Pergamon

Tetrahedron 55 (1999) 3749–3766

TETRAHEDRON

Acceptor Site Recognition of Transglycosylase Inhibitors A β-D-glucopyranosyl-(1→2)-α-D-glucopyranuronamide-derived Moenomycin Analogue

Falk-Thilo Ferse, Kerstin Floeder, Lothar Hennig, Matthias Findeisen, Peter Welzel*

Fakultät für Chemie und Mineralogie der Universität Leipzig, Talstr. 35,

D-04103 Leipzig (Germany)

Dietrich Müller

Fakultät für Chemie der Ruhr-Universität, D-44780 Bochum (Germany)

Jean van Heijenoort

Biochimie Moléculaire et Cellulaire, Université Paris-Sud,

F-91405 Orsay Cedex (France)

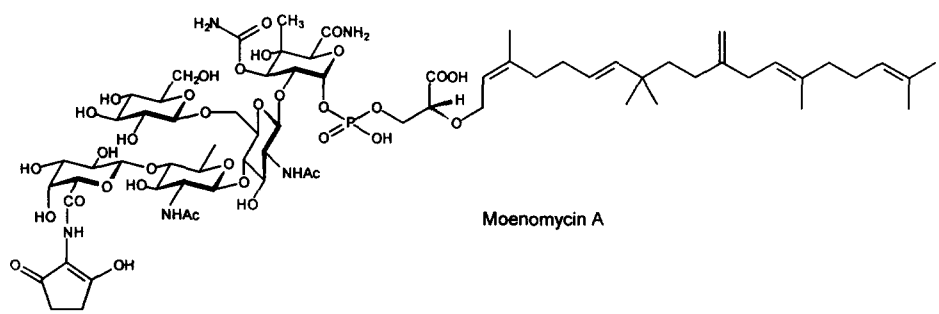
Received 3 September 1998; revised 13 October 1998; accepted 14 October 1998

Abstract - The synthesis, the antibiotic and the transglycosylase inibiting properties of a disaccharide analogue of moenomycin A in which the NHAc group of unit E is replaced by a hydroxyl function are described. It can be concluded that this NHAc group is essential for eliciting transglycosylase inhibiting properties, in agreement with a recently established solution structure of moenomycin A.

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

Introduction

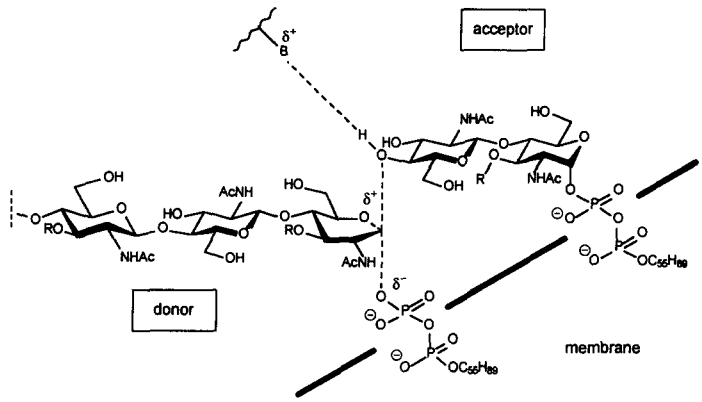
Transglycosylases such as penicillin binding protein 1b (PBP 1b) catalyze the formation of un-crosslinked peptidoglycan from a disaccharide intermediate (Lipid II).[1] This glycosyltransfer reaction is believed to proceed in such a way that the growing peptidoglycan chain is the glycosyl donor substrate whereas lipid II is the glycosyl acceptor (see Scheme 1).[2]



Moenomycin A

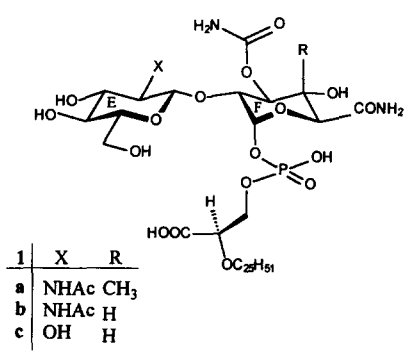
0040-4020/99/$ - see front matter
PII: S0040-4020(98)01181-8

The moenomycin antibiotics have been shown to inhibit PBP 1b and related enzymes.[3] The structure-activity relationships indicate that there are two recognition sites for moenomycin-type transglycosylase inhibitors at the enzyme, one at the donor and one at the acceptor binding site. The moenomycins themselves and struc-

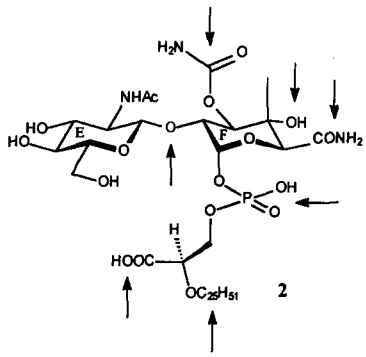


Scheme 1: Transition state of the transglycosylation reaction (speculative)

tural analogues with at least three sugar units bind presumably to the donor binding site.[4,5] They are active *in vivo* (against gram-positive bacteria) as well as in the *in vitro* test systems. Moenomycin analogues with two sugars are antibiotically more or less inactive but they do inhibit the enzyme in the test systems provided that they have the right substitution pattern.[5] Compounds **1a** and **1b** fulfil these conditions.



1	X	R
a	NHAc	CH_3
b	NHAc	H
c	OH	H



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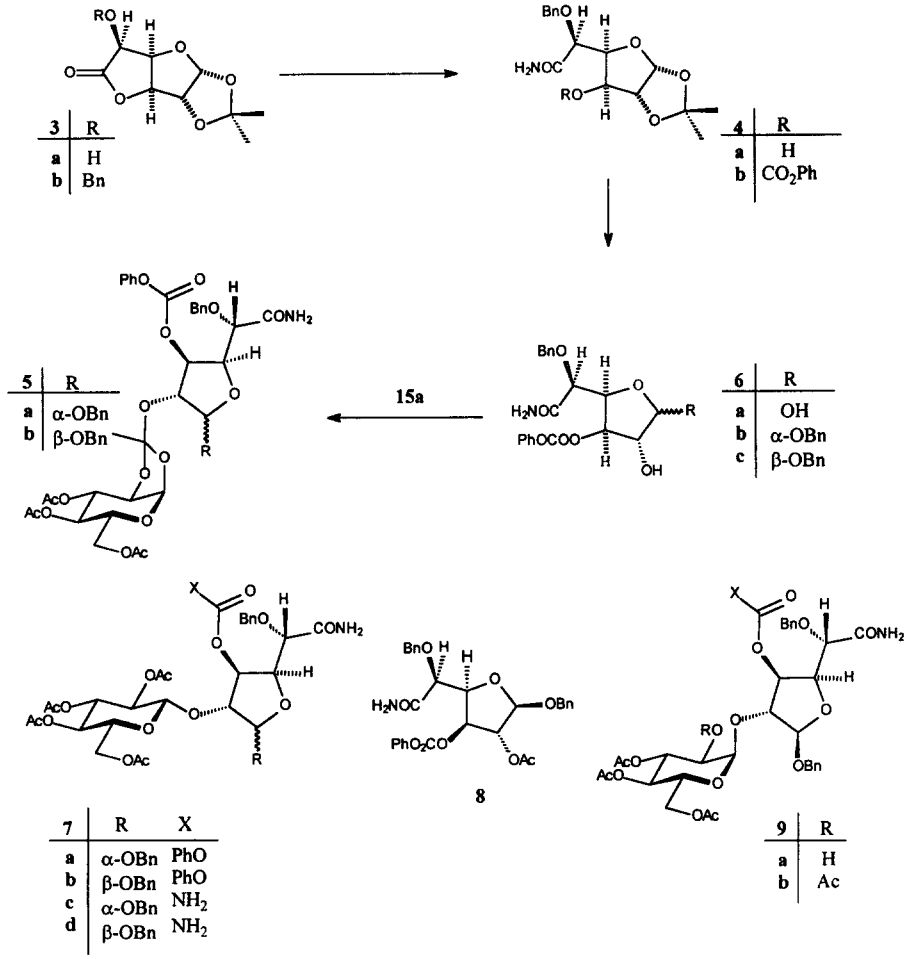
It is believed that these analogues bind to the acceptor binding site. Formula **2** indicates the groups that have been shown with the help of structural analogues to be indispensible for transglycosylase inhibition to be elicited.[5] From a recently determined NMR conformation of moenomycin A in aqueous solution[6] it may be concluded that in addition to the previously established pharmacophoric groups (see arrows in formula **2**) the NHAc group of unit E should also be an obligatory structural feature of transglycosylase inhibitors.

It was the purpose of the work outlined herein to test this conclusion. Described are (i) the synthesis, (ii) the antibiotic, and (iii) the transglycosylase inhibiting properties of compound **1c**, a disaccharide analogue of moenomycin A in which the NHAc group of unit E is replaced by a hydroxyl function.

Synthesis of glycosyl acceptor

The disaccharide part of **1c** was constructed from a suitably functionalized D-glucuronic acid derivative (glycosyl acceptor) and a D-glucose-derived glycosyl donor.



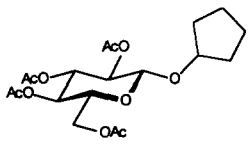
It has been shown previously, that the synthesis of compounds like **1b** is plagued by the high polarity of intermediates with the uronamide and carbamoyl functionalities.[7] A number of measures have been taken to overcome these problems, including development of a new lipophilic protecting group for the anomeric centre.[8] In the present work we used benzyl groups for both the 1- and the 5-position of unit F to render the compounds less polar. Recently, we found out that replacing the carbamoyl group in unit F by a latent functionality, a phenyl carbonate, solved most of the solubility and polarity problems.[5] This method was used for the present synthesis, too, but in addition, we performed some preliminary experiments with a glycosyl donor in which the acetyl were replaced by butyryl groups.

Alkylation[9] of the 5-OH group of **3a** using benzyl trichloroacetimidate[10,11] in a trifluoromethanesulfonic acid-mediated reaction gave benzyl ether **3b** in 60% yield. The lactonic ring in **3b** was opened with NH_3 in THF solution to furnish the uronamide **4a**.[12] **4a** on reaction with phenyl chloroformate in dichloromethane in

the presence of 1.0 eq of DMAP and triethylamine (procedure of McLamore et al.[13]) provided phenyl carbonate **4b**. The acetonide protecting group was then removed with 90 per cent trifluoroacetic acid at 20°C[14,15] to form **6a** in quantitative yield as a mixture of anomers which was in turn converted under Fischer conditions[16] to a mixture of benzyl glycosides **6b** (56%) and **6c** (26%).

Glycosidation experiments

For the synthesis of **1c** we used the known trichloroacetimidate **15a** as glycosyl donor.[17] In a model experiment **15a** was treated with cyclopentanol (0°C, $BF_3{\cdot}Et_2O$-mediated) to provide **10**[18] in an unexceptional reaction (66% yield, not optimized). On the other hand, on reaction of **15a** with the glycosyl acceptor **6c** at -20°C ($BF_3{\cdot}Et_2O$-mediated) in a clean reaction solely orthoester **5b** was formed (93%). The configuration at the orthoester carbon was not determined. When the reaction was repeated at 20°C three products could be isolated. One of them was the desired disaccharide **7b** (17%), the others were



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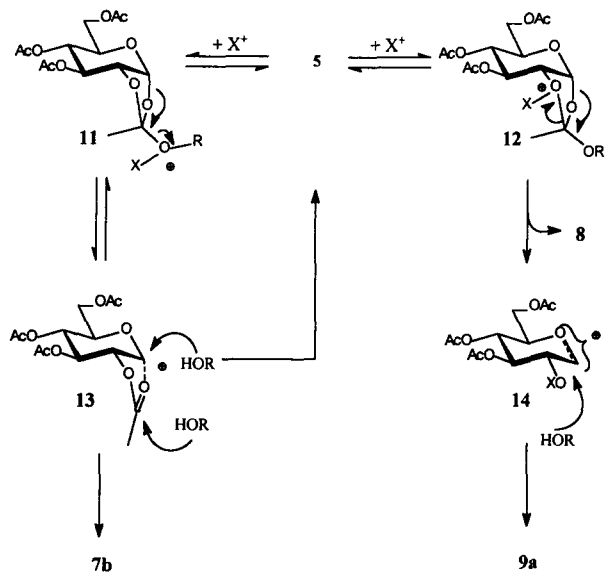
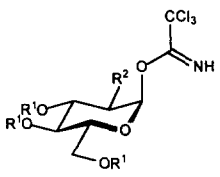
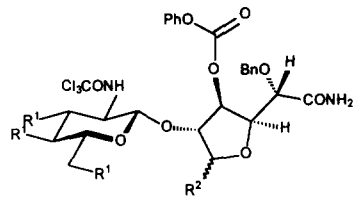


Scheme 2: Proposed mechanism for the formation of **7b**, **8** *and* **9a**

disaccharide **9a** with an α - glucopyranosyl unit lacking the acetyl group of position 2 (29% yield) and glycosyl acceptor derivative **8** in which the accepting OH group of **6c** was acetylated. Scheme 2 indicates how these compounds are probably formed. When the orthoester reacts as indicated in **13** (lower arrow), **7b** is the product. Opening of the bond between the 2-oxygen of the glucose unit and the orthoester carbon leads to the other products. Similar reactions have been found on attempted glycosylation reactions of some steroid aglycons.[19] When the same reaction was repeated with **6b** as glycosyl acceptor **5a** and **7a** were isolated.



15	R^1	R^2
a	OAc	OAc
b	OAc	$NHCOCCl_3$
c	OBut	$NHCOCCl_3$



16	R^1	R^2
a	OAc	α-OBn
b	OAc	β-OBn
c	OBut	α-OBn

In some model experiments glycosyl acceptors **6b** and **6c** were treated with the Beau-Jacquinet glucosamine glycosyl donor **15b**.[20] Here the reactions were again straightforward and provided disaccharides **16a** and **16b**, respectively. As already mentioned above we exchanged **15b** against the butyryl analogue **15c** (for the preparation, see Experimental). The disaccharide formation proceeded as desired and indeed, the disaccharide **16c** was much less polar when compared with **16a**. This example shows that the previously encountered solubility problems can also be solved by the proper choice of acyl protecting groups at least as far as disaccharide intermediates are involved.[5]

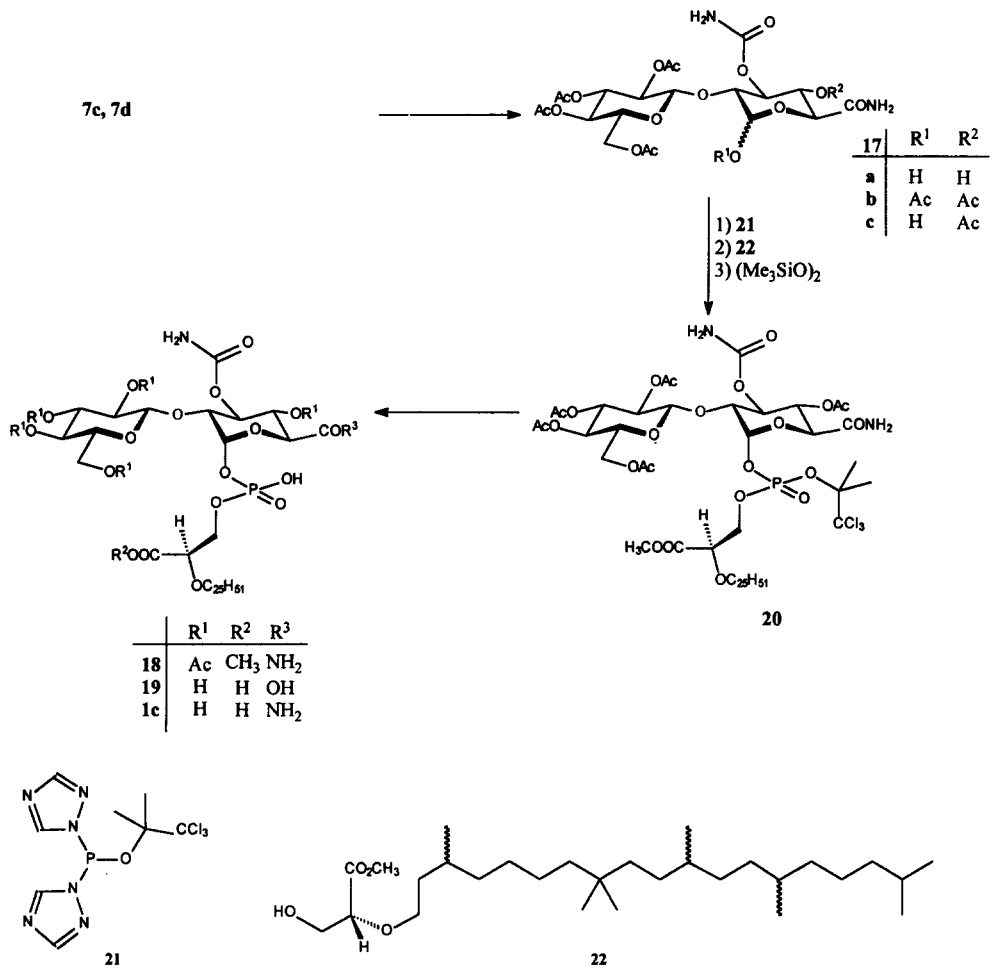
Completion of the synthesis of 1c

Treatment of **7a** and **7b** with NH_3 in THF solution[21] provided **7c** (63%) and **7d** (41%) which on hydrogenation furnished **17a**.[22,23] The anomeric mixture was acetylated to give a mixture of anomeric acetates which was then selectively deacetylated to provide the desired compound **17c**.[24] Treatment of **17c** with reagent **21**, prepared *in-situ* from 1H-1,2,4-triazol and 2,2,2-trichloro-1,1-dimethylethyl dichlorophosphite, followed by reaction with the moenomycin-derived building block **22**[25] furnished a phosphite which was oxidized with bis(trimethylsilyl)peroxide[26] to form phosphoric acid triester **20**.[27] Two diastereoisomers were obtained, the configuration at the phosphate unit was not determined. Deprotection occured in two steps: (i) reductive removal of the phosphate protecting group (zinc-copper couple under Imai conditions)[28] and (ii) base hydrolysis of the ester protecting groups.[29] As usual, the glyceric acid methyl ester reacted most reluctantly. Besides the target compound **1c** uronic acid **19** was isolated. The structure of **1c** was secured by FAB MS and ^{13}C and ^{31}P NMR.

Antibiotic and transglycosylase inhibiting properties of 1c

1c was antibiotically inactive (Staph. aureus SG 511). In van Heijenoort's test system[30] the compound was devoid of activity even at 10 μg / mL.

The results mean that the N-acetyl group of unit E is indeed a prerequisite of transglycosylase inhibiting properties. The suggestion of the NMR analysis is, thus, corroborated by our results. Most probably the NHAc group supplies an important contribution of the binding of moenomycin disaccharide analogues to the enzyme.



EXPERIMENTAL

General

Organic solvent evaporations were performed *in vacuo* at 40 °C using a rotatory evaporator, water was removed by lyophilization (Leybold-Heraeus GT2 or Christ Alpha 1-2). Solvents were purified by standard procedures. If necessary, solvents were degassed by sonication (Bandelin, Sonorex Super RK 106).- 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 Teflon-faced septum (Aldrich).- The instrumentation used was: 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), Bruker DMX 600 spectrometer (^{1}H NMR 600.13 MHz, processed on a SGI O2 workstation using the X-WINNMR program), chemical shifts are given in δ values, the ^{31}P NMR shifts are based on external phosphoric acid; FT-IR: ATI Mattson spectrometer, Genesis series; 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 (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[31] (see formula); melting points (corrected, determined in capillary tubes): Büchi (B-540); analytical TLC: Merck precoated silica gel 60 F_{254} plates (0.2 mm), spots were identified under a UV lamp (λ = 254 nm and λ = 366 nm) and by dipping into a 2.22 mol/L H_2SO_4 solution containing $Ce(SO_4)_2x4H_2O$ (10.0 g/L) and $H_3[PO_4(Mo_3O_9)_4]xH_2O$ (25.0 g/L)[32] and subsequent heating at 140°C or with the phosphate-specific spraying reagent of Dittmer and Lester;[33] flash chromatography (FC)[34]: silica gel (ICN Biomedical Silica 32-63 μm), Optima pump (Model 10007); medium-pressure liquid chromatography (MPLC): silica gel 20-40 μm (Merck), 35-70 μm (Amicon) or 50 μm (Fa. Grace), the samples were applied to a precolumn (3-5 g Kieselgel, 63-100 μm) and eluted at $1\text{-}2\cdot10^5$ Pa using a dosage pump (Promint Dosiertechnik, Heidelberg or Kronlab Chromatographie und Labortechnik, Sinsheim).

1,2-*O*-Isopropylidene-5-*O*-benzyl-α-D-glucofuranosidurono-6,3-lactone (3b)

Trifluoromethanesulfonic acid (96μL) was added to a solution of **3a** (2.3 g, 10.64 mmol) and benzyl trichloroacetimidate[9] (5.5 g, 21.78 mmol) in diethylether – dichloromethane (1:1, 13 mL) at 20°C. The solution was stirred at 20°C for 3 h and then triethylamine (0.2 mL) was added. The mixture was cooled to 0°C, solids were filtered off. The residue was washed several times with petrol-dichloromethane (3:1). Solvent evaporation from the combined solutions and FC (dichloromethane-ethyl acetate 99:1→ ethyl acetate) gave **3b** (1.85 g, 60%).- ^{1}H NMR (200 MHz, ^{1}H-^{1}H COSY, $CDCl_3$): δ = 1.34, 1.51 (6H, 2 s, $O_2C(CH_3)_2$), 4.26 (1H, d, 5-H, $J_{4,5}$ = 4.3 Hz), 4.71 (1H, d, 3-H, $J_{3,4}$ = 2.9 Hz), 4.79 (1H, d, 2-H, $J_{1,2}$ = 3.7 Hz), 4.86 (1H, dd, 4-H, $J_{3,4}$ = 3.0 Hz, $J_{4,5}$ = 4.4 Hz), 4.92 (2H, s, OCH_2Ph), 6.04 (1H, d, 1-H, $J_{1,2}$ = 3.7 Hz), 7.32-7.48 (m, Ph); impurity: 5.30 (CH_2Cl_2).- ^{13}C NMR (50 MHz, $CDCl_3$): δ = 27.0, 27.4 ($O_2C(\underline{C}H_3)_2$), 73.0, 75.0, 77.9, 82.2, 83.0 (C-2, C-3, C-4, C-5, OCH_2Ph), 107.5 (C-1), 113.7 ($O_2\underline{C}(CH_3)_2$), 128.9, 129.1, 136.6 (Ar-Cs), 172.4 (CO).- IR ($CHCl_3$): 1804 cm^{-1}.- $C_{16}H_{18}O_6$ (306.28, 306.11), FAB MS: m/z 329.0 $[M+Na]^+$, 307.0 $[M+H]^+$, 305.0 $[M+H-H_2]^+$.

1,2-*O*-Isopropylidene-5-*O*-benzyl-3-hydroxy-α-D-glucofuranosiduronamide (4a)

Ammonia was slowly bubbled into a solution of **3b** (4.16 g ,13.6 mmol) in dry THF (25 mL) for 1.5 h. The reaction mixture was stirred at 20°C overnight. Solvent evaporation furnished pure **4a** (4.07 g, 93%).- M.p. 136°C, lit.[12] 135°C.- ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.32, 1.47 (6H, 2 s, $C(CH_3)_2$), 4.22 (1H, m, 3-H, $J_{3,OH}$ = 5.4 Hz, $J_{3,4}$ = 3.2 Hz), 4.37 (1H, d, 5-H, $J_{4,5}$ = 2.0 Hz), 4.50 (1H, d, 2-H, $J_{1,2}$ = 3.6 Hz), 4.56 (1H, t, 4-H, $J_{3,4}$ = 3.2 Hz, $J_{4,5}$ = 2.2 Hz), 4.69, 4.77 (2H, AB system, OCH_2Ph, ^{2}J = 11.6 Hz), 5.24 (1H, d, OH, $J_{3,OH}$ = 5.4 Hz), 5.90 (1H, d, 1-H, $J_{1,2}$ = 3.6 Hz), 5.95, 6.82 (2H, 2bs, $CONH_2$), 7.30-7.45 (5H, m, Ph).- ^{13}C NMR (50 MHz, $CDCl_3$): δ = 26.6, 27.4 ($O_2C(\underline{C}H_3)_2$), 74.4, 75.8, 76.9, 81.2, 86.0 (C-2, C-3, C-4, C-5, OCH_2Ph), 105.4 (C-1), 112.5 ($O_2C(\underline{C}H_3)_2$), 128.7, 129.1, 129.3, 136.7 (Ar-Cs), 173.8 ($CONH_2$).- IR (KBr): 1662 cm^{-1}.- $C_{16}H_{21}NO_6$ (323.35, 323.14), FAB MS: m/z 346.0 $[M+Na]^+$, 324.0 $[M+H]^+$.

1,2-*O*-Isopropylidene-5-*O*-benzyl-3-*O*-phenoxycarbonyl-α-D-glucofuranosiduronamide (4b)

To a solution of **4a** (2.1 g, 6.5 mmol) and phenyl chloroformate (1.02 g, 0.82 mL, 1.0 eq.) in dichloromethane (24 mL) DMAP (803 mg, 1.0 eq.) and triethylamine (0.9 mL) were added at 20°C. The solution was stirred at 20°C for 1 h. Dichloromethane (30 mL) was added. The solution was washed twice with a saturated $NaHCO_3$ solution and with water. After drying, solvent evaporation and FC (chloroform-ethyl acetate 7:3) pure **4b** (2.55 g, 88%) was obtained.- M.p. 187°C.- ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.32, 1.50 (6H, 2 s, $O_2C(CH_3)_2$), 4.28 (1H, d, 5-H, $J_{4,5}$ = 6.8 Hz), 4.58 (1H, dd, 4-H, $J_{3,4}$ = 2.8 Hz, $J_{4,5}$ = 6.8 Hz), 4.64, 4.77 (2H, AB system, OCH_2Ph, ^{2}J = 11.4 Hz), 4.68 (1H, d, 2-H), 5.34 (1H, d, 3-H, $J_{3,4}$ = 2.8 Hz), 6.04 (1H, d, 1-H, $J_{1,2}$ = 3.8 Hz), 6.14, 6.41 (2H, 2 bs, $CONH_2$), 6.95-7.38 (10H, m, Ar-Hs).- ^{13}C NMR (50 MHz, $CDCl_3$): δ = 26.7, 27.0 ($O_2C(\underline{C}H_3)_2$), 74.3, 77.3, 79.3, 80.2, 83.3 (C-2, C-3, C-4, C-5, OCH_2Ph), 105.3 (C-1), 113.2 ($O_2\underline{C}(CH_3)_2$), 121.2, 126.7, 128.70, 128,75, 129.1, 130.0, 137.3 (Ar-Cs), 151.4, 153.1 (ipso-Ar-C, carbonate-C), 172.8 ($CONH_2$).- IR (KBr): 1766, 1678, 1380, 1300, 1256, 1213, 1163, 1094, 1077, 1026 cm^{-1}.- $C_{23}H_{25}NO_8$ (443.45, 443.16), FAB MS: m/z 466.1 $[M+Na]^+$, 444.1 $[M+H]^+$.

5-*O*-Benzyl-3-*O*-phenoxycarbonyl-D-glucofuranuronamide (mixture of anomers) (6a)
4b (600 mg, 1.35 mmol) was treated with 90 per cent trifluoroacetic acid (6.6 mL). The solution was stirred at 20°C for 90 min. Lyophilization furnished pure **6a** (545 mg, 100%).- ^{1}H NMR (200 MHz, DMSO-d_6): δ = 3.97 (1H, d, 5-H$^\alpha$, $J_{4,5}$ = 8.1 Hz), 4.03 (1H, m ($w_{½}$ = 2.0 Hz), 2-H$^\beta$), 4.10 (1H, d, 5-H$^\beta$, $J_{4,5}$ = 9.0 Hz), 4.17 (1H, t, 2-H$^\alpha$, J = 4.2 Hz), 4.37-4.62 (6H, m, 2×OCH_2Ph, 4-H$^\alpha$ and 4-H$^\beta$), 5.03 (1H, dd, 3-H$^\beta$, $J_{2,3}$ = 1.4 Hz, $J_{3,4}$ = 4.5 Hz), 5.09 (1H, s, 1-H$^\beta$, $J_{1,2}$ < 1.0 Hz), 5.19 (1H, dd, 3-H$^\alpha$, $J_{2,3}$ = 4.4 Hz, $J_{3,4}$ = 4.8 Hz), 5.26 (1H, d, 1-H$^\alpha$, $J_{1,2}$ = 4.2 Hz), 6.20–6.70 (4H, m, probably OH signals), 7.05-7.50 (10H, m, Ar-Hs), 7.63, 7.69 ($CONH_2$).- ^{13}C NMR (50 MHz, $CDCl_3$): δ = 71.4, 71.7 (OCH_2Ph of both anomers), 73.8, 75.3, 77.9, 78.1, 79.1, 81.6, 82.1 (C-2, C-3, C-4 and C-5 of both anomers), 96.3 (C-1$^\alpha$), 103.3 (C-1$^\beta$), 121.2, 121.3, 126.4, 127,67, 127.75, 127.8, 128.4, 129.8, 129.9, 137.90, 137.93 (Ar-Cs), 150.8, 152.8 (carbonate-C of both anomers), 171.4, 171.5 ($CONH_2$ of both anomers).- $C_{20}H_{21}NO_8$ (403.39, 403.13), FAB MS: m/z 426.1 $[M+Na]^+$, 404.1 $[M+H]^+$, 391.2 $[M+H-H_2O]^+$.

Conversion of 6a to benzyl glycosides 6b and 6c
A mixture of **6a** (294 mg, 0.73 mmol), Dowex *50 WX2*® (H^+ form, ≈ 1 g) and benzyl alcohol (15 mL) was stirred at 20°C for 7 h. After filtration, the resin was washed with ethyl acetate. From the combined solutions ethyl acetate and benzyl alcohol were removed by distillation (benzyl alcohol at 80°C and p = 0.7 Pa). Purification by FC (chloroform-ethyl acetate 7:3) yielded **6b** (92 mg, 26%) and **6c** (202 mg, 56%).

Benzyl 5-*O*-benzyl-3-*O*-phenoxycarbonyl-D-α-glucofuranosiduronamide (6b)
M.p. 176°C (decomp.).- ^{1}H NMR (200 MHz, pyridine-d_5): δ = 4.68 (1H, d, OCH_aH_bPh, ^2J = 11.9 Hz), 4.76 (1H, d, 5-H), 4.87-5.08 (4H, m, 2-H, $J_{1,2}$ = 4.6 Hz, $J_{2,3}$ = 6.8 Hz, OCH_aH_bPh, OCH_2Ph',), 5.41-5.48 (2H, m, 1-H, 4-H), 6.22 (1H, t, 3-H, $J_{2,3}$ = $J_{3,4}$ = 6.8 Hz), 7.09-7.54 (15H, m, Ar-Hs), 8.30, 8.64 (2H, 2 s, $CONH_2$).- ^{13}C NMR (50 MHz, pyridine-d_5): δ = 69.5, 73.5, 75.5, 76.7, 79.9, 81.9 (C-2, C-3, C-4, C-5 and 2×OCH_2Ph), 100.5 (C-1), 121.2, 125.9, 127.6, 127.7, 127.9, 128.0, 128.3, 128.4, 129.3, 138.0 (Ar-Cs), 151.4, 153.7 (OCOOPh, ipso-Ar-C), 172.2 (C-6).- IR (KBr): 1752, 1676, 1657, 1261, 1214 cm^{-1}.- $C_{27}H_{27}NO_8$ (493.51, 493.17), FAB MS: m/z 516.3 $[M+Na]^+$, 494.4 $[M+H]^+$.

Benzyl 5-*O*-benzyl-3-*O*-phenoxycarbonyl-D-β-glucofuranosiduronamide (6c)
M.p. 98°C.- ^{1}H NMR (200 MHz, pyridine-d_5): δ = 4.75, 5.20 (2H, AB system, OCH_2Ph, ^2J = 11.9 Hz), 4.83, 4.99 (2H, AB system, OCH_2Ph, ^2J = 11.4 Hz), 4.90 (1H, d, 5-H, $J_{4,5}$ = 8.6 Hz), 5.08 (1H, bs ($w_{½}$ = 0.4 Hz), 2-H), 5.46 (1H, dd, 4-H, $J_{3,4}$ = 5.1 Hz, $J_{4,5}$ = 8.7 Hz), 5.60 (1H, bs, 1-H), 5.92 (1H, dd, 3-H, $J_{2,3}$ = 1.9 Hz, $J_{3,4}$ = 5.1 Hz), 7.12-7.53 (15H, m, Ar-Hs), 8.52, 8.54 (2H, 2 s, $CONH_2$).- ^{13}C NMR (50 MHz, APT, pyridine-d_5): δ = 70.2, 73.0 (2×OCH_2Ph), 79.3, 80.1, 80.9, 82.6 (C-2, C-3, C-4, C-5), 108.9 (C-1), 121.8, 126.6, 128.2, 128.3, 128.5, 128.7, 128.9, 130.1, 138.6, 138.9 (Ar-Cs), 152.0, 153.9 (OCOOPh, ipso-Ar-C), 173.3 ($CONH_2$).- IR (KBr): 1760, 1683, 1259, 1212, 1093, 1045 cm^{-1}.- $C_{27}H_{27}NO_8$ (493.51, 493.17), FAB MS: m/z 516.2 $[M+Na]^+$, 494.3 $[M+H]^+$.

Cyclopentyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (10)
To a solution of **15a** (327 mg, 0.664 mmol) and cyclopentanol (60 μL, 0.664 mmol) in dichloromethane (3 mL) $BF_3{\cdot}Et_2O$ (21 μL, 0.25 eq.) was added at 0°C. The mixture was stirred at 20°C for 90 min. Triethylamine (0.1 mL) was added. After solvent evaporation, water addition (5 mL) and lyophilization the ^{13}C NMR spectrum of the crude reaction product (397 mg) proved the absence of an orthoester (no signal between 115 and 125 ppm). FC (petroleum ether-ethyl acetate 1:1) furnished **10** (184 mg, 66%).- ^{1}H NMR (200 MHz, homodecoupling, $CDCl_3$): δ = 1.40-1.75 (8H, m, CH_2-2^{cyclopentyl} and CH_2-3^{cyclopentyl} covered by an impurity signal), 1.94, 1.95, 1.96, 2.02 (12H, 4 s, $COCH_3$), 3.62 (1H, ddd, 5-H, $J_{5,6a}$ = 2.6 Hz, $J_{5,6b}$ = 4.8 Hz, $J_{4,5}$ = 9.7 Hz), 4.06, 4.20 (2H, part of an ABX system, CH_2-6, ^2J = 12.3 Hz, $J_{5,6a}$ = 2.6 Hz, $J_{5,6b}$ = 4.8 Hz), 4.21 (1H, m, 1^{cyclopentyl}-H, covered by 6-H), 4.46 (1H, d, 1-H, $J_{1,2}$ = 8.0 Hz), 4.87 (1H, dd, 2-H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.3 Hz), 5.00 (1H, dd, 4-H, $J_{3,4}$ = 9.3 Hz, $J_{4,5}$ = 9.7 Hz), 5.14 (1H, dd, 3-H, J = 9.3 and 9.7 Hz); impurities: δ = 1.98, 2.04, 5.37-5.49 (t), 5.54-5.56 (m), 5.79-5.85 (m), 6.00-6.06 (m).- ^{13}C NMR (50 MHz,

APT, $CDCl_3$): δ = 21.1 (3×), 21.2 ($CO\underline{C}H_3$), 23.5, 23.8 (C-3'), 32.6, 33.6 (C-2'), 62.6 (C-6), 69.1, 71.9, 72.2, 73.4, 82.1 (C-2, C-3, C-4, C-5, C-1'), 100.0 (C-1), 169.7, 169.9, 170.8, 172.2 ($\underline{C}OCH_3$); impurity: δ = 67.8, 69.9.- $C_{19}H_{28}O_{10}$ (416.43, 416.17), FAB MS: m/z 439.1 $[M+Na]^+$, 417.1 $[M+H]^+$.

Benzyl 2-*O*-(2-trichloroacetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-5-*O*-benzyl-3-*O*-phenoxycarbonyl-α-D-glucofuranosiduronamide (16a)

A suspension of **6b** (31 mg, 0.063 mmol), **15b** (49.4 mg, 0.082 mmol), 3 Å molecular sieves (44 mg) in 1,2-dichloroethane (0.6 mL) was stirred at 20°C. After 1 h the mixture was cooled to 0°C and a solution of TMSOTf in toluene (1 M, 12 μL) was added. Stirring was continued for 4.5 h, before the reaction was stopped by adding of triethylamine (11 μL). Ethyl acetate (5 mL) was added and the organic layers were extracted with a saturated $NaHCO_3$ solution, with 10 per cent tartaric acid and with water. The organic layer was dried with $NaSO_4$. Solvent evaporation and FC (chloroform - ethyl acetate 7:3) gave **16a** (40 mg, 68 %).- ^1H NMR (200 MHz, ^{13}C-^1H COSY, DMSO-d_6): δ = 1.91, 1.93, 2.01 (9H, 3 s, $COCH_3$), 3.77-3.93 (2H, m, 2-H^E, 5-H^E), 4.10 (1H, d, 5-H^F, $J_{4,5}$ = 6.5 Hz), 4.15 (2H, d ($w_{½}$ = 0.4 Hz), 6-H^E, $J_{5,6}$ = 2.9 Hz), 4.40-4.73 (6H, m, containing: 2-H^F δ = 4.43, dd, $J_{1,2}$ = 4.3 Hz, $J_{2,3}$ = 7.3 Hz; 2×CH_2Ph, 4-H^F), 4.99 (1H, t, 4-H^E, J = 9.5 Hz), 5.01 (1H, d, 1-H^E, $J_{1,2}$ = 8.5 Hz), 5.17 (1H, d, 1-H^F, $J_{1,2}$ = 4.2 Hz), 5.30-5.44 (2H, m, 3-H^F, 3-H^E), 6.97-7.39 (16H, m, Ph and CONH<u>H</u>'), 7.51 (1H, s, CONH<u>H</u>'), 9.17 (1H, d, NH, $J_{2,NH}$ = 8.9 Hz).- ^{13}C NMR (50 MHz, DMSO-d_6): δ = 20.5, 20.7 (2×) ($CO\underline{C}H_3$), 55.3 (C-2^E), 62.0 (C-6^E), 68.8, 72.7 (2×CH_2Ph), 69.8, 71.4, 71.9, 74.6, 78.3, 79.0, 81.2 (C-3^E, C-4^E, C-5^E, C-2^F, C-3^F, C-4^F, C-5^F), 92.8 (CCl_3), 99.9 (1-H^E), 100.3 (1-H^E), 121.1, 126.4, 127.7, 127.8, 128.0, 128.4, 129.7, 137.8, 138.1 (Ar-Cs), 150.8, 152.6 (ipso-Ar-C, carbonate-C), 161.9, 169.6, 169.7, 170.2, 170.9 ($COCCl_3$, $COCH_3$, $CONH_2$).- $C_{41}H_{43}N_2O_{16}Cl_3$ (926.16, 924.17) FAB MS: m/z 947.1 $[M+Na]^+$, 925.1 $[M+H]^+$.

Benzyl 2-*O*-(2-trichloroacetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-5-*O*-benzyl-3-*O*-phenoxycarbonyl-β-D-glucofuranosiduronamide (16b)

16b was prepared from **6c** and **15b** as described for **16a**. Yield: 83 %.- ^1H NMR (200 MHz, ^{13}C-^1H COSY, DMSO-d_6): δ = 1.92, 1.96, 1.99 (9H, 3 s, $COCH_3$), 3.69-3.96 (2H, m, 2-H^E, 5-H^E), 3.98-4.27 (3H, m, 5-H^F, CH_2-6^E), 4.40-4.59 (5H, m, CH_2Ph, C<u>H</u>$_a$H$_b$Ph', 2-H^F (δ = 4.48, s), 4-H^F), 4.81 (1H, part of an AB system, CH$_a$<u>H</u>$_b$Ph', ^2J = 11.7 Hz), 4.94 (1H, t, 4-H^E, J = 9.6 Hz), 5.137 (1H, s, 1-H^F) 5.144 (1H, d, 1-H^E, $J_{1,2}$ = 8.1 Hz), 5.28 (1H, t, 3-H^E, J = 9.6 Hz), 5.40 (1H, d, 3-H^F, $J_{3,4}$ = 4.8 Hz), 7.05-7.42 (16H, m, Ph and CONH<u>H</u>'), 7.74 (1H, s, CONH<u>H</u>'), 9.22 (1H, d, NH, $J_{2,NH}$ = 9.2 Hz).- ^{13}C NMR (50 MHz, DMSO-d_6): δ = 20.4, 20.6 (2×) ($CO\underline{C}H_3$), 54.8 (C-2^E), 61.8 (C-6^E), 68.6 (C-4^E), 69.0 (CH_2Ph), 71.3 (CH_2Ph'), 71.8 (C-5^E), 72.0 (C-3^E), 77.9 (C-5^F), 78.2 (C-3^F), 79.4 (C-4^F), 83.9 (C-2^F), 92.9 (CCl_3), 98.5 (C-1^E), 105.6 (C-1^F), 121.1, 126.6, 127.7, 127.9, 128.1, 128.4, 128.5, 130.0, 137.7, 137.9 (Ar-Cs), 150.7, 152.4 (ipso-Ar-C, carbonate-C), 162.1, 169.5, 169.6, 170.2, 171.1 ($COCCl_3$, $COCH_3$, $CONH_2$).- $C_{41}H_{43}N_2O_{16}Cl_3$ (926.16, 924.17) FAB MS: m/z 947.1 $[M+Na]^+$, 925.1 $[M+H]^+$.

1,3,4,6-Tetra-*O*-n-butyryl-2-deoxy-2-trichloroacetamido-D-glucopyranose (mixture of anomers, formula not shown)

To a solution of 2-deoxy-2-trichloroacetamido-D-glucopyranose (6.43 g, 19.8 mmol) in pyridine (110 mL) butyryric anhydride (50 mL) was added at 0°C. The mixture was heated to 20°C. After 16 h the reaction was stopped by solvent removal. Repeated FC (petroleum ether-ethyl acetate 2:1) yielded 1,3,4,6-Tetra-*O*-n-butyryl-2-deoxy-2-trichloroacetamido-D-glucopyranose (9.63 g, 80%).- ^1H NMR (200 MHz, $CDCl_3$): δ = 0.82-0.99 (12H, $COCH_2CH_2C\underline{H}_3$ signals), 1.48-1.77 (8H, $COCH_2C\underline{H}_2CH_3$ signals), 2.16-2.42 (8H, $COC\underline{H}_2CH_2CH_3$ signals), 3.95-4.36 (4H, m, 2-H, 5-H, CH_2-6), 5.17 (1H, t, 4-H^β, covered by 4-H^α), 5.23 (1H, t, 4-H^α, J = 9.7 Hz), 5.35 (1H, dd, 3-H, J = 9.5, 10.3 Hz), 5.81 (1H, d, 1-H^β, J = 8.9 Hz), 6.30 (1H, d, 1-H^α, J = 3.7 Hz), 6.89 (1H, d, NH^α, J = 8.1 Hz), 7.17 (1H, d, NH^β); ratio of anomers: α/β = 5:1 (based on the 1 H signal integrals).- ^{13}C NMR (50 MHz, $CDCl_3$): δ = 13.96 (3×), 14.00 ($COCH_2CH_2\underline{C}H_3$), 18.6 (2×), 18.7, 18.8 ($COCH_2\underline{C}H_2CH_3$), 36.20, 36.24, 36.29 (2×) ($CO\underline{C}H_2CH_2CH_3$), 54.0 (C-2), 61.7 (C-6), 67.2, 70.3, 70.5 (C-3, C-4, C-5), 89.7 (C-1), 92.2 ($CO\underline{C}Cl_3$), 162.5, 162.7 (β?) ($\underline{C}OCCl_3$), 171.5, 172.2, 173.8, 175.1

($\underline{C}OCH_2CH_2CH_3$).- $C_{24}H_{36}Cl_3NO_{10}$ (604.91, 603.14).- FAB MS: m/z 626.3 $[M+Na]^+$, 516.3 $[M+H-C_4H_8O_2]^+$.

3,4,6-Tri-*O*-n-butyryl-2-deoxy-2-trichloroacetamido-α-D-glucopyranose (formula not shown)
A solution of 1,3,4,6-tetra-*O*-n-butyryl-2-deoxy-2-trichloroacetamido-D-glucopyranose (2.17 g, 3.59 mmol) and hydrazinium acetate (583 mg, 6.3 mmol) in DMF (20 mL) was stirred at 20°C for 20 min. Ethyl acetate (80 mL) was added. The remaining solution was extracted with water, a saturated $NaHCO_3$ solution and again with water. After drying the organic layer with $MgSO_4$ and solvent removal pure 3,4,6-tri-*O*-n-butyryl-2-deoxy-2-trichloroacetamido-α-D-glucopyranose (1.64 g, 85%) was obtained.- ^1H NMR (200 MHz, $CDCl_3$): δ = 0.83-0.97 (9H, $COCH_2CH_2C\underline{H}_3$ signals), 1.45-1.73 (6H, $COCH_2C\underline{H}_2CH_3$ signals), 2.18-2.37 (6H, $COC\underline{H}_2CH_2CH_3$ signals), 4.12-4.26 (4H, m, 2-H, 5-H, CH_2-6), 5.23 (1H, t, 4-H, *J* = 9.5 Hz), 5.34 (1H, d, 1-H, *J* = 3.5 Hz), 5.41 (1H, dd, 3-H, *J* = 9.7, 10.4 Hz), 7.07 (1H, d, NH, *J* = 9.0 Hz).- ^{13}C NMR (50 MHz, $CDCl_3$): δ = 14.06, 14.11 (2×) ($COCH_2CH_2\underline{C}H_3$), 18.7, 18.8 (2×) ($COCH_2\underline{C}H_2CH_3$), 36.35, 36.42 (2×) ($CO\underline{C}H_2CH_2CH_3$), 54.8 (C-2), 62.1 (C-6), 68.1, 68.4, 70.5 (C-3, C-4, C-5), 91.4 (C-1), 92.5 ($CO\underline{C}Cl_3$), 162.5 ($\underline{C}OCCl_3$), 172.4, 174.0, 174.4 ($\underline{C}OCH_2CH_2CH_3$).- $C_{20}H_{30}Cl_3NO_9$ (534.82, 533.10).- FAB MS: m/z 556.3 $[M+Na]^+$, 516.3 $[M+H-H_2O]^+$.

***O*-(3,4,6-Tri-*O*-n-butyryl-2-deoxy-2-trichloroacetamido-D-glucopyranosyl)-trichloroacetimidate (15c)**
A mixture of 1,3,4,6-tetra-*O*-n-butyryl-2-deoxy-2-trichloroacetamido-D-glucopyranose (1.64 g, 3.07 mmol), trichloroacetonitrile (2.4 mL, 23.2 mmol), dichloromethane (12 mL) and DBU (170 μL) was stirred at 20°C for 30°min. Evaporation and FC (petroleum ether-ethyl acetate 2:1 + 0.1% NEt_3) gave **15c** (1.74 g, 83%).- ^1H NMR (200 MHz, homodecoupling, pyridine-d_5): δ = 0.80-0.89 (9H, $COCH_2CH_2C\underline{H}_3$ signals), 1.53-1.71 (6H, $COCH_2C\underline{H}_2CH_3$ signals), 2.28-2.42 (6H, $COC\underline{H}_2CH_2CH_3$ signals), 4.41-4.75 (3H, m, 5-H, CH_2-6), 5.19 (1H, ddd, 2-H, *J* = 3.1, 8.2, 10.8 Hz), 5.84 (1H, t, 4-H, *J* = 9.6 Hz), 6.04 (1H, dd, 3-H, *J* = 9.6, 10.7 Hz), 7.13 (1H, bs, 1-H, *J* = 3.0 Hz), 8.58 (1H, d, NH, *J* = 8.1 Hz), 10.64 (1H, s, =NH); impurity: 4.77-4.90 (m), 5.59 (dd, *J* = 9.5, 9.9 Hz), 9.35 (d, *J* = 8.8 Hz), 9.79 (d, *J* = 3.1 Hz).- ^{13}C NMR (50 MHz, $CDCl_3$): δ = 13.80, 13.83, 13.9 ($COCH_2CH_2\underline{C}H_3$), 18.7 (3×) ($COCH_2\underline{C}H_2CH_3$), 36.04, 36.07, 36.2 ($CO\underline{C}H_2CH_2CH_3$), 54.7 (C-2), 62.1 (C-6), 68.2, 70.9, 71.5 (C-3, C-4, C-5), 93.1, 94.5 (C-1, $CO\underline{C}Cl_3$, $NHC\underline{C}Cl_3$), 159.6 (C=NH), 163.1 ($\underline{C}OCCl_3$), 172.5, 173.3, 174.3 ($\underline{C}OCH_2CH_2CH_3$). The ^{13}C spectrum contained an other set of signals with low intensity at δ = 13.76, 36.4, 55.7, 62.9, 68.0, 69.7, 71.56 (shoulder), 91.4, 91.8, 172.7, 163.1 (shoulder), 173.4, 173.6 probably belonging to the oxazoline.- $C_{22}H_{30}Cl_6N_2O_9$ (679.21, 676.01).- FAB MS: m/z 699.3 $[M+Na]^+$, 516.3 $[M+H-Cl_3CCONH_2]^+$.-

Benzyl 2-*O*-(3,4,6-tri-*O*-n-butyryl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-5-*O*-benzyl-3-*O*-carbamoyl-α-D-glucofuranosiduronamide (16c)
16c was prepared from **6c** and **15c** as described for **16a**. Yield: 66 % after FC (petroleum ether-chloroform-acetone 1:1:1).- ^1H NMR (400 MHz, ^1H-^1H COSY, ^{13}C-^1H COSY, pyridine-d_5): δ = 0.77-0.90 (9H, $COCH_2CH_2C\underline{H}_3$ signals), 1.51-1.73 (6H, $COCH_2C\underline{H}_2CH_3$ signals), 2.16-2.45 (6H, $COC\underline{H}_2CH_2CH_3$ signals), 3.96 (1H, dt, 5-H^E, $J_{4,5}$ = 9.8 Hz), 4.42-4.59 (3H, m, CH_2-6^E, 2-H^E), 4.73 (1H, d, 5-H^F, $J_{4,5}$ = 4.9 Hz), 4.77/5.00 (2H, AB system, OCH_2Ph, ^2J = 12.1 Hz), 4.89/4.94 (2H, AB system, OCH_2Ph, the second signal was covered by the H_2O signal), ^2J = 11.6 Hz), 5.04 (1H, dd, 2-H^F, $J_{1,2}$ = 4.4 Hz, $J_{2,3}$ = 8.1 Hz), 5.41 (1H, dd, 4-H^F, $J_{3,4}$ = 7.5 Hz, $J_{4,5}$ = 5.1 Hz), 5.52 (1H, d, 1H^E, $J_{1,2}$ = 8.6 Hz), 5.55 (1H, d, 1-H^F, $J_{1,2}$ = 4.4 Hz), 5.58 (1H, d, 4-H^E, the signal was partly covered by 1-H^F, $J_{4,5}$ = 9.7 Hz), 6.15 (1H, dd, 3-H^E, *J* = 9.8, 10.2 Hz), 6.27 (1H, t, 3-H^F, *J* = 7.8 Hz), 7.09-7.35 and 7.49-7.53 (15H, 2 m, Ar-Hs), 8.24, 8.69 (2H, 2s, $CONH_2$), 10.94 (1H, d, NH, $J_{2,NH}$ = 8.3 Hz); impurities: 9.57, 9.75 (2 bs).- ^{13}C NMR (50 MHz, ^{13}C-^1H COSY, DEPT, pyridine-d_5): δ = 13.8 (2×), 14.0 ($COCH_2CH_2\underline{C}H_3$ signals), 18.66, 18.74, 18.84 ($COCH_2\underline{C}H_2CH_3$ signals), 36.04, 36.14, 36.3 ($CO\underline{C}H_2CH_2CH_3$ signals), 57.0 (C-2^E), 62.3 (C-6^E), 69.3 (C-4^E), 70.6 (OCH_2Ph), 72.1 (C-3^E), 72.9 (C-5^E), 74.4 (OCH_2Ph), 76.2 (C-4^F), 79.6 (C-3^F), 81.0 (C-5^F), 82.5 (C-2^F), 94.2 (CCl_3), 100.7, 101.4 (C-1^E, C-1^F), 121.9, 126.6, 128.1, 128.3, 128.5, 128.9, 129.1, 130.0, 138.6, 138.9 (Ar-Cs), 152.0, 154.0 (ipso-Ar-C, carbonate-C), 163.5 ($\underline{C}OCCl_3$), 172.5, 172.6, 173.3, 173.4 ($\underline{C}O(CH_2)_2CH_3$, $CONH_2$).- $C_{47}H_{55}Cl_3N_2O_{16}$ (1010.32, 1008.26).- FAB MS: m/z 1031.3 $[M+Na]^+$, 901.3 $[M+H-BnOH]^+$.

Benzyl 5-*O*-benzyl-3-*O*-(phenoxycarbonyl)-2-*O*-[(Ξ)-1,1-(3,4,6-*O*-triacetyl-α-D-glucopyranose-1-*O*, 2-*O*-diyl)ethyl]-α-D-glucofuranosiduronamide (5a)

To a suspension of **15a** (480 mg, 974 μmol) and **6b** (397 mg, 804 μmol) in dichloromethane (20 mL) a solution of TMSOTf in dichloromethane (0.5 M, 100 μL, 0.06 eq.) was added at -20°C. The mixture was stirred at -20°C for 7 h. After quenching with triethylamine (0.2 mL), solvent evaporation and FC (chloroform-ethyl acetate 2:1) furnished **5a** (505 mg, 76%).- ^{1}H NMR (400 MHz, ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, homodecoupling, pyridine-d$_5$): δ = 1.89, 1.95, 1.99, 2.01 (12H, 4 s, $COCH_3$, CH_3CO_3), 4.30-4.36 (1H, m, 5-H^E), 4.46-4.50 (2H, m, CH_2-6^E), 4.58 (1H, part of an AB system, $OC\underline{H}_aH_bPh$, ^2J = 12.0 Hz), 4.73 (1H, d, 5-H^F, $J_{4,5}$ = 5.3 Hz), 4.76-4.79 (1H, m, 2-H^E), 4.84-5.03 (4H, m, 2-H^F, $OCH_a\underline{H}_bPh$, OCH_2Ph'), 5.30-5.37 (2H, m, 4-H^F, 4-H^E), 5.48 (1H, d, 1-H^F, $J_{1,2}$ = 4.4 Hz), 5.59 (1H, t, 3-H^E, J = 2.6 Hz), 5.89 (1H, d, 1-H^E, $J_{1,2}$ = 5.1 Hz), 6.08 (1H, t, 3-H^F, J = 7.2 Hz), 7.10-7.40 and 7.49-7.55 (15H, 2 m, Ar-Hs), 8.26, 8.63 (2H, 2bs, $CONH_2$).- ^{13}C NMR (50 MHz, APT, ^{13}C-^{1}H COSY, pyridine-d$_5$): δ = 20.17, 20.20 (2×), 20.7 ($CO\underline{C}H_3$, $CO_3\underline{C}H_3$), 63.3 (C-6^E), 67.4 (C-5^E), 68.6 (C-4^F or C-4^E), 69.2 (OCH_2Ph), 70.2 (C-3^E), 73.6 (C-2^E), 73.9 (OCH_2Ph), 75.7 (C-2^F), 75.9 (C-4^F or C-4^E), 79.5 (C-3^F), 80.1 (C-5^F), 97.3 (C-1^E), 99.3 (C-1^F), 121.5 (CO_3), 121.2, 126.0, 127.7, 127.78, 127.82, 128.0, 128.3, 128.4, 128.5, 129.4, 137.7, 137.9, (Ar-Cs), 151.4, 153.4 (ipso-Ar-C, carbonate-C), 168.8, 169.5, 170.2, 171.9 ($\underline{C}OCH_3$, $CONH_2$).- $C_{41}H_{45}NO_{17}$ (823.80, 823.27), FAB MS: m/z 846.3 [M+Na]$^+$, 824.3 [M+H]$^+$, 716.2 [M+H-BnOH]$^+$, 331.1 [e]$^+$.

Benzyl 2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-5-*O*-benzyl-3-*O*-phenoxy-carbonyl-α-D-glucofuranosiduronamide (7a)

i) To a mixture of **6b** (232 mg, 470.1 μmol), **15a** (304 mg, 616.6 μmol) and 1,2-dichloroethane (5.0 mL) at 0°C $BF_3{\cdot}Et_2O$ (8.5 μL, 0.25 eq.) was added. The mixture was stirred at 0°C until the solids dissolved (15 min) and was then left at 20°C for 18 h. Triethylamine was added (0.1 mL). Evaporation at 40°C and MPLC (petroleum ether-chloroform-methanol 10:10:1) furnished **7a** (133 mg, 34%) and a fraction of not identified products (125 mg).

ii) To a mixture of **6b** (153 mg, 310.6 μmol), **15a** (167 mg, 338.2 μmol), 4 Å molecular sieves and dichloromethane (5.0 mL) $BF_3{\cdot}Et_2O$ (5.6 μL, 0.25 eq.) was added at 0°C. The reaction mixture was stirred at 0°C until solid materials dissolved (15 min). The mixture was then left at 20°C for 2 d. Pyridine (0.2 mL) was added. Evaporation at 40°C and HPLC (petrol-chloroform-methanol 10:10:1) furnished **7a** (37 mg, 34%, 9.0 min) and a fraction of not identified products (80 mg, 10.4 min).- HPLC conditions: 10 mL/min, p =1.3 MPa, λ = 254 nm.- ^{1}H NMR (400 MHz, ^{1}H-^{1}H COSY, pyridine-d$_5$): δ = 1.98, 1.99, 2.03, 2.05 (12H, 4s, $COCH_3$), 4.08 (1H, ddd, 5-H^E, $J_{4,5}$ = 10.1 Hz, $J_{5,6a}$ = 4.8 Hz, $J_{5,6b}$ = 2.6 Hz), 4.41/4.52 (2H, part of an ABX system, CH_2-6^E, ^2J = 12.3 Hz, $J_{5,6a}$ = 4.8 Hz, $J_{5,6b}$ = 2.6 Hz), 4.73 (1H, d, 5-H^F, $J_{4,5}$ = 5.3 Hz), 4.73/4.89 (2H, AB system, OCH_2Ph, ^2J = 12.1 Hz), 4.90/4.99 (2H, AB system, OCH_2Ph, ^2J = 11.6 Hz), 4.98 (1H, dd, 2-H^F, $J_{1,2}$ = 4.1 Hz, $J_{2,3}$ = 7.4 Hz), 5.20 (1H, d, 1-H^E, $J_{1,2}$ = 7.9 Hz), 5.34 (1H, dd, 4-H^F, $J_{3,4}$ = 7.5 Hz, $J_{4,5}$ = 5.3 Hz), 5.48-5.54 (3H, m, 1-H^F, 2-H^E, 4-H^E), 5.75 (1H, t, 3-H^E, J = 9.6 Hz), 6.21 (1H, t, 3-H^F, J = 7.6 Hz), 7.13-7.38 (10H, m, Ar-Hs), 7.47-7.56 (5H, m, Ar-Hs), 8.18, 8.66 (2H, 2s, $CONH_2$).- ^{13}C NMR (50 MHz, APT, ^{13}C-^{1}H COSY, pyridine-d$_5$): δ = 19.87, 19.89 (2×), 20.01 ($CO\underline{C}H_3$), 61.8 (C-6^E), 68.5 (C-4^E), 69.6 (OCH_2Ph), 71.5 (C-2^E), 72.0 (C-5^E), 72.7 (C-3^E), 73.8 (OCH_2Ph), 75.7 (C-4^F), 79.2 (C-3^F), 80.0 (C-5^F), 82.6 (C-2^F), 99.5 (C-1^E), 101.4 (C-1^F), 121.2, 126.0, 127.4, 127.6, 127.8, 127.9, 128.2, 128.4, 129.4, 137.9, 138.0 (Ar-Cs), 151.3, 153.4 (ipso-C, carbonate-C), 169.2, 169.4, 169.9, 170.2, 171.9 ($\underline{C}OCH_3$, $CONH_2$).- IR (KBr): 1757, 1688, 1372, 1251, 1073, 1042 cm^{-1}.- $C_{41}H_{45}NO_{17}$ (823.80, 823.27), FAB MS: m/z 846.1[M+Na]$^+$, 824.1 [M+H]$^+$, 716.1 [M+H-BnOH]$^+$.- HR MS: [M+H]$^+$ calc 824.2766, found 824.2794.

Benzyl 5-*O*-benzyl-3-*O*-(phenoxycarbonyl)-2-*O*-[(Ξ)-1,1-(3,4,6-*O*-triacetyl-α-D-glucopyranose-1-*O*,2-*O*-diyl)ethyl]-β-D-glucofuranosiduronamide (5b)

The reaction of **6c** and **15a** under the condition described for the formation of **5a** provided **5b** (93 %).- ^{1}H NMR (200 MHz, ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, homodecoupling, pyridine-d$_5$): δ = 1.95 (2×), 1.98, 2.02 (12H, 3 s, $COCH_3$, CH_3CO_3), 4.37 (1H, dt, 5-H^E, $J_{4,5}$ = 9.2 Hz, $J_{5,6a,6b}$ = 4.0 Hz), 4.48-4.52 (2H, m, CH_2-6^E), 4.67/5.12 (2H, AB, OCH_2Ph, ^2J = 11.7 Hz), 4.78/4.95 (2H, AB, OCH_2Ph, ^2J = 11.4 Hz), 4.80 (1H, d, 5-H^F,

J = 8.4 Hz), 4.84 (1H, dd, 2-H^E, $J_{2,3}$ = 2.9 Hz), 5.04 (1H, bs ($w_{½}$ = 5.6Hz), 2-H^F), 5.25 (1H, dd, 4-H^F, $J_{3,4}$ = 5.1 Hz, $J_{4,5}$ = 8.4 Hz), 5.35 (1H, dd, 4-H^E, $J_{3,4}$ = 2.9 Hz, $J_{4,5}$ = 9.2 Hz), 5.44 (1H, s, 1-H^F), 5.63 (1H, t, 3-H^E, J = 2.9 Hz), 5.79 (1H, dd, 3-H^F, $J_{2,3}$ = 2.2 Hz, $J_{3,4}$ = 5.1 Hz), 6.19 (1H, d, 1-H^E, $J_{1,2}$ = 5.1 Hz), 7.13-7.55 (15H, m, Ar-Hs), 8.43, 8.53 (2H, 2bs, $CONH_2$).- ^{13}C NMR (50 MHz, APT, ^{13}C-^{1}H COSY, pyridine-d_5): δ = 19.27, 19.34 (2×), 21.1 ($CO\underline{C}H_3$, $CO_3\underline{C}H_3$), 62.4 (C-6^E), 66.9 (C-5^E), 67.5 (C-4^E), 68.9 (OCH_2Ph), 69.5 (C-3^E), 71.6 (OCH_2Ph), 73.0 (C-2^E), 78.5 (C-5^F), 78.9 (C-2^F), 79.0 (C-4^F), 79.3 (C-3^F), 96.6 (C-1^E), 105.7 (C-1^F), 121.2 (CO_3), 120.2, 125.2, 126.8, 127.0, 127.2, 127.3, 127.4, 127.5, 127.7, 128.6, 137.0 (Ar-Cs), 150.4, 152.2 (ipso-Ar-C, carbonate-C), 168.1, 168.7, 169.3, 171.4 ($\underline{C}OCH_3$, $CONH_2$).- IR ($CHCl_3$): 1753, 1699, 1371, 1251, 1045 cm^{-1}.- $C_{41}H_{45}NO_{17}$ (823.80, 823.27), FAB MS: m/z 862.0 $[M+K]^+$, 846.2 $[M+Na]^+$, 824.2 $[M+H]^+$, 331.1 $[e]^+$.

Glycosylation of 6c with 15a

To a mixture of **6c** (575 mg, 1.16 mmol) and **15a** (620 mg, 1.26 mmol) in dichloromethane (10.0 mL) $BF_3{\cdot}Et_2O$ (46 μL, 0.29 mmol, 0.25 eq.) was added (TLC indicated the immediate formation of **5b**). The reaction mixture was stirred at 20°C for 27 h. Triethylamine (0.2 mL) was added. After solvent evaporation and MPLC (chloroform-ethyl acetate = 1:1) **7b** (164 mg, 17%), **8** (117 mg, 19%) and **9a** (334 mg, 29%) were obtained.

Benzyl 2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-5-*O*-benzyl-3-*O*-phenoxycarbonyl-β-D-glucofuranosiduronamide (7b)

^{1}H NMR (400 MHz, ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, pyridine-d_5): δ = 1.96, 2.00, 2.02, 2.03 (12H, 4 s, $COCH_3$), 4.09 (1H, ddd, 5-H^E, $J_{4,5}$ = 9.9 Hz, $J_{5,6a}$ = 2.4 Hz, $J_{5,6b}$ = 4.3 Hz), 4.28/4.49 (2H, part of an ABX system, CH_2-6^E, $J_{5,6a}$ = 2.2 Hz, $J_{5,6b}$ = 4.3 Hz, $J_{6a,6b}$ = 12.3 Hz), 4.68/5.14 (2H, AB system, OCH_2Ph, ^2J = 11.8 Hz, signal at 5.14 ppm covered by 2-H^F-signal), 4.80/4.96 (2H, AB system, OCH_2Ph, ^2J = 11.3 Hz), 4.83 (1H, d, 5-H^F, $J_{4,5}$ = 8.5 Hz), 5.16 (1H, bs, 2-H^F, partly hidden), 5.19 (1H, dd, 4-H^F, $J_{3,4}$ = 5.3 Hz, $J_{4,5}$ = 8.4 Hz), 5.41 (1H, d, 1-H^E, $J_{1,2}$ = 8.0 Hz), 5.46-5.56 (3H, m, containing: δ = 5.48, dd, 2-H^E, δ = 5.53, t, 4-H^E, δ = 5.56, s, 1-H^F), 5.76 (1H, t, 3-H^E, J = 9.5 Hz), 5.81 (1H, dd, 3-H^F, $J_{2,3}$ = 1.5 Hz, $J_{3,4}$ = 5.3 Hz), 7.16-7.34 and 7.47-7.55 (15H, 2m, Ar-Hs), 8.45, 8,57 (2H, 2 bs, $CONH_2$); 4.36-4.45 (m, impurity).- ^{13}C NMR (50 MHz, ^{13}C-^{1}H COSY, pyridine-d_5): δ = 20.6 (3×) and 20.7 ($CO\underline{C}H_3$), 62.2 (C-6^E), 69.0 (C-4^E), 70.4 (OCH_2Ph), 72.0 (C-2^E), 72.7 (C-5^E), 73.0 (OCH_2Ph'), 73.5 (C-3^E), 79.5 (C-5^F), 80.0 (C-3^F), 80.5 (C-4^F), 86.3 (C-2^F), 100.8 (C-1^E), 106.8 (C-1^F), 121.8, 126.9, 128.3, 128.4, 128.6, 128.7, 129.0, 130.1, 130.2, 138.51, 138.52 (Ar-Cs), 151.9, 153.8 (ipso-Ar-C, carbonate-C), 170.0, 170.1, 170.6, 170.8, 172.9 ($\underline{C}OCH_3$, $CONH_2$).- $C_{41}H_{45}NO_{17}$ (823.80, 823.27), FAB MS: m/z 846.1 $[M+Na]^+$, 824.1 $[M+H]^+$, 716.1 $[M+H\text{-}BnOH]^+$, 331.0 $[e]^+$.- HR MS: $[M+H]^+$ calc 824.2766, found 824.2775.

Benzyl 2-*O*-acetyl-5-*O*-benzyl-3-*O*-phenoxycarbonyl-β-D-glucofuranosiduronamide (8)

M.p. 88°C.- ^{1}H NMR (200 MHz, pyridine-d_5): δ = 1.92 (3H, s, $COCH_3$), 4.74/5.19 (2H, AB system, OCH_2Ph, ^2J = 12.0 Hz), 4.82/4.96 (2H, AB system, OCH_2Ph, ^2J = 12.8 Hz, signal at 4.96 ppm covered by the water peak), 4.88 (1H, d, 5-H, $J_{4,5}$ = 8.8 Hz), 5.27 (1H, dd, 4-H, $J_{3,4}$ = 5.3 Hz, $J_{4,5}$ = 8.8 Hz), 5.46 (1H, s, 1-H), 5.84 (1H, bs, 2-H), 5.94 (1H, dd, 3-H, $J_{2,3}$ = 1.3 Hz, $J_{3,4}$ = 5.3 Hz), 7.05-7.34 and 7.46-7.60 (15H, 2 m, Ar-Hs), 8.58, 8.63 (2H, 2 s, $CONH_2$).- ^{13}C NMR (50 MHz, pyridine-d_5): δ = 20.0 ($CO\underline{C}H_3$), 69.6, 72.1, 78.9, 79.1, 80.1, 80.5 (C-2^F, C-3^F, C-4^F, C-5^F, 2×OCH_2Ph), 105.4 (C-1^F), 121.1, 126.1, 127.6, 127.7, 127.8, 128.0, 128.3, 129.4, 137.7, 137.8 (Ar-Cs), 151.2, 152.9 (ipso-Ar-C, carbonate-C), 169.2, 172.3 ($\underline{C}OCH_3$, $CONH_2$).- $C_{29}H_{29}NO_9$ (535.55, 535.18), FAB MS: m/z 558.1 $[M+Na]^+$, 536.1 $[M+H]^+$, 428.1 $[M+H\text{-}BnOH]^+$.- HR MS: $[M+H]^+$ calc 536.1921, found 536.1913.

Benzyl 2-*O*-(3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-5-*O*-benzyl-3-*O*-phenoxycarbonyl-β-D-glucofuranosiduronamide (9a)

^{1}H NMR (400 MHz, ^{1}H-^{1}H COSY, DMSO-d_6): δ = 1.83, 1.95, 1.97 (9H, 3 s, $COCH_3$), 3.63 (1H, ddd, 2-H^E, $J_{1,2}$ = 3.8 Hz, $J_{2,3}$ = 9.9 Hz, $J_{2,OH}$ = 6.9 Hz), 3.93-3.98 (2H, m, 5-H^E, $C\underline{H}_a\underline{H}_b$-6^E), 4.07-4.13 (2H, m, 5-H^F, $J_{4,5}$ = 9.4 Hz, $CH_a\underline{H}_b$-6^E), 4.41 (1H, bs, 2-H^F), 4.44/4.57 (2H, AB system, OCH_2Ph, ^2J = 11.5 Hz), 4.53/4.81

(2H, AB system, OCH_2Ph, ^2J = 11.8 Hz), 4.64 (1H, dd, 4-H^F, $J_{3,4}$ = 5.1 Hz, $J_{4,5}$ = 9.2 Hz), 4.80 (1H, t, 4-H^E, J = 9.6 Hz), 5.05-5.09 (2H, m, 1-H^E, 3-H^E), 5.23 (1H, s, 1-H^F), 5.38 (1H, dd, 3-H^F, $J_{2,3}$ = 1.0 Hz, $J_{3,4}$ = 5.1 Hz), 5.45 (1H, d, OH, $J_{2,OH}$ = 6.8 Hz, exchangeable with D_2O), 7.01-7.05 and 7.29-7.41 (15H, 2 m, Ar-Hs) , 7.43, 7.73 (2H, 2 bs, $CONH_2$).- ^{1}H NMR (400 MHz, ^{1}H-^{1}H COSY, pyridine-d_5): δ = 1.98, 2.01, 2.04 (9H, 3 s, $COCH_3$), 4.19-4.26 (1H, m, 2-H^E), 4.38-4.43 (1H, m, $C\underline{H}_a\underline{H}_b$-6^E), 4.51-4.58 (3H, m, 5-H^E, $OC\underline{H}_a\underline{H}_bPh$, $CH_a\underline{H}_b$ -6^E), 4.77/4.94 (2H, AB, $OC\underline{H}_2Ph$, signal at 4.94 ppm hidden by the water signal), 4.79 (1H, d, 5-H^F, $J_{1,2}$ = 8.5 Hz), 5.11 (1H, bs ($w_{½}$ = 4.2 Hz), 2-H^F), 5.18 (1H, d, $OCH_a\underline{H}_bPh$, ^2J = 11.6 Hz), 5.19 (1H, dd, 4-H^F, $J_{3,4}$ = 5.8 Hz, $J_{4,5}$ = 8.4 Hz), 5.47 (1H, dd, 4-H^E, J = 9.6 and 9.9 Hz), 5.56 (1H, d, 1-H^F, $J_{1,2}$ = 1.4 Hz), 5.65 (1H, d, 1-H^E, $J_{1,2}$ = 3.8 Hz), 5.95 (2H, dd and t, 3-H^F, $J_{2,3}$ = 1.9 Hz, $J_{3,4}$ = 5.6 Hz; 3-H^E, J = 9.6 Hz), 7.14-7.33 and 7.47-7.51 (15H, 2 m, Ar-Hs), 8.32, 8.56 (2H, 2 s, $CONH_2$); impurity: 5.68 (CH_2Cl_2).- ^{13}C NMR (50 MHz, APT, pyridine-d_5): δ = 21.0, 20.8, 20.9 ($CO\underline{C}H_3$), 62.9 (C-6^E), 70.4, 73.1 (2×OCH_2Ph), 69.4, 69.8, 70.8, 74.2, 80.1, 80.4, 80.7, 85.8 (C-2^E, C-3^E, C-4^E, C-5^E, C-2^F, C-3^F, C-4^F, C-5^F), 100.3 (C-1^E), 106.8 (C-1^F), 121.8, 126.8, 128.2, 128.4, 128.58, 128.62, 128.98, 129.03, 130.1, 138.5, 138.6 (Ar-Cs), 151.9, 153.7 (ipso-Ar-C, carbonate-C), 170.3, 170.9, 171.0, 172.9 ($\underline{C}OCH_3$, $CONH_2$).- IR ($CHCl_3$): 1749, 1698, 1258, 1237, 1225, 1074, 1039 cm^{-1}.- $C_{39}H_{43}NO_{16}$ (781.77, 781.26), FAB MS: m/z 804.2 $[M+Na]^+$, 782.2 $[M+H]^+$, 674.1 $[M+H-BnOH]^+$.- HR MS: $[M+H]^+$ calc 782.2660, found 782.2663.

Benzyl 2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-5-*O*-benzyl-3-*O*-phenoxycarbonyl-α-D-glucofuranosiduronamide (9b)

9a (55 mg, 70 mmol) was acetylated with acetic anhydride in pyridine. Solvent evaporation and MPLC (petroleum ether-chloroform-methanol 10:10:1) yielded **9b** (59 mg, 98%).- ^{1}H NMR (400 MHz, ^{1}H-^{1}H COSY, pyridine-d_5): δ = 1.95, 2.00, 2.02, 2.04 (12H, 4s, $COCH_3$), 4.35-4.45 (1H, m, $C\underline{H}_a\underline{H}_b$-6^E) 4.49-4.57 (2H, m, 5-H^E, $CH_a\underline{H}_b$-6^E), 4.70/5.20 (2H, AB system, OCH_2Ph, ^2J = 11.7 Hz), 4.80 (1H, d, 5-H^F, $J_{4,5}$ = 8.0 Hz), 4.81/4.94 (2H, AB system, OCH_2Ph, (signal at 4.94 ppm was partly covered by the water peak), ^2J = 11.3 Hz), 5.11 (1H, dd, 2-H^F, $J_{1,2}$ = 1.9 Hz, $J_{2,3}$ = 2.4 Hz), 5.32 (1H, dd, 4-H^F, $J_{4,5}$ = 8.2 Hz, $J_{3,4}$ = 6.0 Hz), 5.36 (1H, dd, 2-H^E , $J_{1,2}$ = 4.0 Hz, $J_{2,3}$ = 10.3 Hz), 5.528 (1H, d, 1-H^F, $J_{1,2}$ = 1.7 Hz), 5.535 (1H, t, 4-H^E, $J_{3,4}$ = 9.6 Hz, $J_{4,5}$ = 9.9 Hz), 5.82 (1H, d, 1-H^E, $J_{1,2}$ = 4.0 Hz), 5.93-5.99 (2H, m, containing: δ = 5.95, dd, 3-H^F J = 2.6 Hz; δ = 5.96, t, 3-H^E, J = 9.9 Hz), 7.14-7.37 and 7.49-7.55 (15H, 2 m, Ar-Hs), 8.35, 8.61 (2H, 2 bs, $CONH_2$).- ^{13}C NMR (50 MHz, pyridine-d_5): δ = 19.8, 19.9, 20.00, 20.04 ($CO\underline{C}H_3$), 61.7 (C-6^E), 68.4, 68.5, 69.8, 70.1, 70.7, 72.6, 79.59, 79.62, 79.68, 84.8 (C-2^E, C-3^E, C-4^E, C-5^E, C-2^F, C-3^F, C-4^F, C-5^F, 2×OCH_2Ph), 96.1 (C-1^E), 105.5 (C-1^F), 121.1, 127.97, 128.03, 128.36, 128.37, 129.4, 137.75, 137.81 (Ar-Cs), 151.2, 153.1 (ipso-Ar-C, carbonate-C), 169.5, 169.9, 170.2, 172.3 ($\underline{C}OCH_3$, $CONH_2$).- $C_{41}H_{45}NO_{17}$ (823.80, 823.27), FAB MS: m/z 846.1 $[M+Na]^+$, 824.1 $[M+H]^+$, 716.1 $[M+H-BnOH]^+$.- HR MS: $[M+H]^+$ calc 824.2766, found 824.2758.

Benzyl 2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-5-*O*-benzyl-3-*O*-carbamoyl-α-D-glucofuranosiduronamide (7c)

Ammonia was slowly bubbled into a solution of **7a** (133 mg, 161.1 μmol) in dry THF (10 mL) for 18 h at 20°C. After solvent removal and FC (chloroform-methanol 10:1) **7c** (76 mg, 63%) and a fraction of compounds with an unprotected OH-function (32 mg) were obtained. The yield of **7c** increased to 85% after acetylation of the 32 mg fraction.- ^{1}H NMR (400 MHz, ^{1}H-^{1}H COSY, homodecoupling, pyridine-d_5): δ = 1.97, 1.99, 2.01, 2.06 (12H, 4s, $COCH_3$), 3.96 (1H, ddd, 5-H^E, $J_{4,5}$ = 9.9 Hz, $J_{5,6a}$ = 4.6 Hz, $J_{5,6b}$ = 2.6 Hz), 4.36/4.49 (2H, part of an ABX system, CH_2-6^E, ^2J = 12.3 Hz, $J_{5,6a}$ = 4.6 Hz, $J_{5,6b}$ = 2.5 Hz), 4.64 (1H, d, 5-H^F, $J_{4,5}$ = 5.3 Hz), 4.69/4.87 (2H, AB system, OCH_2Ph, ^2J = 12.3 Hz), 4.84 (2H, s, OCH_2Ph), 4.95 (1H, dd, 2-H^F, $J_{1,2}$ = 4.4 Hz, $J_{2,3}$ = 7.2 Hz), 5.18 (1H, d, 1-H^E, $J_{1,2}$ = 8.0 Hz), 5.20 (1H, dd, 4-H^F, $J_{3,4}$ = 7.4 Hz, $J_{4,5}$ = 5.3 Hz), 5.45-5.53 (3H, m, containing: δ = 5.472, dd, 4-H^E, J = 9.4, 9.9 Hz, δ = 5.474, d, 1-H^F, J = 4.3 Hz, δ = 5.51 dd, 2-H^E, J = 8.0, 9.6 Hz), 5.66 (1H, t, 3-H^E, $J_{2,3}$ = 9.5 Hz), 6.26 (1H, t, 3-H^F, J = 7.3 Hz), 7.14–7.36 and 7.46–7.54 (10H, 2m, Ar-Hs), 7.71 (2H, s, $OCONH_2$), 7.93, 8.47 (2H, 2s, $CONH_2$).- ^{13}C NMR (50 MHz, ^{13}C-^{1}H COSY, pyridine-d_5): δ = 19.2 (3×), 19.4 ($CO\underline{C}H_3$), 61.1 (C-6^E), 67.9 (C-4^E), 68.7 (OCH_2Ph), 70.7 (C-2^E), 71.1 (C-5^E), 72.2 (C-3^E), 72.8 (OCH_2Ph), 73.9 (C-3^F), 76.0 (C-4^F), 79.4 (C-5^F), 82.2 (C-2^F), 99.1 (C-1^E), 100.6 (C-1^F), 126.5, 126.7, 127.0, 127.4, 127.6, 137.2, 137.5 (Ar-Cs), 156.0

($OCONH_2$), 168.4, 168.6, 169.1, 169.3, 171.5 ($\underline{C}OCH_3$, $CONH_2$).- IR ($CHCl_3$): 1751, 1689, 1376, 1231, 1211, 1042 cm^{-1}.- $C_{35}H_{42}N_2O_{16}$ (746.72, 746.25), FAB MS: m/z 769.2 $[M+Na]^+$, 747.2 $[M+H]^+$, 639.1 $[M+H\text{-}BnOH]^+$.- HR MS: $[M+H]^+$ calc 747.2613, found 747.2607.

Benzyl 2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-5-*O*-benzyl-3-*O*-carbamoyl-β-D-glucofuranosiduronamide (7d)

Ammonia was slowly bubbled into a solution of **7b** (156 mg, 316 μmol) in dry THF (10.0 mL) for 18 h at 20°C. After solvent removal and FC (chloroform-methanol 10:1) **7d** (58 mg, 41%) was isolated.- ^1H NMR (600 MHz, pyridine-d_5): δ = 1.96, 1.97, 1.99, 2.01 (12H, 4s, $COCH_3$), 4.09 (1H, ddd, 5-H^E, $J_{4,5}$ = 10.1 Hz, $J_{5,6a}$ = 2.4 Hz, $J_{5,6b}$ = 4.1 Hz), 4.27/4.51 (2H, part of an ABX system, CH_2-6^E, $J_{5,6a}$ = 2.3 Hz, $J_{5,6b}$ = 4.3 Hz, $J_{6a,6b}$ = 12.3 Hz), 4.69/5.18 (2H, AB system, OCH_2Ph, ^2J = 12.0 Hz), 4.71/4.79 (2H, AB system, OCH_2Ph, ^2J = 10.7 Hz), 4.72 (1H, d, 5-H^F, J = 9.0 Hz), 4.98-5.04 (4-H^F and 2-H^F, hidden by the H_2O signal), 5.45-5.53 (4H, m, containing: δ = 5.46, d, 1-H^E, J = 8.0 Hz and δ = 5.51, s, 1-H^F and 2-H^E, 4-H^E), 5.73 (1H, t, 3-H^E, J = 9.4 Hz), 5.85 (1H, d, 3-H^F, J = 6.0 Hz), 7.21-7.30 and 7.47-7.53 (10H, 2 m, Ar-Hs), 7.90 (2H, s, $OCONH_2$), 8.22, 8.44 (2H, 2 bs, $CONH_2$); impurity: 3.78-3.81 (m), 3.99-4.02 (m).- ^{13}C NMR (50 MHz, pyridine-d_5): δ = 19.0, 19.1 (2×), 19.2 ($CO\underline{C}H_3$), 60.7 (C-6^E), 67.5, 68.7, 70.6, 71.1, 71.6, 72.1, 74.3, 78.4, 79.6, 85.7 (C-2^E, C-3^E, C-4^E, C-5^E, C-2^F, C-3^F, C-4^F, C-5^F, 2×OCH_2Ph), 99.2 (C-1^E), 105.7 (C-1^F), 126.6, 126.9, 127.1, 127.4, 137.1, 137.2 (Ar-Cs), 156.1 ($OCONH_2$), 168.5, 168.6, 169.1, 169.3, 172.1 ($\underline{C}OCH_3$, $CONH_2$).- IR ($CHCl_3$): 1752, 1689, 1231, 1070, 1042 cm^{-1}.- $C_{35}H_{42}N_2O_{16}$ (746.72, 746.25), FAB MS: m/z 769.2 $[M+Na]^+$, 747.2 $[M+H]^+$, 639.1 $[M+H\text{-}BnOH]^+$, 331.1 $[e]^+$.- HR MS: $[M+H]^+$ calc 747.2613, found 747.2618.

2-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-3-*O*-carbamoyl-α-D-glucopyranuronamide (17a)

i) A mixture of **7c** (116 mg, 155.5 μmol) and $Pd(OH)_2/C$ (142 mg, 20%) in THF-acetic acid (10:1, 10 mL) was stirred in a hydrogen atmosphere for 18 h at 20°C. After filtration the catalyst was carefully washed with acetone. Solvent removal from the combined solutions and lyophilization provided **17a** (85 mg, 96%).

ii) A mixture of **7c** (57 mg, 76.5 μmol) and Pd/C (89 mg, 10%) in aqueous THF (4.5 mL) was stirred in a hydrogen atmosphere for 12 h at 20°C. After filtration the catalyst was carefully washed with THF. Solvent evaporation from the combined solutions and FC (chloroform-methanol 4:1) furnished **17a** (40 mg, 91%).- ^1H NMR (600 MHz, ^1H-^1H COSY, ^{13}C-^1H COSY, pyridine-d_5, containing a trace of the β-anomer): δ = 1.92, 1.98, 2.01, 2.31 (12H, 4s, $COCH_3$), 3.91 (1H, ddd, 5-H^E, $J_{4,5}$ = 9.9 Hz, $J_{5,6a}$ = 4.7 Hz, $J_{5,6b}$ = 2.4 Hz), 4.14 (1H, dd, 2-H^F, $J_{1,2}$ = 3.4 Hz, $J_{2,3}$ = 10.1 Hz), 4.34 (1H, part of an ABX system, $C\underline{H_a}H_b$-6^E, $J_{5,6}$ = 2.2 Hz, $J_{6a,6b}$ = 12.1 Hz), 4.43-4.50 (2H, m, $CH_a\underline{H_b}$-6^E, 4-H^F), 5.15 (1H, d, 1-H^E, $J_{1,2}$ = 8.0 Hz), 5.17 (1H, d, 5-H^F, $J_{4,5}$ = 9.9 Hz), 5.40 (1H, t, 4-H^E, J = 9.8 Hz), 5.51 (1H, dd, 2-H^E, $J_{1,2}$ = 8.2 Hz, $J_{2,3}$ = 9.5 Hz), 5.64 (1H, t, 3-H^E, J = 9.6), 6.00 (1H, d, 1-H^F, $J_{1,2}$ = 3.4 Hz), 6.27 (1H, t, 3-H^F, J = 9.6 Hz), 7.52 (2H, bs, $OCONH_2$), 8.50, 8.55 (2H, 2 bs, $CONH_2$).- ^{13}C NMR (50 MHz, ^{13}C-^1H COSY, pyridine-d_5): δ = 19.96 (2×), 20.05, 20.1 ($CO\underline{C}H_3$), 61.9 (C-6^E), 68.7 (C-4^E), 71.17 (C-5^F), 71.24 (C-2^E), 71.7 (C-5^E), 72.1 (C-4^F), 73.3 (C-3^E), 74.2 (C-3^F), 79.8 (C-2^F), 93.1 (C-1^F), 102.2 (C-1^E), 157.7 ($OCONH_2$), 169.5, 169.8, 170.1, 170.2, 173.9 ($\underline{C}OCH_3$, $CONH_2$).- $C_{21}H_{30}N_2O_{16}$ (566.47, 566.16), FAB MS: m/z 589.1 $[M+Na]^+$, 567.1, $[M+H]^+$.- HR MS: $[M+Na]^+$ calc 589.1493, found 589.1493.

1,4-Di-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-*O*-carbamoyl-α-D-glucopyranuronamide (17b)

A mixture of **7d** (48 mg, 64.7 μmol) and $Pd(OH)_2/C$ (51 mg, 20%) in THF-acetic acid (10:1, 5 mL) was stirred in a hydrogen atmosphere for 18 h at 20°C. After filtration the catalyst was carefully washed with THF. After solvent evaporation from the combined solutions the crude product was treated with a mixture of pyridine and acetic anhydride (1:1, 1 mL), and the solution was stirred overnight. Solvent removal by lyophilization and FC (chloroform-methanol 10:1) yielded **17b** (42 mg, 100%).- ^1H NMR (200 MHz, homodecoupling, ^1H-^1H COSY, ^{13}C-^1H COSY, pyridine-d_5): δ = 1.82, 1.98, 2.01, 2.07, 2.11, 2.23 (18H, 6s, $COCH_3$), 3.97 (1H, dt, 5-H^E, $J_{4,5}$ = 9.9 Hz, $J_{5,6}$ = 3.3 Hz), 4.26 (1H, dd, 2-H^F, $J_{1,2}$ = 3.8 Hz, $J_{2,3}$ = 9.7 Hz), 4.43-4.48 (2H, m, CH_2-6^E), 4.81 (1H, d, 5-H^F, $J_{4,5}$ = 9.7 Hz), 5.09 (1H, d, 1-H^E, $J_{1,2}$ = 7.7 Hz), 5.44 (2H, t,

2-H^E, 4-H^E, J = 9.2 Hz), 5.64 (1H, t, 3-H^E, J = 9.4 Hz), 5.90 (1H, dd, 4-H^F, $J_{3,4}$ = 9.5 Hz, $J_{4,5}$ = 9.9 Hz), 6.02 (1H, t, 3-H^F, J = 9.6 Hz), 6.83 (1H, d, 1-H^F, $J_{1,2}$ = 3.9 Hz), 7.79 (2H, bs, OCONH$_2$), 8.32, 8.49 (2H, 2 bs, CONH$_2$); impurity: 5.68 (CH_2Cl_2).- ^{13}C NMR (50 MHz, ^{13}C-^{1}H COSY, pyridine-d$_5$): δ = 19.88, 19.92, 19.95, 20.05, 20.13, 20.4 (CO<u>C</u>H$_3$), 61.6 (C-6^E), 68.4 (C-4^E), 70.1 (C-4^F), 70.9 (C-3^F), 71.1 (C-2^F, C-5^F), 71.7 (C-5^E), 72.8 (C-3^E), 76.9 (C-2^F), 90.6 (C-1^F), 101.7 (C-1^E), 156.8 (OCONH$_2$), 168.9, 169.42, 169.47, 169.55, 170.0, 170.4 (<u>C</u>OCH$_3$, CONH$_2$).- IR ($CHCl_3$): 1755, 1705, 1373, 1228, 1211, 1077, 1041 cm^{-1}.- $C_{25}H_{34}N_2O_{18}$ (650.55, 650.18), FAB MS: m/z 673.1 [M+Na]$^+$, 651.2 [M+H]$^+$, 591.1 [M+H-AcOH]$^+$.- HR MS: [M+Na]$^+$ calc 673.1704, found 673.1718.

2-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-*O*-acetyl-3-*O*-carbamoyl-D-glucopyranuronamide (mixture of anomers) (17c)

A mixture of **17b** (46 mg, 70.68 μmol), hydrazinium acetate (9.3 mg, 100 μmol, 1.4 eq.) and DMF (0.4 mL) was stirred at 20°C for 30 min. Ethyl acetate (0.3 mL), water (0.2 mL) and toluene (3 mL) were added. Solvent removal and FC (chloroform-methanol 10:1) furnished **17c** (26.4 mg, 67%) and a fraction containing non-identified products (5.6 mg).- ^{1}H NMR (400 MHz, homodecoupling, ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, pyridine-d$_5$, containing a trace of the β-anomer): δ = 1.94, 1.98, 2.02, 2.08, 2.28 (15H, 5s, COCH$_3$), 3.94 (1H, ddd, 5-H^E, $J_{4,5}$ = 10.1 Hz, $J_{5,6a}$ = 2.6 Hz, $J_{5,6b}$ = 4.8 Hz), 4.13 (1H, dd, 2-H^F, $J_{1,2}$ = 3.4 Hz, $J_{2,3}$ = 10.1 Hz), 4.36/4.48 (2H, part of an ABX system, CH$_2$-6^E, $J_{5,6a}$ = 2.6 Hz, $J_{5,6b}$ = 4.8 Hz, $J_{6a,6b}$ = 12.3 Hz), 5.12 (1H, d, 1-H^E, $J_{1,2}$ = 8.0 Hz), 5.17 (1H, d, 5-H^F, $J_{4,5}$ = 10.3 Hz), 5.40 (1H, dd, 4-H^E, $J_{4,5}$ = 10.1 Hz, $J_{3,4}$ = 9.4 Hz), 5.48 (1H, dd, 2-H^E, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.6 Hz), 5.64 (1H, t, 3-H^E, J = 9.5 Hz), 5.89 (1H, dd, 4-H^F, $J_{3,4}$ = 9.6 Hz, $J_{4,5}$ = 10.1 Hz), 5.96 (1H, d, 1-H^F, $J_{1,2}$ = 3.3 Hz), 6.24 (1H, t, 3-H^F, J = 9.7 Hz), 7.63 (2H, bs, OCONH$_2$), 8.18, 8.32 (2H, 2 bs, CONH$_2$).- ^{13}C NMR (50 MHz, ^{13}C-^{1}H COSY, pyridine-d$_5$): δ = 20.7 (2×), 20.8 (2×), 21.3 (COCH$_3$), 62.6 (C-6^E), 69.4 (C-4^E), 69.6 (C-5^F), 71.95 (C-2^E), 72.02 (C-4^F), 72.5 (C-3^F, C-5^E), 73.9 (C-3^E), 80.0 (C-2^F), 93.5 (C-1^F), 102.8 (C-1^E), 157.7 (OCONH$_2$), 170.1, 170.3, 170.4, 170.6, 170.8, 171.7 (<u>C</u>OCH$_3$, CONH$_2$).- $C_{23}H_{32}N_2O_{17}$ (608.51, 608.17), FAB MS: m/z 631.0 [M+Na]$^+$.

2-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-*O*-acetyl-3-*O*-carbamoyl-1-*O*-{[(*R*)-2-methoxycarbonyl-2-(3,8,8,11,14,18-hexamethylnonadecyloxy)-ethoxy]-(2,2,2-trichlor-1,1-dimethylethyloxy)-phosphoryl}-α-D-glucopyranuronamide (20)

To a solution of 1H-1,2,4-triazol (44.6 mg, 660.7 μmol) in 1:4 pyridine-dichloromethane (1.2 mL) 2,2,2-trichloro-1,1-dimethylethyl dichlorophosphite (32 μL, 161.7 μmol) was added at 0°C, and the mixture was stirred at 0°C for 30 min. A solution of **17c** (78 mg, 127.5 μmol) in pyridine-dichloromethane (1:1.8, 2.5 mL) was added dropwise. Stirring was continued for 90 min at 0°C. Within 90 min a solution of **22** (181 mg, 384 μmol) in pyridine-dichloromethane (1:4, 2 mL) was added. After another 3 h stirring at 0°C bis(trimethylsilyl)-peroxide (56 μL, 267.4 μmol) was added, and the mixture stirred at 20°C overnight. After solvent removal and FC (chloroform-methanol 20:1) two P-diastereomers of **20** (unpolar product: 51 mg, 31%), polar product: 13 mg, 8%) and a fraction containing both diastereomers (40 mg, 23%) were obtained.

20 (unpolar diastereomer)

^{1}H NMR (400 MHz, ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, pyridine-d$_5$): δ = 0.87-0.97 (23H, m, signals of the lipid part), 1.10-1.60 (32H, m, signals of the lipid part), 1.99, 2.02, 2.07, 2.12, 2.25 (15H, 5s, COCH$_3$), 2.17, 2.18 (6H, 2s, (CH$_3$)$_2$CCCl$_3$), 3.65-3.74, 3.83-3.93 (2H, 2m, CH$_2$-1^l), 3.78 (3H, s, COOCH$_3$), 3.99 (1H, ddd, 5-H^E, $J_{4,5}$ = 9.9 Hz, $J_{5,6a}$ = 2.9 Hz, $J_{5,6b}$ = 3.9 Hz), 4.21 (1H, m, 2-H^F, $J_{1,2}$ = 3.4 Hz), 4.43-4.54 (3H, m, CH$_2$-6^E, 2-H^H), 4.64-4.80 (2H, m, CH$_2$-3^H), 4.93 (5-H^F hidden by the H$_2$O peak), 4.95-5.01 (m, ?), 5.07 (1H, d, 1-H^E, $J_{1,2}$ = 8.0 Hz), 5.47 (1H, dd, 2-H^E, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.7 Hz), 5.52 (1H, t, 4-H^E, J = 9.7 Hz), 5.64 (1H, t, 3-H^E, J = 9.6 Hz), 5.91 (1H, t, 3-H^F or 4H^F, J = 7.1 Hz), 5.93 (1H, t, 3-H^F or 4H^F, J = 7.1 Hz), 6.41 (1H, dd, 1-H^F, $J_{1,2}$ = 3.6 Hz, $J_{1,P}$ = 5.6 Hz), 7.74 (2H, bs, OCONH$_2$), 8.01, 8.59 (2H, 2 bs, CONH$_2$).- ^{13}C NMR (50 MHz, ^{13}C-^{1}H COSY, APT, pyridine-d$_5$): δ = 19.3-41.1 (signals of the lipid part and protecting group signals); 52.0 (OCH$_3$), 61.7 (C-6^E), 68.2 (d, C-3^H, $J_{C,P}$ = 5.5 Hz), 68.6 (C-4^E), 69.7 (C-1^l), 69.8, 70.8 (C-5^F and C-3^F), 70.9 (C-4^F), 71.0 (C-2^E), 71.9 (C-5^E), 73.1 (C-3^E), 77.4 (d, C-2^H, $J_{C,P}$ = 10.1 Hz), 78.0 (d, C-2^F,

$J_{C,P}$ = 9.1 Hz), 90.6 (d, O<u>C</u>$(CH_3)_2CCl_3$, $J_{C,P}$ = 5.5 Hz), 97.1 (d, C-1^F, $J_{C,P}$ = 6.4 Hz), 102.1 (C-1^E), 105.6 (d, CCl_3, $J_{C,P}$ = 14.6 Hz), 156.7 ($OCONH_2$), 168.9, 169.38, 169.43, 169.5, 170.0, 170.1, 170.3 (<u>C</u>OCH_3, $CONH_2$).- ^{31}P NMR (80 MHz, pyridine-d_5): δ = – 4.03 (phosphate).- IR (KBr): 1752 cm^{-1}.- $C_{56}H_{94}Cl_3N_2O_{23}P$ (1300.69, 1298.51), FAB MS: m/z 1321.6 $[M+Na]^+$, 1299.7 $[M+H]^+$, 1179.5 $[M+K-C_4H_5Cl_3]^+$, 1163.5 $[M+Na-C_4H_5Cl_3]^+$.

20 (polar diastereomer)

^{1}H NMR (400 MHz, ^{1}H-^{1}H COSY, pyridine-d_5): δ = 0.80-1.95 (51H, m, signals of the lipid part), 2.02, 2.03, 2.04, 2.06, 2.07, 2.16, 2.27 (21H, 7s, $COCH_3$, $(CH_3)_2CCCl_3$), 3.77-3.84 (1H, m, C<u>H$_a$H$_b$</u>-1^I), 3.96-4.05 (5H, m, containing: δ = 3.96 (d, OCH_3, J ~ 2 Hz, the sample did not contain the other diastereomer, the splitting might be due to ^{1}H, ^{31}P coupling), C<u>H$_a$H$_b$</u>-1^I, 5-H^E), 4.22-4.30 (1H, m, 2-H^F), 4.35-4.44 (m, plasticizer), 4.50-4.66 (3H, m, CH_2-6^E, 2-H^H), 4.79-ca. 5.00 (2H, m, CH_2-3^H, signal partly covered by water), 4.98 (1H, d, 5-H^F, $J_{4,5}$ = 10.4 Hz), 5.12 (1H, d, 1-H^E, $J_{1,2}$ = 8.0 Hz), 5.50-5.57 (2H, m, 2-H^E, 4-H^E), 5.68 (1H, t, 3-H^E, J = 9.6 Hz), 5.84 (1H, dd, 4-H^F, $J_{3,4}$ = 9.8 Hz, $J_{4,5}$ = 10.4 Hz), 5.98 (1H, t, 3-H^F, J = 9.9 Hz), 6.51 (1H, m ($w_{½}$ = 12.8 Hz), 1-H^F), 7.78 (2H, bs, $OCONH_2$), 8.08, 8.55 (2H, 2 bs, $CONH_2$).- ^{13}C NMR (50 MHz, pyridine-d_5): δ = 19.1-41.8 (signals of the lipid part and the protecting group signals); 51.8 (OCH_3), 61.7 (C-6^E), 68.0 (C-3^H, broad signal), 68.5, 69.5, 69.6, 69.8 , 70.5 (2×), 70.9, 71.9, 72.9 (C-2^E, C-3^E, C-4^E, C-5^E, C-3^F, C-4^F, C-5^F, C-1^I), 77.5 (broad signal) and 77.8 (d, J = 8.7 Hz, C-2^H, C-2^F, assignment based on the assignment of the other stereoisomer), 90.3 (d, $(CH_3)_2$<u>C</u>Cl_3, $J_{C,P}$ = 4.5 Hz), 96.4 (d, C-1^F, $J_{C,P}$ = 5.3 Hz), 102.0 (C-1^E), 105.6 (d, CCl_3, $J_{C,P}$ = 15.5 Hz), 156.5 ($OCONH_2$), 169.35, 169.38, 169.5, 169.6, 169.9, 170.2, 170.4 ($COCH_3$, $CONH_2$).- ^{31}P NMR (80 MHz, pyridine-d_5): δ = -4.69 (phosphate).- IR (KBr): 1755 cm^{-1}.- $C_{56}H_{94}Cl_3N_2O_{23}P$ (1300.69, 1298.51), FAB MS: m/z 1321.1 $[M+Na]^+$, 1299.2 $[M+H]^+$, 1179.2 $[M+K-C_4H_5Cl_3]^+$, 1163.2 $[M+Na-C_4H_5Cl_3]^+$, 331 $[e]^+$.

2-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-*O*-acetyl-3-*O*-carbamoyl-1-*O*-{[(*R*)-2-methoxycarbonyl-2-(3,8,8,11,14,18-hexamethylnonadecyloxy)-ethoxy]-hydroxy-phosphoryl}-α-D-glucopyranuronamide (18)

A mixture of both diastereomers of **20** (23 mg, 17.6 μmol) and freshly prepared zinc-copper couple (26 mg), pyridine (1.4 mL) and 2,4-pentandione (30 μL) was stirred at 20°C for 2 h. After filtration the residue was carefully washed with pyridine and ethanol. From the combined solutions the solvent was evaporated. The crude product was dissolved in 8:1 water-ethanol and treated with Dowex *50WX2* (200 mg, H^+-form) for 20 min. The resin was removed by filtration and washed with 8:1 water-ethanol. From the combined solutions after solvent removal (lyophilization) and FC (toluene-chloroform-methanol 1:1:1) **18** (20 mg, 100%) was obtained.- ^{1}H NMR (400 MHz, ^{1}H-^{1}H COSY, pyridine-d_5): δ = 0.85-0.95, 1.10-1.70 (2m, signals of the lipid part), 2.01, 2.02, 2.04 (broad), 2.18 (broad), 2.25 (15H, 5s, $COCH_3$), 3.75-4.03 (6H, m, CH_2-1^I, $COOCH_3$ (δ = 3.87), 5-H^E), 4.07-4.11 (1H, m, 2-H^F), 4.45-4.69 (4H, m, CH_2-6^E, 2-H^H, C<u>H$_a$H$_b$</u>-3^H), 4.76-4.85 (1H, m, C<u>H$_a$H$_b$</u>-3^H, covered by an impurity signal), 5.05 (1H, d, 1-H^E, covered by the H_2O signal), 5.19 (1H, m, 5-H^F), 5.44 (2H, t (broad signal), 2-H^E, 4-H^E, J = 9.4 Hz), 5.63 (1H, t, 3-H^E, J = 9.5 Hz), 5.86 (1H, t, 4-H^F, J = 9.7 Hz), 5.93 (1H, t, 3-H^F, J = 9.5 Hz), 6.36 (1H, bs ($w_{½}$ = 14.5 Hz), 1-H^F), 7.60 (2H, bs, $OCONH_2$), 8.09, 8.30 (2H, m, $CONH_2$)- ^{13}C NMR (50 MHz, ^{13}C-^{1}H COSY, APT, pyridine-d_5): δ = 19.7-42.5 (signals of the lipid part and protecting group signals), 52.3 (OCH_3), 62.5 (C-6^E), 66.7 (C-3^H, broad signal), 69.3 (C-2^E or C-4^E), 69.6 (C-1^I), 70.0 (C-4^F), 70.8 (C-5^F), 71.9 (C-2^E or C-4^E), 72.4 (C-5^E), 72.8 (C-3^F), 73.7 (C-3^E), 78.9 (C-2^F, broad signal), 79.8 (C-2^H, broad signal), 95.3 (C-1^F, broad signal), 102.6 (C-1^E), 157.6 ($OCONH_2$), 170.0, 170.2, 170.5, 170.6, 171.2 (broad signal), 171.6, 172.3 (<u>C</u>OCH_3, <u>C</u>$OOCH_3$, $CONH_2$).- ^{31}P NMR (80 MHz, pyridine-d_5): δ = + 0.78 (phosphate).- $C_{52}H_{89}N_2O_{23}P$ (1141.25, 1140.5594), FAB MS: m/z 1185.5 $[M+2Na-H]^+$, 1179.4 $[M+K]^+$, 1163.5 $[M+Na]^+$.

Deprotection of 18

A degassed solution of **18** (58 mg, 50.65 μmol) in methanol-water (2:1, 4.0 mL) was treated with an aqueous lithium hydroxide solution (degassed, 1 mol·L^{-1}, 100 μL, 2 eq.) at 20°C. After 2 h, 3 h and 6 h further 100μL portions of the lithium hydroxide solution were added. The reaction was quenched by addition of 300 mg

Dowex 50W X2 (H^+-form). Stirring was continued for 30 min. After filtration the resin was washed with ethanol. The combined filtrates were concentrated and the remaining solution was lyophilized. MPLC (chloroform-methanol-water 18:11:1.8→18:11:2.7) and Sephadex® filtration (water) gave **1c** (29.5 mg, 63%) and the uronic acid **19** (13.3 mg, 29%).

2-*O*-(β-D-Glucopyranosyl)-3-*O*-carbamoyl-1-*O*-{[(*R*)-2-carboxy-2-(3,8,8,11,14,18-hexamethylnonadecyloxy)-ethoxy]-hydroxyphosphoryl}-α-D-glucopyranuronamide (1c)

^{13}C NMR (50 MHz, D_2O): signals of the lipid part: δ = 19.8, 20.0, 22.8, 22.9, 24.6, 25.0, 27.2, 28.0, 29.8 (*b*), 32.6, 33.2 (*b*), 34.0, 34.6, 37.4 (*b*), 37.7 (*b*), 39.5, 39.7, 42.6 (*b*) saccharide signals: δ = 60.6 (C-6^E), 69.6 (very broad signal), 71.8 (*s*), 72.9, 75.9 (very broad signal), 78.0, 95.6 (*b*, C-1^F), 104.1 (*b*, C-1^E), 159.1 (*b*, $OCONH_2$), 172.6 (*b*), 174.6? (*b*, COOH, $CONH_2$); impurities or unknown signals: 14.2, 125.5, 129.4 (weak intensity).- ^{31}P NMR (80 MHz, D_2O): δ = - 1.86 (phosphate).- $C_{41}H_{77}N_2O_{18}P$ (916.04, 916.49), FAB MS: m/z 977.4 ($[M+Na+K-H]^+$, 961.5 ($[M+2Na-H]^+$, 955.4 $[M+K]^+$, 939.5 $[M+Na]^+$.- HR MS: $[M+Na]^+$ calc 939.4807, found 939.4799.

2-*O*-(β-D-Glucopyranosyl)-3-*O*-carbamoyl-1-*O*-{[(*R*)-2-carboxy-2-(3,8,8,11,14,18-hexamethylnonadecyloxy)-ethoxy]-hydroxyphosphoryl}-α-D-glucopyranuronic acid (19)

^{13}C NMR (50 MHz, D_2O): signals of the lipid part: δ = 16.9, 22.6, 22.8, 25.6, 27.7 (broad signal), 29.8 (broad signal), 30.8, 32.6, 33.2, 37.5 (broad signal), 40.2, 40.4, 42.3; saccharide signals: δ = 62.0 (C-6^E), 72.6, 78.7, 78.8, 79.0 (no other saccharide carbon signals could be detected).- ^{31}P-NMR (80 MHz, D_2O): δ = – 2.17 (phosphate).- $C_{41}H_{76}NO_{19}P$ (918.02, 917.48), FAB MS: m/z 978.4 $[M+Na+K-H]^+$, 962.4 $[M+2Na-H]^+$, 956.4 $[M+K]^+$, 940.5 $[M+Na]^+$.- HR MS: $[M+Na]^+$ calc 940.4647, found 940.4665.

Acknowledgements - We wish to thank Dr. A. Bonnefoy (Hoechst Marion Roussel, Romainville) for the MIC determination and K. Richter for skillful assistance. Financial support by the Deutsche Forschungsgemeinschaft (Innovationskolleg *Chemisches Signal und biologische Antwort*), the Fonds der Chemischen Industrie, Hoechst Marion Roussel (Frankfurt and Romainville) is gratefully acknowledged by the Leipzig group.

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