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THE FIRST SYNTHESIS OF A MOENOMYCIN-TYPE TRANSGLYCOSYLASE INHIBITOR

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Abstract- Starting from chitobiose octaacetate, D-galacturonic acid derivative **3**, and D-glyceric acid derived building block **7**, the moenomycin trisaccharide analogue **8c** has been synthesized **8c** is an as active transglycosylase inhibitor as moenomycin A

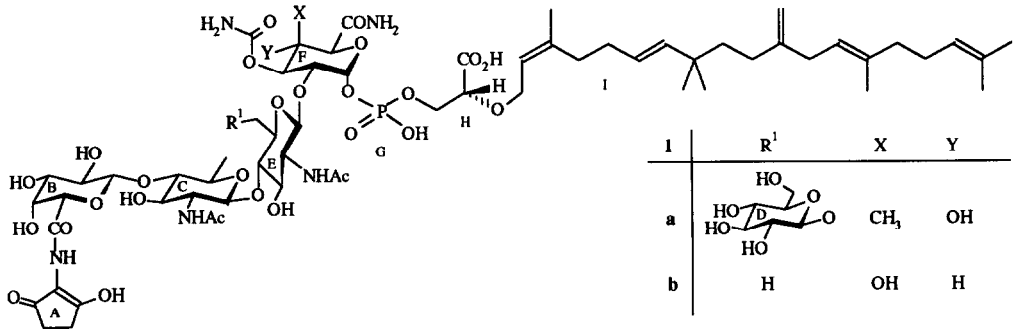
Introduction

The moenomycin-type compounds belong to the rare antibiotics known to interfere with the transglycosylation reaction, one of the key steps in the formation of high-molecular uncrosslinked peptidoglycan from a disaccharide membrane intermediate [1] For moenomycin A (**1a**) it has been demonstrated that units E-F-G-H-I are sufficient to elicit full antibiotic activity, at least in an in-vitro assay Thus, moenomycin A degradation product **2a** was found in this assay to be as active as **1a** [2] On the contrary, in the series of the moenomycin C_1 (**1b**) trisaccharide degradation product **2d** turned out to be the smallest fully active compound whereas disaccharide **2b** was devoid of any antibiotic activity [3] This result is in agreement with our previous finding that the synthetic structural analogue **2c** is antibiotically inactive [4] Comparison of the structures of **2a** and **2c** reveals that both compounds differ only at C-4 in unit F, **2a** having an equatorial OH group (D-gluco configuration) and an axial methyl group in this position, whereas C-4 carries an axial OH group (D-galacto configuration) in **2c** The reason for the dramatic difference in the transglycosylase inhibiting potency of **2a** vs **2c** is at present not understood In contrast to the oligosaccharide analogues derived from moenomycin A, trisaccharide **2d** which is *in vitro* as active as moenomycin A[3] contains only *ordinary* sugar components This observation suggested that one could arrive *synthetically* at transglycosylase inhibitors of this type with reasonable efforts

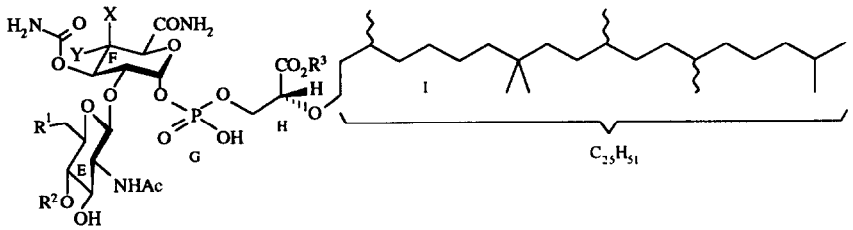
We describe in this article the synthesis of the moenomycin analogue **8c** which differs from **2d** solely by the presence of OH groups in the 6-positions of units C and E This difference seemed of little relevance (as far as the question of antibiotic activity is concerned), since in a series of moenomycin antibiotics OH groups at C-6^C and C-6^E, respectively, did not affect the antibiotic activity [5]

Synthesis of 8c

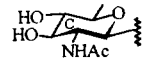
Following the general synthetic scheme developed for compounds of this type[4,6] we planned to assemble trisaccharide **5e** with the moenomycin-derived glyceric acid derivative **7** via the phosphate link Starting materials for the synthesis of **5e** were the known D-galacturonamide derivative **3**[4] and the oxazoline **4**[7] which is available from chitobiose octaacetate[8] Camphorsulfonic acid-promoted coupling of **3** (4 equiv) with **4** furnished **6** in 54% yield (based on **4**) From **6** the acetonide group was removed with 20 per cent acetic acid to give **5a** which turned out to be soluble in 1 1 nitromethane-CH_2Cl_2 Thus, direct reaction with trichloroacetyl isocyanate was possible without the need to form an intermediate tin ether[4] The reaction product was treated with zinc dust in methanol to release the desired urethane **5b** in an overall yield of 62% Based on experience made in the course of the synthesis of **2c**, the 4-OH group in **5b** was protected by formation of the trichloroethoxycarbonyl derivative **5c** Removal of the allyl group from **5c** turned out to be

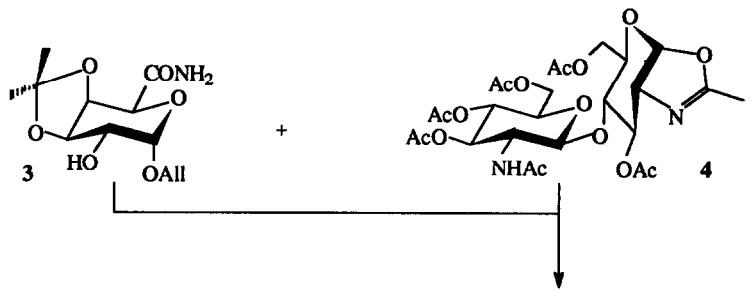


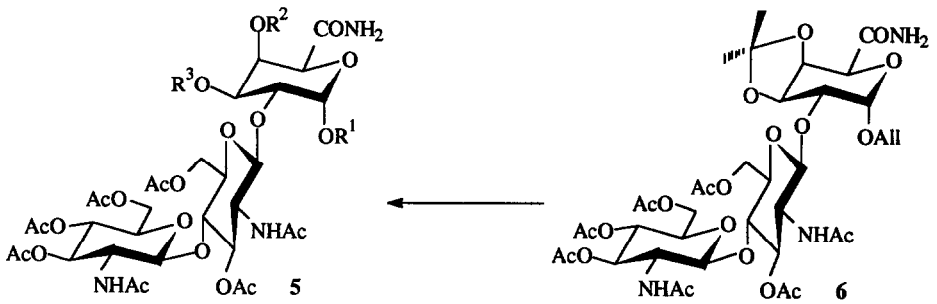
1	R^1	X	Y
a		CH_3	OH
b	H	OH	H



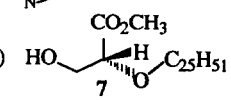
one of the most complicated steps of the synthesis Especially, the standard procedure - isomerization of the allyl into the propenyl group with Wilkinson s catalyst, followed by Hg^{2+}-mediated removal of the C_3 unit - gave very poor results In fact, this method had to be abandoned in the present case Luckily, **5c** was soluble in THF

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



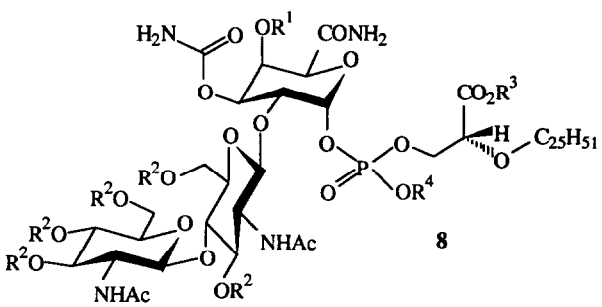


1) $Cl_2POC(CH_3)_2CCl_3$



3) $((CH_3)_3SiO)_2$

5	R^1	R^2	R^3
a	All	H	H
b	All	H	$CONH_2$
c	All	$CO_2CH_2CCl_3$	$CONH_2$
d		$CO_2CH_2CCl_3$	$CONH_2$
e	H	$CO_2CH_2CCl_3$	$CONH_2$



8	R^1	R^2	R^3	R^4
a	$CO_2CH_2CCl_3$	Ac	CH_3	$C(CH_3)_2CCl_3$
b	H	Ac	CH_3	H
c	H	H	H	H

Thus, we could apply the $[Ir(PCH_3(C_6H_5)_2)_2COD]PF_6$-catalyzed isomerization[9,10] of **5c** into **5d** which worked very nicely The ^1H NMR spectrum of the isomerization product revealed that solely the E isomer had been formed In **5d** the propenyl ether was cleaved employing the Hg^{2+} method **5e** was formed in an overall yield of 76%

For the construction of the phosphoric acid diester grouping we used a version of the phosphite methodology adapted to the synthesis of moenomycin analogues [4,6] Thus, the sequence (i) treatment of 2,2,2-trichloro-1,1-dimethylethyl dichlorophosphite with two equivalents of 1H-1,2,4-triazole,[11] (ii) reaction of the thus prepared reagent with **5e,** (iii) subsequent reaction with **7**[4] and (iv) oxidation of the intermediate phosphite triester with bis(trimethylsilyl)peroxide[12] furnished the phosphate triester **8a** (probably mixture of stereoisomers isomeric at the P centre) Removal of the protecting groups with the trichloroethyl unit was achieved under the Imai conditions[13] with freshly prepared Zn-Cu couple[4] to provide **8b.** Finally, hydrolysis of the ester groups converted **8b** into **8c**, the FAB MS and ^{13}C NMR spectra of which were fully in accord with the proposed structure As already observed previously (synthesis of **2b**[3] and **2c**[4]), on deprotection of **8b**, besides **8c** a side product was formed which could be removed by careful chromatographic separations Shortage of material has precluded the structural elucidation of these compounds until now

Antibiotic Activity of 8c

Inhibition of the UDP-N-acetylmuramyl pentapeptide-dependent incorporation of [^{14}C]UDP-N-acetylglucosamine into cross-linked high-molecular weight peptidoglycan was studied with a slightly modified[14] version of the assay described by Izaki, Matsuhashi, and Strominger [15] The results are depicted in Table 1

Table 1 Effect of compound **8c** and of moenomycin A (**1a**, for comparison) on the *in-vitro* UDP-N-acetylmuramyl pentapeptide-dependent incorporation of [^{14}C]UDP-N-acetylglucosamine into cross-linked high-molecular weight peptidoglycan

	%inhibition	
concentration (mg/L)	**8c**	**1a**
10	93	91
1	86	81
0 1	18	72

In addition, the inhibitory effect of **8c** directly on the transglycosylation reaction was determined by the *in vitro* assay developed earlier in one of our laboratories[16] using a crude extract from an over-producer of polymerase PBP1b (*E coli JA200 plc19-19)* and as substrate the lipid intermediate which is the immediate precursor of uncross-linked peptidoglycan (see Table 2) The results in Tables 1 and 2 demonstrate that in the *in-vitro* systems synthetic compound **8c** is an as active inhibitor of the transglycosylation reaction as moenomycin A (**1a**) itself

Table 2 Effect of **8c,** moenomycin C_1 degradation product **2d**, and of moenomycin A (**1a**, for comparison) on the *in-vitro* formation of uncross linked peptidoglycan by transglycosylation

final	%-inhibition		
concentration (mg/L)	**1a**	**8c**	**2d**
10	100	100	100
1	100	93	100
0 1	78	43	51

Finally, the minimum inhibitory concentrations (MIC) of compound **8c** against various microorganisms have been determined by a serial two-fold agar dilution method (Muller Hinton Agar) The results (see Table 3) demonstrate that **8c** like moenomycin A (**1a**) is active only against *gram-positive* bacteria However, **8c** is of distinctly lower activity against *Staph aureus than* moenomycin A (**1a**) This result is in agreement with the previous observation that stepwise shortening of the sugar chain results in an decrease of *in-vivo* activity (cf moenomycin degradation product **2d** in Table 3)

Table 3 Minimum inhibitory concentrations (in mg/L) of compound **8c**, **2d** and of moenomycin A (**1a**, for comparison) against various test organisms

test organism	**1a**	**8c**	**2d**
Staph aureus SG 511	0 05	12 5	6 25
Staph aureus 503	0 05	12 5	6 25
Strept pyogenes A77	<0 01	0 781	0 781
Pseud aerug 1771m	6 25	50	25
E coli DC 2	50	>100	>100

Conclusion

For the first time a synthetic inhibitor (**8c**) of the enzyme, which catalyzes the transglycosylation step of the peptidoglycan biosynthesis, has been obtained With regard to structure activity relations it seems clear now, that in the series containing D-moenuronic acid as unit F, the disaccharide analogue **2a** represents the smallest structure with transglycosylase-inhibiting activity, whereas in the series with a D-galacturonic acid unit F a trisaccharide structure such as **2d** or **8c** constitutes the minimum structural requirement for full (in-vitro) activity

EXPERIMENTAL

O_2- or moisture-sensitive reactions were performed in oven-dried glassware under a positive pressure of argon Liquids and solutions were transferred by syringe Small-scale reactions were performed in Wheaton serum bottles sealed with aluminium caps with open top and Teflon-faced septum (Aldrich) Organic solvent evaporations were performed in vacuo at 40°C using a rotatory evaporator, water was removed by lyophilization using the Leybold-Heraeus GT2 apparatus Solvents were purified by standard techniques - The instrumentation used was ^{1}H NMR AM 400 (Bruker, at 400 MHz), ^{13}C NMR AM 400 (Bruker, at 100 6 MHz), FAB MS MAT 731 (Varian) with a modified Saddle Field Source or VG AUTOSPEC (matrix lactic acid), LC (preparative gravitational liquid chromatography) silica gel (ICN Biomedicals Silica 63-100), MPLC (medium-pressure liquid chromatography) 30 0 cm x 2 5 cm or 40 0 cm x 1 5 cm glass tubes, 50 µm silica gel (Amicon), Duramat pump (CfG), analytical TLC Merck precoated silica gel 60 F_{254} plates (0 2 mm), spots were identified by spraying with a 2 22 mol/L H_2SO_4 solution which contained $Ce(SO_4)_2x4H_2O$ (10 g/L) and $H_3[PO_4(Mo_3O_9)_4]xH_2O$ (25 g/L)[17] and heating at 140°C, or with the phosphate-specific spraying reagent of Dittmer and Lester [18] Carbon and proton numbering in the subunits (see NMR data) follows the moenomycin nomenclature (see formula 1) If not otherwise stated, the following H,H coupling constants were observed allyl unit $|J_{1,1'}|$ = 13 5 Hz, $J_{1,2}$ = 6 Hz, $J_{1',2}$ = 5 Hz, $|J_{3,3'}|$ = 11 Hz, $J_{2,3\text{-trans}}$ = 10 5 Hz, $J_{2,3\text{-cis}}$ = 17 5 Hz, $|^4J|$ = 2 Hz, units C and E, $J_{1,2}$ = 8 5 Hz, $J_{3,4}$ = $J_{4,5}$ = 9 0 Hz, $J_{5,6}$ = 2 Hz, $J_{5,6'}$ = 5 Hz, $|^2J_{6,6'}|$ = 12 5 Hz, $J_{NH,2\text{-}H}$ = 8 5 Hz, unit F, $J_{1,2}$ = 3 5 Hz, $J_{2,3}$ = 10 5 Hz, $J_{3,4}$ = 3 5 Hz, $J_{4,5}$ = 1 5 Hz 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)

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

To a solution of allyl-3,4-O-isopropylidene-α-D-galactopyranosiduronamide (**3**) (2 23 g, 8 16 mmol) in dry CH_2Cl_2 (10 mL) anhydrous camphorsulfonic acid (187 mg, 0 81 mmol) dissolved in CH_2Cl_2 (2 mL) and 2 5 mL of a solution of 2-methyl-{4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-acetyl-1,2-dideoxy-α-D-glucopyrano}-[2,1-d]-2-oxazoline (**4**, 1 25 g, 2 03 mmol) in dry CH_2Cl_2 (5 mL) were added The mixture was stirred at 60°C in a sealed vessel After 3 h the remaining portion of the solution of **4** was added The stirred mixture was left at 60°C for another 3 h Triethylamine (1 mL) was added and stirring was continued for 20 min at 20°C Solvent evaporation followed by LC ($CHCl_3$-CH_3OH 30 1) furnished **6** (967 mg, 54%, based on **4**) - ^{1}H NMR ($CDCl_3$) δ = 5 20 and 5 15 (dd, 3-H^C and 3-H^E), 5 02 (dd, 4-H^E), 4 97 (d, 1-H^F), 4 82 and 4 63 (2d's, 1-H^C and 1-H^E), 4 54 (dd, 4-H^F), 4 48 (d, 5-H^F), 4 35 (dd, 6-H^C and 6-H^E), 4 30 (dd, 3-H^F) 4 20 and 4 15 (2dd's, 6-H'^C and 6-H'^E), 3 68 and 3 53 (5-H^C and 5-H^E), 2 05-1 90 (7s's, $COCH_3$), 1 46 and 1 30 (2s's, $C(CH_3)_2$) - ^{13}C-NMR ($CDCl_3$) δ = 171 6-170 6 ($COCH_3$ signals), 169 6 (C-6^F), 133 5 ($OCH_2CH=CH_2$), 118 0 ($OCH_2CH=CH_2$), 109 8 ($C(CH_3)_2$), 101 3 and 101 1 (C-1^C and C-1^E), 97 8 (C-1^F), 76 2 (C-2^F), 62 4 and 62 0 (C-6^C and C-6^E), 55 2 and 54 5 (C-2^C and C-2^E) - $C_{38}H_{55}N_3O_{21}$ (889 86, 889 33), FAB MS m/z 890 1 ($[M+H]^+$), 617 1 ($[e]^+$), 330 ($[c]^+$)

Allyl 2-O-{2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl}-α-D-galactopyranosiduronamide (5a)

A solution of **6** (1 146 g, 1 29 mmol) in acetic acid (20 per cent, 35 mL) was stirred at 60°C for 4½ h Water (200 mL) was added and most of the acetic acid was removed by evaporation of 25% of the solvent Water (50 mL) addition and subsequent lyophilization yielded pure **5a** (1 02 g, 93%) - ^{1}H NMR (pyridine-d_5) δ = 9 25 (d, $NHCOCH_3^C$), 9 12 (d, $NHCOCH_3^E$), 8 37 and 7 82 (2s's, $CONH_2$), 6 10 (dd, 3-H^C), 5 94 ($OCH_2CH=CH_2$), 5 72 (dd, 3-H^E) , 5 53 (d, 1-H^F), 5 50 (d, 1-H^C), 5 40 (dd, 4-H^C), 5 36 and 5 07 ($OCH_2CH=CH_2$), 5 25 (d, 1-H^E), 5 03 (dd, 4-H^F), 4 86 (dd, 2-H^F), 4 78 (d, 5-H^F), 4 68 (dd, 3-H^F), 4 47 (dd, 6-H'^E), 4 26 and 4 15 ($OCH_2CH=CH_2$), 3 76 (ddd, 5-H^E), 2 22 and 2 18 (2s's, $NHCOCH_3$), 2 08 - 1 95 (5s's, $COCH_3$) - ^{13}C NMR (pyridine-d_5) δ = 172 5-170 5 ($COCH_3$ signals), 169 9 (C-6^F), 135 0 ($OCH_2CH=CH_2$), 116 8 ($OCH_2CH=CH_2$), 103 8 and 101 1 (C-1^C and C-1^E), 99 4 (C-1^F), 79 5 (C-2^F), 62 6 and 62 3 (C-6^C and C-6^E), 56 7 and 55 2 (C-2^C and C-2^E), 23 3 ($NHCOCH_3$) - $C_{35}H_{51}N_3O_{21}$ (849 80, 849 30), FAB MS m/z 850 ($[M+H]^+$), 617 ($[e]^+$), 330 ($[c]^+$)

Allyl 2-O-{2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl}-3-O-carbamoyl-α-D-galactopyranosiduronamide (5b)

A solution of **5a** (922 mg, 1 09 mmol) in dry 1 1 CH_2Cl_2-CH_3NO_2 (45 mL) was cooled to 0°C Slowly trichloroacetyl isocyanate (141 µL, 1 19 mmol) was added, and the mixture was stirred at 0°C for 4 5 h Excess reagent was destroyed by addition of methanol (1 mL) and allowing the mixture to warm to 20°C After solvent evaporation the residue was redissolved in methanol (50 mL), Zn dust (800 mg) was added, and the mixture was stirred at 20°C for 4h Filtration, washing the solid with 4 1 methanol-water, solvent evaporation and MPLC (B column, toluene-$CHCl_3$-methanol 1 1 0 2) provided **5b** (603 mg, 62%) - ^{1}H NMR (H,H COSY, pyridine-d_5) δ = 9 10 (d, $NHCOCH_3^C$), 8 62 (d, $NHCOCH_3^E$), 8 40 and 7 85 (2s's, $CONH_2$), 6 06 (dd, 3-H^C), 5 88 ($OCH_2CH=CH_2$), 5 82 (dd, 3-H^E) , 5 82 (dd, 3-H^F), 5 53 (d, 1-H^F), 5 45 (d, 1-H^C),

5 42 (dd, 4-H^F), 5 37 (dd, 4-H^C), 5 30 and 5 03 ($OCH_2CH=C\underline{H}_2$), 4 83 (dd, 2-H^F), 4 81 (d, 5-H^F), 4 65 (dd, 6-H'^{C}), 4 45 (dd, 6-H'^{E}), 4 22 and 4 10 ($OC\underline{H}_2CH=CH_2$), 3 65 (ddd, 5-H^E), 2 21 and 2 19 (2s's, $NHCOC\underline{H}_3$), 2 17 - 2 01 ($COCH_3$ signals) - ^{13}C NMR (pyridine-d_5) δ = 172 0-170 5 ($\underline{C}OCH_3$ signals), 169 8 (C-6^F), 157 6 ($OCONH_2$), 134 7 ($OCH_2\underline{C}H=CH_2$), 116 8 ($OCH_2\underline{C}H=CH_2$), 102 5 and 101 0 (C-1^C and C-1^E), 99 2 (C-1^F), 76 7 (C-2^F), 62 6 and 62 3 (C-6^C and C-6^E), 56 4 and 55 8 (C-2^C and C-2^E), 23 3 and 23 4 ($NHCO\underline{C}H_3$), 23 4 - 20 5 ($CO\underline{C}H_3$ signals) - $C_{36}H_{52}N_4O_{22}$ (892 82, 892 31), FAB MS m/z 893 4 ([M+H]$^+$), 617 ([e]$^+$), 330 ([c]$^+$)

Allyl 2-O-{2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl}-4-O-(2,2,2-trichloroethoxycarbonyl)-3-O-carbamoyl-α-D-galactopyranosiduronamide (**5c**)

To a solution of **5b** (315 mg, 0 35 mmol) in dry pyridine (20 mL) 2,2,2-trichloroethylchloroformate (75 μL, 0 53mmol) was added and the mixture was stirred at 20°C for 2h Excess reagent was destroyed by addition of methanol (0 5 mL) Solvent evaporation and LC ($CHCl_3$-CH_3OH 10 1) furnished **5c** (368 mg, 98%) - ^{1}H NMR (pyridine-d_5) δ = 9 22 (d, $N\underline{H}COCH_3$^{C}), 8 72 (d, $N\underline{H}COCH_3$^{E}), 8 70 and 8 05 (2s's, $CONH_2$), 6 58 (dd, 4-H^F), 6 07 (dd, 3-H^C), 5 96 (dd, 3-H^F), 5 88 (dd, 3-H^E), 5 85 ($OCH_2C\underline{H}=CH_2$), 5 53 (d, 1-H^F), 5 50 (d, 1-H^C), 5 39 (dd, 4-H^C), 5 33 (d, 1-H^E), 5 32 and 5 05 ($OCH_2CH=C\underline{H}_2$), 4 95 (d, 5-H^F), 4 95 and 4 87 (2d's, $|J_{gem}|$ = 12 5 Hz, CCl_3CH_2OCO), 4 90 (dd, 2-H^F), 4 66 (dd, 6-H^C and 6-H'^{E}), 3 73 (ddd, 5-H^E), 2 22 and 2 18 (s, $NHCOC\underline{H}_3$), 2 16 - 1 97 (5s's, $COC\underline{H}_3$) -^{13}C NMR (pyridine-d_5) δ = 171 2-169 9 ($\underline{C}OCH_3$ signals), 169 7 (C-6^F), 157 0 ($OCONH_2$), 154 3 ($CCl_3CH_2O\underline{C}O$), 134 4 ($OCH_2CH=\underline{C}H_2$), 117 2 ($OCH_2\underline{C}H=CH_2$), 102 6 and 101 1 (C-1^C and C-1^E), 99 0 (C-1^F), 96 0 ($\underline{C}Cl_3CH_2OCO$), 77 2 (C-2^F), 64 4 ($CCl_3\underline{C}H_2OCO$), 62 5 and 62 3 (C-6^C and C-6^E), 56 4 and 55 6 (C-2^C and C-2^E), 23 3 ($NHCO\underline{C}H_3$), 20 6 - 20 1 ($CO\underline{C}H_3$ signals) - $C_{39}H_{53}N_4O_{24}Cl_3$ (1068 22, 1066 21), FAB MS m/z 1168 ([M+H+NEt_3]$^+$), 1067 ([M+H]$^+$), 617 ([e]$^+$), 330 ([c]$^+$)

(E)-1-Propen-1-yl-2-O-{2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-gluco-pyranosyl)-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl}-3-O-carbamoyl-4-O-(2,2,2-trichloroethoxycarbonyl)-α-D-galactopyranosiduronamide (**5d**)

To a solution of **5c** (107 mg, 0 1 mmol) in dry THF (4 mL) at 20°C a suspension of $[Ir(PCH_3(C_6H_5)_2)_2COD]PF_6$ (3 7 mg, 0 0044 mmol, 4 4 mol%) in dry THF (1 mL) was added and the mixture was stirred for 2 min at 20°C The solution was degassed as described in ref [9a] and the reagent was hydrogenated until the colour of the solution turned from reddish orange to pale yellow After 3 further degassing cycles the reaction mixture was stirred under nitrogen at 20°C for 3 5 h Solvent evaporation and LC (toluene-$CHCl_3$-CH_3OH 1 1 0 15→0 4) provided **5d** (100 7 mg, 94%) - ^{1}H NMR (pyridine -d_5) δ = 9 30 (d, $N\underline{H}COCH_3$^{C}), 9 00 (d, $N\underline{H}COCH_3$^{E}), 8 85 and 8 16 (2s s, $CONH_2$), 6 53 (dd, 4-H^F), 6 32 (dq, $OC\underline{H}=CHCH_3$, $J_{1,2}$ = 12 Hz, $|^4J|$ = 1 5 Hz), 6 12 (dd, 3-H^C), 6 07 (dd, 3-H^F), 5 98 (dd, 3-H^E), 5 80 (d, 1-H^F), 5 52 (d, 1-H^C), 5 42 (dd, 4-H^C), 5 37 (d, 1-H^E), 5 05 (dq, $OCH=C\underline{H}CH_3$, $J_{2,3}$ = 7 Hz), 5 02 (d, 5-H^F), 5 00 and 4 75 (2d's, $|J_{gem}|$ = 12 Hz, CCl_3CH_2OCO), 3 75 (ddd, 5-H^E), ≈2 20 (2s, $NHCOC\underline{H}_3$), 2 15 - 2 00 (5s's, $COCH_3$), 1 38 (dd, $OCH=CHC\underline{H}_3$)

2-O-{2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-acetyl-2-deoxy-ß-D-glucopyranosyl}-3-O-carbamoyl-4-O-(2,2,2-trichloroethoxycarbonyl)-α-D-galactopyranuronamide (5e)

20 0 mg (0 019 mmol) of **5c** were isomerisized into **5d** as described above After solvent evaporation the residue was redissolved in 9 1 acetone-water (2 mL) After treatment with HgO (20 mg, 0 095 mmol) to the stirred suspension a solution of $HgCl_2$ (20 mg, 0 076 mmol) in 9 1 acetone-water (0 5 mL) was added dropwise The stirred reaction mixture was left at 20°C for 1 5 h Solids were removed by filtration and into the clear solution gaseous H_2S was passed carefully The precipitates were removed by centrifugation and the residue was carefully washed with acetone From the combined filtrates acetone was distilled off and water was then removed by lyophilization LC (toluene-$CHCl_3$-CH_3OH 1 1 0 4) provided pure **5e** (14 5 mg, 76%) - ^1H NMR (pyridine-d_5) δ = 9 25 (d, N<u>H</u>$COCH_3{}^C$), 8 65 (d, N<u>H</u>$COCH_3{}^E$), 8 68 and 8 05 (2s, $CONH_2$), 6 67 (dd, 4-H^F), 6 10 (d, 1-H^F), 6 08 (dd, 3-H^C), 6 23 (dd, 3-H^F), 5 85 (dd, 3-H^E), 5 47 (d, 1-H^C), 5 40 (dd, 4-H^C), 5 33 (d, 1-H^E), 5 38 (d, 5-H^F), 4 97 and 4 85 (2d, CCl_3CH_2OCO), 4 86 (dd, 2-H^F), 4 66, 4 56, 4 43 and 4 22 (4dd's, CH_2-6^C and CH_2-6^E), 3 65 (ddd, 5-H^E), 2 20 and 2 19 (s, $NHCOCH_3$), 2 15 - 1 94 (5s $COCH_3$) - ^{13}C NMR (pyridine-d_5) δ = 171 2-169 9 (<u>C</u>OCH_3 signals), 169 7 (C-6^F), 157 0 ($OCONH_2$), 154 3 (CCl_3CH_2O<u>C</u>O), 102 6 and 101 1 (C-1^C and C-1^E), 99 0 (C-1^F), 96 0 (<u>C</u>Cl_3CH_2OCO), 77 2 (C-2^F), 64 4 (CCl_3<u>C</u>H_2OCO), 62 5 and 62 3 (C-6^C and C-6^E), 56 4 and 55 6 (C-2^C and C-2^E), 23 4 and 23 3 ($NHCOCH_3$), 20 6 - 20 1 ($COCH_3$ signals) - $C_{36}H_{49}N_4O_{24}Cl_3$ (1028 15, 1026 18), FAB MS m/z 1027 ([M+H]$^+$), 617 ([e]$^+$), 330 ([c]$^+$)

2-O-{2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl}-3-O-carbamoyl-4-O-(2,2,2-trichloroethoxycarbonyl)-1-O-{[(R)-2-methoxycarbonyl-2-(3,8,8,11,14,18-hexamethylnonadecyloxy)-ethoxy]-(2-trichloromethyl-2-propyloxy)-phosphoryl}-α-D-galactopyranuronamide (8a)

To a solution of 1H-1,2,4-triazole (46 mg, 0 67 mmol) in 1 4 pyridine-CH_2Cl_2 (3 mL) 2,2,2-trichloro-1,1-dimethylethyl dichlorophosphite (24 7 µL, 0 12 mmol) was added at 0°C The mixture was stirred at 0°C for 20 min **5e** (98 mg, 0 095 mmol), dissolved in 1 4 pyridine-CH_2Cl_2 (3 mL), was added and the reaction mixture stirred for 3 5 h at 0°C After addition of 7 (134 mg, 0 28 mmol) in three portions over a period of 2 h the mixture was stirred for 2 h at 0°C Bis(trimethylsilyl)peroxide (28 2 µL, 0 13 mmol) was injected into the reaction flask and the stirred mixture was maintained at 0°C for 12 h, then 1h at 20°C The reaction mixture was filtered and solvent evaporation followed by LC (toluene-$CHCl_3$-CH_3OH 1 1 0 2) yielded **8a** (127 mg, 78%) - ^1H NMR (pyridine-d_5) δ = 9 26 (d, N<u>H</u>$COCH_3{}^C$), 8 76 (d, N<u>H</u>$COCH_3{}^E$), 8 90 and 8 20 (2s's, $CONH_2$), 6 63 (dd, 4-H^F), 6 56 (dd, 1-H^F, $J_{P,H}$ = 6 Hz), 6 10 (dd, 3-H^C), 6 05 (dd, 3-H^F), 5 88 (dd, 3-H^E), 5 55 (d, 1-H^C), 5 42 (dd, 4-H^C), 5 37 (d, 5-H^F), 5 30 (d, 1-H^E), 4 97 and 4 75 (2d's, CCl_3CH_2OCO, $|J_{gem}|$ =12 Hz), 4 90 (dd, 2-H^F), 4 65 (dd, 6-H^C or 6-H^E), 2 20 and 2 18 (2s's, $NHCOCH_3$), 2 14 - 1 98 (5s's $COCH_3$) - ^{13}C NMR (pyridine-d_5) δ = 170 9-170 3 (<u>C</u>OCH_3signals), 169 9 ($CONH_2$), 156 6 ($OCONH_2$), 154 1 (CCl_3CH_2O<u>C</u>O), 102 6 and 101 0 (C-1^C and C-2^E), 97 8 (C-1^F), 95 1 (<u>C</u>Cl_3CH_2OCO), 90 6 ($C(CH_3)_2$<u>C</u>Cl_3), 78 2 (C-2^H), 75 2 (broad signal, C-2^F), 62 5 and 62 2 (C-6^C and C-6^E), 56 6 and 55 6 (C-2^C and C-2^E), 52 1 (CO_2<u>C</u>H_3), 42 3-20 1 (CH, CH_2, CH_3 signals) - $C_{69}H_{111}N_4O_{30}Cl_6P$ (1720 33, 1716 51), FAB MS m/z 1717 ([M+H]$^+$), 1011 0 ([f]$^+$), 617 1 ([e]$^+$), 330 0 ([c]$^+$)

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

To a solution of triester **8a** (126 mg, 0 074mmol) in dry pyridine (6 mL) Zn-Cu couple (freshly prepared, 90 mg) and 2,4-pentanedione (120 µl) were added and the mixture was stirred at 20°C for 2 h Excess Zn-Cu couple was removed by filtration (washing with ethanol) After solvent evaporation the residue was redissolved in 8 1 water-ethanol (15 mL), and Zn^{2+} ions were removed by treatment with Dowex WX 50/200 resin (H^+ form) Filtration, lyophilization, and LC (toluene-$CHCl_3$-CH_3OH 1 1 0 4→0 6) provided **8b** (66 mg, 64%) as a mixture of two compounds (≈ 1 1 mixture as judged from the ^{13}C NMR spectrum) with slightly different R_f values, which could not be separated - ^{13}C NMR ($CDCl_3$-CD_3OD-D_2O 18 13 2 7) In the region of the sugar carbon signals only one set of signals was observed whereas the chemical shifts of some of the unit H and I carbons differed slightly, δ = 172 4-171 1 ($\underline{C}OCH_3$ signals), 170 2 (C-6^F), 157 3 ($OCONH_2$), 101 5 and 100 8 (C-1^C and C-1^E), 94 8 (d, C-1^F, $^2J_{P,C}$ = 6 Hz), 78 5 and 78 4 (2d's, C-2^H of the two diastereoisomers, $^3J_{P,C}$ = 8 Hz), 73 7 (d, C-2^F, $^3J_{P,C}$ = 8 Hz), 65 5 (d, C-3^H, $^2J_{P,C}$ = 5 Hz), 62 2 and 61 4 (C-6^C and C-6^E), 54 0 and 53 8 (C-2^C and C-2^E), 51 8 ($CO_2\underline{C}H_3$), 41 7-18 8 (CH, CH_2, CH_3 signals) - $C_{62}H_{105}N_4O_{28}P$ (1385 49, 1384 66), FAB MS m/z 1423 5 ([M+K]$^+$), 1407 6 ([M+Na]$^+$), 1385 6 ([M+H]$^+$), 835 2 ([f]$^+$), 617 2 ([e]$^+$), 589 3 ([M-f+K+H]$^+$), 4o9 1 ([M-g]$^+$), 330 1 ([c]$^+$)

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

A solution of **8b** (58 mg, 0 042 mmol) in 2 5 1 THF-water (bidist , 6 mL) was flushed with argon and then at 0°C 1 mol/L LiOH (310 µl) was added The mixture was stirred at 20°C for 2 h, then the reaction was stopped by addition of Dowex WX 50/200 resin (H^+ form) Stirring at 20°C for 30 min, filtration and lyophilization yielded a mixture of two compounds which was separated by MPLC (SiO_2, isopropanol-2 mol/L NH_3 4 1) Both compounds were then subjected to a further MPLC (RP-18, CH_3OH-CH_3CN-H_2O 8 4 1) to give a main product which is assigned structure **8c** (23 mg, 47%) and a minor product (12 9 mg, 26%) -Spectra of **8c** ^{13}C NMR ($CDCl_3$-CD_3OD-D_2O 18 13 2 7) δ = 172 6 ($\underline{C}OCH_3$), 171 8 (C-6^F), 156 9 ($OCONH_2$), 102 1 and 101 2 (C-1^C and C-1^E), 95 3 (broad signal, C-1^F), 79 0 (C-2^H), 70 9 (broad signal), 64 9 (broad signal, C-3^H), 60 5 and 59 2 (C-6^C and C-1^E), 55 0 and 54 6 (C-2^C and C-2^E), 41 5-18 0 (CH, CH_2, CH_3 signals) - $C_{51}H_{93}N_4O_{23}P$ (1161 31, 1160 60), FAB MS m/z 1199 5 ([M+K]$^+$), 1183 5 ([M+Na]$^+$), 1161 5 ([M+H]$^+$), 625 1 ([f]$^+$), 407 1 ([e]$^+$), 204 0 ([c]$^+$)

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References and Notes

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