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MOENOMYCIN A - STRUCTURE-ACTIVITY RELATIONS

SYNTHESIS OF THE D-GALACTURONAMIDE ANALOGUE OF THE SMALLEST ANTIBIOTICALLY ACTIVE DEGRADATION PRODUCT OF MOENOMYCIN A

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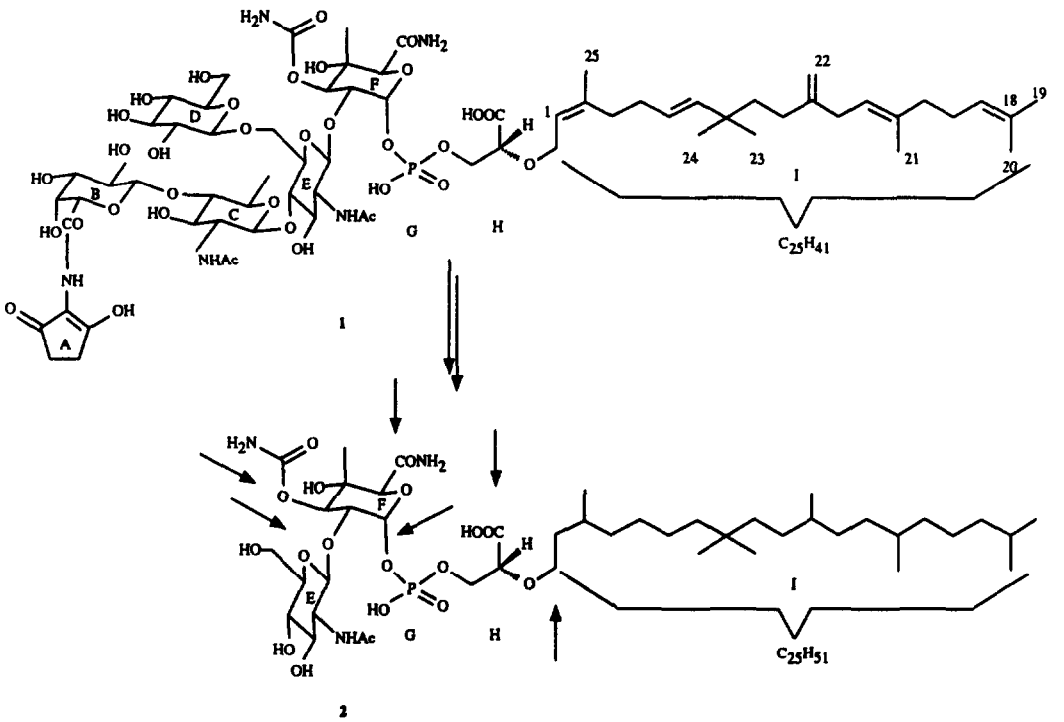
Abstract- Compound **10c** which is the galacturonamide analogue of **2**, the smallest degradation product of moenomycin A (**1**) with full antibiotic activity, has been synthesized. **10c** is devoid of antibiotic activity.

Introduction

Among the different constituents of the bacterial cell wall, the most important for the survival and integrity of the cell is peptidoglycan, a β-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-acetylglucosaminyl-N-acetylmuramyl-(pentapeptide)-pyrophosphoryl-undecaprenol are (i) the trans-glycosylation 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. With cell-free systems from *E.coli* it was demonstrated that the antibiotic moenomycin A (**1**)[2] inhibits



selectively the transglycosylation step by its inhibitory effect on penicillin-binding protein 1b (PBP 1b). Both with the cell-free systems and purified PBP 1b moenomycin A was inhibitory at concentrations between 10^{-8} and 10^{-7} mol/l.[3] Moenomycin A, its derivatives, and related antibiotics[4] belong to the rare compounds known to inhibit efficiently the transglycosylation reaction. On the contrary, a large number of antibiotics, notably the β-lactam antibiotics, are well-known inhibitors of the transpeptidation step.

A systematic stepwise degradation of moenomycin A in conjunction with assaying the degradation products for antibiotic activity both *in vivo* and in the *E.coli* cell-free system has shown, that (i) units E, F, G, H, and I are essential, (ii) the moenocinol part I may be saturated, (iii) the carboxyl group in unit H must be free and that in unit F in the carboxamide form, and (iv) the carbamoyl group in unit F must be present. Thus, compound 2 was demonstrated to be the smallest moenomycin A derivative with full antibiotic activity. The arrows in formula 2 indicate further cleavage reactions that lead to antibiotically less active or inactive compounds.[5,6,7] It appears hardly conceivable that a deeper insight into the structure - activity relations can be gained by further degradative work. Thus, compounds structurally related to 2 are needed which are prepared by chemical synthesis. One of the most significant questions to be answered is how the configuration at C-4 in the uronic acid part F and the presence (or absence) of the 4-methyl group are related to the antibiotic activity. Especially the last mentioned matter merits special attention since the synthesis of moenuronic acid derived structural analogues of 2 (with

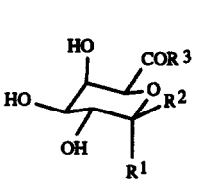
the 4-methyl group) would be (at least with the presently available methodologies[8]) of an unacceptable complexity. Some time ago we have, therefore, synthesized the galacturonic acid derived compound **10d** which turned out to be antibiotically inactive.[9] However, later it was found, that the amide function in unit F is a prerequisite of antibiotic activity.[10] In the present paper we report on the synthesis and antibiotic activity of compound **10c**, which is the galacturonamide analogue of **2** and should provide an answer to some of the questions raised above. We could follow the general synthetic plan developed for the synthesis of **10d**,[9] but as will be apparent below, the presence of the uronamide functionality neccessiated some major modifications.

Synthesis of **10b** and attempted deprotection

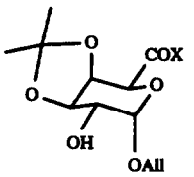
As described previously,[9] D-galacturonic acid was treated with anhydrous allyl alcohol in the presence of Dowex 50 resin (H^+ form)[11] to yield a mixture of **3a** and **3b** and the corresponding furanoid derivatives. Whereas the separation of **3a**/**3b** from the furanoid isomers was simple, the separation of **3a** from **3b** was difficult and required careful medium-pressure chromatography (MPLC). The yield of **3a** was 25%. Acetonide **4** was obtained from **3a** by acid-catalyzed transacetalysation.[12] 2,2-Dimethoxypropane was used both as reagent and solvent.[13] Finally, treatment of **4** with methanolic ammonia[14] provided building block **4b**.

Recently, we have demonstrated that a modified version of Boullanger's glycosylation with tetra-O-acetyl-N-allyloxycarbonyl-2-deoxy-ß-D-glucose[15] is well suited to prepare disaccharides of type **7**.[16] In the present case, however, oxazolin **5** was found to be of sufficient reactivity to permit disaccharide formation.[17] The oxazolin method has the advantage that it installs directly the correct functionality at C-2 of the amino sugar moiety. Glycosyl donor **5** was prepared using the excellent method of Nakabayashi et al.[18] (treatment of 1,3,4,6-tetra-O-acetyl-2-acetamido-2-deoxy-α-D-glucopyranose with trimethylsilyl triflate). The glycosylation reaction was performed at 60°C in CH_2Cl_2 solution (sealed vessel) with a threefold excess of **5** and camphorsulfonic acid as catalyst. The yield (based on consumed **4b**) was 91%. Structure **7** is in agreement with all spectroscopic results. The coupling constant $J_{1,2\ (E)}$[19] = 8.6 Hz confirms the ß glycosidic bond which is also indicated by the ^{13}C chemical shift (δ = 101) of C-1^E. The signal of C-2^F is shifted to higher frequencies (about 9 ppm) when compared with **4b** (ß effect).

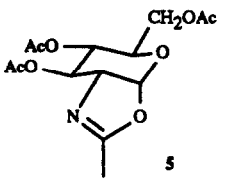
Removal of the acetonide protecting group from **7** (with 20 per cent acetic acid at 50°C, quantitative yield) led to highly polar **6a**. We wished to introduce the carbamoyl group into the 3-position by reaction with trichloroacetyl isocyanate (TAI) and subsequent removal of the trichloroacetyl group.[20] Non-nucleophilic solvents have to be used for the reaction of an alcohol with highly reactive TAI. Unfortunately, **6a** turned out to be very little soluble in suitable solvents such as toluene, chloroform, ethyl acetate, nitromethane and acetonitrile. Recourse was, therefore, made to tributylstannyl ether formation[21] with the hope both to increase the solubility[21a] and the selectivity of the acylation reaction.[21b] Indeed, heating of **6a** in chloroform in the presence of 0.5 equivalents of bis(tributyltin)oxide and continuous removal of water led to a clear solution which was treated at 0°C with one equivalent of TAI. For the removal of the trichloroacetyl



3	R^1	R^2	R^3
a	OAll	H	OAll
b	H	OAll	OAll



4	X
a	All
b	NH_2



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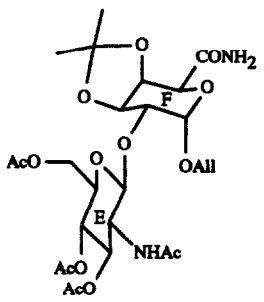

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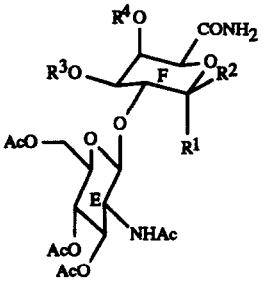

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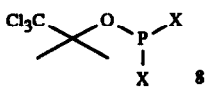

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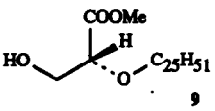
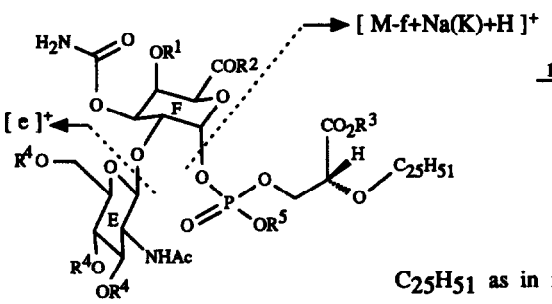


9

$Me_3SiOOSiMe_3$

Zn - Cu

6	R^1	R^2	R^3	R^4
a	OAll	H	H	H
b	OAll	H	$CONHCOCCl_3$	H
c	OAll	H	$CONH_2$	H
d	OAll	H	$CONH_2$	Ac
e	H, OH		$CONH_2$	Ac
f	OCH_2COCH_3	H	$CONH_2$	Ac
g	OAll	H	$CONH_2$	$CO_2CH_2CCl_3$
h	H, OH		$CONH_2$	$CO_2CH_2CCl_3$



10	R^1	R^2	R^3	R^4	R^5
a	Ac	NH_2	Me	Ac	CMe_3CCl_3
b	Ac	NH_2	Me	Ac	H
c	H	NH_2	H	H	H
d	H	OH	H	H	H
e	$CO_2CH_2CCl_3$	NH_2	Me	Ac	CMe_2CCl_3
f	H	NH_2	Me	Ac	H
g	H	NH_2	Me	H	H

$C_{25}H_{51}$ as in formula 2

group from the resulting trichloroacetyl urethane (cf. **6b**) a number of hydrolytic procedures aided by K_2CO_3,[20d,8c] Al_2O_3,[20e,9] or an anion exchange resin in its OH^- form[20h] have been recommended. In the present case, removal by an Zn dust induced elimination process in methanol[20f, 20g] gave by far the best results. The overall yield of **6c** (based on **6a**) was 78%. The Al_2O_3 method was completely unsuitable since the urethane could only partly be eluted from the stationary phase.[22,20h] The formation of **6c** was indicated by the appearance of a ^{13}C signal at δ = 157.7 (urethane C) and the downfield shift of the 3-H^F signal from about δ = 5.1 (in **6a**) to δ = 5.83.

The free OH group in unit F of **6c** was protected by acetylation (quantitative yield of **6d**). For the cleavage of allyl ethers exist a number of methods.[23] Most of them are two-step procedures consisting of (i) the rearrangement of the allyl ether to a propenyl ether grouping induced by KO^tBu[24] or an Rh(I)[25] or Ir(I)[26] catalyst[27] and (ii) cleavage of the enol ether either by oxidation–hydrolysis[28] or via a very labile hemiacetal formed on electrophilic addition of XOH to the double bond.[29] It has also been reported that allyl glycosides can be cleaved via allylic oxidation with SeO_2,[30] with palladium in the presence of strong acid,[31] and with Pd(II) via an intermediate π-allyl complex.[32] The last-mentioned method when applied to **6d** was not very efficient.[33] Besides the desired **6e** which was obtained in an unsatisfactory yield (44%), up to 30% of the Wacker product **6f** were formed[34]. **6e** and **6f** could be separated only with great difficulties. The Rh(I) mediated isomerisation in ethanol[25,35], followed by $HgCl_2$/HgO cleavage in acetone–water, converted **6d** into **6e** in 74% yield. For the double bond isomerisation it was found necessary to use freshly prepared catalyst.[36] Older specimen were inactive and could not be reactivated on treatment with triphenylphosphine.[37]

For the construction of the phosphoric acid diester grouping of moenomycin analogues the Ugi variant[38] of the phosphite methodology[39] has proven its merits.[9] Reagent **8** (X = 1,2,4-triazol-1-yl) permits the selective sequential reaction with the two different alcohol components[9,38,40] and the bulky 2,2,2-trichloro-1,1-dimethyl-ethyl protecting group[38,41] renders the intermediate phosphite and phosphate triesters quite stable. Thus, treatment of the phosphitylating reagent **8** (X = 1,2,4-triazol-1-yl), prepared *in situ* from the dichlorophosphite **8** (X = Cl) first with the disaccharide **6e** and then with the moenomycin-derived primary alcohol **9**,[42] followed by oxidation with bis(trimethylsilyl)peroxide[9,43,44] provided phosphoric acid triester **10a** which was directly converted into phosphoric acid diester **10b** by Zn–Cu couple induced removal[45,46] of the protecting group. The overall yield (based on **6e**) was 47%. The structural assignment was based on ^1H NMR (1-H^F: δ = 6.67, dd, $J_{1,2}$ = 3.1 Hz, $J_{1,P}$ = 6.0 Hz) and ^{13}C NMR results (δ = 102.0 (C-1^E), 95.9 (C-1^F), 79.5 (C-2^H), 157.2 (urethane C)) as well as the expected FAB MS molecular ion peaks (see Experimental). Attempted deblocking of **10b** by cleavage of the ester groups with 0.1 mol/l aqueous lithium hydroxide gave disappointing results. A mixture of four products was formed that could only partly be separated. The most polar compound turned out to be the galacturonic acid derivative **10d** (identified by FAB MS and TLC comparison with an authentic sample[9]). A compound slightly less polar was (according to FAB MS) presumably the desired **10c**. We believe that the other two compounds

were the 4^F-O-acetyl derivatives of **10c** and **10d**, respectively, since we have previously observed that the hydrolysis of the ester grouping in the 4-position of the galacturonic acid moiety proceeds sluggishly.[9] In the present case, hydrolysis of the 4^F-O-acetyl group seems to compete with cleavage of the uronamide. As will be reported below, replacing this O-acetyl group by a more suitable protecting group, avoids all the problems associated with the deprotection of **10a**.

Synthesis of **10c**

In order to circumvent the deblocking difficulties reported above, the free OH group in **6c** was protected with the trichloroethoxycarbonyl (TrOC[47]) group (**6c** --> **6g**, 90% yield). The ^{13}C NMR spectrum of **6g** displayed the CO signal of the TrOC group at δ = 154.2 and that of the CCl_3 carbon at δ = 95.1. The next steps of the synthesis, i.e. allyl group removal (**6g** --> **6h**, two-step procedure, 49% yield) and successive reactions with (i) **8** (X = 1,2,4-triazol-1-yl), (ii) **9**, (iii) bis(trimethylsilyl)peroxide were performed as described above and yielded phosphoric acid triester **10a** in 54% yield (based on **6h**). The structural assignment of **10a** is fully consistent with the ^1H and ^{13}C NMR data (see Experimental). The removal of the protecting groups containing the trichloroethyl unit needed optimisation. Finally, it was found, when the Imai protocol[45] was followed (with pyridine as solvent instead of DMF) and Zn-Cu couple freshly prepared in the absence of moisture and oxygen was used both the phosphate and the 4^F-OH protecting groups were removed in one operation to provide **10f** in 90% yield. Removal of the remaining protecting groups was uneventful. We observed that hydrolysis of the acetate groups at the amino sugar part (E) proceeded faster than cleavage of the glyceric acid methyl ester. In one experiment the intermediate methyl ester **10g** was isolated. Structural assignment rests mainly on the presence of a FAB MS peak at m/z = 589.1 which we assume to correspond to fragment $[M-f+K+H]^+$ (see formula **10**). Normally, alkaline ester hydrolysis was carried on until **10c** was the sole reaction product (TLC control). In the FAB MS besides the correct molecular ion peaks at m/z = 958.4 ($[M+H]^+$), 980.4 ($[M+Na]^+$), 996.8 ($[M+K]^+$), the presence of signals at m/z = 559.3 ($[M-f+Na+H]^+$) and 204.1 ($[e]^+$, see formula **10**) were fully in accord with structure **10c**.

Antibiotic activity of **10c**

The inhibition of the polymerization of the peptidoglycan sugar chains was studied with a slightly modified version of the assay described by Izaki[48] using UDP-N-acetylmuramyl pentapeptide isolated from *Bacillus cereus T* and cell membranes of *E.coli* JE5684. At concentrations of 10 mg/l and 1 mg/l **10c** was practically inactive (39% and 10% inhibition). This result though disappointing, stresses at the same time the high specificity of the interaction of moenomycin (**1**) and degradation products such as **2** with the binding site at the transglycosylating enzyme that forms the basis of the antibiotic activity. It has to be determined wether it is the equatorial hydroxy, the axial methyl group, or the combination of both structural features that exerts this striking effect on the structure-activity relations.

EXPERIMENTAL

General
All 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). Solvent evaporations were performed in vacuo at 40°C using a rotatory evaporator. Solvents were purified by standard techniques. Molecular sieves were activated at 320°C and 13 Pa for 14 h. The instrumentation used was: ^1H NMR: WP 80 (Bruker), AM 400 (Bruker); ^{13}C NMR: AM 400 (Bruker at 100.6 MHz); IR: Perkin Elmer 1310 for solution spectra (solvent given in parenthesis; diffuse reflectance infrared Fourier transform (DRIFT) spectra: Perkin Elmer FT/IR, model 1710; EI MS: MAT CH5 (Varian); FAB MS: (i) MAT 731 (Varian), (ii) VG AUTOSPEC, (iii), VG Analytical ZAB2-SEQ (BEQQ configuration); LC (preparative gravitational liquid chromatography): silica gel (ICN Biomedicals Silica 63-100); MPLC (medium-pressure liquid chromatography): 40.0 cm x 4.5 cm glass tubes, 50 µm silica gel (Amicon), Duramat pump (CfG), Thomachrom UV detector (Reichelt); analytical TLC: Merck precoated silica gel 60 F_{254} plates (0.2 mm), spots were identified under a UV lamp (Camag 29 200) and 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)[49] and heating at 140°C or with the phosphate-specific spraying reagent of Dittmer and Lester[50], lyophilization: Leybold-Heraeus GT2.
Carbon and proton numbering in the subunits (see NMR and MS spectral data) follows the moenomycin nomenclature (see formula 1). With one exception, the NMR signals of the allyl protecting group are not reported, cf. ref.[9] Two molecular masses are always communicated, the first was calculated using the International Atomic Masses, the second refers to ^{12}C, ^1H, ^{16}O, ^{14}N, ^{31}P (mono-isotopic masses).

Allyl (allyl α-D-galactopyranosid)uronate (3a)
D-Galacturonic acid monohydrate (25.5 g, 0.12 mol) was treated with anhydrous allyl alcohol in the presence of Dowex 50 W X2 resin (H^+ form) as described in ref. [9] LC (680 g of SiO_2, petrol-ethyl acetate-ethanol 1:1:0.2) of the resulting mixture of furanosid- and pyranosiduronates (36.6 g) yielded two fractions, one containing the furanosiduronates (14.6 g, 44%), the other the pyranosiduronates. The latter fraction was separated by MPLC (petrol-ethyl acetate-ethanol 1:1:0.2) and provided pure fractions of **3a** (8.3 g, 25%) and **3b** (1.8 g, 5%).- ^{13}C NMR ($CDCl_3$) of **3a**: allyl groups: δ= 65.9 (C-1), 68.9 (C-1), 131.5 (C-2), 133.5 (C-2), 117.7 (C-3), 118.6 (C-3); sugar part: δ = 98.1 (C-1), 68.1 (C-2), 69.7 (C-3), 70.3 (C-4), 70.4 (C-5), 168.7 (C-6).- For all other data, see ref. [9].

Allyl (allyl 3,4-O-isopropylidene-α-D-galactopyranosid)uronate (4a)
A mixture of **3a** (1.758 g, 6.42 mmol), p-toluenesulfonic acid (20 mg), and 2,2-dimethoxypropane (40 ml) was stirred for 45 min at 20°C. Quenching by addition of triethylamine (0.2 ml), solvent evaporation, and MPLC (petrol-ethyl acetate 5:1) yielded **4a** (1.632 g, 81%).- ^1H NMR (400 MHz, $CDCl_3$): δ = 5.01 (d, 1-H), 3.93 (dd, 2-H), 4.34 (dd, 3-H), 4.55 (dd, 4-H), 4.67 (d, 5-H), 1.31 (s, CH_3), 1.46 (s, CH_3); $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ ≈ 5 Hz, $J_{3,4}$ ≈ 6 Hz, $J_{4,5}$ = 2.0 Hz.- ^{13}C NMR ($CDCl_3$): δ = 96.3 (C-1), 68.1 (C-2), 68.7 (C-3), 73.5 (C-4), 75.2 (C-5), 167.8 (C-6), 110.1 $\underline{C}(CH_3)_2$, 27.2 and 25.6 (CH_3 signals).- IR ($CHCl_3$): 1750, 1725, 1600 cm^{-1}. Anal calcd for $C_{15}H_{22}O_7$ (314.3): C 57.32, H 7.05, found C 57.37, H 7.25.

Allyl (3,4-O-isopropylidene-α-D-galactopyranosid)uronamide (4b)
A solution of **4a** (35 mg, 0.11 mmol) in methanol, saturated at 0°C with dry ammonia (28 ml) was stirred at 0°C for 24 h to give **4b** as the sole reaction product (TLC control: petrol-ethyl acetate-ethanol 1:1:1). Solvent evaporation yielded **4b** (29.0 mg, 96%).- ^1H NMR (400 MHz, $CDCl_3$, H,H COSY): δ = 4.98 (d, 1-H), 3.92 (dd, 2-H), 4.35 (dd, 3-H), 4.62 (dd, 4-H), 4.52 (dd, 5-H), 1.31 (s, CH_3), 1.46 (s, CH_3), 5.90-5.98 and 6.45-6.55 ($CONH_2$); $J_{1,2}$ = 3.8 Hz, $J_{2,3}$ = 5.7 Hz, $J_{3,4}$ = 5.7 Hz, $J_{4,5}$ = 2.3 Hz.- ^{13}C NMR, $CDCl_3$, C,H COSY, DEPT): δ = 96.3 (C-1), 68.3 (C-2), 75.0 (C-3), 73.0 (C-4), 69.7 (C-5) 171.0 (C-6), 109.9 ($\underline{C}(CH_3)_2$), 25.4 and 27.2 (CH_3 signals).- IR ($CHCl_3$): 3580 (OH), 3540 und 3420 (NH), 1695

(amide I), 1575 (amide II), 1460 cm^{-1}.- $C_{12}H_9NO_6$ (273.29, 273.12), FAB MS (glycerol): m/z (%) 274 (14, $[M+H]^+$), 216 (44), 198 (18), 158 (100), 140 (62).

Allyl 2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4-O-isopropylidene-α-D-galactopyranosiduronamide (7)

To **4b** (1.005 g, 3.68 mmol) solutions of 2-methyl-(3,4,6-tri-O-acetyl-1,2-dideoxy-α-D-glucopyrano)[2,1-d]oxazoline (**5**) (1.25 g, 3.80 mmol) CH_2Cl_2 (10 ml) and anhydrous camphorsulfonic acid (170 mg, 0.73 mmol) in CH_2Cl_2 (10 ml) were added and the mixture was stirred in a sealed vessel at 60°C. After 4 h and 6 h further portions of **5** (1.00 g, 3.04 mmol and 1.28 g, 3.89 mmol, respectively), dissolved in CH_2Cl_2, were added. Stirring at 60°C was continued for altogether 22 h. The reaction was stopped by addition of triethylamine (1 ml), and the mixture was stirred at 20°C for 30 min and then passed through a column containing Florisil (10 g, elution with CH_2Cl_2-ethanol 5:1). Solvent evaporation and MPLC ($CHCl_3$-ethanol-triethylamine 50:1:0.2) yielded **7** (1.68 g, 76 %, 91% based on consumed **4a**), 167 mg (0.61 mmol) of **4a** were recovered.- ^1H NMR, 400 MHz, $CDCl_3$, C,H COSY, H,H COSY): unit E: δ = 4.98 (d, 1-H), 3.79-3.87 (m, 2-H), 5.31 (dd, 3-H), 5.04 (t, 4-H), 3.63-3.68 (ddd, 5-H), 4.14-4.18 (CH_2-6), 1.92, 1.99, 2.00, 2.05 (4 CH_3 s's), 5.73 (d, NHCOCH$_3$), $J_{NH,2-H}$ = 8.5 Hz, $J_{1,2}$ = 8.6 Hz , $J_{2,3}$ = 10.6 Hz; $J_{3,4}$ = 9.3 Hz, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ and $J_{5,6'}$ = 2.9 and 4.3 Hz; unit F: δ = 4.99 (d, 1-H), 3.77 (dd, 2-H), 4.29 (dd, 3-H), 4.54 (dd, 4-H), 4.49 d (5-H), 5.79 and 6.47 ($CONH_2$, J_{gem} = 3.5 Hz); $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 8.1 Hz, $J_{3,4}$ = 5.3 Hz, $J_{4,5}$ = 2.8 Hz.- ^{13}C NMR ($CDCl_3$, DEPT, C,H COSY): unit E: δ = 101.0 (C-1), 54.9 (C-2), 72.3 (C-3), 68.6 (C-4). 72.0 (C-5), 62.1 (C-6), 23.3 and 20.61-20.72 (CH_3 s's), 170.2, 170.4, 170.6, 170.7 ($\underline{C}OCH_3$ signals); unit F: δ = 97.5 (C-1), 77.5 (C-2), 75.2 (C-3), 73.6 (C-4), 68.3 (C-5), 169.4 (C-6), 109.6 ($\underline{C}(CH_3)_2$), 26.4 and 28.3 (acetonide CH_3 signals).- FAB MS (glycerol): m/z (%) 603 (1.5 $[M+H]^+$), 487 (5), 445 (5), 330 (40, $[e]^+$), 210 (40), 168 (98) 150 (100).- Anal calc for $C_{26}H_{38}N_2O_{14}$ (602.59, 602.29): C 51.82, H 6.36, found C 51.64, H 6.39.

Allyl 2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-α-D-galactopyranosiduronamide (6a)

To **7** (351.0 mg, 0.583 mmol) aqueous acetic acid (20 per cent, 15 ml) was added and the mixture was stirred at 50°C for 9.5 h (TLC control: petrol-ethyl acetate-ethanol 1:1:1). After solvent evaporation (codistillation with toluene) pure **6a** (307.4 mg, 94%) was obtained.- ^1H NMR, (400 MHz, pyridine-d_5, H,H COSY): unit E: δ = 5.45 (d, 1-H), 4.54-4.60 (m, 2-H), 5.85 (dd, 3-H), 5.45 (dd, 4-H), 3.75-3.82 (ddd, 5-H), 4.31 (dd, 6-H), 4.48 (dd, 6-H'), 1.96, 1.98-2.02 (CH_3 signals), 9.34 (d, NHCOCH$_3$), $J_{NH,2} \approx 7$ Hz, $J_{1,2}$ = 8.4 Hz, $J_{2,3} = J_{3,4}$ = 9.8 Hz, $J_{5,6}$ = 4.3 Hz, $J_{5,6'}$ = 2.4 Hz, $J_{6,6'}$ = 11.6 Hz; unit F: δ = 5.52 (d, 1-H), 4.65 (dd, 2-H), 4.61 (dd, 3-H), 5.04 (dd, 4-H), 4.80 (d, 5-H), 7.85 (d) and 8.45 (d, $J_{gem} \approx 2.8$ Hz, $CONH_2$), $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 3.1 Hz, $J_{4,5}$ = 1.2 Hz.- ^{13}C NMR (pyridine-d_5, C,H COSY, DEPT): unit E: δ = 103.6 (C-1), 55.5 (C-2), 74.0 (C-3), 69.6 (C-4), 72.1 (C-5), 62.5 (C-6), 23.2, 20.56, 20.71, 20.67 ($COCH_3$ signals), 170.6, 170.72, 170.75, 172.50 ($\underline{C}OCH_3$ signals); unit F: δ = 99.6 (C-1), 79.3 (C-2), 69.6 (C-3), 71.4 (C-4), 73.2 (C-5), 169.8 (C-6).- IR: 3600-3100 (OH), 1745, 1737 (ester C=O), 1663, 1647, 1549 cm^{-1} (amide bands).- FAB MS (glycerol): m/z (%) 563 (6 $[M+H]^+$), 330 (80, $[e]^+$), 210 (50), 168 (90), 150 (100).- Anal calc for $C_{23}H_{34}N_2O_{14}$ (562.53, 562.20): C 49.11, H 6.09, found C 49.16, H 6.25.

Allyl 2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-carbamoyl-α-D-galactopyranosiduronamide (6c)

A mixture of **6a** (232.4 mg, 0.413 mmol), bis(tributyltin)oxide (123 mg, 0.206 mmol), and $CHCl_3$ (60 ml) was heated under reflux for 7 h. Water was continously removed by passing the condensed solvent through a layer of 4Å molecular sieves. After cooling to 0°C trichloroacetyl isocyanate (48.9 µl, 0.41 mmol) was added and the mixture was stirred at 0°C for 1 h. Excess reagent was destroyed by addition of methanol (0.5 ml) and stirring at 0°C for 10 min. After solvent evaporation the residue was redissolved in methanol (40 ml), Zn dust (260 mg) was added and the reaction mixture was stirred at 20°C for 3 h. Filtration, washing the solid with methanol and methanol-water, evaporation of the combined liquid phases and MPLC of the residue (petrol-$CHCl_3$-methanol 1:1:0.35) provided **6c** (195.8 mg, 78%), 34.4 mg (15%) of **6a** were recovered.- ^1H NMR (400 MHz, pyridine-d_5, H,H

COSY): unit E: δ = 5.67 (d, 1-H), 4.09 (2-H, overlapping with the 1-$H_{allyl\ signal}$), 6.09 (dd, 3-H), 5.39 (dd, 4-H), 3.78 (ddd, 5-H), 4.30 (dd, 6-H), 4.48 (dd, 6-H'), 1.98, 1.99, 2.03, 2.14 (4 $COCH_3$ s's), 9.08 (d, $NHCOCH_3$), $J_{1,2}$ = 8.4 Hz, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, $J_{4,5}$ = 10 Hz, $J_{5,6}$ = 2.6, $J_{5,6'}$ = 4.4 Hz , $J_{6,6'}$ = 12.3 Hz, $J_{NH,2}$ ≈ 8 Hz; unit F: δ = 5.55 (d, 1-H), 4.92 (dd, 2-H), 5.83 (dd, 3-H), 5.46 (dd, 4-H), 4.84 (d, 5-H), 7.90 and 8.51 (2 d's, J_{gem} = 2.1 Hz, $CONH_2$), $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 3.1 Hz, $J_{4,5}$ = 1.4 Hz.- ^{13}C NMR, (pyridine-d_5, C,H COSY), unit E: δ = 102.2 (C-1), 56.3 (C-2), 72.9 (C-3), 69.8 (C-4), 72.0 (C-5), 62.3 (C-6), 20.56, 20.66, 20.70, 23.42 ($COCH_3$ signals), 170.5, 170.6, 171.0, 172.0 ($COCH_3$ signals); unit F: δ = 99.1 (C-1), 75.8 (C-2), 72.8 (C-3), 68.9 and 69.1 (C-4 and C-1_{allyl}), 73.0 (C-5), 169.9 (C-6), 157.7 ($OCONH_2$).- IR: 3475, 3340, 1748, 1716, 1675, 1595,1539 cm^{-1}.- $C_{24}H_{35}N_3O_{15}$ (605.55, 605.21), FAB MS (glycerol): m/z (%) 606.4 (2.5 ($[M+H]^+$), 330.1 (100, $[e]^+$), 168 (16), 150 (80).

Allyl 2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-4-O-acetyl-3-O-carbamoyl-α-D-galactopyranosiduronamide (**6d**)

A mixture of **6c** (18.2 mg, 0.03 mmol), DMAP (8.7 mg, 0.071 mmol), pyridine (2 ml), and acetic anhydride was stirred at 0°C for 45 min (TLC control: petrol-$CHCl_3$-methanol 1:1:0.75). Filtration through a SiO_2 layer, solvent evaporation (codistillation with toluene, 3 x 3 ml), MPLC (petrol-$CHCl_3$-ethanol 5:1:0.5) yielded pure **6d** (19.1 mg, 98%).- The ^1H NMR spectrum was practically identical with that of **6c**, differences: δ = 1.84 (additional $COCH_3$ signal), 6.66 (4-H^F).- ^{13}C NMR: Practically identical with that of **6c**, additional signals at δ = 20.5 (CH_3) and 170.0 (C=O).- IR 3100-3600 (OH, NH), 1748 (ester C=O), 1691 ($CONH_2$), 1595, 1548 cm^{-1} (NHAc).- $C_{26}H_{37}N_3O_{16}$ (647.59, 647.22), FAB MS (glycerol): m/z (%) 670 (0.8, $[M+Na]^+$), 648 (2.8 $[M+H]^+$), 589 (1.1), 330 (60, $[e]^+$), 210 (43), 150 (100).

Deallylation of **6d**

a) A mixture of **6d** (93.4 mg, 0.144 mmol), tris(triphenylphosphin)rhodium-(I) chloride (freshly prepared, 13.3 mg, 0.0144 mmol), diazabicyclo [2.2.2]octan (DABCO, 4 mg, 0.036 mmol), and ethanol (7 ml) was stirred at 80°C for 2 d (sealed vessel). The catalyst was removed by filtration. After solvent evaporation the residue was redissolved in 9:1 acetone-water, HgO (160 mg, 0.74 mmol), and $HgCl_2$ (180 mg, 0.66 mmol) were added, and the mixture was stirred at 20°C for 4 h. Insoluble matter was removed by centrifugation. Into the clear solution H_2S gas was passed. The precipitated inorganic salts were removed by centrifugation, and the precipiate was washed several times with acetone. The combined liquid phases after solvent evaporation and MPLC (petrol-$CHCl_3$-methanol 1:1:0.2) yielded **6e** (64.5 mg, 74 %).- b) To **6d** (330 mg, 0.51 mmol) and $PdCl_2$ (126 mg, 0.71 mmol) acetate buffer (0.1 mol/l NaOAc in acetic acid-water 20:1, 50 ml) was added and the mixture was stirred at 20°C for 24 h. Filtration, evaporation of the filtrate and MPLC (RP-18, water-methanol 5:1) gave **6e** (137 mg, 44 %) and **6f** (95.3, 28 %).

2-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-4-O-acetyl-3-O-carbamoyl-α-D-galactopyranuronamide (**6e**)

^1H NMR (400 MHz, pyridine- d_5, spectral assignment by comparison with **6c**): unit E: δ = 5.74 (d, 1-H), 4.02-4.10 (2-H), 6.15 (dd, 3-H), 5.35 (4-H, hidden by the 5-H^F signal, 3.95 (ddd, 5-H), 4.30 (dd, 6-H), 4.43 (dd, 6-H'), 9.05 (d, $NHCOCH_3$), $J_{1,2}$ = 8.3 Hz, $J_{2,3}$ = $J_{3,4}$ = $J_{4,5}$ = 10.1 Hz, $J_{5,6}$ = 2.6, $J_{5,6'}$ = 4.7 Hz, $J_{6,6'}$ = 12.0 Hz , $J_{NH,2}$ ≈ 8 Hz; unit F: δ = 6.18 (d, 1-H), 4.66 (dd, 2-H), 6.19 (3-H, hidden by the signals of 1-H^F) and 3-H^E), 6.72 (dd, 4-H), 5.35 (d, 5-H), 7.94 and 8.45 ($CONH_2$), 7.48 (broad signal ($OCONH_2$), $J_{1,2}$ = 3.3 Hz, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.3 Hz, $J_{4,5}$ = 1.4 Hz.- ^{13}C NMR (pyridine-d_5, DEPT, C,H COSY): δ = 102.4 (C-1^E), 93.8 (C-1^F), 77.2 (C-2^F), 72.8 and 72.0 (C-3^E and C-3^F), 71.1, 70.2, 70.1 (C-4^E, C-5^E, C-4^F and C-5^F), 62.6 (C-6^E), 56.4 (C-2^E), 157.4 ($OCONH_2$), 169.8 (C-6^F).- IR 3700, 3478, 3340, 1747, 1691, 1682, 1595, 1550, 1537 cm^{-1}.- $C_{23}H_{33}O_{16}N_3$ (607.53, 607.19), FAB MS (glycerol): m/z (%) 630 (3.7 $[M+Na]^+$), 608 (11.8 $[M+H]^+$), 330 (80, $[e]^+$), 210 (41), 150 (100).

2-Oxopropyl 2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-gluco-pyranosyl)-4-O-acetyl-3-O-carbamoyl-α-D-galactopyranosiduronamide (**6f**)

6f could not be obtained completely pure (impurity: **6e**).- ^{1}H NMR (400 MHz, pyridine-d_5, C,H COSY, spectral assignment by comparison with **6c**): unit E: δ = 5.75 (d, 1-H), 3.92-4.05 (2-H and 5-H), 6.21 (3-H), 5.42 (dd, 4-H), 4.40-4.46 (CH_2-6), 9.30 (d, NHCOCH$_3$), $J_{1,2}$ = 8.5 Hz, $J_{3,4}$ = $J_{4,5}$ ≈ 9.5 Hz, $J_{NH,2}$ ≈ 8 Hz; unit F: δ = 5.63 (d, 1-H), 4.63 (dd, 2-H), 6.03 (dd, 3-H), 6.66 (dd, 4-H), 5.10 (d, 5-H), 4.28 and 4.32 (AB system, J_{AB} = 17 Hz, OCH$_2$COCH$_3$), 2.17 (s,OCH$_2$COCH$_3$), 7.58 (OCONH$_2$), 8.05 and 8.65 (CONH$_2$), $J_{1,2}$ = 3.5 Hz , $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.5 Hz, $J_{4,5}$ = 1.5 Hz.- ^{13}C NMR (pyridine-d_5, DEPT, C,H COSY): unit E: δ = 102.2 (C-1). 56.4 (C-2), 72.5 (C-3), 69.6 (C-4), 72.1 (C-5), 62.0 (C-6), 170.2-171.2 (4 OCOCH$_3$ signals); unit F δ = 100.5 (C-1), 76.4 (C-2), 69.4 (C-3), 70.4 (C-4), 70.9 (C-5), 169.6 (C-6), 74.6 (OCH$_2$COCH$_3$), 206.9 (OCH$_2$COCH$_3$), 26.8 (OCH$_2$COCH$_3$), 157.2 (OCONH$_2$).- $C_{28}H_{37}O_{17}N_3$ (663.59, 663.21), FAB MS (glycerol): m/z (%) 686 (7, [M+Na]$^+$), 664 (4, [M+H]$^+$), 330 (90, [e]$^+$), 228 (18), 168 (100), 140 (55), 124 (25).

2-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-4-O-acetyl-3-O-carbamoyl-1-O-[[(R)-2-methyloxycarbonyl-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxyl-hydroxy-phosphoryl]-α-D-galactopyranuronamide, triethylammonium salt (**10b**)

To a solution of 1H-1,2,4-triazole (41.3 mg, 0.6 mmol) in 4:1 THF-pyridine (2 ml) 1,1,1-trichloro-2-methyl-prop-2-yl dichlorophosphite (**8**, X = Cl, 29.6 µl, 0.15 mmol) were added and the mixture was stirred at 0°C for 20 min (colourless precipitate). A solution of **6e** (90 mg, 0.148 mmol) in 4:1 THF-pyridine (2 ml) was added and stirring at 0°C was continued for 1.5 h. TLC control (petrol-CHCl$_3$-methanol 1:1:0.75) then indicated quantitative reaction. A solution of **9** (70 mg, 0.15 mmol) in 4:1 THF-pyridine (1 ml) was added and the reaction mixture was stirred at 0°C for a further 6 h. Then bis(trimethylsilyl)peroxide (32 µl, 0.15 mmol) was added and the mixture was stirred at 0°C for 15 h. The solvent was evaporated in a stream of argon until only 0.5 ml of a solution remained (which was shown to be free of peroxides). Pyridine (5 ml) was added and the stirred solution was treated with Zn-Cu couple (130 mg) and 2,4-pentanedione (100 µl) for 6 h at 20°C. Filtration, solvent evaporation and MPLC (CHCl$_3$-methanol 2.5:1) furnished **10b** (78.1 mg, 47%).- ^{1}H NMR (400 MHz, pyridine-d_5, assignment by comparison with the data in ref.[9]): δ = 6.68 (4-H^F), 6.57 (dd, 1-H^F), 5.91-5.98 (3-H^E and 3-H^F), 5.68 (d, 1-H^E), 5.42 (t, 4-H^E), 5.36 (broad s, 5-H^F), 4.35-4.44, 4.54, 4.67, 4.62-4.80 (CH_2-6^E, 2-H^F, 2-H^H, CH_2-3^H), 3.90-3.98, 3.75-3.85, 3.60-3.73 (2-H^E, 5-H^E, and CH_2-1^I), 7.55 (OCONH$_2$), 8.08 and 8.50 (CONH$_2$), 9.07 (NHCOCH$_3$), 3.79 (CO$_2$CH$_3$), 1.24 (t) and 3.1 (triethylammonium), $J_{1,2^E}$ = 8.5 Hz, $J_{3,4^E}$ = $J_{4,5^E}$ = 9.2 Hz, $J_{1,2^F}$ = 3.1 Hz, J_{1,P^F} = 6.0 Hz.- ^{13}C NMR (the following signals could be assigned): unit E: δ = 102.0 (C-1), 55.4 (C-2); unit F: δ = 95.9 (C-1, C,P coupling), 75.9 (C-2, $J_{C,P}$ ≈ 8 Hz), 157.2 (OCONH$_2$); unit H: δ = 79.5 (C-2, $J_{C,P}$ = 8.0 Hz), 66.5 (CH_2-3), 55.4 (CO$_2$CH$_3$), unit I: δ = 69.5 (CH_2-1); 171.7, 171.1, 170.8, 170.6, 170.3, 169.8, 169.7 (CO signals), 45.9 and 8.7 (triethylammonium).- $C_{52}H_{90}N_3O_{22}P$ (1140.07, 1139.58), FAB MS (glycerol): m/z (%) 1178.5 (4, [M+K]$^+$), 1140.5 (1.5 [M+H]$^+$), 589 (6), 548 (5), 330 (45, [e]$^+$), 288 (20), 168 (95), 150 (100).

Attempted deprotection of **10b**

To **10b** (19 mg, 1.67x10^{-5}mol) 0.1 mol/l aqueous lithium hydroxide (830 µl, 8.3x10^{-5} mol) and THF (2.1 ml) were added, and the mixture was stirred at 20°C. After 30 min TLC (CHCl$_3$-methanol-water 18:11:2.7 and ethyl acetate-CH$_3$OH-H$_2$O 1:1:0.2) indicated the formation of four main products (according to increasing r_f values p_1 to p_4). After longer reaction times (up to 12 h) the same four products were observed the most polar compound giving the strongest spot. Complete LC separation of the compounds failed. The compound with the lowest r_f value (p_1) could be enriched. According to FAB MS (matrix: methanol-nitrobenzyl alcohol), signals at 1003.5 and 981.5, see ref.[9]) and TLC comparison (for solvent systems, see above) with a reference sample[9] it was **10d**. The fraction p_3 contained probably the desired compound **10c**. The FAB MS (methanol-trifluoroacetic acid-nitrobenzyl alcohol) showed signals at m/z 980.5 ([M+Na]$^+$, 986.6 ([M+Na+Li-H]$^+$), and 1002.4 ([M+2Na-H$^+$).

Allyl 2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-4-O-(2,2,2-trichloroethoxy)carbonyl-3-O-carbamoyl-α-D-galactopyranosiduronamide (**6g**)

To a solution of **6c** (324.0 mg, 0.54 mmol) in pyridine (29 ml) at 0°C 2,2,2-trichloroethyl chloroformate (111.0 µl, 0.80 mmol) was added, and the mixture was stirred at 20°C for 17 h. After addition of water (9 ml) solvents were removed by lyophilization. LC (petrol-$CHCl_3$-methanol 1:1:0.5) furnished **10f** (373.8 mg, 90%).- ^1H NMR (400 MHz, pyridine-d_5) unit E: δ = 5.52 (d, 1-H), 3.95-4.04 (2-H), 6.02 (dd, 3-H), 5.36 (dd, 4-H), 3.82-3.88 (ddd, 5-H), 4.38 (dd, 6-H), 4.43-4.48 (6-H'), 9.01 (d, NHCOCH$_3$), 1.98-2.12 (signals of 4 OCCH$_3$ groups), $J_{1,2} \approx 8$ Hz, $J_{3,4} = J_{4,5} \approx 10$ Hz, $J_{5,6} = 2.5$ Hz, $J_{5,6'} = 4.0$ Hz, $J_{6,6'} = 13$ Hz, $J_{NH,2} = 7$ Hz; unit F: δ = 5.50 (d, 1-H), 4.46 (2-H, overlapping with the 6-H'^{E} signal), 5.92 (dd, 3-H), 6.52 (dd, 4-H), 4.98 (5-H, overlapping with one of the OCOCH$_2$CCl$_3$ signals), 4.76 (AB, one of the OCOCH$_2$CCl$_3$ signals, $J_{gem} = 12.5$ Hz), 7.98 und 8.65 (OCONH$_2$), $J_{1,2} \approx 3$ Hz, $J_{2,3} = 11.0$ Hz; $J_{3,4} = 3.5$ Hz , $J_{4,5} \approx 1.5$ Hz.- ^{13}C NMR (pyridine-d_5): unit E: δ = 102.0 (C-1), 56.2 (C-2), 62.3 (C-6), unit F: δ = 98.7 (C-1), 77.0 (C-2), 154.2 and 156.84 (OCOCH$_2$CCl$_3$ and OCONH$_2$), 95.1 (OCOCH$_2$CCl$_3$), 169.7-170.9 (5 CO signals), 20.4-23.2 (4 COCH$_3$ signals).- FAB MS (lactic acid): m/z (%) 780 (3.5 [M+H]$^+$), 330 (90, [e]$^+$), 210 (60), 145 (80), 135 (100).- Anal calc for $C_{27}H_{36}O_{17}N_3Cl_3$ (780.95, 779.11): C 41.53, H 4.65, Cl 13.62, found C 41.62, H 4.79, Cl 13.77.

2-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-4-O-(2,2,2-trichloroethoxy)carbonyl-3-O-carbamoyl-α-D-galactopyranuronamide (**6h**)

A mixture consisting of **6g** (60.8 mg, 0.078 mmol), tris(triphenyl-phosphin)rhodium-(I) chloride (7.2 mg, 0.0078 mmol), DABCO (2.6 mg, 0.0234 mmol), and dry ethanol (1.8 ml) was heated to 80°C for 6h in a sealed vessel. Solid material was removed by filtration and the filtrate evaporated. The residue was taken up in 9:1 acetone-water and treated with HgO (84.5 mg, 0.390 mmol) and $HgCl_2$ (84.7 mg, 0.312 mmmol). The mixture was stirred at 20°C for 2h. Solids were removed by centrifugation. Into the clear solution gaseous H_2S was passed. The precipitates were removed by centrifugation and the solid material was washed with acetone (3 x 7 ml). The combined solutions were evaporated. LC ($CHCl_3$-ethanol 5:1) furnished pure **6h** (28.2 mg, 49%).- ^1H NMR (400 MHz, pyridine-d_5): unit E: δ = 5.60 (d, 1-H), 4.08 (2-H), 6.08 (dd, 3-H), 5.35 (dd, 4-H), 3.85 (ddd, 5-H) , 4.30 (dd, 6-H), 4.46 (dd, 6-H'), 9.05 (d, NHCOCH$_3$), $J_{1,2} = 8.5$ Hz, $J_{2,3} = J_{3,4} = 10$ Hz, , $J_{5,6} = 2.5$ Hz, $J_{5,6'} = 4.5$ Hz, $J_{6,6'} = 13$ Hz, $J_{NH,2} = 8.5$ Hz; unit F: δ = 6.10 (d, 1-H), 4.60 (dd, 2-H), 6.22 (dd, 3-H), 6.65 (dd, 4-H), 5.38 (d 5-H, ?) 4.80 and 5.00 (AB, $J_{gem} = 12.5$ Hz, OCOCH$_2$CCl$_3$), $J_{1,2} = 3.5$ Hz, $J_{2,3} = 10.5$ Hz, $J_{3,4} = 3.5$ Hz, $J_{4,5} = 1.5$ Hz.- ^{13}C NMR (pyridine-d_5): unit E: δ = 102.3 (C-1), 56.3 (C-2), 62.5 (C-6); unit F: δ = 93.6 (C-1), 77.1 (C-2), 154.5 and 157.2 (OCOCH$_2$CCl$_3$ and OCONH$_2$), 95.3 (OCOCH$_2$CCl$_3$).- $C_{24}H_{32}O_{17}N_3Cl_3$ (740.88, 739.08), FAB MS (lactic acid): m/z 740, 742, 744 ([M+H]$^+$), 541, 469, 397, 330 ([e]$^+$), 307, 235, 217, 163, 135.

2-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-carbamoyl-4-O-(2,2,2-trichloroethoxy)carbonyl-1-O-{[(R)-2-methoxycarbonyl-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxyl-(2-trichloromethyl-2-propyloxy)-phosphoryl]-α-D-galacto-pyranuronamide (**10e**)

To a solution of 1H-1,2,4-triazole (22.5 mg, 0.325 mmol) in 4:1 CH_2Cl_2-pyridine (1.0 ml) 1,1,1-trichloro-2-methyl-prop-2-yl dichlorophosphite (**8**, X = Cl, 22.5 mg, 0.081 mmol) was added at 0°C and the mixture was stirred at 0°C for 20 min. Disaccharide **6h** (53.6 mg, 0.072 mmol), dissolved in 4:1 CH_2Cl_2-pyridine (0.5 ml), was added and the mixture was stirred at 0°C for 4 h. Then a solution of methyl (R)-3-hydroxy-2-(3.8.8.11.14.18-hexamethyl-nonadecyloxy)-propionate (101.7 mg, 0.216 mmol) in 4:1 CH_2Cl_2-pyridine (1.5 ml) was added in 3 portions (within 2 h). Stirring at 0°C was continued for a total of 3 h. Then bis(trimethylsilyl)peroxide (18.0 mg, 0.101 mmol) was added at 0°C and the reaction mixture was stirred at 20°C for 15 h. After solvent evaporation the residue was purified by LC (hexanes-ethylacetate-ethanol 2.5:1:0.5 + 0.1% triethylamine) to give (41.0 mg, 54%).- ^1H NMR (400 MHz, pyridine-d_5): δ = 0.90-1.50 (H's from the moenocinol unit), 1.95-2.20 (6 CH$_3$ signals), 3.58-3.68 (1-H^I), 3.73 (s, OCH$_3$), 3.78-3.87 (1-H^I); 3.92-3.98 (5-H^E); 3.99-4.10 (2-H^E); 4.40-4.47 (2-H's); 4.52-4.79 (6-H's, 2-H^F, CH$_2$-6^E, 1 H of OCH$_2$CCl$_3$ (d, J = 12 Hz)); 5.00 (d, J = 12 Hz, 1 H of OCH$_2$CCl$_3$); 5.38 ($W_{1/2} = 4$ Hz, 5-H^F); 5.46 (t, J= 10.0 Hz, 4-H^E); 5.59 (d, J= 8.5 Hz, 1-H^E); 6.05 (dd, $J_{2,3} = 10.5$ Hz, $J_{3,4} = 3.5$ Hz; 3-

H^F); 6.10 (t, J = 10 Hz, 3-H^E), 6.57 ($W_{1/2}$ = 12-H, 1-H^F); 6.60 (dd, $J_{3,4}$ = 3.5 Hz, $J_{4,5}$ = 1.5 Hz); 8.12 and 8.90 (NH_2); 9.20 (d, J = 8 Hz, NH^E).- ^{13}C NMR (400 MHz, pyridine-d_5): δ = 52.2 (OCH_3), 56.2 (C-2^E); 62.4 (CH_2-6^E); 68.4 (C-2^I); 68.6; 69.5; 69.8; 71.8; 72.0; 72.5; 75.7 (C-2^F); 77.1; 78.2 (C-2^H); 90.7 (CCl_3, phosphate protecting group); 95.1 (CCl_3, TROC group); 97.8 (C-1^F); 102.5 (C-1^E); 154.1 and 156.6 (O<u>C</u>OOCCl_3 and $OCONH_2$); 168.7; 169.9; 170.3; 170.4; 170.7; 170.9 (CO signals). No mass spectrum could be obtained.

<u>2-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-carbamoyl-1-O-{[(R)-2-methyloxycarbonyl-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethyloxy]-hydroxy-phosphoryl}-α-D-galactopyranuronamide (10f)</u>

To a solution of triester **10e** (42.0 mg, 0.030 mmol) in pyridine (1.25 ml) Zn-Cu couple (freshly prepared[51], 24 mg) and 2,4-pentanedione[45] (57 µl, 0.56 mmol) were added and the mixture was stirred at 20°C for 5 h. After filtration and solvent evaporation the residue was dissolved in 10:1 water-methanol (5 ml), and Zn^{2+} ions were removed by addition of Dowex 50 W X10 resin (H^+ form, stirred for 1 h). Filtration, lyophilization, and LC ($CHCl_3$-methanol 2:1) gave **10f** (29.7 mg, 90%).- ^{13}C NMR (pyridine-d_5): δ = 51.9 (OCH_3); 54,9 (C-2^E); 62.3 (C-6^E); 66.4; 68.9; 69.4; 69.5; 69.6; 71.8; 72.5; 73.9; 75.2 (C-2^F); 79.5 (C-2^H); 95.9 (d, $J_{13C,31P}$ = 5.8 Hz, C-1^F); 102.2 (C-1^E); 157.8 ($OCONH_2$); 169.8; 170.4; 170.7; 171.0; 171.6; 172.0 (CO signals).- $C_{50}H_{88}N_3O_{21}P$ (1098.230, 1097.565), FAB MS (lactic acid): m/z 1136.7 ($[M+K]^+$), 589.4 ($[M-f+K+H]^+$), 330.3 ($[e]^+$).

<u>2-O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-3-O-carbamoyl-1-O-{[(R)-2-methoxycarbonyl-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethoxy]-hydroxy-phosphoryl}-α-D-galactopyranuronamide (10g)</u>

A solution of **10f** (5 mg, 0.0046 mmol) in 2:1 methanol-water (bidist., 0.5 ml) was flushed with argon and then at 0°C 0.3 mol/l LiOH (92.0 µl, 0.0276 mmol) was added. The mixture was stirred at 0°C for 30 min. The reaction was stopped by addition of Dowex 50 W X2 (H^+ form). Stirring at 20°C for 30 min, filtration, lyophilization, LC (gradient $CHCl_3$-methanol 2:1-->$CHCl_3$-methanol-water 20:10:1.5) gave **10g** (2.0 mg, 45%). This sample was dissolved in water, and passed through a small column with Dowex 50 W X2 (H^+) to remove inorganic ions, and then freed from solvent by lyophilization.- $C_{44}H_{82}N_3O_{18}P$ (972.118, 971.533), FAB MS (lactic acid): m/z 1010.9 ($[M+K]^+$), 589.1($[M-f+K+H]^+$), 422.0, 204.2 ($[e]^+$).

<u>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]-hydroxy-phosphoryl}-α-D-galactopyranuronamide (10c)</u>

A solution of **10f** (20.2 mg, 0.0184 mmol) in 2:1 methanol-water (bidist., 2 ml) was flushed with argon, and then at 0°C 0.3 mol/l LiOH (371.7 µl, 0.112 mmol) was added. The mixture was stirred at 20°C for 13 h, then the reaction was stopped by addition of Dowex 50 W X2 (H^+ form). Stirring at 20°C for 30 min, filtration, and lyophilization gave a sample of 16.7 mg which according to ^{13}C NMR contained about 30% of a second compound with a similar structure. Separation was achieved by LC (SiO_2, isopropanol - 2 mol/l NH_3 7:3), the fraction containing pure **10c** were combined, lyophilized, redissolved in water, and passed through of column of RP-18 (40-63 µm, methanol-water-acetonitrile 8:1:4) to remove inorganic ions. 5.4 mg of pure **10c** were obtained.- ^{13}C NMR ($CDCl_3$-CD_3OD-D_2O 18:11:2.7): δ = 173.2 (C-1^H), 172.0 ($NHCOCH_3$ and $CONH_2$), 157.1 ($OCONH_2$), 102.4 (C-1^E), 94.8 (C-1^F), 77.2 (?, C-2^H), 75.6 (C-2^F (C,P coupling) and another signal), 75.0, 73.6, 71.0 (C-1^I, C,P coupling), 70.3, 69.7 (broad signal), 69.5, 69.0 (broad signal), 67.6 (C-5^F), 65.7 (C-3^H, C,P coupling), 60.3 (C-6^E), 55.2 (C-2^E).- $C_{43}H_{80}N_3O_{18}P$ (958.091, 957.517), FAB MS (lactic acid): m/z 1002.35 ($[M-H+2Na]^+$), 996.8 ($[M+K]^+$), 980.4 ($[M+Na]^+$), 958.4 ($[M+H]^+$), 559.3 ($[M-f+Na+H]$), 204.1 ($[e]^+$).

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