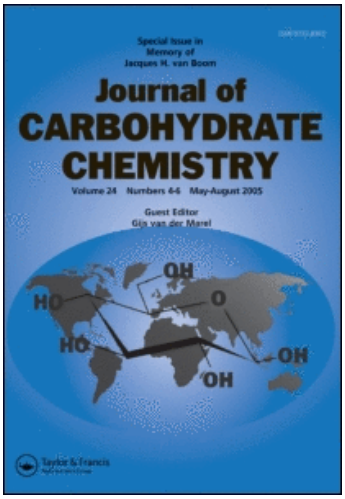

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Stereoselective Synthesis of a Tetrameric Fragment of *Streptococcus Pneumoniae* Type 1 Containing an α-Linked 2-Acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose (SUG*p*) Unit

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STEREOSELECTIVE SYNTHESIS OF A TETRAMERIC FRAGMENT OF *STREPTOCOCCUS PNEUMONIAE* TYPE 1 CONTAINING AN α-LINKED 2-ACETAMIDO-4-AMINO-2,4,6-TRIDEOXY-D-GALACTOPYRANOSE (SUG*p*) UNIT

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ABSTRACT

Block condensation of fully protected donor ethyl 1,2,3,4-tetra-*O*-benzyl-D-Rib-(5→P→6)-2,3,4-tri-*O*-benzoyl-1-thio-β-D-Glc*p* (**2**), having a (5→6)-phosphotriester union between the ribitol and the glucopyranosyl moieties, with the free 3'-OH group in the acceptor methyl 2-acetamido-4-*O*-(2-acetamido-4-(benzyloxycarbonyl)amino-2,4,6-trideoxy-α-D-Gal*p*)-3,6-di-*O*-benzyl-2-deoxy-α-D-Gal*p* (**3**), under the agency of *N*-iodosuccinimide and triflic acid, gave the fully protected tetrameric fragment **22**. Elimination of the 2-cyanoethyl group from the phosphotriester and subsequent debenzoylation, followed by hydrogenolysis of the benzyl and benzyloxycarbonyl groups provided the target tetramer methyl D-Rib-(5→P→6)-D-Glc*p*-β(1→3)-Sug*p*-α(1→4)-α-D-Gal*p*NAc (**1**).

INTRODUCTION

The cell-wall associated and antigenic complex polysaccharide, the so-called C-substance, from *Streptococcus pneumoniae* type 1 is a teichoic acid[1,2] (see Figure 1), the trisaccharide-ribitol-phosphate repeating unit of which contains the rare sugar 2-acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose (Sug*p*). In a recent study,[3] we disclosed a route of synthesis to a Sug*p* glycosyl donor (*i.e.*, compound

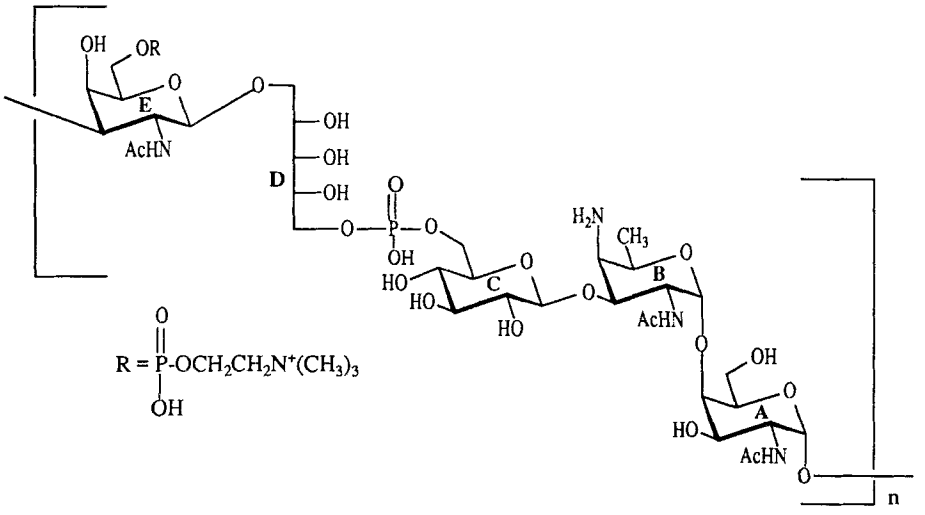


Figure 1. Repeating unit of the subcapsular polysaccharide C-substance from *Streptococcus pneumoniae* type 1.

18) which could be coupled stereoselectively with 1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy-β-D-galactopyranose to give an α(1→4) linked dimer.

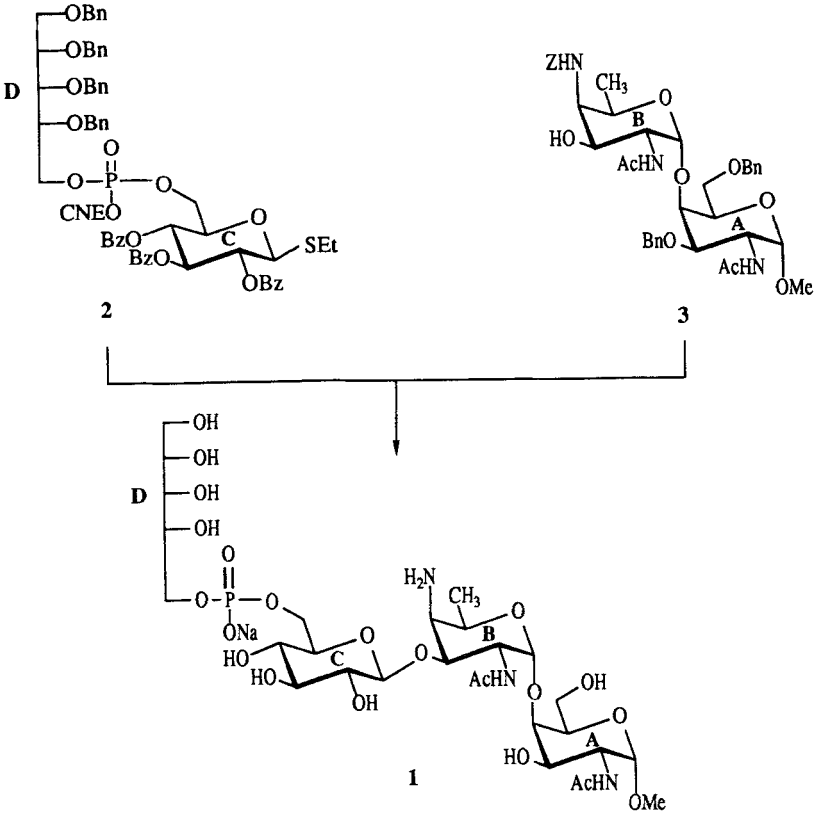
As part of a programme[3,4] to study the immunological properties of C-substance fragments, we here report the synthesis of the tetrameric fragment methyl D-Rib-(5→P→6)-D-Glc*p*-β(1→3)-Sug*p*-α(1→4)-α-D-Gal*p*NAc (*i.e.*, compound **1** in Scheme 1).

RESULTS AND DISCUSSION

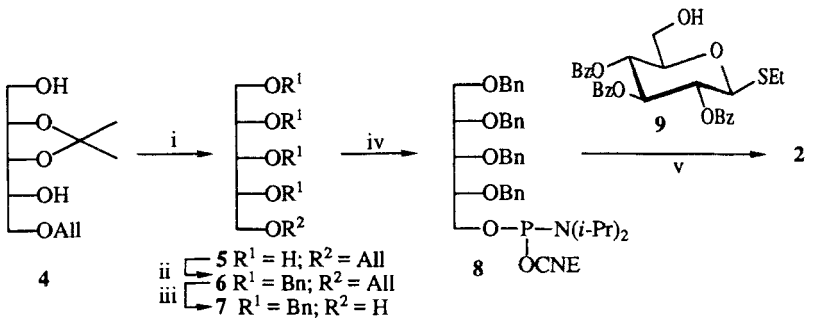
Retrosynthetic analysis combined with our knowledge and experience in this particular field[3,4] indicated that a route of synthesis as depicted in Scheme 1 would be desirable. Thus, glycosylation of the secondary C-3-OH of the α(1→4)-linked BA-dimer **3** with DC-dimer **2** containing an intermediate (5→P→6)-interglycosidic phosphodiester union will lead, after removal of the protecting groups, to the target tetrameric fragment **1**.

The preparation of intermediate **2** is outlined in Scheme 2 and commenced with acidic hydrolysis of the isopropylidene group from known[5] 5-*O*-allyl-2,3-*O*-isopropylidene-D-ribitol (**4**). Conventional benzylation (**5**→**6**) and subsequent deblocking of

Scheme 1



Scheme 2[a]



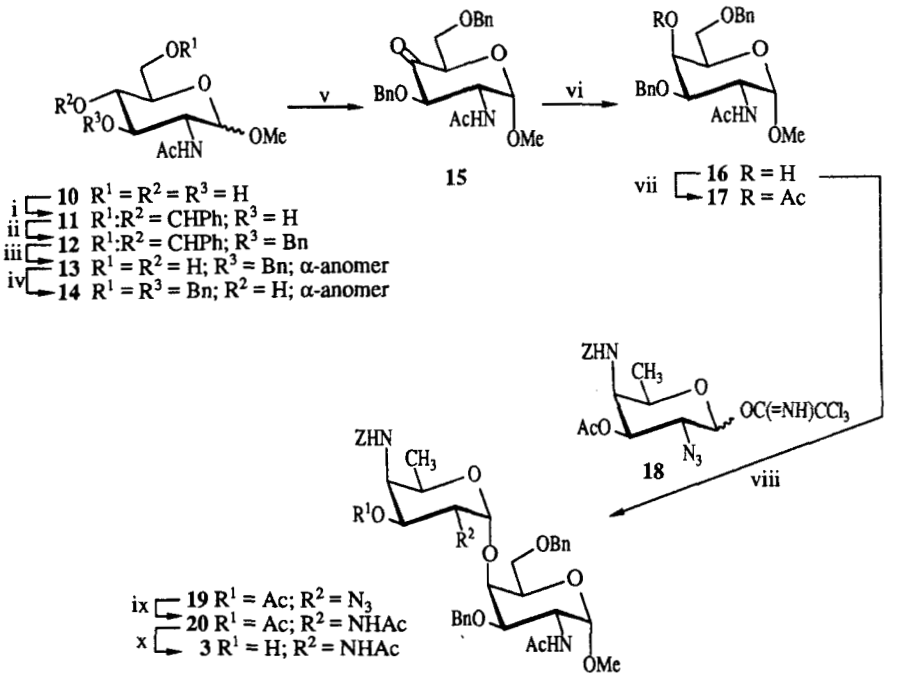
Key[a] : i) $AcOH/H_2O$, 4/1, v/v, 50 °C, 1h; ii) BnBr, NaH in DMF (58%, based on **4**); iii) $Ru(II)H_2[P(Ph)_3]_4$, EtOH, reflux, 3h, then 0.5 M HCl in MeOH (76%); iv) DIPEA, chloro 2-cyanoethyl (*N,N*-diisopropyl)phosphoramidite, CH_2Cl_2 (84%); v) 1*H*-tetrazole, CH_3CN, followed by *t*BuOOH (72%).

the allyl protecting group by isomerization with dihydridotetrakis(triphenylphosphine) ruthenium(II),[6] followed by acidic hydrolysis of the resulting propen-1-yl group, afforded **7** in 44% yield over the four steps. Phosphitylation of the deblocked C-5-OH with the well-established[7] reagent chloro 2-cyanoethyl (*N,N*-diisopropyl)-phosphoramidite in the presence of *N,N*-diisopropylamine (DIPEA) afforded, after purification by silica gel chromatography, the intermediate phosphoramidite **8** as a mixture of diastereoisomers (δ_P 148.7 and 149.0 ppm) in an excellent yield. 1*H*-Tetrazole-mediated phosphitylation of the partially benzoylated ethyl thioglucosyl acceptor[8] **9** with **8** gave an intermediate phosphite triester, which was oxidized *in situ* with *t*-butyl hydroperoxide to provide, after purification, homogeneous **2** (δ_P -0.98 and -1.44 ppm) as a diastereomeric mixture in 60% yield based on **7**.

The sequence of steps in the route to the partially protected methyl 2-acetamido-2-deoxy-α-D-galactopyranoside **16**, which serves as the glycosyl acceptor in the assembly of the key Sug*p*-containing dimer **3**, is shown in Scheme 3. Thus, acid-catalyzed acetalation of easy accessible[9] methyl 2-acetamido-2-deoxy-α(ß)-D-glucopyranoside (**10**), followed by benzylation (**11**→**12**) and subsequent acidic hydrolysis of the 4,6-*O*-benzylidene function gave, after purification on silica gel, the α-diol derivative **13** in 32% yield based on **10**. Regioselective benzylation[10] of **13** could be effected by treating its 4,6-*O*-dibutylstannylidene acetal with benzyl bromide in the presence of cesium fluoride, to provide homogeneous **14** in 53% yield. Swern oxidation of (**14**→**15**) and reduction of the generated ketone function with sodium borohydride led, after purification of the resulting mixture of D-*gluco* and D-*galacto* epimers **14** and **16**, to the isolation (50%) of the D-galactopyranoside **16**, as evidenced by NMR and mass spectroscopy. In addition, the *galacto*-configuration of **16** was firmly established by ^{13}C- and ^{1}H NMR spectroscopy (H-4, 5.59 ppm, $J_{3,4}$ = 3.2 Hz, $J_{4,5}$ = 1.1 Hz) of its acetylated derivative **17**.

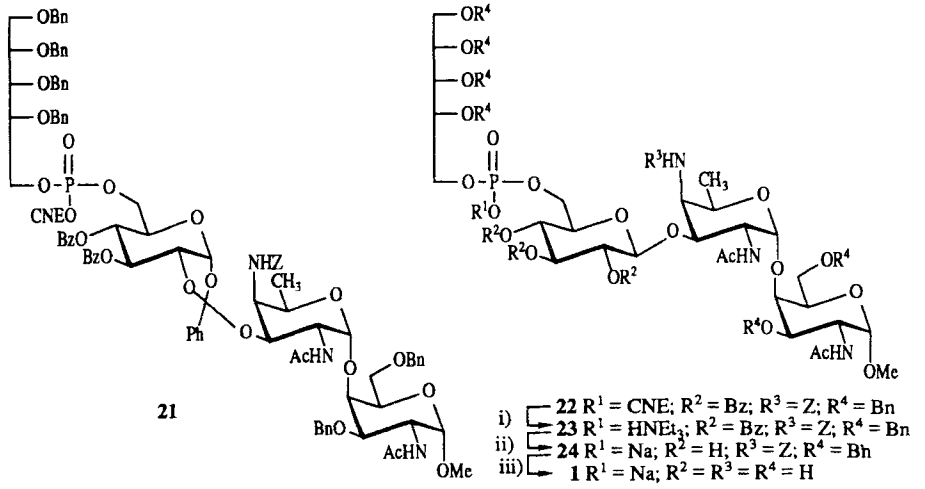
Glycosylation of acceptor **16** with Sug*p*-trichloroacetimidate donor **18**, prepared earlier by us, under the agency of trimethylsilyl triflate,[11] proceeded smoothly and stereoselectively to give, after purification by LH-20 chromatography, the expected α(1→4) linked dimer **19** in a good yield. Dimer **19** was transformed into the requisite acceptor **3** by the following two steps. First, the azido group was converted into the acetamido function by prolonged treatment with thioacetic acid[12] to yield, after silica gel chromatography, homogeneous **20**. Saponification of the acetyl ester and silica gel purification gave dimer **3** in 60% yield for the two steps.

Scheme 3[a]



Key[a] : i) benzaldehyde dimethyl acetal, *p*TsOH in acetonitrile; ii) NaH, BnBr, DMF; iii) $AcOH/H_2O$, 4/1, v/v reflux, 1 h (32%, based on **10**); iv) dibutyl stannic oxide, MeOH, reflux, 3 h, then BnBr, CsF in DMF, 16 h, 40 °C (53%); v) oxalyl chloride, DMSO, CH_2Cl_2, Et_3N; vi) $NaBH_4$ in $CHCl_3/EtOH/H_2O$, 2/2/1, v/v/v (yield **14/16**, 1/2, 78%); vii) Ac_2O/pyridine, 2/1, v/v (87%); viii) **18**, TMSTriflate, CH_2Cl_2, 0 °C, 3h (69%); ix) AcSH, 72h, 20 °C; x) *t*BuOK in MeOH.

At this stage, having the glycosyl acceptor **3** and donor **2** in hand, the assembly of the target molecule **1** was undertaken. In a first attempt, the condensation of **3** with **2** under standard conditions[13] using excess *N*-iodosuccinimide and a catalytic amount of trifluoromethanesulfonic acid (TfOH) gave exclusively, as evidenced by NMR spectroscopy, the orthoester derivative **21** in 56% yield. The outcome of the latter glycosylation reaction is probably due to protonation of the protected amino function at C-4 of the Sug*p* moiety in the acceptor molecule by TfOH, thus favouring the formation of the orthoester derivative **21**. A similar observation was also made by Kunz *et al.*[14] in a preliminary attempt to employ glucopyranosyl donors having an *N*-phenylcarbamoyl at C-4 in glycosylation processes. As a consequence, execution of the above glycosylation in the presence of an increased amount of TfOH (0.4 equiv. with respect to acceptor **3**), resulted in the exclusive formation of the fully protected tetrameric fragment **22** which was isolated as a



Reagents and conditions : i) $Et_3N/C_5H_5N/H_2O$, 3/1/1, v/v/v; ii) NH_3 in methanol, 40 h, then Dowex (Na^+-form); iv) $Pd(OH)_2/C$, H_2, dioxane/*t*BuOH/AcOH/H_2O, 5/8/1/1, v/v/v/v, 48 h, 20 °C.

mixture of diastereoisomers (δ_P -0.6 and -1.3 ppm) and in a yield of 55%. Furthermore, it is also of interest to note that the rearrangement of orthoester **21** into **22** using trimethylsilyl triflate[15] or TfOH was unsuccessful.

Complete deblocking of **22** to afford the C-substance fragment **1** started with the elimination of the cyanoethyl group from the phosphotriester function under mild basic conditions. Thus, treatment of **22** with triethylamine/pyridine/water furnished, after work-up and purification, the phosphodiester derivative **23** (δ_P 0.9 ppm). Debenzoylation of **23** by ammonolysis and subsequent hydrogenolysis of the benzyl and benzyloxycarbonyl groups from purified **24** (Na^+-salt) gave tetramer **1** in 60% overall yield. The structural integrity and homogenicity of **1** was in complete accord with the recorded ^{13}C-, ^1H NMR (see Figure 2) and mass spectroscopic data.

The synthetic approach described in this paper may open the way to the assembly of C-substance fragments suitable for the future design and development of a synthetic vaccine against pneumococcal infections.[16]

EXPERIMENTAL

General Procedures. Dioxane and pyridine were dried by refluxing with CaH_2 (5 g/L) for 6 h and then distilled. Dichloromethane, 1,2-dichloroethane and toluene were distilled from P_2O_5. *N,N*-Dimethylformamide was stirred with CaH_2 at room

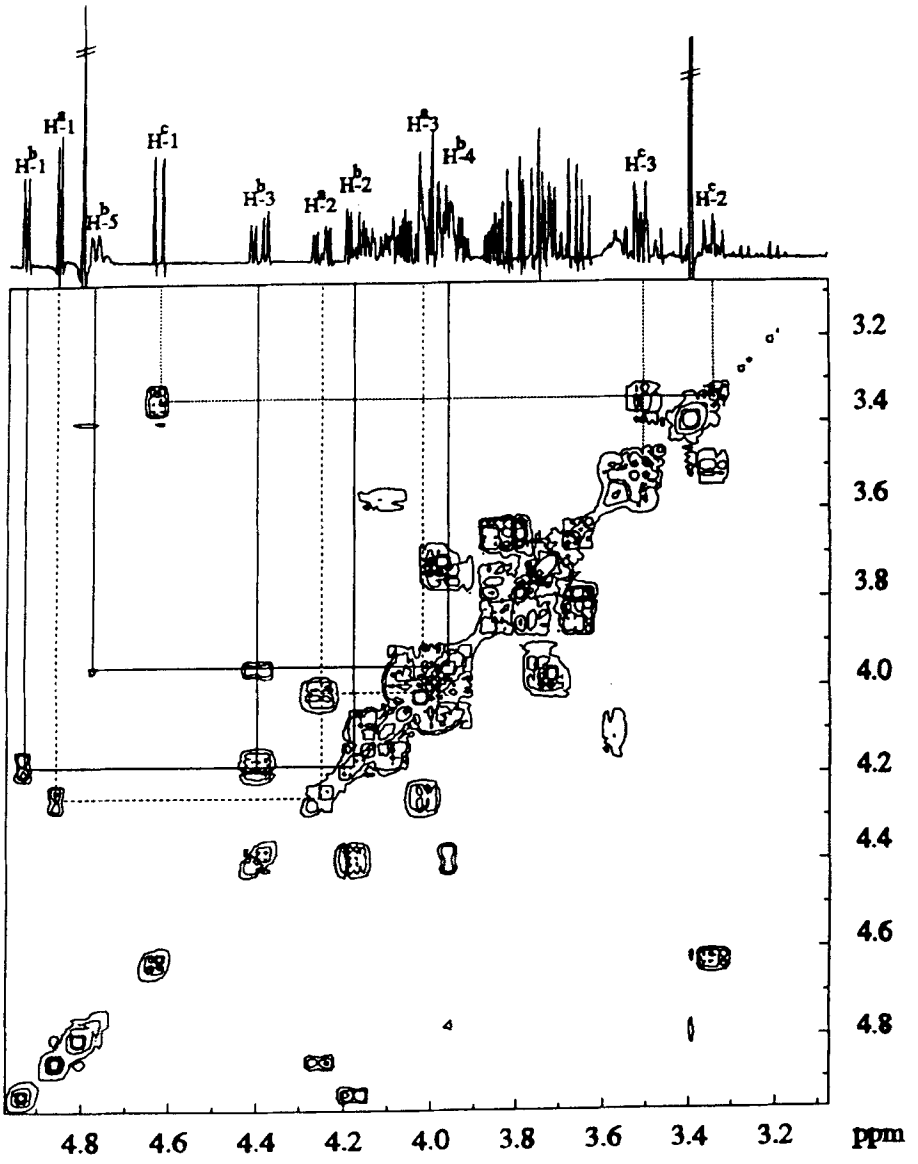


Figure 2. 400 MHz 2D ^{1}H-^{1}H shift-correlation spectrum (region 3.0-5.0 ppm) of **1**. The lines in the spectrum indicate the connectivities for the respective residues (Gal*p*NAc^a -----; Sug*p*^{b} ___ ;Glc*p*^{c}.....).

temperature for 16 h and distilled under reduced pressure. Diethyl ether was distilled from $LiAlH_4$. Dioxane, pyridine and *N,N*-dimethylformamide were stored over molecular sieves 4 Å (Aldrich). Toluene and diethyl ether were stored over sodium wire, dichloromethane and 1,2-dichloroethane over alumina. Schleicher and Schüll DC Fertigfolien F1500 LS 254 were used for TLC analysis. The following eluents were used: System A (dichloromethane/methanol, 9/1, v/v), System B (diethyl ether/hexane, 1/4, v/v), System C (diethyl ether/hexane, 1/2, v/v), System D (dichloromethane/acetone, 97/3, v/v), System E (diethyl ether/methanol, 99/1, v/v), System F (diethyl ether/methanol, 95/5, v/v), System G (diethyl ether/methanol, 92/8, v/v), System H (ethyl acetate/methanol, 85/15, v/v), System I (ethyl acetate/methanol, 2/1, v/v). Compounds were detected by charring with 10% sulphuric acid in water, containing $(NH_4)_6Mo_7O_{24}$ x $4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4$ x $2H_2O$ (10 g/L). Compounds **4-7** were detected by charring with 20% sulphuric acid in methanol. Optical rotations were recorded at 20 °C with a Perkin-Elmer 241 polarimeter. Column chromatography was performed on silica gel 60, 70-230 mesh (Merck). Gel filtration was performed on Sephadex LH-20 (Pharmacia). NMR spectra were recorded with a JEOL JNM-FX 200 [^{13}C NMR (50.1 MHz), internal standard chloroform or methanol (respectively 77 and 49 ppm relative to Me_4Si); ^{31}P NMR (80.7 MHz)] and a Bruker WM-300 spectrometer equipped with an Aspect-2000 computer (^1H, 300 MHz, internal standard Me_4Si). Mass spectra were obtained with a Finnigan MAT SSQ 710 (Finnigan MAT, San José) equipped with an electrospray interface (Finnigan MAT). Mass spectra were recorded in the constant infusion mode with a flow rate of 1 µL/min at a concentration of 100 pmol/µL in both the positive and negative ion mode. The positive ion mode spectra were dominated by the $[M+H]^+$ and $[M+Na]^+$ ions and in the negative ion mode the spectra were dominated by $[M-H]^-$ ions.

5-*O*-Allyl-1,2,3,4-tetra-*O*-benzyl-D-ribitol (6). Compound **4**[5] (6.5 g, 28 mmol) was dissolved in acetic acid/water (4/1, v/v, 100 mL) and stirred for 1 h at 50 °C, when TLC analysis (System A) indicated complete conversion of **4** into **5** (Rf 0.45). The mixture was concentrated with toluene (5 x 50 mL), redissolved in DMF and cooled to 0 °C. To the cooled solution was added sodium hydride (80% dispersion in mineral oil, 3.5 g, 123 mmol) and benzyl bromide (14.6 mL, 123 mmol). The mixture was stirred for 16 h, while it was allowed to reach room temperature. TLC analysis (System B) showed complete conversion of **5** into **6**. Methanol was added (2 mL) and after stirring for 1 h, the mixture was concentrated *in vacuo*, redissolved in

dichloromethane (100 mL), washed with water (3 x 75 mL), dried ($MgSO_4$) and concentrated. The residue was chromatographed on silica gel (eluent: diethyl ether/hexane, 1/6 to 1/4, v/v) to give pure **6** (9.1 g, 58%) as an oil; ^{13}C NMR data ($CDCl_3$) δ 138.3-138.6 (C-quat., arom); 134.85 (CH, allyl); 127.3-128.3 (C-arom); 116.6 (CH_2, allyl); 78.6, 78.3 (C-2, C-3, C-4); 70.0-73.7 (4 x CH_2-benzyl, CH_2-All, C-1, C-5).

1,2,3,4-Tetra-*O*-benzyl-D-ribitol (7). A solution of compound **6** (8.5 g, 15.3 mmol) in toluene (50 mL) and ethanol (50 mL) was concentrated, the process repeated and the residue redissolved in ethanol (75 mL). To the solution was added dihydridotetrakis(triphenylphosphine) ruthenium(II)[6] (176 mg, 0.153 mmol) in portions of 17.6 mg. After the mixture was refluxed for 3 h, the reaction mixture was concentrated *in vacuo* and the residue was redissolved in dioxane (20 mL) and a solution of hydrochloric acid in methanol (0.5 M, 10 mL) was added. After the mixture was stirred for 30 min, TLC analysis (System C) showed the reaction to be complete. Triethylamine (2 mL) was added and the mixture was concentrated to dryness. The residue was purified on silica gel (eluent: diethyl ether/hexane, 1/3 to 1/1, v/v) to give **7** (5.9 g, 76%); Rf 0.25 (System C); $[\alpha]_D^{20}$ +14.6° (*c* 1, $CHCl_3$); ^{13}C NMR data ($CDCl_3$) δ 137.9-138.0 (C-quat, arom); 127.1-127.9 (C-arom); 77.9, 78.4, 78.7 (C-2, C-3, C-4); 60.8-73.4 (C-1, C-5, 4x CH_2, benzyl).

Anal. Calcd for $C_{33}H_{36}O_5$ (M = 512.65): C, 77.32, H, 7.08. Found: C, 77.38, H, 7.11.

1,2,3,4-Tetra-*O*-benzyl-D-ribityl 2-Cyanoethyl (*N,N*-Diisopropyl)phosphoramidite (8, R/S). A solution of compound **7** (750 mg, 1.46 mmol) in toluene (3 x 5 mL) was concentrated and redissolved in dichloromethane (5 mL). To the solution was added diisopropylethylamine (0.63 mL, 3.65 mmol) and chloro 2-cyanoethyl (*N,N*-diisopropyl)-phosphoramidite[7] (450 mg, 1.90 mmol). After the solution was stirred for 15 min under a blanket of argon, TLC analysis (System C + 1% triethylamine) showed the formation of the two diastereisomers of **8** (Rf 0.40, 0,45) to be complete. The solution was diluted with dichloromethane (30 mL), washed with a saturated NaCl solution containing 5% triethylamine (2 x 25 mL), 2 M TEAB (25 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified on silica gel (eluent: diethyl ether/hexane/triethylamine, 5/19/1 to 5/14/1, v/v/v) to yield pure **8** (870 mg, 84%); ^{13}C NMR data ($CDCl_3$) δ 138.5-138.8 (C-quat, arom); 127.4-128.3 (C-arom); 117.7 (CN); 78.6-79.2 (C-2, C-3, C-4); 72.4-73.7 (4 x CH_2, benzyl); 70.2 (C-1); 63.2 (C-5); 58.2-

58.6 (OCH_2, CNE); 42.9, 43.2 (2 x CH, isopropyl); 24.6, 24.7 (2 x CH_3, isopropyl); 20.2 (CH_2-CN); ^{31}P NMR data ($CDCl_3$) δ 149.0, 148.7.

(Ethyl 2,3,4-Tri-*O*-benzoyl-1-thio-ß-D-glucopyranosyl) 6-(1,2,3,4-Tetra-*O*-benzyl-D-ribityl 2-Cyanoethyl 5'-Phosphate) (2, R/S). A solution of compound **8** (800 mg, 1.12 mmol) and ethyl 2,3,4-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside[8] **9** (560 mg, 1.04 mmol) in toluene (3 x 10 mL) was concentrated and the remaining oil was redissolved in acetonitrile (15 mL). 1*H*-Tetrazole (140 mg, 2.0 mmol), previously concentrated from acetonitrile solution (3 x 5 mL), was added to the solution of **8** and **9** and the mixture was stirred for 1 h, when TLC analysis showed complete conversion of the phophoramidite **8** (System D, Rf 0.5) into one product. The reaction mixture was cooled to 0 °C and *t*-butyl hydroperoxide (0.3 mL, 2.9 mmol) was added. The mixture was stirred for 30 min at room temperature, when TLC analysis (System E) showed the oxidation to be complete. The mixture was diluted with dichloromethane, washed with 2 M TEAB (10 mL) and water (10 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified on silica gel (eluent: diethyl ether/methanol, 100/0 to 99/1) to furnish homogeneous **2** (865 mg, 72%) as a mixture of diastereisomers (ratio: 1/1); $[\alpha]_D^{20}$ +8.1° (*c* 1, $CHCl_3$); ^{13}C NMR data ($CDCl_3$) δ 164.9-165.5 (3x C=O, benzoyl); 138.0-138.3 (C-quat., arom); 127.4-133.4 (C-arom); 116.2 (CN); 83.5, 83.6 (C-1); 23.8, 24.1 (CH_2, SEt); 19.0 (CH_2-CN); 14.7 (CH_3, SEt); ^{31}P NMR data ($CDCl_3$) δ -0.98, -1.44.

Methyl 2-Acetamido-4,6-*O*-benzylidene-2-deoxy-α,ß-D-glucopyranoside (11). To a solution of methyl 2-acetamido-α(ß)-D-glucopyranoside[9] (**10**, α/ß 2/1, 9 g, 38.3 mmol) in acetonitrile (100 mL) was added benzaldehyde dimethyl acetal (17.3 mL, 115.2 mmol) and *p*-toluenesulfonic acid monohydrate (7.3 g, 38.4 mmol). After stirring for 18 h at 20 °C, the mixture was neutralized with triethylamine (15 mL) and concentrated *in vacuo*. The residue was crystallized from diethyl ether to give **11** as a yellow solid, which was sufficiently pure to be used for the synthesis of **12** without further purification; mp 135-143 °C.

Methyl 2-Acetamido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (13). To a cooled (0 °C) solution of compound **11** (9.0 g, 27.9 mmol) in DMF (100 mL) was added benzyl bromide (3.6 mL, 30.3 mmol) and sodium hydride (80% dispersion in mineral oil, 881 mg, 30.6 mmol) and the mixture was stirred for 2 h at 20 °C, when TLC analysis (System F, **12:** Rf 0.4) showed the reaction to be complete. The solution was quenched with methanol, neutralized with acetic acid and concentrated *in vacuo*. The

remaining oil was dissolved in acetic acid/water (4/1, v/v, 100 mL) and the solution was refluxed for 1 h, when TLC analysis (System G) showed complete conversion of compound **12** into one major product. The mixture was concentrated *in vacuo* and then concentrated again from toluene (4 x 50 mL) and ethanol (2 x 50 mL) solution. The residue was chromatographed on silica gel (eluent: diethyl ether/methanol, 95/5 to 92/8, v/v) to give pure **13** (3.9 g, 32% based on **10**); Rf 0.6 (System H); ^{13}C NMR data ($MeOD_4$) δ 171.9 (C=O, acetyl); 139.0 (C-quat., arom); 127.9-128.6 (C-arom); 98.96 (C-1, $J_{C,H}$ = 171.4 Hz); 80.2 (C-3); 74.6 (CH_2, benzyl); 70.8, 72.4 (C-4, C-5); 61.7 (C-6); 55.2 (OMe); 52.9 (C-2); 22.9 (CH_3, acetyl).

Methyl 2-Acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (14). A solution of compound **13** (1.5 g, 4.6 mmol) in toluene (3 x 25 mL) was concentrated and the residue dissolved in methanol (50 mL). To this solution was added dibutyltin oxide (1.3 g, 5.2 mmol) and the mixture was refluxed for 3 h. The reaction mixture was concentrated *in vacuo*, a solution of the remaining oil in toluene (2 x 25 mL) was concentrated and this oil subsequently redissolved in DMF (50 mL). To this solution was added benzyl bromide (0.65 mL, 5.4 mmol) and cesium fluoride (1.5 g, 9.9 mmol) and the mixture was stirred for 18 h at 40 °C. The solution was concentrated and the remaining oil was dissolved in dichloromethane (75 mL), extracted with aq $NaHCO_3$ (50 mL) and water (2 x 50 mL), dried ($MgSO_4$) and concentrated. The residue was chromatographed on silica gel (eluent: diethyl ether/methanol, 95/5, v/v) to give **14** (1.02 g, 53%). Further elution (diethyl ether/methanol, 9/1, v/v) resulted in the recovery of unreacted **13** (300 mg, 20%). Compound **14**: Rf 0.45 (System F); $[\alpha]_D^{20}$ +63.0° (*c* 1.5, $CHCl_3$); ^{13}C NMR data ($CDCl_3$) δ 169.9 (C=O, acetyl); 137.7, 138.4 (2 x C-quat., arom); 127.3-128.2 (C-arom); 98.3 (C-1, $J_{C,H}$ = 167 Hz); 79.5 (C-3); 73.3, 73.4 (2 x CH_2, benzyl); 70.3, 71.4 (C-4, C-5); 69.7 (C-6); 54.7 (OMe); 51.7 (C-2); 23.0 (CH_3, acetyl); ^1H NMR data ($CDCl_3$): δ 7.3-7.5 (H-arom); 5.41 (bd, 1 H, $J_{NH,H-2}$ = 9.3 Hz, NH); 4.67 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1); 4.53-4.78 (2 x AB, 4 H, 2 x CH_2-benzyl); 4.19-4.26 (ddd, 1 H, $J_{2,3}$ = 10.6 Hz, H-2); 3.69-3.76 (m, 4 H, H-4, H-5, 2 x H-6); 3.51-3.58 (dd, 1 H, $J_{3,4}$ = 8.0 Hz, H-3); 3.34 (s, 3 H, OMe); 1.9 (s, 3 H, CH_3, acetyl).

Anal. Calcd for $C_{23}H_{29}O_6N$ (M = 415.49): C, 66.49, H, 7.03, N, 3.37. Found: C, 66.58, H, 7.01, N, 3.41.

Methyl 2-Acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-galactopyranoside (16). To a cooled (-70 °C) solution of oxalyl chloride (0.22 mL, 2.5 mmol) in dichloromethane (5 mL) was added methyl sulfoxide (1.92 M in dichloromethane, 1.5 mL) and the

mixture was stirred for 5 min, when a solution of compound **14** (0.93 g, 2.2 mmol) in dichloromethane (5 mL) was added dropwise. After stirring for 30 min at -70 °C, triethylamine (1.6 mL, 11.1 mmol) was added and the reaction mixture was allowed to reach room temperature. This solution was concentrated *in vacuo* and a solution of the residue in toluene (2 x 40 mL) was concentrated to give crude **15** (Rf 0.6, System F). Compound **15** was dissolved in chloroform/ethanol/water (2/2/1, 20 mL) and sodium borohydride (190 mg, 5 mmol) was added. After stirring for 10 min at room temperature, TLC analysis (System F) showed complete conversion of ketone **15** into two products. The mixture was diluted with dichloromethane (50 mL) and the organic layer was washed with aq $NaHCO_3$ (25 mL) and water (2 x 25 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed on silica gel (eluent: diethyl ether/methanol, 95/5, v/v). The first product to be eluted was the D-*gluco*-epimer **14** (260 mg, 28%). The second product to be eluted was the D-galactosamine derivative **16** (465 mg, 50%); Rf 0.5 (System G); $[\alpha]_D^{20}$ +105.5° (*c* 4.3, $CHCl_3$); ^{13}C NMR data ($CDCl_3$) δ 169.8 (C=O, acetyl); 137.6, 137.8 (2 x C-quat., arom); 127.3-128.2 (C-arom); 98.5 (C-1); 75.8 (C-3); 70.7, 73.3 (2 x CH_2, benzyl); 69.4 (C-6); 65.7, 68.7 (C-4, C-5); 54.8 (OMe); 48.0 (C-2); 23.1 (CH_3, acetyl); ^1H NMR data ($CDCl_3$) δ 7.2-7.4 (H-arom); 5.40 (bd, 1 H, $J_{NH,H-2}$ = 9.4 Hz, NH); 4.69 (d, 1 H, $J_{1,2}$ = 3.9 Hz, H-1); 4.4-4.75 (2 x AB, 4H, 2 x CH_2-benzyl); 4.46-4.58 (m, 1 H, H-2); 4.1 (dd, 1H, $J_{3,4}$ = 3.2 Hz, $J_{4,5}$ = 1 Hz, H-4); 3.8-3.9 (dq, 1H, H-5); 3.68-3.82 (m, 2H, 2 x H-6); 3.49 (dd, 1H, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 3.1 Hz, H-3); 3.32 (s, 3H, OMe); 1.93 (s, 3H, CH_3, acetyl); Mass spectroscopy: $[M+H]^+$ ion m/z 416.

Anal. Calcd for $C_{23}H_{29}O_6N$ (M = 415.49): C, 66.49, H, 7.03, N, 3.37. Found: C, 66.51, H, 7.05, N, 3.40.

Methyl 2-Acetamido-4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-α-D-galactopyranoside (17). Compound **16** (104 mg, 0.25 mmol) was dissolved in a mixture of pyridine/acetic anhydride (2/1, v/v, 3 mL) and the solution was stirred overnight at 20 °C, when TLC analysis (System F) showed complete conversion of **16** into one product. The mixture was concentrated *in vacuo* and the solution of the residue in toluene (3x 20 mL) was concentrated. The residue was chromatographed on silica gel (eluent: diethyl ether), to give **17** (100 mg, 87%); ^{13}C NMR ($CDCl_3$) δ 169.7, 170.2 (2 x C=O, acetyl); 137.5, 137.7 (2 x C-quat., arom); 127.5-128.1 (C-arom); 98.4 (C-1); 75.0 (C-3); 70.4, 74.9 (2 x CH_2, benzyl); 68.2 (C-6); 66.1, 67.6 (C-4, C-5); 55.0 (OMe); 48.6 (C-2); 20.6, 23.0 (2 x CH_3, acetyl); ^1H NMR data: δ 7.2-7.4 (H-arom); 5.59 (dd, 1H, $J_{3,4}$ = 3.2 Hz, $J_{4,5}$

= 1.1 Hz, H-4); 5.25 (bd, 1 H, $J_{NH,H-2}$ = 8.9 Hz, NH); 4.77 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1); 4.32-4.74 (2 x AB, 4 H, 2 x CH_2-benzyl); 4.38-4.44 (ddd, 1 H, H-2); 4.0 (dq, 1H, H-5); 3.48-3.68 (m, 3H, H-3, 2x H-6); 3.32 (s, 3H, OMe); 1.92, 2.09 (2 x s, 2 x CH_3, acetyl).

Methyl 2-Acetamido-4-*O*-(3-*O*-acetyl-2-azido-4-(benzyloxycarbonyl)amino-2,4,6-trideoxy-α-D-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-α-D-galactopyranoside (19). To a thoroughly dried mixture of Sug(*p*) glycosyl donor[3] **18** (750 mg, 1.47 mmol) and acceptor **16** (480 mg, 1.15 mmol) in dichloromethane (4 mL) was added molecular sieves 4Å (0.5 g). After stirring for 1 h under a blanket of nitrogen, the mixture was cooled to 0 °C. To the cooled solution was added a catalytic amount of trimethylsilyl triflate (0.1 M in CH_2Cl_2, 1.4 mL). After stirring for 3 h at room temperature, TLC analysis (System H) indicated the reaction to be complete. The solution was diluted with dichloromethane (50 mL) and filtered. The solution was washed with 10% aq $NaHCO_3$ (25 mL) and water (2 x 50 mL). The organic layer was dried ($MgSO_4$), concentrated *in vacuo* and the residue was purified by LH-20 chromatography (eluent: dichloromethane/methanol, 1/1, v/v) to give homogeneous **19** (600 mg, 69%); Rf 0.45 (System G); $[\alpha]_D^{20}$ +139.8° (*c* 2.1, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 169.3 (C=O, acetyl); 156.3 (C=O, Z); 136.2-137.7 (C-quat., arom); 127.0-128.1 (C-arom); 98.3 (2 x C-1); 75.4 (C^a-3); 71.2, 73.1 (CH_2, benzyl); 64.6-75.4, (C^a-4, C^a-5, C^b-3, C^b-5); 67.1 (C^a-6); 66.4 (CH_2, Z); 48.6, 52.5, 54.9, 58.1 (C^a-2, C^b-2, C^b-4, OMe); 23.0, 20.5 (2 x CH_3, acetyl); 16.0 (C^b-6); ^1H NMR ($CDCl_3$) δ 7.1-7.4 (m, 15H, H-arom); 5.23-5.28 (dd, 1H, $J_{2,3}$ = 11.2 Hz, $J_{3,4}$ = 3.8 Hz, H^b-3); 5.2 (bd, 1H, $J_{NH,H-2}$ = 9.4 Hz, NH); 5.02-5.18 (AB, 2H, CH_2, Z); 4.93 (bd, 1H, $J_{NH,Hb-4}$ = 9.6 Hz, NH-C^b-4); 4.88 (d, 1H, $J_{1,2}$ = 4.0 Hz, H^b-1); 4.78 (d, 1H, $J_{1,2}$ = 3.3 Hz, H^a-1); 4.74-4.78, 4.36-4.41 (AB, 2H, CH_2, benzyl); 4.50-4.60 (AB, 2H, CH_2, benzyl); 4.62-4.68 (dq, 1H, H^b-5); 4.44-4.54 (m, 1H, H^a-2); 4.10-4.25 (m, 2H, H^b-4, H^a-4); 3.80-4.0 (m, 2H, H^a-6, H^a-6'); 3.45-3.59 (m, 2H, H^a-3, H^a-5); 3.40 (dd, 1H, $J_{1,2}$ = 4.0 Hz, $J_{2,3}$ = 11.3 Hz, H^b-2); 3.29 (s, 3H, OCH_3); 1.97, 1.90 (2 x s, 6H, 2 x CH_3, acetyl); 0.91 (d, 3H, $J_{5,6}$.3 Hz, CH_3). $[M + H]^+$ ion m/z 762.4; $[M+Na]^+$ ion m/z 784.6; dimerisation: 2 x $[M + H]^+$ m/z 1524).

Anal. Calcd for $C_{39}H_{47}O_{11}N_5$ (M = 761.84): C, 61.49, H, 6.22, N, 9.19. Found: C, 61.56, H, 6.31, N, 9.29.

Methyl 2-Acetamido-4-*O*-(2-acetamido-3-*O*-acetyl-4-(benzyloxycarbonyl)amino-2,4,6-trideoxy-α-D-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-α-D-galactopyranoside (20). Compound **19** (600 mg, 0.79 mmol) was dissolved in thioacetic acid (5 mL) and

the mixture was stirred for 3 days at 20 °C, when TLC analysis (System G) showed the formation of one major product. The mixture was concentrated *in vacuo* and the crude product was purified by silica gel chromatography (eluent: diethyl ether/methanol, 97/3 to 9/1, v/v) to yield pure **20** (490 mg, 80%) as an oil, which solidified upon standing; ^{13}C NMR data ($CDCl_3$) δ 170.8, 171.1, 171.2 (3 x C=O, acetyl); 156.8 (C=O, Z); 137.0-137.4 (C-quat., arom); 125.9-128.0 (C-arom); 97.1, 98.1 (2 x C-1); 74.5 (C^a-3); 71.2, 72.9 (2 x CH_2, benzyl); 64.3-70.5 (C^a-4, C^a-5, C^b-3, C^b-5); 66.4, 67.1 (C^a-6, CH_2-Z); 54.8 (OMe); 47.5, 48.8, 52.1 (C^a-2, C^b-2, C^b-4); 20.2, 22.2, 22.4 (3 x CH_3, acetyl); 16.0 (C^b-6).

Methyl 2-Acetamido-4-*O*-(2-acetamido-4-(benzyloxycarbonyl)amino-2,4,6-trideoxy-α-D-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-α-D-galactopyranoside (3). Compound **20** (530 mg, 0.68 mmol) was dissolved in methanol (15 mL) and KO*t*Bu (15 mg, 0.13 mmol) was added. The mixture was stirred for 1 h, when TLC analysis (System H) indicated the reaction to be complete. The solution was neutralized with acetic acid, concentrated *in vacuo* and the residue was chromatographed on silica gel (eluent: ethyl acetate/methanol, 19/1 to 17/3, v/v) to give homogeneous **3** (360 mg, 72%) as an oil; Rf 0.5 (System H); $[\alpha]_D^{20}$ +116.9° (*c* 0.85, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 170.9, 171.1 (2 x C=O, acetyl); 157.5 (C=O, Z); 136.3-137.7 (C-quat., arom); 127.0-128.1 (C-arom); 98.4 (C^a-1); 97.6 (C^b-1); 74.9 (C^a-3); 65.4-73.1 (2 x CH_2-benzyl, C^a-4, C^a-5, C^b-3, C^b-5, C^a-6, CH_2-Z); 48.7, 50.6, 55.0, 55.4 (C^a-2, C^b-2, C^b-4, OMe); 23.0, 23.1 (2 x CH_3, acetyl); 16.5 (C^b-6).

(Methyl 2-Acetamido-4-*O*-(2-acetamido-3-*O*-(2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl)-4-(benzyloxycarbonyl)amino-2,4,6-trideoxy-α-D-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyl) 6''-(1,2,3,4-Tetra-*O*-benzyl-D-ribityl 2-Cyanoethyl 5'''-Phosphate) (22, R/S).

Method A. A solution of glycosyl donor **2** (207 mg, 0.18 mmol) and acceptor **3** (87 mg, 0.12 mmol) together in toluene (2 x 5 mL) and 1,2-dichloroethane (3 x 5 mL) was concentrated and the residue redissolved in a mixture of diethyl ether/1,2-dichloroethane (1/1, v/v, 4 mL). To the stirred solution was added molecular sieves 4Å under a blanket of argon. After 1 h, *N*-iodosuccinimide (60 mg, 0.27 mmol) was added. To the reaction mixture was injected a catalytic amount of trifluoromethanesulfonic acid (3 µl, 0.03 mmol) and stirring was continued for 4 h, when TLC analysis (System G) showed complete conversion of **2** into one major product. The solution was diluted with dichloromethane, washed with aq $Na_2S_2O_3$ (1M, 2 x 30 mL) and water

(30 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified on silica gel (eluent: diethyl ether/methanol, 97/3 to 92/8, v/v) to give homogeneous orthoester **21 (R/S)** (120 mg, 56%); Rf 0.6 (System G); ^{13}C NMR data ($CDCl^3$) δ 169.4, 169.7 (2 x C=O, acetyl); 164.6, 164.8 (2x C=O, benzoyl); 156.9 (C=O, Z); 136.0-138.4 (C-quat., arom); 126.3-133.3 (C-arom); 120.9 (C-quat., orthoester); 116.8 (CN); 97.1, 97.9, 98.6 (C^a-1, C^b-1, C^c-1); 48.5, 48.6, 55.2, 55.4 (C^a-2, C^b-2, C^b-4, OMe); 23.4 (2 x CH_3, acetyl); 19.2 (t, OCH_2, CNE); 16.6 (C^b-6); ^{31}P NMR data δ -1.0, -1.22.

Method B. To a solution of donor **2** (410 mg, 0.35 mmol) and acceptor **3** (215 mg, 0.29 mmol) in 1,2-dichloroethane (10 mL) was added, under a blanket of argon, a solution of *N*-iodosuccinimide (158 mg, 0.7 mmol) and trifluoromethanesulfonic acid (10.8 μl, 0.12 mmol) in 1,2-dichloroethane (4 mL). Stirring was continued for 5 h, when TLC analysis (System F) showed complete conversion of the donor **2** in one major product having a different Rf value than **21**. Work-up and purification as described by method A gave homogeneous tetramer **22** (268 mg, 55%); Rf 0.5 (System G); ^{13}C NMR data δ 169.8, 169.9 (2 x C=O, acetyl); 164.8, 164.9, 165.6 (3 x C=O, benzoyl); 156.7 (C=O, Z); 136.8-138.3 (C-quat., arom); 127.4-133.2 (C-arom); 117.3 (CN); 100.6 (C^c-1, J_{CH} = 156.8); 98.4 (C^a-1); 97.3 (C^b-1); 48.9, 54.0, 55.2 (C^a-2, C^b-2, C^b-4, OMe); 22.6, 23.4 (2 x CH_3, acetyl); 19.4 (OCH_2, CNE); 16.6 (C^b-6); ^{31}P NMR data ($CDCl_3$) δ -0.63, -1.34.

(Methyl 2-Acetamido-4-*O*-(2-acetamido-3-*O*-(2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl)-4-(benzyloxycarbonyl)amino-2,4,6-trideoxy-α-D-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyl) 6''-(1,2,3,4-Tetra-*O*-benzyl-D-ribityl Triethylammonium 5'''-Phosphate) (23). Compound **22** (260 mg, 0.14 mmol) was dissolved in a mixture of triethylamine (6 mL), pyridine (2 mL) and water (2 mL). The solution was stirred for 5 h at 20 °C, when TLC analysis (System H) showed complete conversion of **22** to **23**. The solution was concentrated *in vacuo* and the residue was purified by LH-20 column chromatography (eluent: dichloromethane/methanol, 1/1, v/v) to yield **23** (240 mg, 90%) as an oil; Rf 0.3 (System H); ^{13}C NMR data (CD_3OD) δ 172.8, 173.2 (2 x C=O, acetyl); 166.4, 166.9 (3 x C=O, benzoyl); 158.6 (C=O, Z); 138.9-140.1 (C-quat., arom); 127.5-134.4 (C-arom); 102.5 (C^c-1); 98.6, 100.2 (C^a-1, C^b-1); 50.7, 55.8, 56.2 (C-2, C^b-2, C^b-4, OMe); 46.9 (CH_2, Et_3NH^+); 22.6, 22.8 (2 x CH_3, acetyl); 17.2 (C^b-6); 9.2 (CH_3, Et_3NH^+); ^1H NMR data δ 7.0-8.0 (m, 50H, H-arom); 5.8 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz, H^c-3); 5.6 (t, 1 H, $J_{4,5}$ = $J_{3,4}$ = 9.6 Hz, H^c-4); 5.38 (dd, 1 H, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.6 Hz, H^c-2); 5.01 (d, 1H, H^c-1); 3.23 (s, 3H, OMe); 1.6, 1.9 (2 x s, 6H, 2 x Ac); 0.93 (d, 3H, $J_{5,6}$.27 Hz, H^b-6); ^{31}P NMR data δ 0.90.

(Methyl 2-Acetamido-4-*O*-(2-acetamido-3-*O*-(β-D-glucopyranosyl)-4-(benzyloxycarbonyl)amino-2,4,6-trideoxy-α-D-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyl) 6''-(1,2,3,4-Tetra-*O*-benzyl-D-ribityl Sodium 5'''-Phosphate) (24). Compound **23** (220 mg, 0.12 mmol) was dissolved in a saturated solution of methanolic aq ammonia (25%, 15 mL) and the solution stirred at room temperature for 40 h, when TLC analysis (System H) showed complete reaction. The solution was concentrated *in vacuo* and the residue was purified by LH-20 column chromatography (eluent: methanol). The appropriate fractions were concentrated *in vacuo* to an oil and the residue was redissolved in methanol/water (1/1, v/v, 5 mL) and treated for 30 min with Dowex 50WX4 (Na^+-form). The mixture was filtered and the filtrate concentrated to yield pure **23** (150 mg, 87%); ^{13}C NMR data (CD_3OD) δ 173.5, 174.0 (2 x C=O, acetyl); 159.3 (C=O, Z); 138.4-140.0 (C-quat., arom); 128.0-129.3 (C-arom); 105.0 (C^c-1); 99.3, 100.3 (C^a-1, C^b-1); 55.8 (OMe); 48.0-56.5 (C^a-2, C^b-2, C^b-4, OMe); 22.8, 23.3 (2 x CH_3, acetyl); 17.1 (C^b-6); ^1H NMR data ($CDCl_3/CD_3OD$, 1/1, v/v) δ 7.2-7.5 (m, 35H, H-arom); 4.97 (d, 1H, $J_{1,2}$ = 3.4 Hz, H^b-1); 4.71 (d, 1H, $J_{1,2}$ = 3.4 Hz, H^a-1); 4.47 (d, 1H, $J_{1,2}$ = 7.7 Hz, H^c-1); 3.26 (s, 3H, OMe); 1.9, 2.0 (2 x s, 6H, 2 x Ac); 0.85 (d, 3H, $J_{5,6}$.2 Hz, H^b-6); ^{31}P NMR data δ 0.36.

(Methyl 2-Acetamido-4-*O*-(2-acetamido-3-*O*-(β-D-glucopyranosyl)-4-amino-2,4,6-trideoxy-α-D-galactopyranosyl)-2-deoxy-α-D-galactopyranosyl) 6''-(D-Ribityl Sodium 5'''-Phosphate) (1). Compound **24** (60 mg, 0.04 mmol) was dissolved in dioxane (5 mL) and added to a suspension of $Pd(OH)_2$ on charcoal (100 mg) in *t*BuOH/AcOH/water (8/1/1, v/v/v, 10 mL). The mixture was shaken under a hydrogen atmosphere (P_{H2} 0.5 mPa) for 48 h at ambient temperature. TLC analysis (System I) showed complete disappearance of the starting material. The palladium catalyst was removed by filtration and the filtrate was treated with Amberlist IRA-400 (OH^-) (0.2 g). The ion-exchange resin was filtered off and the filtrate was concentrated *in vacuo*. The residue was purified by hiload Sephadex S100 HR 26/60 column chromatography (eluent: 2M TEAB). The appropriate fractions were concentrated *in vacuo* and a solution of the residue in water (10 x 5 mL) was concentrated under reduced pressure to remove the TEAB salts. The remaining oil was dissolved in water (5 mL) and Dowex 50WX4 (100 mg, Na^+-form) was added. After the mixture was stirred for 15 min, the solution was filtered and concentrated to give homogeneous **1** (25 mg, 76%); $[\alpha]_D^{20}$ +33.2° (*c* 0.37, H_2O); ^{13}C NMR data (D_2O) δ 175.4, 175.5 (2 x C=O, acetyl); 104.8 (C^c-1); 99.0 (C^a-1); 98.8 (C^b-1); 78.1 (C^a-4); 76.1 (C^c-3); 75.4 (C^b-3); 75.1, 75.2

(C^c-5); 73.5 (C^c-2); 72.8 (C^d-2); 72.4 (C^d-3); 72.0 (C^a-3); 71.64, 71.71 (C^d-4); 69.6 (C^c-4); 67.8 (C^a-5); 67.3, 67.4 (C^d-5); 65.0, 65.06 (C^c-6); 63.8 (C^b-5); 63.1 (C^d-1); 61.1 (C^a-6); 56.1 (OMe); 55.5 (C^b-4); 50.7 (C^a-2); 49.1 (C^b-2); 22.6, 22.7 (2 x CH_3, acetyl); 16.0 (C^b-6); ^{1}H NMR data (D_2O): δ 4.93 (d, 1H, $J_{1,2}$ = 4.1 Hz, H^b-1); 4.85 (d, 1H, $J_{1,2}$ = 3.6 Hz, H^a-1); 4.76-4.80 (m, 1H, H^b-5); 4.62 (d, 1H, $J_{1,2}$ = 7.9 Hz, H^c-1); 4.37-4.41 (dd, 1H, $J_{2,3}$ = 11.5 Hz, $J_{3,4}$ = 4.4 Hz, H^b-3); 4.23-4.27 (ddd, 1H, H^a-2); 4.15-4.19 (dd, 1H, $J_{1,2}$ = 4.1 Hz, $J_{2,3}$ 11.5 Hz, H^b-2); 4.04-4.17 (m, 3H, H^c-6, H^c-6', H^d-5); 3.91-4.03 (m, 6 H, H^a-3, H^a-4, H^a-5, H^b-4, H^d-4, H^d-5'); 3.83-3.87 (ddd, 1H, $J_{1,2}$ = 3.0 Hz, $J_{1',2}$ = 7 Hz, $J_{2,3}$ = 6 Hz, H^d-2); 3.78-3.81 (dd, 1H, $J_{1,2}$ = 3 Hz, $J_{1,1'}$ = 12 Hz, H^d-1); 3.67-3.77 (m, 3H, H^a-6, H^a-6', H^d-3); 3.62-3.67 (dd, 1H, $J_{2,3}$ = 7 Hz, $J_{1,1'}$ = 11.5 Hz, H^d-1'); 3.54-3.58 (m, 1H, H^c-5); 3.45-3.54 (m, 2H, H^c-3, H^c-4); 3.39 (s, 3H, OMe); 3.31-3.36 (dd, 1H, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.3 Hz, H^c-2); 2.04, 2.08 (2 x s, 6H, 2 x Ac); 1.21-1.23 (d, 3H, $J_{5,6}$ = 6.7 Hz, H^b-6); ^{31}P NMR data δ 1.6; Mass spectroscopy $[M+H]^+$ 798 g/mol; $[M+Na]^+$ 820 g/mol; $[M-H]^-$ 796 g/mol; calculated mass 796.7 g/mol.

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