvent evaporation and MPLC (petrol-ethyl acetate-ethanol-triethylamine 7:4:5:0.01) furnished **15a** (9.7 mg, 25%) and **15b** (18.8 mg, 49%).

Methyl [allyl-2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-ß-D-glucopyranosyl)-5-O-allyl-3-O-carbamoyl-ß-D-glucofuranosid]uronate (14b)

15b, oxazoline **5** (1.5 equiv, another portion (1.0 equiv) after 1 h), and p-toluenesulfonic acid were heated in 1,2-dichloroethane solution at 65°C for 10 h. The reaction was stopped by addition of pyridine (0.5 mL) and stirring at 20°C for 30 min. After filtration, solvent evaporation, and MPLC ($CHCl_3$-methanol 25:1), then further separation of the **14b** containing fractions by MPLC (petrol-ethyl acetate-ethanol 10:10:1) and the **15b** containing fractions by MPLC (petrol-ethyl acetate-ethanol 5:5:1) yielded **14b** (29%), 38% of **15b** were recovered. Yield, based on consumed **15b**: 46%.- M.p.: 192 - 193°C (ethyl acetate-petrol).- ^{1}H NMR (400 MHz, pyridine-d_5, H,H COSY): unit E: δ = 5.59 (1-H), 4.58 (2-H), 5.83-5.97 (3-H), 5.46 (4-H), 3.93 (5-H), 4.25 (6-H), 4.45 (6-H'), 9.46 (NH), $J_{1,2}$ = 8.6 Hz, $J_{2,3}$ = 9.2 Hz, $J_{3,4}$ = 9.2 Hz, $J_{4,5}$ = 10.0 Hz, $J_{5,6}$ = 2.4 Hz, $J_{5,6'}$ = 4.3 Hz, $|J_{6,6'}|$ = 12.2 Hz, $J_{NH,2}$ = 8.8 Hz; unit F: 5.34 (1-H), 4.90 (2-H), 5.79 (3-H), 4.94 (4-H), 4.52 (5-H), 3.72 (OCH_3), 7.81, 8.23 ($CONH_2$), $J_{1,2}$ < 1 Hz, $J_{2,3}$ < 1 Hz, $J_{3,4}$ = 5.1 Hz, $J_{4,5}$ = 9.4 Hz.- ^{13}C NMR (100.6 MHz, pyridine-d_5, DEPT, H,C COSY): δ = 169.76 (C-6^F), 157.10 ($CONH_2$), 107.21 (C-1^F), 100.82 (C-1^E), 86.20 (C-2^F), 80.50 (C-4^F), 77.46 (C-5^F), 75.02 (C-3^F), 73.46 (C-3^E), 72.33 (C-5^E), 69.39 (C-4^E), 69.11, 71.89 (C-1^{allyl}), 62.18 (C-6^E), 54.82 (C-2^E), 51.85 (OCH_3).- FAB MS: (matrix: glycerol); m/z = 603 ([M-OAll]$^+$), 330 ([e]$^+$), 288, 270, 210, 168, 150, 126, 108.- $C_{28}H_{40}O_{16}N_2$ (660.63, 660.28) calc C 50.91, H 6.10, found C 50.90, H 6.15.

Methyl [allyl-2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-ß-D-glucopyranosyl)-5-O-allyl-3-O-carbamoyl-α-D-glucofuranosid]uronate (14a)

15a, oxazoline **5** (1.5 equiv, further portions 1.0 equiv, after 1 h and 2.2 equiv after 23 h), and p-toluenesulfonic acid (another portion 0.15 equiv after 23 h) were heated in 1,2-dichloroethane solution at 70°C for 31 h. The reaction was stopped by addition of pyridine (0.5 mL) and stirring at 20°C for 30 min. After filtration, solvent evaporation, and MPLC ($CHCl_3$-methanol 25:1), then further separation of the **14a** containing fractions by MPLC (petrol-ethyl acetate-ethanol 10:10:1) and the **15a** containing fractions by MPLC (petrol-ethyl acetate-ethanol 5:5:1) yielded **14a** (14%), 43% of **15a** were recovered.- M.p.: 228 - 229°C (ethyl acetate-petrol).- ^{1}H NMR (400 MHz, pyridine-d_5): unit E: δ = 5.58 (1-H), 4.22 (2-H), 6.03 (3-H), 5.39 (4-H), 3.91 (5-H), 4.12-4.34 (6-H), 4.46 (6-H'), 9.37 (NH), $J_{1,2}$ = 8.5

Hz, $J_{2,3}$ = 9.2 Hz, $J_{3,4}$ = $J_{4,5}$ = 10.2 Hz, $J_{5,6}$ = 2.5 Hz, $J_{5,6'}$ = 4.5 Hz, $|J_{6,6'}|$ = 12.4 Hz, $J_{NH,2}$ = 8.3 Hz; unit F: 5.38 (1-H), 4.84 (2-H), 6.20 (3-H), 4.99 (4-H), 4.47 (5-H), 3.72 (OCH_3), $J_{1,2}$ = 4.5 Hz, $J_{2,3}$ = $J_{3,4}$ = 6.9 Hz, $J_{4,5}$ = 6.8 Hz.- ^{13}C NMR (100.6 MHz, pyridine-d_5): δ = 169.86 (C-6^E), 157.09 ($CONH_2$), 101.32 (C-1^F), 100.52 (C-1^E), 82.72 (C-2^F), 78.00 (C-5^F), 76.27 and 74.90 (C-4^F and C-3^F), 73.07 (C-3^E), 72.59 (C-5^E), 72.20, 69.14 (C-1^{allyl}), 69.70 (C-4^E), 62.53 (C-6^E), 55.58 (C-2^E), 51.93 (OCH_3).- $C_{28}H_{40}O_{16}N_2$ (660.63, 660.28), FAB MS: (matrix: glycerol): m/z = 661 ($[M+H]^+$), 603 ($[M-OAll]^+$), 330 ($[e]^+$), 210, 168, 150, 108.

Methyl 2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-ß-D-glucopyranosyl)-3-O-carbamoyl-α-D-gluco-pyranuronate (16)

(i) **14a** (17.4 mg, 26.3 µmol) and palladium(II) chloride (23.7 mg, 133.6 µmol) in 0.1 mol/L sodium acetate in 20:1 acetic acid/water (0.5 mL) were stirred at 20°C for 42 h. The resulting mixture was separated by gel filtration (Sephadex G-10, 18 g, elution with water, 50 mL/h, fraction volume 10-12 mL). Fractions 2 and 3 were combined. Lyophilization followed by LC (petrol-ethyl acetate-ethanol 2:2:1) furnished **16** (7.5 mg, 49%).

(ii) The allyl protecting group was removed from **14b** as described for **14a**. Yield: 40% - ^1H NMR (400 MHz, pyridine-d_5): unit E: δ = 5.58 (1-H), 4.53 (2-H), 6.10 (3-H), 5.30 (4-H), 3.98 (5-H), 4.25 (6-H), 4.42 (6-H'), 9.07 (NH), $J_{1,2}$ = 8.5 Hz, $J_{2,3}$ = 9.2 Hz, $J_{3,4}$ = $J_{4,5}$ = 10.0 Hz, $J_{5,6}$ = 2.5 Hz, $J_{5,6'}$ = 5.5 Hz, $|J_{6,6'}|$ = 12.2 Hz, $J_{NH,2}$ = 8.3 Hz; unit F: 6.08 (1-H), 4.19 (2-H), 6.25 (3-H), 4.17 (4-H), 5.28 (5-H), 3.71 (OCH_3), 7.49 ($CONH_2$), $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10.1 Hz; $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz.- ^{13}C NMR (100.6 MHz, pyridine-d_5): δ = 169.98 (C-6^F), 158.38 ($CONH_2$), 102.72 (C-1^E), 94.04 (C-1^F), 80.90 (C-2^F), 74.77 (C-3^F), 73.07 (C-3^E), 72.55, 72.11, 71.99 (C-5^E, C-4^F, C-5^F), 69.93 (C-4^E), 62.68 (C-6^E), 55.97 (C-2^E), 52.11 (OCH_3).- $C_{22}H_{32}O_{16}N_2$ (580.5)

1,2-O-Isopropylidene-5-O-allyl-α-D-glucofuranosiduronamide (13b)

Methanol was saturated with ammonia at 0°C. A solution of **12b** (1.182 g, 4.62 mmol) in methanolic ammonia was stirred at 0°C for 80 min. After solvent evaporation and drying pure **13b** (1.242 g, 99%) was obtained.- M.p. 131°C ($CHCl_3$-petrol).- ^1H NMR (80 MHz, $CDCl_3$): δ = 5.88 (1-H), 5.80-5.95, 6.78 (NH_2), $J_{1,2}$ = 3.6 Hz.- IR ($CHCl_3$): 3520, 3405, 3370 - 3090 (NH_2, OH), 1685 (amide I), 1570 cm^{-1} (amide II).- $C_{12}H_{19}O_6N$ (273.3) , calc C 52.74, H 7.01, found C 52.96, H 6.96.

1,2-O-Isopropylidene-5-O-allyl-3-O-trichloroacetylcarbamoyl-α-D-glucofuranosiduronamide (13c)

At -15°C trichloroacetyl isocyanate (527 µL, 4.45 mmol) was added to a solution of **13b** (1.216 g, 4.45 mmol) in dry CH_2Cl_2 (30 mL). After 20 min at -15°C methanol (1 mL) was added. Solvent evaporation left pure **13c** (2.047 g, 100%) .- ^1H NMR (80 MHz, $CDCl_3$) :δ = 8.87 (broad s, NH), 5.97 (1-H), 5.70-5.85, 6.45 (NH_2), 5.68 (3-H), 4.62 (2-H), 4.51 (4-H), 4.19 (5-H), $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ < 1Hz, $J_{3,4}$ = 3.0 Hz, $J_{4,5}$ = 6.0 Hz.- IR ($CHCl_3$): 3520, 3410 (NH_2), 3370 - 3095 (NH_2, NH), 1810 ($COCCl_3$), 1755 (OCONH; amide I), 1695 ($CONH_2$; amide I), 1575 ($CONH_2$; amide II), 1490 cm^{-1} (OCONH; amide II).- $C_{15}H_{19}O_8N_2Cl_3$ (461.7).

1,2-O-Isopropylidene-5-O-allyl-3-O-carbamoyl-α-D-glucofuranosiduronamide (13d)

Amberlite IRA 93 (≈ 4 g, OH^- form) was added to a solution of **13c** (2.047 g, 4.43 mmol) in CH_2Cl_2 (60 mL), and the mixture was stirred at 20°C for 24 h. Filtration, careful washing of the resin with methanol, and solvent removal from the combined filtrates gave **13d** (1.345 g, 96%).- M.p. 256°C (water; melting with decomposition).- ^1H NMR: (80 MHz, pyridin-d_5): δ = 8.10 (broad s, NH_2), 7.63 (broad s, NH_2), 6.13 (1-H), 5.79 (3-H), 4.96 (4-H), 4.82 (2-H), 4.53 (5-H), $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ < 1Hz, $J_{3,4}$ = 3.0 Hz, $J_{4,5}$ = 9.0 Hz.- ^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 171.51 (C-6), 155.40 ($OCONH_2$), 111.13 ($C(CH_3)_2$), 104.51 (C-1), 82.64 (C-2), 78.25 (C-5), 76.42, 74.95 (C-4 and C-3), 70.36 (C-1^{allyl}).- IR (DMSO): 3520 - 3100, 3460, 3190 (NH_2), 1735 ($OCONH_2$; amide I), 1690 ($CONH_2$; amide I), 1630, 1585 cm^{-1} (amide II).- EI MS: m/z (%) = 301 (4), 272 (14), 258 (7), 226 (18), 202 (17), 182 (63), 159 (20), 144 (32), 140 (33), 115 (41), 100 (100).- $C_{13}H_{20}O_7N_2$ (316.3), calc C 49.36, H 6.37, found C 49.46, H 6.40.

5-O-Allyl 3-O-carbamoyl-D-glucofuranuronamide (15c), mixture of anomers

13d (110.0 mg, 348 µmol) was treated with 90 per cent trifluoroacetic acid (0.8 mL), and the mixture was stirred at 20°C for 40 min. Water (170 mL) was added and solvents were removed by lyophilization to give pure **15c** (95.1 mg,

100%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 6.22, 6.51, 7.12, 7.32 (broad signals, NH_2, OH), α-anomer: 5.15 (d, 1-H, $J_{1,2}$ = 4.0 Hz), 4.99 (dd, 3-H, $J_{3,4}$ = 4.8 Hz), 4.18 (dd, 4-H, $J_{4,5}$ = 9.0 Hz), 3.84-3.88 (2-H, $J_{2,3}$ = 3.6 Hz), 3.82 (d, 5-H). β-anomer: 4.98 (broad s, 1-H, $J_{1,2}$ < 1 Hz), 4.84 (dd, 3-H, $J_{2,3}$ = 1.6 Hz), 4.23 (dd, 4-H, $J_{3,4}$ = 4.8 Hz, $J_{4,5}$ = 8.5 Hz), 3.77 (broad s, 2-H), 3.67 (d, 1H, 5-H).- $C_{10}H_{16}O_7N_2$ (276.3)

Allyl glycoside formation of 15c

(i) A mixture of **15c** (103.5 mg, 375 μmol), Dowex 50WX8® (H^+ form, ≈ 300 mg), and allyl alcohol (5 mL) were stirred at 20°C for 50 min. Filtration, washing the resin first with allyl alcohol, then with methanol-triethylamine, solvent removal from the combined filtrates, and LC (petrol-ethyl acetate-ethanol 2:2:1) gave **15d** (29.1 mg, 25%), **15e** (25.0 mg, 21%), and a fraction containing both **15d** and **15e** (9.2 mg, 8%).

(ii) A solution of **15c** (481.0 mg, 1.74 mmol) and camphorsulfonic acid (420.0 mg, 1.81 mmol) in allyl alcohol (20 mL) was stirred at 20°C for 50 min. After adddition of pyridine (3 mL), solvent removal and subsequent LC (petrol-ethyl acetate-ethanol 3:3:1) furnished **15d** (187.2 mg, 34%), **15e** (210.8 mg, 38%), and a fraction containing **15d** and **15e** (22.1 mg, 4%).

Allyl 5-O-allyl-3-O-carbamoyl-α-D-glucofuranosiduronamide (15d)

^{1}H NMR (400 MHz, pyridine-d_5): 5.38 (1-H), 4.94 (2-H), 6.18 (3-H), 5.22 (4-H), 4.49 (5-H), 7.97 and 8.39 (NH_2), $J_{1,2}$ = 4.4 Hz, $J_{2,3}$ = $J_{3,4}$ = 6.5 Hz, $J_{4,5}$ = 6.2 Hz.- ^{13}C NMR (100.6 MHz, pyridine-d_5): δ = 173.15 (C-6), 157.59 ($OCONH_2$), 101.42 (C-1), 80.17 (C-5), 77.99 and 77.67 (C-3, C-4), 75.88 (C-2), 69.10, 72.62 (C-1^{allyl}).- FAB MS (matrix: DMSO-glycerol): m/z = 339 ($[M+Na]^+$), 317 ($[M+H]^+$), 259 ($[M-OAll]^+$)- $C_{13}H_{20}O_7N_2$ (316.3) calc C 49.36, H 6.37, found C 49.42, H 6.46.

Allyl 5-O-allyl-3-O-carbamoyl-ß-D-glucofuranosiduronamide (15e)

^{1}H NMR (400 MHz, pyridine-d_5) δ = 5.48 (1-H), 4.91 (2-H), 5.95 (3-H), 5.21 (4-H), 4.60 (5-H), 7.68 (NH_2), 8.10 and 8.27 (NH_2), $J_{1,2}$ < 1 Hz, $J_{2,3}$ < 1 Hz, $J_{3,4}$ = 5.5 Hz, $J_{4,5}$ = 8.7 Hz.- ^{13}C NMR (100.6 MHz, pyridine-d_5): δ = 173.65 (C-6), 157.45 ($OCONH_2$), 109.29 (C-1), 81.13, 79.81, 79.56, 78.11 (C-2–C-5), 69.04, 71.81 (C-1^{allyl}).- $C_{13}H_{20}O_7N_2$ (316.3), FAB MS (matrix: DMSO-glycerol): m/z = 339 ($[M+Na]^+$), 317 ($[M+H]^+$), 259 ($[M-OAll]^+$).

Allyl 4,6-O-benzylidene-2,3-di-O-^{t}butyldimethylsilyl-β-D-glucopyranoside (17b)

A solution of allyl 4,6-O-benzylidene-β-D-glucopyranoside (**17a**, 8.87 g, 28.7 mmol) ^{t}butyldimethylsilyl chloride (14.3 g, 95 mmol), and imidazole (13.1 g, 190 mmol) in dimethylformamide (110 mL) was stirred at 40°C for 5 d. Usual work-up (ethyl acetate) and LC (petrol - ethyl acetate 20:1) gave **17b** (14.1 g, 92%).- ^{1}H NMR (80 MHz, $CDCl_3$): 5.40 (s, 1H, acetal H), 4.35 (d, 1-H, $J_{1,2}$ = 7 Hz).- IR ($CHCl_3$): 1690 cm^{-1} (C=C).- EI MS: m/z (%) = 479 (0.085), 179 (100), 135 (22), 105 (42), 77 (28).- $C_{28}H_{48}O_6Si_2$ (536.85, 536.29) calc C 62.64, H 9.01, found C 63.22, H 9.11.

Allyl 4-O-benzyl-2,3-di-O-^{t}butyldimethylsilyl-β-D-glucopyranoside (18a)

To a solution of **17b** (846 mg, 1.57 mmol) in methylene chloride (20 mL) and ether (20 mL) lithium aluminum hydride (149 mg, 3,9 mmol) and subsequently a solution of aluminum trichloride (635 mg, 4.73 mmol) in ether (30 mL) were added. The mixture was stirred at 20°C for 30 min, ethyl acetate and saturated aq. ammonium sulfate were added. Solids were removed by filtration. Usual work-up (ethyl acetate) and LC (petrol - ethyl acetate 10:1) furnished **18a** (425 mg, 50%).- ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.84 and 4.57 (CH_2-Ph, J_{AB} = 12 Hz), 4.34-4.28 (2*1H, d, 1-H, $J_{1,2}$ = 7 Hz and ddt, 1-H^{allyl}), 3.75 (ddd, 1H, 6-H, $J_{5,6}$ = 2,5 Hz, $|J_{6,6'}|$ = 8,5 Hz), 3.69-3.59 (2*1H, t, 3-H, $J_{2,3}$ = $J_{3,4}$ = 7.5 Hz and ddd, 6-H', $J_{5,6'}$ = 6.5 Hz), 3.50-3.38 (3*1H, t, 2-H, m, 5-H and t, 4-H, $J_{4,5}$ = 7.5 Hz).- IR ($CHCl_3$): 3300-3660 (OH), 1050-1120 cm^{-1} (C-O-C).- EI MS m/z (%): 423 (3.2), 288 (7.1), 231 (7.4), 199 (5.2), 115 (8), 91 (100), 73 (46), 41 (9).- $C_{28}H_{50}O_6Si_2$ (538.87, 538.31) calc C 62.41, H 9.35, found C 62.39, H 9.09.

Allyl 4-O-benzyl-2,3-di-O-^{t}butyldimethylsilyl-β-D-glucopyranosiduronic acid (18b)

(i) Swern oxidation: To a solution of oxalyl chloride (29 μl, 0.329 mmol) in methylene chloride (0.4 mL) at -78°C a solution of DMSO (45 μl, 0,634 mmol) in methylene chloride (0.4 mL) was added. The mixture was stirred at -78°C for 2 min, then a solution of **18a** (68.1 mg, 0.126 mmol) in methylene chloride (1.2 mL) was added. After 15 min of

additional stirring at -78°C triethylamine (160 µl) was added, and the mixture was stirred at -78°C for 5 min and at 20°C for 15 min. After usual work-up (CH_2Cl_2), the aldehyde was immediately used for the next step.
(ii) Sodium chlorite oxidation: To a solution of the above Swern oxidation product and 2-methyl-2-butene (600 µl) in ^{t}butanol (2.4 mL) a solution of sodium chlorite (104 mg, 1.155 mmol) and sodium dihydrogen phosphate (122 mg, 0.888 mmol) in water (1 mL) was added dropwise within 10 min. After 30 min at 20°C solvents were mostly evaporated, the mixture diluted with water and extracted with hexane. Then the aqueous phase was adjusted with dilute HCl to pH 3. After work-up (ether) and solvent evaporation crude **18b** (72.5 mg) was obtained.- IR ($CHCl_3$): 3000-3580 (COOH), 1610 cm^{-1} (C=O).

Allyl 4-O-benzyl-2,3-di-O-^{t}butyldimethylsilyl-β-D-glucopyranosiduronamide (18c)
To a stirred mixture containing **18b** (38.5 mg, 0.07 mmol), methylene chloride (1 mL), and 4 Å molecular sieves a solution of 1,1'-carbonyldiimidazole (13.6 mg, 0.08 mmol) in methylene chloride (0.5 mL) was added. Stirring was continued at 20°C for 4 h. Then, at 0°C, gaseous, dry ammonia was passed through the reaction flask for 40 min. Usual work-up (methylene chloride, washing with dilute aq NH_3 solution) and LC (petrol - ethyl acetate 3:1) provided **18c** (34.4 mg, 89%, based on **18a**).- M.p. 74°C ($CHCl_3$-petrol).- ^{1}H NMR (400 MHz, benzene-d_6): δ = 6.05 (broad s, NH), 4.95 (broad s, 1H, NH), 4.83 (d, 1-H, $J_{1,2}$= 7 Hz), 4.56 und 4.60 (CH_2-Ph, J_{AB} = 11.5 Hz), 4.42 (d, 5-H, $J_{4,5}$ =1.5 Hz), 4.15-4.22 (2H, 1-H^{allyl} and 4-H), 4.08 (3-H, $J_{2,3}$ = 1.5 Hz, $J_{3,4}$ = 4 Hz), 3.88 (2-H).- IR ($CHCl_3$): 3500, 3400 (NH), 1680 (C=O), 1550 cm^{-1} (C=C).- EI MS, m/z (%): 494 (12), 436 (10), 386 (9), 362 (19), 346 (12), 254 (8), 214 (6), 172 (7), 115 (10), 91 (100), 73 (50).- $C_{28}H_{49}O_6NSi_2$ (551.87, 551.30) calc C 60.94, H 8.94, found C 60.93, H 8.79.

Treatment of 18c with TBAF in THF-water
To a solution of **18c** (534 mg, 0.97 mmol) in THF (32 mL) and water (1.6 mL) 8.1 mL of a 0.1 mol/L THF solution of TBAF was added in three equal portions (interval: 1h). Stirring at 20°C was then continued for 1 h. Usual work-up (ethyl acetate) and LC (petrol - ethyl acetate 3:1) furnished **18d** (295 mg,70%) and diol **18e** (60 mg, 19%).-

Allyl 4-O-benzyl-2-O-^{t}butyldimethylsilyl-β-D-glucopyranosiduronamide (18d)
M.p. 153°C-155°C (ethyl acetate - petrol).- ^{1}H NMR (400 MHz, pyridine-d_5): δ = 6.30 (broad s, NH), 5.52 (broad s, OH), 4.79 and 4.68 (CH_2-Ph, J_{AB} = 11.5 Hz), 4.38-4.32 (2*1H, ddt, 1-H^{allyl} and d, 1-H, $J_{1,2}$ = 7.5 Hz), 3.82 (d, 5-H, $J_{4,5}$ = 9.5 Hz), 3.63 (t, 3-H, $J_{3,4}$ = $J_{2,3}$ = 9.5 Hz), 3.48 (t, 4-H), 3.46 (dd, 2-H).- IR (KBr): 3382 (OH), 1684 (C=O), 1646 cm^{-1} (C=C).- EI MS: m/z (%) = 380 (0.2), 362 (8), 322 (7), 214 (11), 117 (3), 91 (100).- $C_{22}H_{35}O_6NSi$ (437.60, 437.22) calc C 60.38, H 8.06, found C 60.52, H 7.96.

Allyl 4-O-benzyl-β-D-glucopyranosiduronamide (18e)
M p. 176°C (methanol).- ^{1}H NMR (400 MHz, pyridine-d_5, H,H COSY): δ = 8.59 and 8.39 (NH_2), 5.41-5.32 (2*1H, dq, 3-H$_{cis}^{allyl}$ and d, CH_2-Ph), 5.24 (d, 1H, CH_2-Ph, J_{AB} = 11 Hz), 4.82 (d, 1H, 1-H, $J_{1,2}$ = 7.5 Hz), 4.55-4.47 (ddt, 1-H^{allyl} and d, 5-H, $J_{4,5}$ = 9 Hz), 4.38-4.29 (2t, 4-H, $J_{3,4}$ = 9 Hz and bt, 3-H, $J_{2,3}$ = 9 Hz), 4.04 (broad t, 2-H).- IR (KBr): 3220-3600 (OH, NH), 1698 (amide I), 1575 cm^{-1} (amide II).- EI MS, m/z (%): 265 (0.18), 217 (2.1), 155 (4), 118 (10), 100 (24), 91 (100), 41 (34).- $C_{16}H_{21}O_6N$ (323.34, 323.13) calc C 59.43, H 6.54, found C 59.38, H 6.49.

Allyl 4-O-benzyl-2-O-^{t}butyldimethylsilyl-3-O-carbamoyl-β-D-glucopyranosiduronamide (18f)
To a solution of **18d** (567 mg, 1.30 mmol) in methylene chloride (25 mL) TAI (200 µl, 1.69 mmol) was added at 20°C. The mixture was left at 20°C for 14 h. Excess reagent was destroyed by addition of methanol (4 mL). After stirring at 20°C for 1 h and solvent evaporation, the residue was taken up in methanol (16 mL). Zn dust (810 mg, 13.0 mmol) was added and the reaction mixture was stirred at 20°C for 5 h. Filtration, followed by carefully washing the residue with 8:2 methanol - water, solvent evaporation, and LC (petrol - ethyl acetate 2:1) yielded **18f** (567 mg, 90%).- M.p. 191-193°C (ethyl acetate - petrol).- ^{1}H NMR (400 MHz, pyridine-d_5): δ = 6.28 (broad s, 1H, NH), 5.53 (bs, 1H, NH), 5.01 (t, 3-H, $J_{2,3}$ = $J_{3,4}$ = 9 Hz), 4.68 (d, 1H, CH_2-Ph, J_{AB} = 11 Hz), 4.58 (2*1H, d, CH_2-Ph and bs, NH), 4.41 (d, 1-H, $J_{1,2}$ = 7.5 Hz), 3.96 (d, 5-H), 3.63 (t, 4-H, $J_{4,5}$ = 9 Hz), 3.52 (dd, 2-H).- IR (KBr): 3000-3600 (OH, NH), 1686, 1610 cm^{-1} (C=O).- EI MS, m/z (%): 423 (2), 362 (7), 214 (7), 131 (4), 118 (20), 91 (100).- $C_{23}H_{36}O_7N_2Si$ (480.63, 480.22) calc C 57.47, H 7.55, found C 57.13, H 7.80.

Allyl 4-O-benzyl-3-O-carbamoyl-β-D-glucopyranosiduronamide (18g)

At 20°C to a solution of **18f** (499 mg, 1.04 mmol) in 95:5 THF-water (30 mL) 0.1 mol/L TBAF in THF (3 mL) was added in small portions within 1 h. The mixture was stirred at 20°C for 4 h. Work-up (3:1 ethyl acetate - ^{n}butanol, washing with saturated aq. NaCl), LC ($CHCl_3$ - MeOH 20:1), and subsequent removal of ammonium salts by passing a methanolic solution through a column with Dowex 50WX2-200 (H^+ form) provided **18g** (216 mg, 57%).- M.p. 176-177°C (methanol).- ^{1}H NMR (400 HMz, pyridine-d_5): δ = 8.65 (broad s, 1H, NH), 8.43 (broad s, 1H, NH), 5.85 (t, 3-H, $J_{2,3} = J_{3,4} = 9.5$ Hz), 5.17-5.08 (3*1H, dq, 3-H_{trans}^{allyl} and 2*d, CH_2-Ph, $J_{AB} = 11$ Hz), 4.88 (d, 1-H, $J_{1,2} = 7.5$ Hz), 4.56-4.48 (2*1H, ddt, 1-H^{allyl} and d, 5-H, $J_{4,5} = 9.5$ Hz), 4.38 (t, 4-H), 4.10 (dd, 2-H).- IR (KBr): 3100-3700 (OH, NH), 1704, 1686 (C=O), 1615 cm^{-1} (C=C).- FAB MS (lactic acid): m/z = 733 ($[2M+H]^+$), 367 ($[M+H]^+$).- $C_{17}H_{22}O_7N_2$ (366.37, 366.14), calc C 55.73, H 6.05, found C 55.64, H 5.90.

Allyl 4-O-benzyl-3-O-carbamoyl-2-O-(N-acetylglycyl)-β-D-glucopyranosiduronamide (20a)

Solutions of **18g** (33.1 mg, 0.09 mmol), N-acetylglycine (15.9 mg, 0.13 mmol), 4-dimethylaminopyridine (11 mg, 0.09 mmol), and DCC (20.5 mg, 0.10 mmol), each in DMF (0.5 mL), were combined, and the mixture stirred at 20°C for 24 h. After addition of solid sodium hydrogen carbonate the content of the reaction flask was directly transferred onto the top of a chromatography column (LC). Elution with toluene-$CHCl_3$-EtOH 10:6:2) provided pure **20a** (35.5 mg, 0.08 mmol, 84%).- ^{1}H NMR (400 MHz, pyridine-d_5): δ = 9.20 (broad t, 1H, NH), 8.68 (broad s, 1H, NH), 8.58 (broad s, 1H, NH), 7.78 (broad s, 2H, NH_2), 5.95-5.82 (2*1H, 2-H ^{allyl} and t, 3-H, $J_{2,3} = J_{3,4} = 9.5$ Hz), 5.61 (dd, 1H, 2-H, $J_{1,2} = 8$ Hz), 5.14-4.93 (3-H_{trans}^{allyl}, CH_2-Ph and 1-H), 4.55-4.32 (5-H, $J_{4,5} = 9.5$ Hz, $C\underline{H}_2$-NHAc, 4-H and 1-H^{allyl}), 2.09 (s, 3H, CH_3).- IR (KBr): 1759, 1717, 1685, 1633 (C=O), 1620 cm^{-1} (C=C).- FAB-MS (lactic acid): m/z = 931 ($[2M+H]^+$), 466 ($[M+H]^+$).- $C_{21}H_{27}O_9N_3$ (465.46, 465.17), calc C 54.19, H 5.84, found C 51.71, H 5.80.

Removal of the allyl protecting group from 20a

(i) A mixture containing **20a** (105 mg, 0.224 mmol), 0.1 mol/L aq sodium acetate in 20:1 acetic acid-water (5 mL), and palladium(II) chloride (58 mg, 0.328 mmol) was stirred at 20°C for 24 h. Water was added and inorganic cations were removed by stirring with Dowex 50WX2-200 (H^+ form). After filtration and careful washing of the residue solvents were removed by evaporation and lyophilzation, respectively. LC ($CHCl_3$-methanol 10:1) provided **20c** (30.6 mg, 32%) and methyl ketone **20b** (not completely pure, 10 mg, 9%).

(ii) To a solution of **20a** (100 mg, 0.213 mmol) in DMF (8 mL) and water (1.4 mL) palladium(II) chloride (9.5 mg, 0.053 mmol) and copper(I) chloride (63 mg, 0.636 mmol) were added. At 20°C oxygen was passed into the stirred mixture by means of a syringe. After 8 h a further portion of palladium(II) chloride (5 mg, 0.028 mmol) was added, and the mixture was stirred for another 28 h. For working up the products were partitioned between 3:1 ethyl acetate-1-butanol and water. The organic phase was washed with brine. Solvent evaporation, followed by LC ($CHCl_3$-methanol 10:1) furnished **20c** (12 mg) and methyl ketone **20b** (59 mg). The latter fraction was dissolved in acetonitrile (140 mL); the solution purged with argon (30 min), then triethylamine (87 µl, 0.629 mmol) was added and the mixture then exposed to UV light (Philips HPK 125, quartz vessel) for 35 min. Solvent evaporation and subsequent LC ($CHCl_3$-methanol 10:1) provided **20c** (39 mg). The overall yield was 56%.

4-O-Benzyl-3-O-carbamoyl-2-O-(N-acetylglycyl)-α-D-glucopyranuronamide (20c)

^{1}H NMR (400 MHz, pyridine-d_5): δ = 9.18 (broad t, 1H, NH), 8.78 (broad s, 1H, NH), 8.49 (broad s, 1H, NH), 6.42 (t, 3-H, $J_{2,3} = J_{3,4} = 9.5$ Hz), 5.99 (d, 1-H, $J_{1,2} = 3$ Hz), 5.49 (dd, 2-H), 5.25 (d, 5-H, $J_{4,5} = 9.5$ Hz), 5.07 and 5.11 (CH_2-Ph, $J_{AB} = 10.5$ Hz), 2.08 (s, CH_3).- $C_{18}H_{23}O_9N_3$ (425.39)

2-Oxopropyl 4-O-benzyl-3-O-carbamoyl-2-O-(N-acetylglycyl)-β-D-glucopyranosiduronamide (20b)

^{1}H NMR (400 MHz, pyridine-d_5): δ = 9.22 (broad t, 1H, NH), 8.68 (broad s, 1H NH), 8.58 (broad s, 1H, NH), 7.80 (broad s, 1H, OH), 5.92 (t, 1H, 3-H, $J_{2,3} = J_{3,4} = 9.5$ Hz), 5.65 (t, 1H, 2-H, $J_{1,2} = 8.5$ Hz), 2.25 (s, 3H, methyl ketone), 2.09 (s, 3H, CH_3-NHAc).- $C_{21}H_{27}O_{10}N_3$ (481.46).

Benzyl (R)-3-hydroxy-2-(3,8,8,11,14,18-hexamethylnonadecyloxy)-propanoate (9c)

(i) A mixture of methyl ester **9b** (62 mg, 0.132mmol), benzyl alcohol (0.5 mL), and titanium(IV) isopropoxide (30 µl)

were stirred at 60°C for 5 d. SC (petrol - ethyl acetate 15:1) furnished **9c** (54.7 mg, 76%).
(ii) Acetyl chloride (50 µl, 0.703 mmol) was added to a solution of **9a** (212 mg, 0.465 mmol), in benzyl alcohol (0.5 mL). The solution was stirred at 60°C for 5 d. Dowex 50WX2-200 (H^+ form) served equally well as acid catalyst. TLC indicated only the presence of **9c** besides starting material **9a**. LC (petrol - ethyl acetate 15:1) provided **9c** (149 mg, 58%).- ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.41-7.31 (Ar-H's), 5.17, 5.21 (CH_2-Ph, J_{AB} = 12.5 Hz), 4.01-3.42 (5H, 2-H^H, CH_2-3^H, CH_2-1^I), 2,11 (bt, 1H, OH), 1.71-0.81 (alkyl-H's).- ^{13}C NMR (100.6 MHz, $CDCl_3$)[55]: δ = 170.89 (C-1^H), 135.63, 128.85, 128.65, 128.44 (Ar-C's), 79.88 (C-2^H), 79.78 (C-2^H), 70.03 (C-1^I), 69.91 (C-1^I), 66.91 (C-3^H), 63.70 (CH_2-Ph), 42.20-19.79 (C-2^I -C-25^I).- IR (nujol): 3368 (OH), 1746 cm^{-1} (C=O).- $C_{35}H_{62}O_4$ (546.87), EI MS: m/z (%) = 516 (0.25), 243 (4), 180 (18), 111 (16), 91 (100), 71 (56), 57 (76), 43 (38) .

4-O-Benzyl-3-O-carbamoyl-2-O-(N-acetylglycyl)-1-O-{[(R)-2-benzyloxycarbonyl-2-(3,8,8,11,14,18-hexamethylnonadecyloxy)-ethoxy]-(2-trichlormethyl-2-propyloxy)-phosphoryl}-α-D-glucopyranuronamide (19a), (P diastereomers)
To a solution of 1H-1,2,4-triazole (37 mg, 0.528 mmol) in 1:4 pyridine-CH_2Cl_2 (1 mL) 2,2,2-trichloro-1,1-dimethylethyl dichlorophosphite (19 µl, 0.094 mmol) was added at 0°C. The mixture was stirred at 0°C for 20 min. A slurry of **20c** (32 mg, 0.075 mmol) in 1:4 pyridine-CH_2Cl_2 (2 mL), was added and the reaction mixture stirred for 4 h at 0°C. After addition **9c** (122 mg, 0.226 mmol) dissolved in CH_2Cl_2 (1.5 mL) in three portions over a period of 2 h the mixture was stirred for 1 h at 0°C. Bis(trimethylsilyl)peroxide (23 µl, 0.105 mmol) was injected into the reaction flask and the stirred mixture was maintained at 0°C for 15 h. The reaction mixture was filtered, and solvent evaporation followed by LC ($CHCl_3$-MeOH 30:1) yielded **19a** (46 mg, 52%).- ^{13}C NMR (100.6 MHz, pyridine-d_5): δ = 157.19 ($OCONH_2$), 95.56 (C-1^F), 90.83/90.77/90.72 (CCl_3^G), 78.44/78.34/78.10; 75.16/75.06; 71.77/71.72; 71.65; 70.05/69.99; 68.69; 67.23 (C-2^H; C-2^F; CH_2-Ph; C-3^F; C-1^I; C-5^F; C-3^H), 61.76 (CH_2NHAc), 42.30-19.57 (C-2^I - C-25^I and CH_3 signals).- ^{1}H NMR (400 MHz, pyridine-d_5): δ = 9.15/9.05 (2*bt, 2*1H, 2*NH, 2:1 mixture of diastereoisomers isomeric at P), 7.59-7.15 (Ar-H), 6.52/6.49 (2*dd, 2*1H, 2*1-H, $J_{1,2}$ = 3.5 Hz, $J_{1,P}$ = 6.5 Hz).- $C_{57}H_{89}O_{15}N_3$ (1193.00).

4-O-Benzyl-3-O-carbamoyl-2-O-(N-acetylglycyl)-1-O-{[(R)-2-benzyloxycarbonyl-2-(3,8,8,11,14,18-hexamethylnonadecyloxy)-ethoxy]-hydroxy-phosphoryl}-α-D-glucopyranuronamide (19b)
To a solution of triester **19a** (49 mg, 0.041 mmol) in dry pyridine (2 mL) Zn-Cu couple (freshly prepared, 27 mg, 0.41 mmol) and 2,4-pentanedione (48 µl, 0.41 mmol) were added and the mixture was stirred at 20°C for 4 h. Excess Zn-Cu couple was removed by filtration (washing with ethanol). After solvent evaporation the residue was redissolved in 10:1 water-ethanol (70 mL), and Zn^{2+} ions were removed by treatment with Dowex 50WX2-200 (H^+ form). Filtration, lyophilization, and MPLC ($CHCl_3$-methanol-1-butanol 4:1.25:1) yielded **19b** (27.8 mg, 0.027 mmol, 66%).- ^{13}C NMR (100.6 MHz, $CDCl_3$-methanol-d_4-D_2O 18:13:2.7): δ = 156.72 ($OCONH_2$), 91.66 (C-1^F), 78.36/78.32; 74.34; 71.71/71.49/71.08; 70.48; 69.81/69.61; 66.73; 65.22 (C-2^H; C-2^F; CH_2-Ph; C-3^F; C-1^I; C-5^F; C-3^H), 60.51 (CH_2-NHAc), 48.56-18.73 (C-2^I -C-25^I and CH_3).- $C_{53}H_{84}O_{15}N_3P$ (1034.23, 1033.56), FAB MS (matrix: lactic acid): m/z = 1078 ([M+2Na-H]$^+$), 1072 ([M+K]$^+$), 1056 ([M+Na]$^+$).

4-O-Benzyl-3-O-carbamoyl-2-O-(N-acetylglycyl)-1-O-{[(R)-2-carboxy-2-(3,8,8,11,14,18-hexamethylnonadecyloxy)-ethoxy]-hydroxy-phosphoryl}-α-D-glucopyranuronamide (19c)
19b (27.8 mg, 0.027 mmol) dissolved in ethanol (4 mL) was hydrogenated over 10 per cent Pd/C (51 mg) for 5 d at 20°C. Filtration, washing the residue with water, methanol, and ethanol, followed by solvent evaporation and LC ($CHCl_3$-methanol-water 16:7:1) gave **19c** (12 mg, 52%).- ^{13}C NMR (100.6 MHz, $CDCl_3$-methanol-d_4-D_2O 18:13:2.7): δ = 157.58 ($OCONH_2$), 92.05 (C-1^F), 71.52; 70.85; 69.33 (C-2^F; C-3^F; C-1^I), 48.46-18.74 (C-2^I -C-25^I and CH_3).- $C_{39}H_{72}O_{15}N_3P$ (853.98, 853.47), FAB MS (matrix: lactic acid): m/z = 898 ([M+2Na-H]$^+$), 892 ([M+K]$^+$), 876 ([M+Na]$^+$).

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