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A Chemoenzymatic Approach towards Moenomycin Structural Analogues

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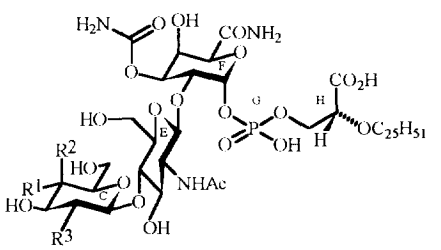
Abstract - The trisaccharide moenomycin analogue **1c** has been synthesized. One starting material was N-acetyllactosamine obtained by an enzyme-catalyzed transglycosylation. **1c** differs from moenomycin degradation product **1a** only in two positions of unit C. In contrast to **1a** the synthetic **1c** is antibiotically inactive.

When the antibiotics moenomycin A_{12}[1] and C_1[2] are degraded to trisaccharide derivatives of type **1a**[3] the antibiotic activity is fully retained. However, inactive disaccharide products result when unit C is removed from **1a** and similar structures.[1,2,4] With the aim of defining the role of unit C with respect to structure-activity relations

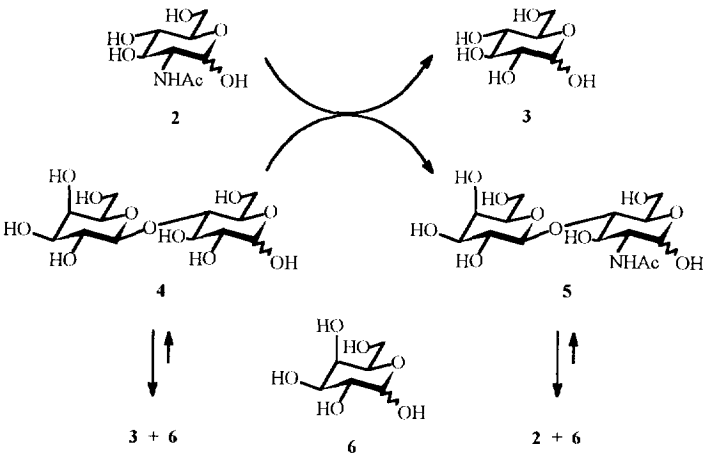


1	R^1	R^2	R^3
a	OH	H	NHAc
b	OH	H	OH
c	H	OH	OH

Scheme 1

we synthesized compound **1b** which differs from **1a** solely at C-2 of unit C. **1b** was found to be antibiotically inactive. The implications of this important result are discussed in the preceding paper.[5]

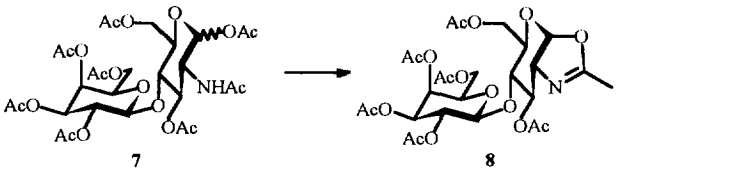
Parallel with the synthesis of **1b** we set out to prepare **1c**, the unit C D-*galacto* isomer of **1b** planning to make use of N-acetyllactosamine (**5**) as starting material. **5** is readily available by a *Bacillus circulans* β-galactosidase-catalyzed transglycosylation[6] in a membrane reactor.[7] In this reaction the galactose unit is transferred from D-lactose (**4**) to N-acetylglucosamine (**2**) to yield **5** and D-glucose (**3**). In a parallel reaction the enzyme catalyses the hydrolysis of **4** to give **3** and D-galactose (**6**). Since **5**, too, is a substrate of the enzyme, the desired **5** under the reaction conditions is partly hydrolyzed to monosaccharides **2** and **6**. Thus, a rather complicated reaction mixture results, containing monosaccharides **2**, **3**, and **6** and disaccharides **4** and **5**. The formation of the 1→6 isomer of **5** has also been detected. The first objective of the present study was to develop a protocol for the separation of this mixture.[8]



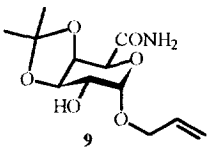
Scheme 2

Repeated silica gel chromatography allowed to isolate pure **5** from the mixture. In a much more convenient way the mixture was first peracetylated. Then simple flash chromatography (silica gel, $CHCl_3$-acetone-ethanol 95:5:0.5→95:10:0.5) permitted the isolation of **7** (0.112 g from 1.248 g of the mixture of **2** - **6**). Since **7** is readily hydrolyzed to provide **5**[9] the detour via **7** seems to be justified.

7 was converted into oxazoline **8** (method of Nakabayashi et al. [10]) and this was used to form trisaccharide **11** in an acid-catalyzed reaction with the known glycosyl acceptor **9**[11] (69% yield). Acetonide cleavage (**11**→**10a**),



7 8



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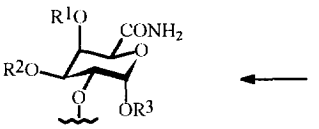
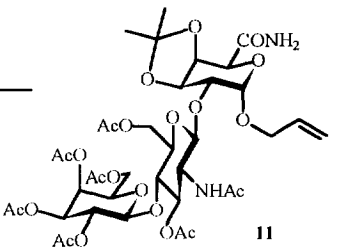
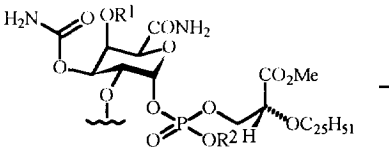


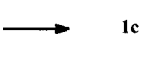
10	R^1	R^2	R^3
a	H	H	All
b	H	$CONH_2$	All
c	$CONH_2$	$CONH_2$	All
d	$CO_2CH_2CCl_3$	$CONH_2$	All
e	$CO_2CH_2CCl_3$	$CONH_2$	
f	$CO_2CH_2CCl_3$	$CONH_2$	OH



11



12	R^1	R^2
a	$CO_2CH_2CCl_3$	$C(CH_3)_2CCl_3$
b	H	H



1c

Scheme 3

followed by (i) reaction with trichloroacetyl isocyanate and (ii) zinc in methanol provided **10b** (78%) and a small amount of the dicarbamoyl derivative **10c**.

The free hydroxy group of **10b** was protected with the TROC group[12] (**10b→10d**) and the allyl group was then removed in a two-step procedure including allyl → (E)-propenyl isomerization with a hydrogen-activated cationic iridium complex[13,14,15] and subsequent treatment with $HgCl_2$/HgO in aqueous acetone[16] (**10d→10e→10f**).

For the construction of the phosphoric acid diester grouping we used a version of the phosphite procedure as adapted to the synthesis of moenomycin analogues.[17] Thus, the sequence (i) treatment of 2,2,2-trichloro-1,1-dimethylethyl dichlorophosphite with two equivalents of 1H-1,2,4-triazole,[18] (ii) reaction of the thus prepared reagent with **10f**, (iii) subsequent reaction with moenomycin degradation product methyl (R)-2-(3,8,8,11,14,18-hexamethylnonadecyloxy)-3-hydroxypropionate,[19] and (iv) oxidation of the intermediate phosphite triester with bis(trimethylsilyl)peroxide[20] furnished a 6:1 mixture of two compounds. The main component according to all spectroscopic data was a triester of the general formula **12a**. It was clearly an α-phosphate with $J_{1,2}$ in unit F of 3.7 Hz. The chemical shift of C-1^F was δ = 97.33, as expected. The spectra of the side product are almost identical with those of the main compound. For C-1^F δ = 96.86 was observed. Probably the two compounds are stereoisomeric at the P centre.

Removal of the protecting groups with the trichloroethyl unit from the main phosphate triester was achieved under the Imai conditions[21] with freshly prepared Zn-Cu couple[4] to provide **12b** (41%). 4% of a side product were isolated the structure of which could not be determined. Finally, hydrolysis of the ester groups converted **12b** into **1c** (32% of the pure compound were isolated). Again, a side product was formed the structure of which could not be determined because only trace amounts were isolated. The FAB MS of **1c** showed the correct $[M+H]^+$ peak at m/z=1120.3. The ^{13}C NMR spectrum was not of high quality because of the small amount which was prepared but displayed all the expected signals. Solely the signal of C-1^F was somewhat broader than expected.

Antibiotic activity of 1c

The biological activities of **1c** were tested in the following assays:

(i) Inhibition of the UDP-N-acetylmuramyl pentapeptide-dependent incorporation of [^{14}C]UDP-N-acetylglucosamine into cross-linked high-molecular weight peptidoglycan (studied with a slightly modified[22] version of the assay described by Izaki, Matsuhashi, and Strominger[23])

(ii) the inhibitory effect of **1c** directly on the transglycosylation reaction (determined by the *in vitro* assay developed earlier in one of our laboratories[24] using a crude extract from an over-producer of polymerase PBP1b (*E.coli JA200 plc19-19)* and as substrate lipid II which is the immediate precursor of uncrosslinked peptidoglycan)

(iii) the minimum inhibitory concentrations (MIC) of compound **1c** against various microorganisms (determined by a serial two-fold agar dilution method, Müller Hinton Agar).

Table 1: Effect of compound **1c**, **1a**,[3] and of moenomycin A (for comparison) on the *in-vitro*UDP-N-acetylmuramyl pentapeptide-dependent incorporation of [^{14}C]UDP-N-acetyl-glucosamine into cross-linked high-molecular weight peptidoglycan.

concentration (mg/L)	% inhibition moenomycin A	**1a**	**1c**
10	95	93	37
1	87	86	0
0.1	24	18	0

Table 2: Effect of **1c**, **1a**,[3] and of moenomycin A (for comparison) on the *in-vitro* formation of uncross-linked peptidoglycan by transglycosylation.

final concentration (mg/L)	% inhibition moenomycin A	**1a**	**1c**
10	100	100	62
1	100	93	0
0.1	78	43	0

Table 3: Minimum inhibitory concentrations (in mg/L) of compound **1c**, **1a**,[3] and of moenomycin A (for comparison) against various test organisms.

test organism	moenomycin A	**1a**	**1c**
Staph.aureus SG 511	0.025	12.5	>50
Staph.aureus 503	0.049	12.5	>50
Strept. pyogenes A77	<0.002	0.781	6.25
Pseud. aerug. 1771m	3.125	50	>50
E. coli DC 2	50	>100	>50

The results (see Tables 1-3) demonstrate that **1c** is neither active in the *in-vitro* nor in the *in-vivo* test systems. This result is in agreement with those discussed in the preceding paper.

EXPERIMENTAL

For equipment and general methods, see the preceding publication. If not stated otherwise, the H,H coupling constants, when observed, were within 0.2 Hz of the following values: unit C: $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.5 Hz, $J_{4,5}$ = 1.0 Hz, $J_{5,6}$ = 8.0 Hz, $J_{5,6'}$ = 7.0 Hz; unit E: $J_{1,2}$ = 8.5 Hz, $J_{2,NH}$ = 9.0 Hz, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ =

8.5 Hz, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 5.4 Hz, $J_{5,6'}$ = 2.0 Hz, $|J_{6,6'}|$ = 12.0 Hz; unit F: $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.5 Hz; allyl unit, $|J_{1,1'}|$ = 13.4 Hz, $J_{1,2}$ = 5.6 Hz, $J_{1',2}$ = 5.3 Hz, $J_{2,3cis}$ = 10.5 Hz, $J_{2,3trans}$ = 17.0 Hz, $|^4J|$ = 1.4 Hz, $|J_{3cis,3trans}|$ = 3.6 Hz; trichloroethoxycarbonyl unit, $|J_{gem}|$ = 12.0 Hz.

Separation of the enzyme-catalyzed transgalactosylation reaction mixture

a) After acetylation: An aqueous solution of the reaction mixture[7] (5 mL) was freeze-dried. The residue (1.248 g), pyridine (27 mL), and acetic anhydride (27 mL) were stirred at 20°C for 4h. After solvent evaporation and lyophilization a yellow solid (2.6 g) was obtained. This was dissolved in CH_2Cl_2, Celite® was added and the mixture was evaporated. This material was transferred onto the top of a flash column (40 cm, ∅ = 2.5 cm, 50 g of silica gel). Elution with $CHCl_3$-acetone-ethanol 95:5:0.5 gave a mixture of the pentaacetyl derivatives of D-glucose and D-galactose (both anomers, 0.189 g), the peracetylated anomers of D-lactose (0.259 g), a mixture of the peracetylated anomers of D-lactose and D-glucosamine (0.664 g) and 1,3,4,6-tetra-O-acetyl-2-acetamido-2-desoxy-D-glucopyranose (both anomers, 0.894 g). Then the polarity of the solvent was increased ($CHCl_3$-acetone-ethanol 90:10:0.5) to elute the desired **7** (0.112 g). Final traces of this material were eluted with $CHCl_3$-acetone 60:40.

b) Direct separation: The crude aqueous reaction mixture[7] (vide supra) was freeze-dried. 25 g of the residue were dissolved in water, Celite® (75 g) was added, and the solvent was removed by lyophilization. The product mixture adsorbed on Celite® was applied onto the top of a chromatographic column (silica gel, 800 g, elution with ethyl acetate-ethanol-water 8:4:1). The following fractions were obtained (increasing polarity, TLC: ethyl acetate-ethanol-water 2:4:1 and 8:4:1, three times developed): monosaccharides (5.99 g), monosaccharides and small amounts of disaccharides (2.90 g), a fraction containing all components (3.68 g), a fraction containing mainly **4** and **5** (3.56 g) and almost pure **4** (2.40 g). From the 3.56 g fraction 1.75 g were separated by MPLC ($CHCl_3$-methanol-water 12:7:1, the sample was dissolved in pyridine and then applied onto the top of the column) to give **5** (109.7 mg), a mixture containing **5** with some **4** (168.0 mg), a mixture containing **5** and mainly **4** (330.9 mg), and **4** (680.5 mg).

2-Acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-glucopyranose (7)

To a solution of N-acetyllactosamine (**5**) (154.0 mg, 0.402 mmol) in pyridine (5.0 mL) acetic anhydride (6.0 mL) was added and the mixture was stirred at 20°C for 4 h. Solvent evaporation and LC ($CHCl_3$-EtOH 50:1) yielded a mixture of α- and β-**7** (220.8 mg, 81%). A separation of α- and β-**7** using the same solvent system was possible.- α-**7**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.90 - 2.10 (8 s's, $COC\underline{H}_3$), 3.79 - 3.92 (m, 4-H^E, 5-H^E, 5-H^C), 4.03 - 4.16 (m, 6-H^E, 6-H^C, 6'-H^C), 4.30 - 4.43 (m, 2-H^E, 6'-H^E), 4.51 (d, 1-H^C), 4.95 (dd, 3-H^C), 5.10 (dd, 2-H^C), 5.22 (br dd, 3-H^E), 5.35 (dd, 4-H^C), 5.61 (br d, N$\underline{H}$COCH$_3$), 6.07 (d, 1-H^E); coupling constants: unit E, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 11.0 Hz.- β-**7**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.90 - 2.14 (8 s's, $COC\underline{H}_3$), 3.74 (ddd, 5-H^E), 3.81 (dd, 4-H^E), 3.84 - 3.92 (m, 5-H^C), 4.02 - 4.17 (m, 6-H^E, 6-H^C, 6'-H^C), 4.24 (ddd, 2-H^E), 4.42 (dd, 6'-H^E), 4.46 (d, 1-H^C), 4.95 (dd, 3-H^C), 5.03 (dd, 3-H^E), 5.08 (dd, 2-H^C), 5.33 (dd, 4-H^C), 5.61 (d, 1-H^E), 5.88 (br d, N$\underline{H}$COCH$_3$); coupling constants: unit E, $J_{1,2}$ = 8.0 Hz, $J_{2,NH}$ = 9.5 Hz, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 8.0 Hz, $J_{4,5}$ = 8.0 Hz, $J_{5,6}$ = 5.0 Hz, $J_{5,6'}$ = 3.0 Hz.- Spectra of a α/β-**7**: mixture: IR ($CHCl_3$): 3420 (NH), 1745 (C=O), 1685 (amide I), 1510 (amide II), 1370 cm^{-1} (CH_3CO).- FAB MS: m/z = 1355.5 ([2M+H]$^+$), 700.3 ([M+Na]$^+$), 678.3 ([M+H]$^+$), 618.2 ([M+H-HOAc]$^+$), 331.1 ([c]$^+$).- Anal. calc. for $C_{28}H_{39}O_{18}N$ (677.61, 677.22): C 49.63, H 5.80, found C 49.61, H 5.84.

2-Methyl-{3,6-di-*O*-acetyl-1,2-dideoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyrano}-[2,1-d]-oxazoline (8)

To a solution of a mixture of α- and β-**7** (3.578 g, 5.280 mmol) in dry 1,2-dichloroethane (21 mL) TMSOTf (1.15 mL, 6.34 mmol) was added and the mixture was stirred at 50°C. After 4.5 h another portion of TMSOTf (0.29 mL, 1.60 mmol) was added. After a further 1.5 h, the reaction was stopped with triethylamine (1.5 mL). Solvent evaporation, followed by LC (toluene-ethyl acetate-triethylamine 100:150:1), gave **8** (2.583 g, 79%).- ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.92 - 2.17 (7 s's, $COC\underline{H}_3$ and $C\underline{H}_3$), 3.44 - 3.49 (m, 4-H^E), 3.63 (br d, J = 9.0 Hz, 5-H^E), 3.94 (ddd, 5-H^C), 4.01 - 4.15 (m, 6-H^C, 6-H'^C, 6-H^E, 2-H^E), 4.18 (dd, 6-H'^E), 4.62 (d, 1-H^C),

4.98 (dd, 3-H^{C}), 5.15 (dd, 2-H^{C}), 5.34 (dd, 4-H^{C}), 5.62 (br d, J = 2.5 Hz, 3-H^{E}), 5.90 (d, 1-H^{E}); coupling constants: unit C, $J_{5,6}$ = 7.5 Hz, $J_{5,6'}$ = 6.5 Hz; unit E, $J_{1,2}$ = 7.5 Hz, $J_{5,6'}$ = 2.5 Hz.- IR ($CHCl_3$): 1745 (C=O), 1670 (C=N), 1365 (CH_3CO), 1045 cm^{-1}(C-O ether).- Anal. calc. for $C_{26}H_{35}O_{16}N$ (617.56, 617.20): C 50.57, H 5.71, found C 50.40, H 5.77.

Allyl 2-*O*-{2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl}-3,4-*O*-isopropylidene-α-D-galactopyranosiduronamide (11)

To a solution of allyl 3,4-*O*-isopropylidene-α-D-galactopyranosiduronamide (**9**) (4.574 g, 16.74 mmol) in dry CH_2Cl_2 (3.8 mL) solutions of camphorsulfonic acid (195 mg, 0.838 mmol) in dry CH_2Cl_2 (7.75 mL) and of oxazoline **8** (1.292 g, 2.092 mmol) in dry CH_2Cl_2 (3.0 mL) were added and the mixture was stirred 3 h at 60°C. Then further portions of **8** (1.292 g, 2.092 mmol) in dry CH_2Cl_2 (5.0 mL) and camphorsulfonic acid (50 mg, 0.215 mmol) in dry CH_2Cl_2 (2.0 mL) were added. After stirring for another 3.5 h at 60°C, the reaction was stopped by addition of triethylamine (1.0 mL). After 15 min the solvents were evaporated. LC ($CHCl_3$-EtOH 30:1) gave **11** (2.589 g, 69%); 2.844 g (10.41 mmol) of **9** were recovered.- ^{1}H NMR (400 MHz, $CDCl_3$, H,H COSY): δ = 1.33 and 1.48 (2 s's, C(C$\underline{H}_3$)$_2$), 1.90 - 2.16 (7 s's, COC$\underline{H}_3$), 3.55 (ddd, 5-H^{E}), 3.75 (dd, 2-H^{F}), 3.79 (dd, 4-H^{E}), 3.85 (ddd, 5-H^{C}), 3.92 - 4.14 (m, 6-H^{C}, 6-H'^{C}, 6-H^{E}, 2-H^{E}, 1-H^{allyl}, 1'-H^{allyl}), 4.27 (dd, 3-H^{F}), 4.48 (d, 5-H^{F}), 4.50 (d, 1-H^{C}), 4.51 - 4.57 (m, 6-H'^{E}, 4-H^{F}), 4.77 (d, 1-H^{E}), 4.94 (dd, 3-H^{C}), 4.95 (d, 1-H^{F}), 5.08 (dd, 2-H^{C}), 5.12 (dd, 3-H^{E}), 5.16 (m, 3_{cis}-H^{allyl}), 5.28 (m, 3_{trans}-H^{allyl}), 5.32 (dd, 4-H^{C}), 5.68 (br s, $CONH_2$), 5.73 (d, N$\underline{H}$COCH$_3$), 5.77 - 5.89 (m, 2-H^{allyl}), 6.46 (br d, J = 3.5 Hz, $CONH_2$); coupling constants: unit E, $J_{5,6}$ = 4.5 Hz; unit F, $J_{2,3}$ = 8.0 Hz, $J_{3,4}$ = 5.5 Hz, $J_{4,5}$ = 3.0 Hz; allyl unit, $|J_{3cis,3trans}|$ = 3.0 Hz.- ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 20.70 - 21.07 (OCO$\underline{C}$H$_3$ signals), 23.42 (NHCO$\underline{C}$H$_3$), 26.62 and 28.48 (C(C$\underline{H}_3$)$_2$), 54.20 (C-2^{E}), 60.99 and 62.12 (C-6^{C} and C-6^{E}), 66.84, 68.46, 69.43, 69.64, 70.90, 71.10, 73.10, 73.82, 75.53, 76.19, 77.35, 77.48, 97.85 (C-1^{F}), 101.23 and 101.39 (C-1^{C} and C-1^{E}), 109.73 (C(CH$_3$)$_2$), 117.93 (C-3^{allyl}), 133.44 (C-2^{allyl}), 169.42 - 170.77 (COCH$_3$ signals and C-6^{F}).- IR ($CHCl_3$): 3520 and 3410 (NH amide), 1750 (C=O acetyl), 1695 (amide I), 1575 cm^{-1} (amide II).- $C_{38}H_{54}O_{22}N_2$ (890.85, 890.32), FAB MS: m/z = 891.3 ([M+H]$^+$), 618.2 ([e]$^+$), 331.1 ([c]$^+$).

Allyl 2-*O*-{2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl}-α-D-galactopyranosiduronamide (10a)

A solution of **11** (2.460 g, 2.762 mmol) in acetic acid (20 per cent, 79 mL) was stirred at 60°C for 4 h. After addition of water (500 mL), most of the solvents were removed by evaporation. Lyophilization and LC ($CHCl_3$-EtOH 6:1) yielded **10a** (2.026 g, 86%).- ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.87 - 2.10 (7 s's, COC$\underline{H}_3$), 3.52 - 3.57 (m, 5-H^{E}), 3.74 - 3.79 (m, 4-H^{E}, 2-H^{F}), 3.84 (m, 5-H^{C}), 3.92 - 4.15 (m, 6-H^{C}, 6-H'^{C}, 2-H^{E}, 6-H^{E}, 3-H^{F}, 4-H^{F}, 5-H^{F}, 1-H^{allyl}, 1'-H^{allyl}), 4.19 (s, OH), 4.33 (br s, OH), 4.47 (d, 1-H^{C}), 4.55 - 4.62 (m, 1-H^{E}, 6-H'^{E}), 4.92 (dd, 3-H^{C}), 4.96 (dd, 2-H^{C}), 5.00 (dd, J =10.4 Hz, J = 7.7 Hz, 3-H^{E}), 5.05 (d, 1-H^{F}), 5.10 (m, 3_{cis}-H^{allyl}), 5.24 (m, 3_{trans}-H^{allyl}), 5.29 (dd, 4-H^{C}), 5.76 - 5.88 (m, 2-H^{allyl}), 6.61 and 6.77 (2 br s's, $CONH_2$), 7.15 (br d, N$\underline{H}$COCH$_3$); coupling constants: unit E, $J_{2,NH}$ = 9.5 Hz; allyl unit, $|J_{3cis,3trans}|$ = 3.0 Hz.- ^{13}C NMR (100.6 MHz, $CDCl_3$, C,H COSY): δ = 20.68 - 21.13 (OCO$\underline{C}$H$_3$ signals), 23.25 (NHCO$\underline{C}$H$_3$), 53.75 (C-2^{E}), 60.87 (C-6^{C}), 61.8 (C-6^{E}), 66.84, 68.36, 69.50 (C-1^{allyl}), 69.66 (C-5^{C}), 70.07, 70.88, 71.10, 71.39, 72.88, 73.89, 76.37 (C-4^{E}), 77.5, 78.73, 98.51 (C-1^{F}), 101.45 (C-1^{C}), 103.23 (C-1^{E}), 117.72 (C-3^{allyl}), 133.94 (C-2^{allyl}), 169.53 - 172.69 (COCH$_3$ signals and C-6^{F}).- IR ($CHCl_3$): 3600-3150 (OH), 1750 (C=O acetyl), 1685 (amide I), 1570 cm^{-1} (amide II).- $C_{35}H_{50}O_{22}N_2$ (850.78, 850.29), FAB MS: m/z = 851.4 ([M+H]$^+$), 618.2 ([e]$^+$), 331.1 ([c]$^+$).

Allyl 2-*O*-{2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl}-3-*O*-carbamoyl-α-D-galactopyranosiduronamide (10b)

To a solution of **10a** (2.103 g, 2.472 mmol) in dry CH_2Cl_2 (200 mL) at -15°C trichloroacetyl isocyanate (0.30 mL, 2.5 mmol) was added and the mixture was stirred for 2 h at this temperature. After destruction of excess reagent with MeOH (10 mL) and stirring for 15 min, the solvent was evaporated. The residue was redissolved in MeOH (120 mL), Zn dust (1.615 g, 24.70 mmol) was added, and the mixture was stirred at 20°C for 2 h 45 min. After filtration and washing of the residue with MeOH (20 mL), the residue (zinc and zinc salts) were extracted with CH_2Cl_2 (340 mL) in a Soxhlet apparatus for 15.5 h. Evaporation of the combined filtrates,

followed by LC ($CHCl_3$-MeOH 10:1), then MPLC (B column, $CHCl_3$-MeOH 10:1) furnished 668.3 mg of recovered **10a** (0.786 mmol) and the carbamoylated product **10b** (1.181 g, 53%, 78% based on consumed **10a**).- ^{1}H NMR (400 MHz, pyridine-d_5, H,H COSY): δ = 1.92 - 2.27 (7 s's, $COC\underline{H}_3$), 3.54 (ddd, 5-H^E), 4.10 (br t, J≈ 8.8 Hz, 4-H^E), 4.11 (ddt, 1-H^{allyl}), 4.19 - 4.29 (m, 2-H^E, 1'-H^{allyl}), 4.39 (dd, 6-H^E), 4.41 - 4.49 (m, 5-H^C, 6-H^C, 6-H'^{C}), 4.74 (br dd, 6-H'^{E}), 4.83 (s, 5-H^F), 4.85 (dd, 2-H^F), 5.03 (m, 3_{cis}-H^{allyl}), 5.10 (d, 1-H^C), 5.29 (m, 3_{trans}-H^{allyl}), 5.32 (d, 1-H^E), 5.44 (br s, 4-H^F), 5.56 (d, 1-H^F), 5.60 - 5.70 (m, 2-H^C, 3-H^C), 5.78 (br t, J≈ 9.5 Hz, 3-H^E), 5.80 - 5.85 (m, 3-H^F, 4-H^C), 5.86 - 5.93 (m, 2-H^{allyl}), 7.91 (d, 1H, J = 2.2 Hz, $CONH_2$), 8.52 (br s, 1H, $CONH_2$), 8.67 (d, N$\underline{H}$$COCH_3$); coupling constants: unit C, $J_{1,2}$ = 7.5 Hz; unit F, $J_{2,3}$ = 11.0 Hz; allyl unit, $J_{2,3trans}$ = 16.5 Hz.- ^{13}C NMR (100.6 MHz, pyridine-d_5): δ = 19.57 - 20.43 ($OCO\underline{C}H_3$ signals), 22.69 ($NHCO\underline{C}H_3$), 55.03 (C-2^E), 60.87 and 61.81 (C-6^C and C-6^E), 67.15, 68.31, 68.48, 69.50, 70.53, 71.12, 72.09, 72.38, 72.47, 73.11, 75.46, 76.97 (C-2^F), 98.51 (C-1^F), 101.10 and 101.82 (C-1^C and C-1^E), 116.23 (C-3^{allyl}), 134.12 (C-2^{allyl}), 157.04 ($O\underline{C}ONH_2$), 169.23 - 171.43 ($\underline{C}OCH_3$ signals and C-6^F).- IR ($CHCl_3$): 3600-3150 (OH and NH), 1750 (C=O acetyl), 1720 (C=O carbamoyl), 1680 (amide I), 1600, 1570 (amide II), 1325 cm^{-1}.- $C_{36}H_{51}O_{23}N_3$ (893.81, 893.29), FAB MS: m/z = 916.2 ([M+Na]$^+$), 894.2 ([M+H]$^+$), 618.1 ([e]$^+$), 331.1 ([c]$^+$).

Allyl 2-*O*-{2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl}-3,4-di-*O*-carbamoyl-α-D-galacto-pyranosiduronamide (10c)

10c was isolated as a side product (3% yield, 7% based on consumed **10a**) in another experiment that was carried out under conditions practically identical to those described above.- ^{1}H NMR (400 MHz, pyridine-d_5): δ = 1.91 - 2.27 (7 s's, $COC\underline{H}_3$), 3.47 (ddd, 5-H^E), 4.09 (br t, J≈ 9.4 Hz, 4-H^E), 4.13 (ddt, 1-H^{allyl}), 4.22 (ddt, 1'-H^{allyl}), 4.22 - 4.28 (m, 2-H^E), 4.33 (dd, 6-H^E), 4.40 - 4.49 (m, 5-H^C, 6-H^C, 6-H'^{C}), 4.76 (dd, 6-H'^{E}), 4.92 (d, 5-H^F), 5.03 (m, 3_{cis}-H^{allyl}), 5.12 (m, 1H), 5.13 (d, 1-H^C), 5.29 (m, 3_{trans}-H^{allyl}), 5.57 (d, 1-H^F), 5.61 - 5.70 (m, 2-H^C, 3-H^C), 5.75 (dd, 3-H^E), 5.81 - 5.85 (m, 4-H^C), 5.84 - 5.92 (m, 2-H^{allyl}), 6.02 (dd, 3-H^F), 6.70 (dd, 4-H^F), 7.90 (d, 1H, J≈ 2 Hz, $CONH_2$), 8.58 (br s, 1H, $CONH_2$), 8.72 (m, N$\underline{H}$$COCH_3$); coupling constants: unit E, $J_{3,4}$ = 9.1 Hz, $J_{4,5}$ = 10.0 Hz, $J_{5,6}$ = 4.8 Hz; allyl unit, $|J_{1,1'}|$ = 13.1 Hz.- ^{13}C NMR (100.6 MHz, pyridine-d_5): δ = 19.25 - 20.13 ($OCO\underline{C}H_3$ signals), 22.33 ($NHCO\underline{C}H_3$), 54.48 (C-2^E), 60.47 and 61.35 (C-6^C and C-6^E), 66.78, 68.36, 69.22, 69.42, 69.86, 70.07, 70.18, 70.79, 72.08, 72.90, 75.91, 76.49 (C-2^F), 98.07 (C-1^F), 100.79 and 101.82 (C-1^C and C-1^E), 116.06 (C-3^{allyl}), 133.59 (C-2^{allyl}), 156.34 ($O\underline{C}ONH_2$, very large signal), 168.90 - 169.72 ($\underline{C}OCH_3$ signals and C-6^F).- IR ($CHCl_3$): 3600-3100 (NH), 1745 (C=O acetyl), 1680 (amide I), 1600 (carbamoyl), 1550 cm^{-1} (amide II).- $C_{37}H_{52}O_{24}N_4$ (936.83, 936.30), FAB MS: m/z = 959.6 ([M+Na]$^+$), 937.6 ([M+H]$^+$), 618.4 ([e]$^+$), 331.2 ([c]$^+$).

Allyl 2-*O*-{2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl}-3-*O*-carbamoyl-4-*O*-(2,2,2-trichloro-ethyloxy)carbonyl-α-D-galactopyranosiduron-amide (10d)

To a solution of **10b** (1.520 g, 1.700 mmol) in dry pyridine (104 mL) 2,2,2-trichloroethyl chloroformate (0.35 mL, 2.44 mmol) was added and the mixture was stirred at 20°C for 1 h. Excess reagent was destroyed with MeOH (5.0 mL). Solvent evaporation and LC ($CHCl_3$-MeOH 25:1) provided **10d** (1.518 g, 84%).- ^{1}H NMR (400 MHz, pyridine-d_5): δ = 1.92 - 2.25 (7 s's, $COC\underline{H}_3$), 3.64 (ddd, 5-H^E), 4.11 (br t, J≈ 9.5 Hz, 4-H^E), 4.11 - 4.19 (m, 2-H^E, 1-H^{allyl}), 4.23 (ddt, 1'-H^{allyl}), 4.40 (dd, 6-H^E), 4.41 - 4.48 (m, 5-H^C, 6-H^C, 6'-H^C), 4.81 (d, H^{TrOC}), 4.85 (dd, 6-H'^{E}), 4.97 (d, 5-H^F), 4.98 (d, H'^{TrOC}), 5.04 (m, 3_{cis}-H^{allyl}), 5.13 (br d, 1-H^C), 5.24 (d, 1-H^E), 5.30 (m, 3_{trans}-H^{allyl}), 5.56 (d, 1-H^F), 5.61 - 5.70 (m, 2-H^C, 3-H^C), 5.79 - 5.84 (m, 3-H^E, 4-H^C), 5.84 - 5.94 (m, 2-H^{allyl}), 6.02 (dd, 3-H^F), 6.60 (dd, 4-H^F), 8.09 (br s, 1H, $CONH_2$), 8.79 (s, 1H, $CONH_2$), 8.80 (d, N$\underline{H}$$COCH_3$); coupling constants: unit C, $J_{1,2}$ = 7.6 Hz; unit E, $J_{2,NH}$ ≈ 10 Hz, $J_{4,5}$ = 10.1 Hz.- ^{13}C NMR (100.6 MHz, pyridine-d_5, C,H COSY): δ = 19.83 - 20.66 ($OCO\underline{C}H_3$ signals), 22.88 ($NHCO\underline{C}H_3$), 55.30 (C-2^E), 61.08 (C-6^C), 62.01 (C-6^E), 67.37 (C-4^C), 69.15 (C-3^F), 69.76, 70.76 (C-5^C), 71.35, 72.76 (C-5^E), 73.06 (C-3^E), 75.78 (C-4^F), 75.87, 76.78 (C-2^F), 77.11 (C-4^E), 79.5, 94.9 ($OCOOCH_2\underline{C}Cl_3$), 98.53 (C-1^F), 101.32 (C-1^C), 102.00 (C-1^E), 116.80 (C-3^{allyl}), 134.03 (C-2^{allyl}), 153.93 ($O\underline{C}OOCH_2CCl_3$), 156.54 ($O\underline{C}ONH_2$), 169.44 - 170.35 ($\underline{C}OCH_3$ signals and C-6^F).- IR ($CHCl_3$): 3650-3100 (NH), 1752 (C=O acetyl), 1600 cm^{-1} (carbamoyl).- $C_{39}H_{52}O_{25}N_3Cl_3$ (1069.21, 1067.20), FAB MS: m/z = 1068.1 ([M+H]$^+$), 618.1 ([e]$^+$), 331.1 ([c]$^+$).

(E)-1-Propen-1-yl 2-*O*-{2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galacto-pyranosyl)-β-D-glucopyranosyl}-3-*O*-carbamoyl-4-*O*-(2,2,2-trichloroethyloxy)carbonyl-α-D-galacto-pyranosiduronamide (10e)

A solution of $[Ir(I)(cod)(PMePh_2)_2]PF_6$ (5.8 mg, 0.007 mmol) in dry THF (3.8 mL) and **10d** (75.8 mg, 0.071 mmol was carefully degassed, then the mixture was hydrogenated at 0.13 MPa until the colour of the solution turned from red to pale yellow. After three further degassing cycles the mixture was stirred under Ar at 20°C for 4 h. Solvent evaporation yielded 81.7 mg of crude **10e** which was only slightly impure (^{1}H NMR and TLC) and used without further purification.- ^{1}H NMR (400 MHz, pyridine-d_5): δ = 1.36 (dd, 3-$CH_3^{propenyl}$), 1.91 - 2.23 (7 s's, COC$\underline{H}_3$), 3.67 (ddd, 5-H^E), 4.05 - 4.13 (m, 2-H^E, 4-H^E), 4.37 (dd, 6-H^E), 4.41 - 4.48 (m, 5-H^C, 6-H^C, 6-H'^C), 4.77 (d, H^{TrOC}), 4.91 (dd, 6-H'^E), 4.97 (d, H'^{TrOC}), 5.00 (d, 5-H^F), 5.09 (dq, 2-$H^{propenyl}$), 5.12 (d, 1-H^C), 5.30 (d, 1-H^E), 5.61 - 5.68 (m, 2-H^C, 3-H^C), 5.79 (d, 1-H^F), 5.80 - 5.83 (m, 3-H^E, 4-H^C), 6.05 (dd, 3-H^F), 6.31 (dq, 1-$H^{propenyl}$), 6.58 (dd, 4-H^F), 8.09 and 8.77 (2 br s's, 1H each, $CONH_2$), 8.86 (d, N$\underline{H}$COCH$_3$); coupling constants: unit C, $J_{1,2}$ = 7.6 Hz; unit E, $J_{1,2}$ = 8.1 Hz, $J_{2,NH}$ = 8.5 Hz, $J_{4,5}$ = 10.1 Hz, $J_{5,6}$ = 4.9 Hz; propenyl unit: $J_{1,2}$ = 12.1 Hz, $J_{1,3}$ = 1.5 Hz, $J_{2,3}$ = 6.6 Hz.- ^{13}C NMR (100.6 MHz, pyridine-d_5): δ = 11.96 (C-$3^{propenyl}$), 19.77 - 20.60 (OCO$\underline{C}$H$_3$ signals), 22.82 (NHCO$\underline{C}$H$_3$), 55.36 (C-2^E), 61.03 (C-6^C), 61.66 (C-6^E), 67.33 (C-4^C), 68.85 (C-3^F), 69.74, 70.74 (C-5^C), 71.30, 72.73 (C-5^E), 72.93 (C-3^E), 75.31, 75.70 (C-4^F), 76.75 (C-2^F), 76.97, 94.80 (OCOOCH$_2\underline{C}$Cl$_3$), 98.37 (C-1^F), 101.26 and 101.88 (C-1^C and C-1^E), 104.65, 143.60 (C-$1^{propenyl}$), 153.9 (O$\underline{C}$OOCH$_2$CCl$_3$), 156.43 (O$\underline{C}$ONH$_2$), 169.00 - 170.28 ($\underline{C}$OCH$_3$ signals and C-6^F).- IR ($CHCl_3$): 3650-3100 (NH), 1752 (C=O acetyl), 1600 (carbamoyl), 1574 cm^{-1} (amide II).- $C_{39}H_{52}O_{25}N_3Cl_3$ (1069.21, 1067.20), FAB MS: m/z = 1068.1 ([M+H]$^+$), 618.1 ([e]$^+$), 331.1 ([c]$^+$).

2-*O*-{2-Acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl}-3-*O*-carbamoyl-4-*O*-(2,2,2-trichloroethoxy)carbonyl-α-D-galactopyranuronamide (10f)

To a solution of crude **10e** (39.6 mg, see above) in 9:1 acetone-water (1.5 mL) HgO (11.4 mg, 0.053 mmol) and $HgCl_2$ (18.9 mg, 0.070 mmol) were added, and the mixture was stirred at 20°C for 2.5 h. After removal of solids by filtration through celite, gaseous H_2S was passed carefully into the clear solution. The precipitates were removed by centrifugation and carefully washed with acetone. From the combined filtrates, acetone was distilled off, and the remaining water removed by lyophilization. LC ($CHCl_3$-MeOH 12:1) gave 6.8 mg of a mixture of **10d** and **10e** (0.006 mmol) and **10f** (27.8 mg, 79%, 96% based on recovered **10d** and **10e**).- ^{1}H NMR (400 MHz, pyridine-d_5): δ = 1.87 - 2.23 (7 s's, COC$\underline{H}_3$), 3.54 - 3.60 (m, 5-H^E), 4.06 (br t, J≈ 9.3 Hz, 4-H^E), 4.28 (ddd, 2-H^E), 4.33 (dd, 6-H^E), 4.38 - 4.44 (m, 5-H^C, 6-H^C, 6-H'^C), 4.53 (dd, 2-H^F), 4.70 - 4.76 (m, 6-H'^E), 4.84 (d, H^{TrOC}), 4.96 (d, H'^{TrOC}), 5.06 (1-H^C), 5.21 (d, 1-H^E), 5.36 (d, 5-H^F), 5.54 - 5.66 (m, 2-H^C, 3-H^C), 5.71 (dd, 3-H^E), 5.79 (dd, 4-H^C), 6.09 (d, 1-H^F), 6.18 (dd, 3-H^F), 6.64 (m, 4-H^F), 7.99 (br s, 1H, $CONH_2$), 8.56 (d, N$\underline{H}$COCH$_3$), 8.63 (br s, 1H, $CONH_2$); coupling constants: unit C, $J_{3,4}$ = 3.2 Hz, $J_{4,5}$< 1.0 Hz; unit E, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 9.3 Hz; unit F, $J_{1,2}$ = 3.2 Hz, $J_{4,5}$ = 0.9 Hz.- ^{13}C NMR (100.6 MHz, pyridine-d_5)*: δ = 19.71 - 20.57 (OCO$\underline{C}$H$_3$ signals), 22.79 (NHCO$\underline{C}$H$_3$), 55.01 (C-2^E), 60.97 and 62.08 (C-6^C and C-6^E), 67.25, 70.65, 71.24, 72.61, 73.23, 76.28, 76.43, 76.67, 77.19, 93.16 (C-1^F), 94.81 (OCOOCH$_2\underline{C}$Cl$_3$), 101.21 and 102.12 (C-1^C and C-1^E), 154.00 (O$\underline{C}$OOCH$_2$CCl$_3$), 156.66 (O$\underline{C}$ONH$_2$), 169.37 - 170.34 ($\underline{C}$OCH$_3$ signals and C-6^F).- IR ($CHCl_3$): 3650-3100 (OH, NH), 1752 (C=O acetyl), 1574 (amide II), 1371 cm^{-1} (CH_3CO).- $C_{36}H_{48}O_{25}N_3Cl_3$ (1029.14, 1027.16), FAB MS: m/z = 1028.0 ([M+H]$^+$), 618.1 ([e]$^+$), 331.0 ([c]$^+$).

*Signals at 69.27, 69.44, 69.60 probably belong to an impurity.

2-*O*-{2-Acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl}-3-*O*-carbamoyl-1-*O*-{[(R)-2-methyloxycarbonyl-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethyloxy]-(2-trichloromethyl-2-propyloxy)-phosphoryl}-4-*O*-(2,2,2-trichloroethoxy)carbonyl-α-D-galactopyranuronamide (12a)

To a solution of 1H-1,2,4-triazole (32.2 mg, 0.466 mmol) in dry CH_2Cl_2-pyridine 4:1 (1.4 mL) 1,1,1-trichloro-2-methyl-prop-2-yl dichlorophosphite (22.5 μL, 0.112 mmol) was added at 0°C. After stirring at 0°C for 25 min, **10f** (105.8 mg, 0.103 mmol), dissolved in dry CH_2Cl_2-pyridine 4:1 (0.8 mL), was added and the mixture was stirred at 0°C for 4 h. A solution of methyl (R)-2-(3,8,8,11,14,18-hexamethylnonadecyloxy)-3-hydroxy-

propionate[17] (145.4 mg, 0.309 mmol) in dry CH_2Cl_2-pyridine 4:1 was added in three portions (first 97.0 mg in 1.00 mL, after 40 min 24.2 mg in 0.25 mL, and after another 50 min 24.2 mg in 0.25 mL). Stirring of the mixture at 0°C was continued for 1.5 h. Bis(trimethylsilyl)peroxide (32 µL, 95% as determined by 80 MHz ^1H NMR, 0.144 mmol) was injected into the reaction flask, the cooling bath was removed and the mixture stirred for 15.5 h at 20°C. Solvent evaporation and MPLC ($CHCl_3$-MeOH 26:1) yielded **12a** (82.1 mg, 46%) and a slightly less polar side product (15.0 mg, 8%).- Spectra of **12a**: ^1H NMR (400 MHz, pyridine-d_5, H,H COSY): δ = 0.85 - 1.83 (lipid part), 1.93 - 2.25 (9 s's, $COC\underline{H}_3$ and $OC(C\underline{H}_3)_2CCl_3$), 3.58 - 3.64 (m, 1-$H^I$), 3.68 (ddd, 5-$H^E$), 3.72 (s, $COOC\underline{H}_3$), 3.76 - 3.86 (m, 1'-H^I), 4.20 - 4.30 (m, 2-H^E, 4-H^E), 4.39 - 4.44 (m, 5-H^C, 6-H^C, 6-H'^{C}, 6-H^E, 2-H^H), 4.55 (dt, 2-H^F), 4.59 - 4.68 (m, 3-H^H), 4.69 - 4.76 (m, 3'-H^H), 4.78 (d, H^{TrOC}), 4.78 - 4.85 (m, 6-H'^{E}, partially hidden), 4.97 (d, H'^{TrOC}), 5.16 (d, 1-H^C), 5.24 (d, 1-H^E), 5.36 (s, 5-H^F), 5.60 (dd, 3-H^C), 5.65 (dd, 2-H^C), 5.76 (t, J≈ 9.6 Hz, 3-H^E), 5.81 (d, 4-H^C), 6.01 (dd, 3-H^F), 6.56 (dd, 1-H^F), 6.61 (dd, 4-H^F), 8.09 (br s, 1H, $CONH_2$), 8.58 (d, $N\underline{H}COCH_3$), 8.78 (br s, 1H, $CONH_2$); coupling constants: unit C, $J_{1,2}$ = 7.3 Hz, $J_{4,5}$< 0.6 Hz; unit E, $J_{2,NH}$ = 8.5 Hz, $J_{4,5}$ = 9.9 Hz, $J_{5,6}$ and $J_{5,6'}$ = 5.2 and 2.6 Hz; unit F, $J_{1,P}$ = 5.2 Hz, $J_{2,P}$ ≈ 3.3 Hz, $J_{4,5}$< 0.7 Hz.- ^{13}C NMR (100.6 MHz, pyridine-d_5, C,H COSY): δ = 19.13 - 41.82 (CH, CH_2, CH_3 signals), 51.68 ($COO\underline{C}H_3$), 54.91 (C-2^E), 60.97 and 62.02 (C-6^C and C-6^E), 67.27 (C-4^C), 67.91, 68.33 (C-3^F), 69.36, 69.39, 69.68 (C-2^C), 70.71 (C-5^C), 71.26 (C-3^C), 71.39 (C-5^F), 72.62 (C-5^E), 73.15 (C-3^E), 74.84 (d, $^3J_{P,C}$ = 9.1 Hz, C-2^F), 75.29 (C-4^F), 76.75, 77.01, 77.85 (d, $^3J_{P,C}$ = 7.6 Hz, C-2^H), 90.34 (d, $^3J_{P,C}$≈ 5 Hz, $C(CH_3)_2\underline{C}Cl_3$), 94.70 ($OCOOCH_2\underline{C}Cl_3$), 97.33 (d, $^2J_{P,C}$ = 6.1 Hz, C-1^F), 101.26 (C-1^C), 102.20 (C-1^E), 153.64 ($O\underline{C}OOCH_2CCl_3$), 156.08 ($O\underline{C}ONH_2$), 168.11, 169.29, 169.63, 169.79 (very large signal), 169.88, 170.00, 170.08 and 170.43 (8 signals for $\underline{C}OCH_3$, C-6^F and C-1^H).- IR ($CHCl_3$): 3520 (NH), 3410 (NH), 1750 (C=O acetyl), 1705 (ester), 1580 (amide II), 1045 cm^{-1} (P-O-alkyl). $C_{69}H_{110}O_{31}N_3Cl_6P$ (1721.32, 1717.50, FAB MS: m/z = 1718.5 ($[M+H]^+$), 1009.9 ($[f]^+$), 618.0 ($[e]^+$), 331.0 ($[c]^+$).- Spectra of of the minor product: ^1H NMR (400 MHz, pyridine-d_5, H,H COSY): δ = 0.83 - 1.87 (lipid part), 1.91 - 2.28 (9 s's, $COC\underline{H}_3$ and $OC(C\underline{H}_3)_2CCl_3$), 3.69 - 3.78 (m, 5-$H^E$, 1-$H^I$), 3.90 - 3.99 (m, 1'-$H^I$), 3.95 and 3.96 (2 s's, $COOC\underline{H}_3$)*, 4.20 (br t, J ≈ 9.4 Hz, 4-H^E), 4.31 (ddd, 2-H^E), 4.41 - 4.59 (m, 5-H^C, 6-H^C, 6-H'^{C}, 6-H^E, 2-H^F, 2-H^H), 4.72 and 4.75 (2 d's, H^{TrOC})*, 4.78 - 4.87 (m, 6-H'^{E}, 3-H^H, 3'-H^H), 4.96 (d, H'^{TrOC}), 5.14 (d, 1-H^C), 5.20 (d, 1-H^E), 5.37 (br s, 5-H^F), 5.62 (dd, 3-H^C), 5.67 (dd, 2-H^C), 5.77 (dd, 3-H^E), 5.83 (d, 4-H^C), 6.06 (dd, 3-H^F), 6.62 - 6.68 (m, 1-H^F, 4-H^F), 8.33 (br s, 1H, $CONH_2$), 8.83 and 8.86 (2 d's, $N\underline{H}COCH_3$)*, 8.93 (br s, 1H, $CONH_2$); coupling constants: unit C, $J_{1,2}$ = 7.4 Hz, $J_{3,4}$ = 3.0 Hz, $J_{4,5}$ < 0.6 Hz; unit E, $J_{3,4}$ = 9.2 Hz; unit F, $J_{2,3}$ = 10.3 Hz, $J_{4,5}$ < 0.7 Hz.- ^{13}C NMR (100.6 MHz, pyridine-d_5): δ = 18.93 - 41.61 (CH, CH_2, CH_3 signals), 51.67 ($COO\underline{C}H_3$), 54.56 (C-2^E), 60.71*, 61.03 and 61.99 (C-6^C and C-6^E), 67.01, 67.79, 68.27, 69.42, 70.46, 71.04, 71.19, 72.41, 72.86, 74.72, 75.10, 76.48, 76.89, 77.67 (d, $^3J_{P,C}$ = 9.0 Hz, C-2^H), 89.93 (d, $^3J_{P,C}$ = 4.6 Hz, $C(CH_3)_2\underline{C}Cl_3$), 94.43 ($OCOOCH_2\underline{C}Cl_3$), 96.86 (br s, C-1^F), 101.17 and 101.95 (C-1^C and C-1^E), 153.42 ($O\underline{C}OOCH_2CCl_3$), 155.82 ($O\underline{C}ONH_2$), 168.16, 169.10, 169.42, 169.59, 169.81, 169.91, 170.10, 170.38 and 174.40 (9 signals for $COCH_3$, C-6^F and C-1^H).- $C_{69}H_{110}O_{31}N_3Cl_6P$ (1721.32, 1717.50), FAB MS: m/z = 1756.7 ($[M+K]^+$), 1718.5 ($[M+H]^+$), 1009.9 ($[f]^+$), 618.0 ($[e]^+$), 331.0 ($[c]^+$).

*These signals indicate an impurity.

2-*O*-{2-Acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl}-3-*O*-carbamoyl-1-*O*-{[(R)-2-methyloxycarbonyl-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethyloxy]-hydroxyphosphoryl}-α-D-galactopyranuronamide (12b)

To a solution of **12a** (74.6 mg, 0.043 mmol) in dry pyridine (3.9 mL) Zn-Cu couple (freshly prepared, 50 mg, ca. 0.77 mmol) and 2,4-pentandione (70 µL, 0.68 mmol) were added and the mixture was stirred vigorously at 20°C for 2.5 h. After filtration and washing of the solid residue with EtOH, the combined solutions were evaporated. The residue was redissolved in 8:1 EtOH-H_2O (10 mL), and Zn^{2+} ions were removed by stirring with Dowex 50 W X 2 (H^+ form, ca. 2.0 g) at 20°C for 40 min, filtration and washing of the resin with EtOH-H_2O 8:1 (ca. 40 mL). Solvent evaporation, followed by LC furnished **12b** (38.1 mg, 63%) contaminated by small amounts of a slightly less polar side product. In another experiment we succeeded to separate both compounds by repeated MPLC ($CHCl_3$-MeOH 15:4) In this experiment the two compounds were formed in a ratio of 10:1- Spectra of **12b**: ^1H NMR (400 MHz, pyridine-d_5): All signals were extremely broad. The signals

of the lipid portion, the acetyl groups and the methyl ester group are present, whereas no signals for either the phosphate protecting group or the TrOC group could be found.- ^{13}C NMR (100.6 MHz, pyridine-d_5, APT): δ = 19.55 - 42.39 (CH, CH_2, CH_3 signals), 51.96 ($COO\underline{C}H_3$), (55.13)**, 55.29 (C-2^E), 61.34 and 62.69* (C-6^C and C-6^E), 66.45*, 67.66, 68.51*, 69.53*, 69.65*, 70.03, 71.04, 71.76, 72.88, 73.37*, 73.65*, 77.66, 79.48, 95.89 (br, C-1^F), 101.48 and 101.70* (C-1^C and C-1^E), 157.68 ($O\underline{C}ONH_2$), 169.73 - 171.72 ($\underline{C}OCH_3$ signals, C-6^F and C-1^H).- $C_{62}H_{104}O_{29}N_3P$ (1386.48, 1385.65), FAB MS: m/z = 1423.9 ($[M+K]^+$), 1408.0 ($[M+Na]^+$), 618.0 ($[e]^+$), 589.1 ($[M\text{-}f+K+H]^+$), 573.2 ($[M\text{-}f+Na+H]^+$), 331.0 ($[c]^+$).

* These signals are very broad.

** This signal probably is an artefact.

2-*O*-{2-Acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl}-3-*O*-carbamoyl-1-*O*-{[(R)-2-methyloxycarbonyl-2-(3,8,8,11,14,18-hexamethyl-nonadecyloxy)-ethyloxy]-hydroxyphosphoryl}-α-D-galactopyranuronamide (1c)

A solution of **12b** (34.2 mg, 0.025 mmol) in 5:2 THF- bidist. H_2O (3.5 mL) was flushed with Ar for 10 min, then at 0°C 1 mol/L LiOH (215 µL, in H_2O bidist., 0.215 mmol) was slowly added. The mixture was stirred at 0°C for 30 min and another 2 h at 20°C, then the reaction was stopped by addition of Dowex 50 WX2 resin (H^+ form) (ca. 1.5 g). Stirring at 20°C for 30 min, filtration, washing of the resin with 2:1 THF-H_2O (bidist.), lyophilization and separation by MPLC (SiO_2, 20-45 µm, 60 Å, isopropanol-2 mol/L NH_3 4:1) followed by a further MPLC (RP-18, MeOH-CH_3CN-H_2O 8:4:1) gave **1c** (8.9 mg, 32%) and traces of a slightly less polar compound.- Spectra of **1c**: ^1H NMR (400 MHz, $CDCl_3$-CD_3OD-D_2O 18:13:2.7): O-acetyl signals were absent.- ^{13}C NMR (100.6 MHz, $CDCl_3$-CD_3OD-D_2O 18:13:2.7): δ = 18.72 - 41.59 (CH, CH_2, CH_3 signals), (52.43)*, 54.83 (C-2^E), 59.54 and 60.85 (C-6^C and C-6^E), 67.54, 68.48, 70.31, 70.34, 70.75, 71.89, 72.68, 74.39, 75.06, 78.12 (C-2^H), 94.92/95.58 (br, C-1^F), 102.05 and 102.83 (C-1^C and C-1^E), 157.01 ($O\underline{C}ONH_2$), 171.01, 172.02 and 173.19 (3 signals for $NH\underline{C}OCH_3$, C-6^F and C-1^H).- FAB MS: m/z 1180.3 ($[M\text{-}H+K+Na]^+$), 1164.3 ($[M\text{-}H+2Na]^+$), 1158.3 ($[M+K]^+$), 1142.3 ($[M+Na]^+$), 1120.3 ($[M+H]^+$), 559.2 ($[M\text{-}f+Na+H]^+$), 366.0 ($[e]^+$).- $C_{49}H_{90}O_{23}N_3P$ (1120.23, 1119.57).- FAB MS of the minor products: The spectrum appeared to contain two sets of signals: A) 1158.3 ($[M+K]^+$), 1142.5 ($[M+Na]^+$), 1120.5 ($[M+H]^+$), and B) 1115.5 ($[M+K]^+$), 1099.4 ($[M+Na]^+$), 1077.5 ($[M+H]^+$). Set A) could derive from a contamination with compound **1c**, whereas set B) would be correct for a substance with a formula of $C_{48}H_{89}O_{22}N_2P$; this would indicate a loss of the carbamoyl group.

*It is unclear wether this signal is an artefact or indicates the presence of an impurity.

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