Articles

Synthesis of S-Linked Thiooligosaccharide Analogues of Nodulation Factors. 2.[1] Synthesis of an Intermediate Thiotrisaccharide

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An *S*-linked thiotrisaccharide analogue of chitin that carries different *N*-protecting groups at the reducing and nonreducing end glucosamine residues was prepared as an intermediate for the synthesis of thioanalogues of nodulation factors. 1,6-Anhydro-2-azido-3-*O*-benzoyl-2-deoxy-*β*-D-glucopyranose (**3**) was prepared either through the nucleophilic displacement of the 4-triflate *galacto* analogue **1** with potassium chloroacetate or via the selective acylation of the analogous *gluco* diol **4**. Condensation of **3** with the *N*-phthalimido trichloroacetimidate **9** led to the disaccharide **10**, which was converted in four steps to the glucosyl bromide **15**. Nucleophilic displacement of the anomeric bromide by a 4-thiolate derivative of glucosamine (**16**) bearing a trichloroethoxycarbamate at C-2 was performed in anhydrous oxygen-free THF and led to the desired thiotrisaccharide precursor of thioanalogues of nodulation factors. Alternatively, the disaccharide **10** was prepared by regioselective glycosylation of the 1,6-anhydro diol **4** followed by benzoylation of the remaining free hydroxyl group.

Introduction

The infection of leguminous plants with soil bacteria of the genera *Rhizobium*, *Bradyrhizobium*, and *Azorhizobium* was shown[2] to allow the plant to use atmospheric nitrogen previously fixed and metabolized into ammonia by the bacteria. At an early stage of the host-specific infection, the bacteria produces extracellular signaling molecules called nodulation factors (Nod factors) which induce deformations and nodule organogenesis on the plant roots.[3] Nod factors were shown[4] to be in all cases lipooligosaccharides constituted of a tetra- (*ABCD*) or pentasaccharidic (*ABCDE*) backbone of chitin variably substituted as represented in Figure 1. Even though only very small concentrations (10^{-6}−10^{-12} mol L^{-1}) of natural Nod factors are required to initiate nodulation, their activity is limited by the action of chitinases[5] that cleave the oligosaccharides at the glycosidic bond linking units *B* and *C*. Therefore, we have developed a synthetic strategy to prepare thioanalogues of Nod factors in which a sulfur atom replaces the oxygen atom of the interglycosidic bond *B*−*C*. These analogues, which should be resistant[6] to the action of glycosidases, will be used to investigate the structure/activity relationship of Nod factors. We have reported[1] that both the nucleophilic displacement of a 4-triflate 1,6-anhydro derivative of galactosamine by an anomeric thiolate and the base-catalyzed glycosylation of a 4-thiol derivative of glucosamine with an anomeric bromide led efficiently to thiodisaccharide derivatives of chitin. We report here the application of the later strategy to the preparation of an intermediate protected thiotrisaccharide.

Results and Discussion

Nucleophilic displacement of the triflate group in the 1,6-anhydro derivative **1** with sodium chloroacetate gave (Scheme 1) the *gluco* analogue **2** (50%), which was subsequently deprotected at C-4 with thiourea to give the glycosyl acceptor **3** in 87% yield. In view of the poor yield obtained for the preparation of the chloroacetate **2** from the triflate **1**, the synthesis of **3** was also attempted starting from the known[7] 1,6-anhydro diol **4**. Selective chloroacetylation of diol **4** with 1.4 equiv of chloroacetyl chloride at −18 °C gave (Scheme 2) the dichloroacetate

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(1) Part 1: Auzanneau, F.-I.; Bennis, K.; Fanton, E.; Promé, D.; Defaye, J.; Gelas, J. *J. Chem. Soc., Perkin Trans. 1*, in press.

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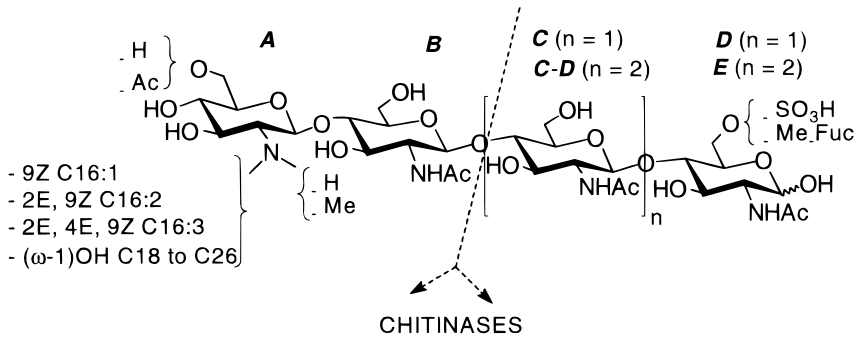
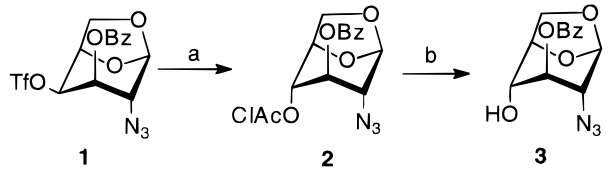


Figure 1. Schematic representation of natural Nod factors.[4]

Scheme 1[a]



[a] Legend: (a) $ClAcO^-$, Na^+−DMF, 50 °C; (b) $S{=}C(NH_2)_2$.

5 (7%), the chloroacetates **6** (43%) and **7** (12%), and the unreacted starting diol **4** (12%). The same reaction using 1.2 equiv of chloroacetyl chloride and followed by in situ benzoylation of the crude mixture gave a mixture of dibenzoylated and monochloroacetylated derivatives which were not separated. After workup, the crude acylation mixture was treated with thiourea and the dibenzoate **8** (15%) was separated from the desired alcohol **3**, which was obtained in 48% yield from the diol **4**. In turn, glycosylation of the acceptor **3** with the known[8] trichloroacetimidate **9** under catalysis with trimethylsilyl trifluoromethanesulfonate (TMSOTf) gave (Scheme 3) the disaccharide **10**, which was obtained in 90% yield (43% overall yield from the diol **4**).

The observed higher reactivity of the C-4 hydroxyl group in diol **4** prompted us to attempt the selective glycosylation of this diol by the trichloroacetimidate **9** under catalysis with TMSOTf (Scheme 4). In all the reactions, both the disaccharide **11** and the trisaccharide **12** were isolated (Table 1). The structure of disaccharide **11** and thus the selectivity of the glycosylation were confirmed by comparison of the ^{1}H NMR spectrum of the corresponding benzoylated disaccharide with an authentic sample of **10** that had been prepared from **3** and also by the analysis of the COSY spectrum of the disaccharide **14**. The reaction conditions were optimized and, as shown in Table 1, best results were obtained when the reaction was carried out at a high dilution of acceptor **4** in CH_2Cl_2 (entries 2−4). Lower temperatures limited the degradation of the glycosyl donor **9**, but precipitation of the starting diol from the reaction mixture at these lower temperatures also resulted in formation of a higher percentage of the undesired trisaccharide **12**. The best yields of the desired disaccharide **11** (∼50%) were obtained by dropwise addition of **9** at room temperature (entries 3 and 4). The ^{1}H NMR spectrum of disaccharide **11** (Figure 2A) showed unusual coupling constants between H-2**B** and H-3**B** (5 Hz) and between H-3**B** and H-4**B** (4.5 Hz). In addition, these coupling constants, which were close to 0 Hz in the analogous 4-chloroacetylated monosaccharide **6**, disappeared upon benzoylation of the free hydroxyl group to give the disaccharide **10**. Thus, we postulate that a hydrogen bond between HO-3**B** and O-5**A** is responsible for the conformational change observed in disaccharide **11**. Benzoylation of the disaccharide **11** gave the disaccharide **10** (90%), which was thus obtained in two steps from diol **4** with a 42% overall yield.

The azido group in disaccharide **10** was reduced (H_2−Pd/C), and the intermediate amino disaccharide was treated with trichloroethyl chloroformate to give the carbamate **13** (60%, Scheme 5). Opening of the anhydro ring in **13** (CF_3CO_2H−Ac_2O) gave the anomeric mixture of the diacetate **14** which was converted to the bromide **15** that was obtained anomerically pure as the α-anomer.

Under the reaction conditions described previously,[1] the bromide **15** was allowed to react with the thiolate **16** in anhydrous oxygen-free THF and gave the thiotrisaccharide **17** in 49% yield (Scheme 6). Contamination of the trisaccharide with less than 10% (molar) of a monosaccharidic impurity was assessed by ^{1}H NMR and resulted in considerable loss of product upon repeated silica gel chromatography. The trisaccharide **17**, which was obtained pure by gel permeation chromatography (30%), is the first thiotrisaccharide derivative of chitin to be described that carries different protecting groups at C-2 of the two terminal glucosamine residues. Thus, it can be used in chain extension reactions at the reducing end and can also be selectively deprotected and *N*-acylated at its nonreducing end, providing the first access to thio Nod factors.

Experimental Section

General Methods. NMR spectra were recorded at 400.13 MHz (^{1}H) and 100.6 MHz (^{13}C). First-order chemical shifts and coupling constants were obtained from one-dimensional spectra, and assignments of proton resonances were based on COSY experiments. Mass spectra were obtained by liquid secondary ionization. TLC was performed on precoated aluminum plates with Kieselgel silica gel 60 F_{254} (E. Merck) and detected with UV light and/or charred with a 10% H_2SO_4 solution in EtOH. Compounds were purified by flash[9] or atmospheric pressure chromatography with silica gel 60 (230−400 mesh or 70−120 mesh, respectively). Solvents were distilled and dried according to standard procedures,[10] and when necessary they were obtained oxygen-free by purging with argon. Organic solutions were dried on Na_2SO_4 and concentrated below 40 °C under reduced pressure. When

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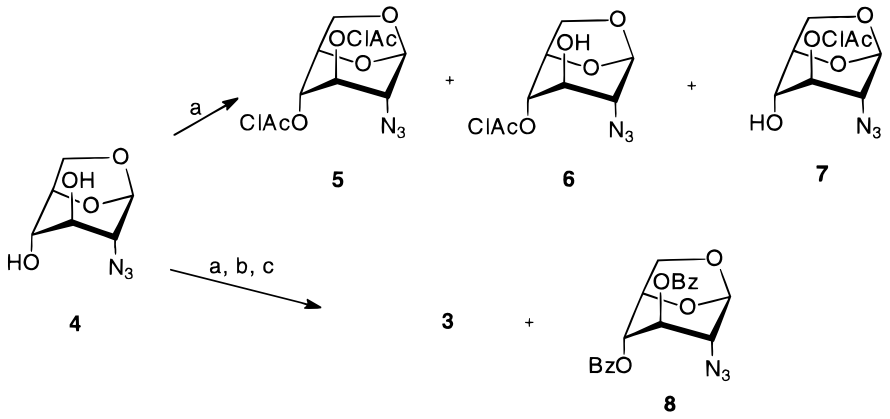
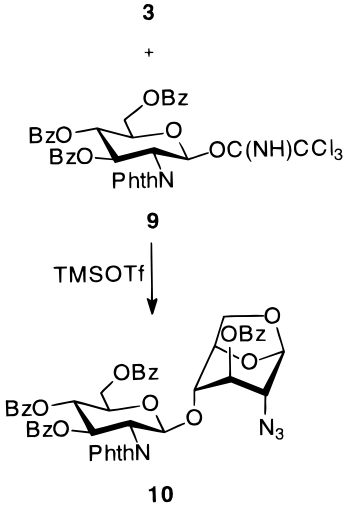
Scheme 2[a]

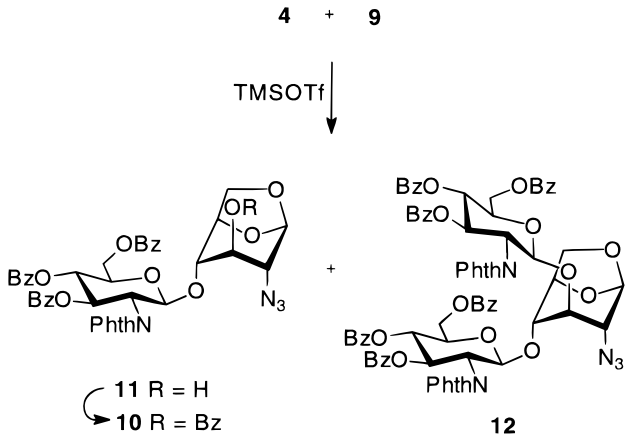


[a] Legend: (a) $ClCH_2COCl$, −18 °C; (b) BzCl; (c) $S{=}C(NH_2)_2$.

Scheme 3



Scheme 4



necessary for analytical purposes, permeation gel chromatography of protected compounds was performed using a Sephadex LH20 column (1.5 × 100 cm) eluted with 1:1 $CHCl_3$−MeOH. Elemental analyses were performed at the "Service Central d'Analyse du CNRS, Lyon" or alternatively at the "Service d'Analyse de la Faculté de Pharmacie, Châtenay-Malabry".

1,6-Anhydro-2-azido-3-*O*-benzoyl-4-*O*-chloroacetyl-2-deoxy-*β*-D-glucopyranose (2). A mixture of 1,6-anhydro-2-azido-3-*O*-benzoyl-2-deoxy-4-*O*-trifluoromethanesulfonyl-*β*-D-galactopyranose[1] (**1**; 206 mg, 0.49 mmol) and sodium chloroacetate (220 mg, 1.9 mmol) in DMF (20 mL) was stirred at room temperature for 3 h. The solution was diluted with CH_2Cl_2 (30 mL) and washed successively with H_2O (20 mL) and saturated aqueous NaCl (20 mL). The washings were reextracted with CH_2Cl_2 (2 × 20 mL), and the combined organic phases were dried and concentrated. Flash chromatography of the residue (8:2 cyclohexanes−EtOAc) gave the chloroacetate **2** (90 mg, 50%) as a colorless glass. $[\alpha]^{20}_D = +13$ (*c* 1.1, CH_2Cl_2). ^{1}H NMR: δ 3.38 (bs, 1 H, H-2), 3.95 (dd, 1 H, $J_{6,6'} =$ 8 Hz, $J_{6,5} = 5.5$ Hz, H-6), 4.25 (s, 2 H, $ClCH_2CO$), 4.30 (d, 1 H, H-6′), 4.78 (bd, 1 H, H-5), 4.92 (bs, 1 H, H-4), 5.23 (m, 1 H, H-3) 5.63 (bs, 1 H, H-1), 7.50, 7.70, 8.05 (3 m, 5 H, aromatics). Anal. Calcd for $C_{14}H_{12}N_3F_3O_7S$: C, 49.0; H, 3.8; N, 11.4. Found: C, 48.9; H, 3.9; N, 11.2.

1,6-Anhydro-2-azido-3-*O*-benzoyl-2-deoxy-*β*-D-glucopyranose (3) and 1,6-Anhydro-2-azido-3,4-di-*O*-benzoyl-2-deoxy-*β*-D-glucopyranose (8). Method A. Thiourea (135 mg, 1.8 mmol) was added to a solution of the chloroacetate **2** (595 mg, 1.6 mmol) in a mixture of pyridine (40 mL) and EtOH (8 mL). The reaction mixture was stirred overnight at room temperature and concentrated and the residue was dissolved in CH_2Cl_2 (60 mL); this solution was washed successively with 10% aqueous $KHSO_4$ (50 mL), 1 M HCl (50 mL), saturated aqueous $NaHCO_3$ (50 mL) and saturated aqueous NaCl (50 mL). The washings were reextracted with CH_2Cl_2 (2 × 40 mL), and the combined organic solutions were dried and concentrated. Flash chromatography (6:4, 250 mL, 1:1, 200 mL, cyclohexanes−EtOAc) of the residue gave the alcohol **3** (412 mg, 87%), which crystallized on standing.

Method B. A stirred solution of 1,6-anhydro-2-azido-*β*-D-glucopyranoside[7] (**4**; 100 mg, 0.534 mmol) in anhydrous CH_2Cl_2 (30 mL) containing pyridine (0.3 mL) and activated 4 Å molecular sieves (0.5 g) was cooled to −18 °C under N_2, and chloroacetyl chloride (50 *μ*L, 0.62 mmol) was added. After the mixture was stirred at −17 °C for 1.5 h, benzoyl chloride (120 *μ*L, 1.0 mmol) was added and the reaction mixture was stirred at room temperature for 30 min. Anhydrous pyridine (3 mL), DMAP (50 mg, 0.4 mmol), and benzoyl chloride (250 *μ*L, 2.1 mmol) were added and the mixture was stirred overnight at room temperature. Methanol (1 mL) was added, solvents were removed in vacuo, and a solution of the residue in CH_2Cl_2 (15 mL) was washed with 1 M HCl (20 mL) and saturated aqueous $NaHCO_3$ (20 mL). The washings were reextracted with CH_2Cl_2 (3 × 15 mL), and the combined organic extracts were dried and concentrated. The residual oil was dissolved in a mixture of pyridine (13 mL) and EtOH (3 mL), thiourea (56 mg, 0.73 mmol) was added, and the reaction mixture was stirred overnight at room temperature and worked up as described above for the preparation of **3** using method A. Chromatography (8:2, then 7:3, and finally 6:4 cyclohexanes−EtOAc) gave first the dibenzoate **8** (33 mg, 15%), which crystallized on standing, and then the alcohol **3** (74.7 mg, 48%). Alternatively, the alcohol **3**, which crystallized on standing, was obtained pure by filtration from 1:1 cyclohexanes−EtOAc.

Analytical Data for 3. Mp: 143−145 °C. $[\alpha]^{20}_D = +24$ (*c*

Table 1. Selective Glycosylation of Diol 4 with the Trichloroacetimidate 9

entry	temp (°C)	concn of **4** (mg/mL)	amt of donor **9** (equiv)	amt of TMSOTf (equiv)	yield of **11** (%)	yield of **12** (%)
1	−70	10	1	0.09	24	11
2	−70 → room temp	3.3	1[a] + 0.2[b]	0.09	35	11
3	room temp	3.3	1.2[b]	3 × 0.09	50	6
4	room temp	3.3	1.5[b]	4 × 0.09	47	7

[a] Dropwise at −70 °C. [b] Dropwise at room temperature.

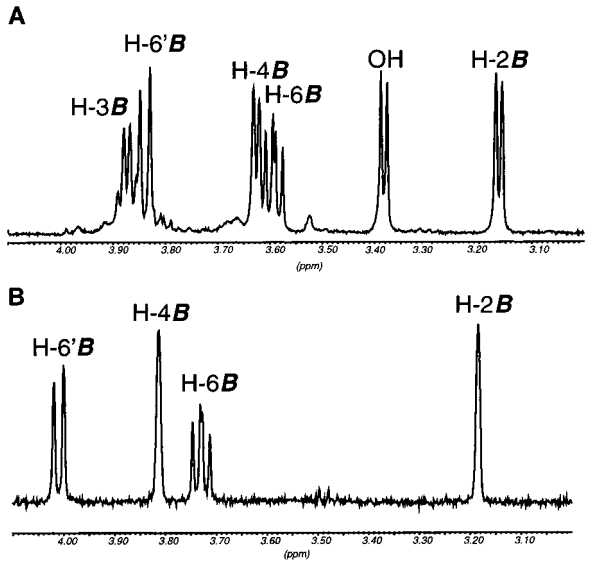
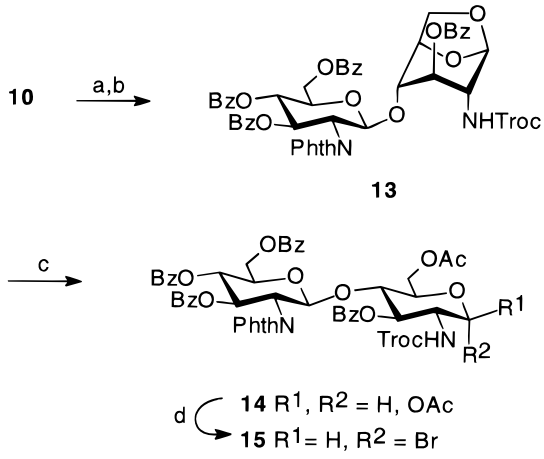


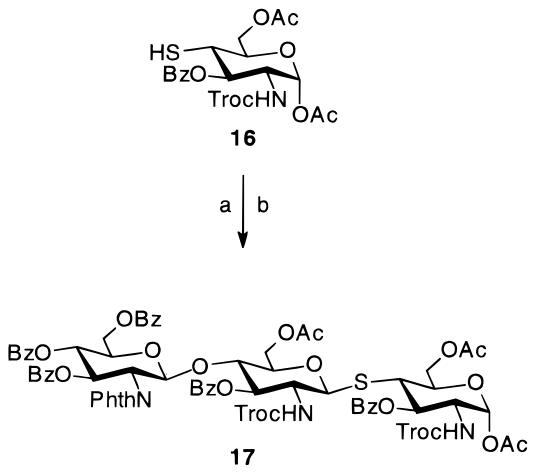
Figure 2. Sections (3.0−4.1 ppm) of the ^{1}H NMR spectra of disaccharides **11** (**A**) and **10** (**B**).

Scheme 5[a]



[a] Legend: (a) H_2, Pd/C; (b) Cl_3CCH_2OCOCl; (c) $CF_3CO_2H-Ac_2O$; (d) HBr−AcOH.

Scheme 6[a]



[a] Legend: (a) NaH; (b) **15**.

0.9, CH_2Cl_2). ^{1}H NMR: δ 2.90 (d, 1 H, $J_{OH,4}$ = 10 Hz, OH), 3.65 (bs, 1 H, H-2), 3.79 (bd, 1 H, H-4), 3.93 (dd, 1 H, $J_{6,6'}$ = 7.5 Hz, $J_{6,5}$ = 6 Hz, H-6), 4.27 (d, 1 H, H-6′), 4.67 (bd, 1 H, H-5), 5.16 (m, 1 H, H-3), 5.52 (bs, 1 H, H-1), 7.51, 7.63, 8.04 (3 m, 5 H, aromatics). Anal. Calcd for $C_{13}H_{13}N_3O_5$: C, 53.6; H, 4.5; N, 14.4. Found: C, 53.0; H, 4.5; N, 13.9.

Analytical Data for 8. Mp: 108−109 °C. $[\alpha]^{20}_D$ = −89 (*c* 1.0, CH_2Cl_2). ^{1}H NMR: δ 3.47 (bs, 1 H, H-2), 3.97 (dd, 1 H, $J_{6,6'}$ = 7.5 Hz, $J_{6,5}$ = 5.5 Hz, H-6), 4.37 (d, 1 H, H-6′), 4.87 (bd, 1 H, H-5), 5.11 (bs, 1 H, H-4), 5.37 (m, 1 H, H-3), 5.66 (bs, 1 H, H-1), 7.49, 7.62, 8.04, 8.18 (4 m, 10 H, aromatics). Anal. Calcd for $C_{20}H_{17}N_3O_6$: C, 60.7; H, 4.3; N, 10.6. Found: C, 60.2; H, 4.3; N, 10.2.

1,6-Anhydro-2-azido-3,4-di-*O*-chloroacetyl-2-deoxy-β-D-glucopyranose (5), 1,6-Anhydro-2-azido-4-*O*-chloroacetyl-2-deoxy-β-D-glucopyranose (6), and 1,6-Anhydro-2-azido-3-*O*-chloroacetyl-2-deoxy-β-D-glucopyranose (7). A stirred solution of the diol **4** (100 mg, 0.534 mmol) in anhydrous CH_2Cl_2 (30 mL) containing pyridine (0.3 mL) was cooled to −18 °C under N_2, and chloroacetyl chloride (40 μL, 0.503 mmol) was added. The reaction mixture was stirred between −18 and −10 °C for 2 h, during which time more chloride was added portionwise (2 × 10 μL, 0.251 mmol). Methanol was added, solvents were evaporated, and residual pyridine was coevaporated with toluene. Chromatography (gradient 8:2 to 0:1 cyclohexanes−EtOAc) gave first the dichloroacetate **5** (16.6 mg, 7%), followed by the monochloroacetate **6** (59.7 mg, 43%) and then the chloroacetate **7** (17 mg, 12%), and finally the unreacted starting diol **4** (12 mg, 12%).

Analytical Data for 5. $[\alpha]^{20}_D$ = +30 (*c* 1.1, CH_2Cl_2). ^{1}H NMR: δ 3.24 (bs, 1 H, H-2), 3.87 (dd, 1 H, $J_{6,6'}$ = 8 Hz, $J_{6,5}$ = 5.5 Hz, H-6), 4.12 (s, 2 H, $ClCH_2CO$), 4.18 (d, 1 H, H-6′), 4.21 (s, 2 H, $ClCH_2CO$), 4.72 (bd, 1 H, H-5), 4.80 (bs, 1 H, H-4), 5.00 (bs, 1 H, H-3), 5.57 (bs, 1 H, H-1). Anal. Calcd for $C_{10}H_{11}N_3ClO_6$: C, 35.3; H, 3.3; N, 12.3. Found: C, 35.3; H, 3.3; N, 12.1.

Analytical Data for 6. $[\alpha]^{20}_D$ = −6 (*c* 1.1, CH_2Cl_2). ^{1}H NMR: δ 3.28 (bs, 1 H, H-2), 3.82 (dd, 1 H, $J_{6,6'}$ = 7.5 Hz, $J_{6,5}$ = 5.5 Hz, H-6), 3.92 (bs, 1 H, H-3), 4.20 (s, 2 H, $ClCH_2CO$), 4.22 (d, 1 H, H-6′), 4.66 (bd, 1 H, H-5), 4.77 (bs, 1 H, H-4), 5.57 (bs, 1 H, H-1). Anal. Calcd for $C_8H_{10}N_3ClO_5$: C, 36.4; H, 3.8; N, 15.9. Found: C, 36.3; H, 4.0; N, 15.7.

Analytical Data for 7. $[\alpha]^{20}_D$ = −3 (*c* 1.0, CH_2Cl_2). ^{1}H NMR: δ 3.51 (bs, 1 H, H-2), 3.67 (bs, 1 H, H-4), 3.84 (dd, 1 H, $J_{6,6'}$ = 7.5 Hz, $J_{6,5}$ = 6 Hz, H-6), 4.12 (s, 2 H, $ClCH_2CO$), 4.14 (d, 1 H, H-6′), 4.61 (bd, 1 H, H-5), 4.93 (m, 1 H, H-3) 5.47 (bs, 1 H, H-1). Anal. Calcd for $C_8H_{10}N_3ClO_5$: C, 36.4; H, 3.8; N, 15.9. Found: C, 36.4; H, 4.0; N, 15.6.

1,6-Anhydro-2-azido-3-*O*-benzoyl-4-*O*-(3,4,6-tri-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranose (10). Method A. A mixture of the acceptor **3** (567 mg, 1.95 mmol) and the trichloroacetimidate[8] **9** (1.83 g, 2.38 mmol) in anhydrous CH_2Cl_2 (50 mL) containing 4 Å activated molecular sieves (5 g) was stirred under N_2 for 1 h at room temperature and cooled to −78 °C. TMSOTf (30 μL, 0.17 mmol) was added, and the stirred reaction mixture

was allowed to reach +10 °C in 7 h under N_2. Triethylamine (50 μL, 0.36 mmol) was added, and the solids were filtered off and washed with CH_2Cl_2 (70 mL). The combined filtrate and washings were washed successively with H_2O (100 mL), 1 M HCl (100 mL), saturated aqueous $NaHCO_3$ (100 mL), and saturated aqueous NaCl (100 mL). The aqueous washings were reextracted with CH_2Cl_2 (2 × 100 mL), and the combined organic phases were dried and concentrated. Flash chromatography (100:5, 1 L; 100:7, 0.5 L; 100:8, 0.5 L; toluene−EtOAc) gave the disaccharide **10** (1.33 g, 76%). Impure fractions containing mostly **10** were pooled, concentrated, and submitted to chromatography to give more disaccharide **10** (164 mg, 9%). Since the disaccharide thus obtained was contaminated with trichloroacetamide, an analytical sample was purified by gel permeation and crystallized on standing.

Method B. Benzoyl chloride (83 μL, 0.71 mmol) was added to a solution of the disaccharide **11** (100.3 mg, 0.127 mmol) in anhydrous CH_2Cl_2 (9 mL) containing anhydrous pyridine (1 mL) and DMAP (10 mg). The reaction mixture was stirred at room temperature for 3 days and worked up as described for the preparation of **3** and **8** using method B. Chromatography (9:1 and then 5:1 toluene−EtOAc) gave the disaccharide **10** (101.8 mg, 90%).

Analytical Data for 10. Mp: 116−118 °C. $[\alpha]^{20}_D = +26$ (*c* 1.0, CH_2Cl_2). ^{1}H NMR: δ 3.19 (bs, 1 H, H-2*B*), 3.73 (dd, 1 H, $J_{6,6'} = 7.5$ Hz, $J_{6,5} = 6$ Hz, H-6*B*), 3.82 (bs, 1 H, H-4*B*), 4.01 (bd, 1 H, H-6′*B*), 4.48 (m, 2 H, H-5*A* and H-5*B*), 4.56 (dd, 1 H, $J_{6,6'} = 12.5$ Hz, $J_{6,5} = 6$ Hz, H-6*A*), 4.71 (dd, 1 H, $J_{6',5} = 3$ Hz, H-6′*A*), 4.75 (dd, 1 H, $J_{2,1} = 8.5$ Hz, $J_{2,3} = 10.5$ Hz, H-2*A*), 5.40 (bs, 1 H, H-3*B*), 5.63 (bs, 1 H, H-1*B*), 5.74 (t, 1 H, $J_{4,3+4,5} = 19$ Hz, H-4*A*), 6.09 (d, 1 H, H-1*A*), 6.24 (dd, 1 H, $J_{3,4} = 10$ Hz, H-3*A*), 7.20−8.05 (m, 24 H, aromatics). Anal. Calcd for $C_{48}H_{38}N_4O_{14}$: C, 64.4; H, 4.3; N, 6.3. Found: C, 64.3; H, 4.3; N, 5.9.

1,6-Anhydro-2-azido-4-*O*-(3,4,6-tri-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranose (11) and 1,6-Anhydro-2-azido-3,4-di-*O*-(3,4,6-tri-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranose (12). A suspension of the diol **4** (100 mg, 0.53 mmol) in anhydrous CH_2Cl_2 (30 mL) containing 4 Å activated molecular sieves (3 g) was stirred under N_2 for 1 h at room temperature. A solution of TMSOTf in CH_2Cl_2 (0.16 M, 100 μL, 0.016 mmol) followed by a solution of the trichloroacetimidate[8] **9** (41 mg, 0.054 mmol) in CH_2Cl_2 (100 μL) were added, and the reaction was monitored by TLC (3:1 toluene−EtOAc; R_f(**11**) 0.16, R_f(**12**) 0.37). More TMSOTf in CH_2Cl_2 (0.16 M, 3 × 100 μL, 0.048 mmol) and more donor **9** (14 × 41 mg, 0.75 mmol) in CH_2Cl_2 (100 μL) were added portionwise over 3 h, and the stirred reaction mixture was then left for 18 h under N_2 at room temperature. Triethylamine (18 μL, 0.13 mmol) was added, and the molecular sieves were decanted and washed with CH_2Cl_2 (10 mL). The combined supernatant and washings were concentrated, and chromatography (9:1 followed by 5:1 and 1:1 toluene−EtOAc) gave the trisaccharide **12** contaminated with trichloroacetamide, which was removed by gel permeation chromatography (55 mg, 7%), and the disaccharide **11** (200 mg, 47%). For analytical purposes an aliquot of **11** was also submitted to gel permeation while the remaining disaccharide was used directly in the benzoylation step.

Analytical Data for 11. White powder. Mp: 103−105 °C. $[\alpha]^{20}_D = +17$ (*c* 1.0, CH_2Cl_2). ^{1}H NMR: δ 3.16 (bd, 1 H, $J_{2,3} =$ 5 Hz, H-2*B*), 3.36 (bd, 1 H, $J_{OH,3} = 4.5$ Hz, OH-3*B*), 3.59 (dd, 1 H, $J_{6,6'} = 7$ Hz, $J_{6,5} = 5.5$ Hz, H-6*B*), 3.62 (bd, 1 H, $J_{4,3} = 4.5$ Hz, H-4*B*), 3.83 (d, 1 H, H-6′*B*), 3.87 (m, 1 H, H-3*B*), 4.30 (m, 1 H, H-5*A*), 4.32 (bd, 1 H, H-5*B*), 4.45 (dd, 1 H, $J_{6,6'} = 12.5$ Hz, $J_{6,5} = 6$ Hz, H-6*A*), 4.66 (dd, 1 H, $J_{2,1} = 8.5$ Hz, $J_{2,3} = 11$ Hz, H-2*A*), 4.82 (dd, 1 H, $J_{6',5} = 3$ Hz, H-6′*A*), 5.25 (bs, 1 H, H-1*B*), 5.70 (t, 1 H, $J_{4,3+4,5} = 19.5$ Hz, H-4*A*), 5.84 (d, 1 H, H-1*A*), 6.21 (dd, 1 H, $J_{3,4} = 9.5$ Hz, H-3*A*), 7.20−8.05 (m, 19 H, aromatics). Anal. Calcd for $C_{41}H_{34}N_4O_{13}$: C, 62.3; H, 4.3; N, 7.1. Found: C, 62.3; H, 4.4; N, 6.9.

Analytical Data for 12. White powder. Mp: 145−147 °C. $[\alpha]^{20}_D = +43$ (*c* 1.0, CH_2Cl_2). ^{1}H NMR: δ 2.72 (bs, 1 H, H-2*B*), 3.30 (dd, 1 H, $J_{6,6'} = 7$ Hz, $J_{6,5} = 6$ Hz, H-6*B*), 3.77 (bd, 1 H, H-6′*B*), 3.97 (bs, 1 H, H-4*B*), 4.23 (bs, 1 H, H-3*B*), 4.27 (m, 2 H, H-5*A*, H-5*A*′), 4.36 (bd, 1 H, H-5*B*), 4.45 (m, 3 H, H-6*A*, H-2*A*′, H-6*A*′), 4.60 (dd, 1 H, $J_{2,1} = 8.5$ Hz, $J_{2,3} = 11$ Hz, H-2*A*), 4.77 (dd, 1 H, $J_{6',6} = 12.5$ Hz, $J_{6',5} = 3$ Hz, H-6′*A*), 4.80 (dd, 1 H, $J_{6',6} = 12.5$ Hz, $J_{6',5} = 2.5$ Hz, H-6′*A*′), 5.03 (bs, 1 H, H-1*B*), 5.63 (m, 3 H, H-4*A*, H-1*A*′, H-4*A*′), 5.92 (d, 1 H, H-1*A*), 6.16 (dd, 1 H, $J_{3,4} = 11$ Hz, H-3*A*), 6.24 (dd, 1 H, $J_{3,2} = 9.5$ Hz, $J_{3,4} = 11$ Hz, H-3*A*′), 7.22−8.15 (m, 38 H, aromatics). Anal. Calcd for $C_{76}H_{59}N_5O_{22}$: C, 65.5; H, 4.3; N, 5.0. Found: C, 65.7; H, 4.4; N, 5.0.

1,6-Anhydro-4-*O*-(3,4,6-tri-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-deoxy-2-(((trichloroethoxy)carbonyl)amino)-β-D-glucopyranose (13). Palladium-on-carbon catalyst (10%-on-C, 50% in H_2O, 100 mg) was added to a solution of the azide **10** (88 mg, 0.098 mmol) in MeOH (10 mL), and the reaction mixture was stirred under H_2 at room temperature for 4 h. The catalyst was filtered off and rinsed with MeOH (3 × 4 mL), and the combined filtrate and washings were concentrated. Residual traces of water and MeOH were coevaporated with anhydrous toluene (4 × 10 mL), and the residual white solid was dissolved in anhydrous CH_2Cl_2 (10 mL) and treated with NEt_3 (150 μL, 1.08 mmol) and trichloroethyl chloroformate (20 μL, 0.15 mmol). The reaction mixture was stirred under N_2 at room temperature for 0.5 h, and more trichloroethyl chloroformate (14 μL, 0.1 mmol) was added. The reaction was left to proceed for 18 h at room temperature and quenched by addition of MeOH (50 μL). The solution was diluted with CH_2Cl_2 (15 mL) and washed successively with 1 M HCl (10 mL), saturated aqueous $NaHCO_3$ (15 mL), and brine (15 mL). The aqueous washings were reextracted with CH_2Cl_2 (3 × 10 mL), and the combined organic phases were dried and concentrated. Chromatography of the residue (64:36 hexanes−EtOAc) gave the carbamate **13** (62 mg, 60%) as a white powder. Mp: 125−127 °C. $[\alpha]^{20}_D = -11$ (*c* 1.05, CH_2Cl_2). ^{1}H NMR: δ 3.70 (dd, 1 H, $J_{6,6'} = 8$ Hz, $J_{6,5} = 6$ Hz, H-6*B*), 3.75 (bs, 1 H, H-4*B*), 3.86 (bd, 1 H, $J_{2,NH} =$ 9.5 Hz, H-2*B*), 4.00 (bd, 1 H, H-6′*B*), 4.35 (bd, 1 H, H-5*B*), 4.47 (dd, 1 H, $J_{6,6'} = 12$ Hz, $J_{6,5} = 7.5$ Hz, H-6*A*), 4.54 (m, 1 H, H-5*A*), 4.63 (d, 1 H, $J = 12$ Hz, OC*H*HCCl$_3$), 4.70 (dd, 1 H, $J_{6',5} = 2$ Hz, H-6′*A*), 4.75 (dd, 1 H, $J_{2,1} = 8.5$ Hz, $J_{2,3} = 11$ Hz, H-2*A*), 4.85 (d, 1 H, OC*H*HCCl$_3$), 5.38 (bs, 1 H, H-1*B*), 5.57 (bs, 1 H, H-3*B*), 5.66 (t, 1 H, $J_{4,3+4,5} = 19$ Hz, H-4*A*), 5.69 (d, 1 H, NH*B*), 6.04 (d, 1 H, H-1*A*), 6.29 (dd, 1 H, $J_{3,4} = 9$ Hz, H-3*A*), 7.11−8.00 (m, 24 H, aromatics). Anal. Calcd for $C_{51}H_{41}Cl_3N_2O_{16}$: C, 58.7; H, 4.0; N, 2.7. Found: C, 58.8; H, 4.2; N, 2.7.

1,6-Di-*O*-acetyl-4-*O*-(3,4,6-tri-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-deoxy-2-(((trichloroethoxy)carbonyl)amino)-α,β-D-glucopyranose (14). The anhydro compound **13** (62 mg, 0.059 mmol) was dissolved in 9:1 Ac_2O−CF_3CO_2H (20 mL), and the solution was stirred for 18 h at room temperature. Solvents were evaporated, and residual traces of acid were coevaporated with toluene. Chromatography (6:4 hexanes−EtOAc) of the dry residue gave the anomeric mixture of diacetate **14** (62 mg, 91%). ^{1}H NMR in $CDCl_3$ showed an α:β ratio of 75:25. ^{1}H NMR for the α-anomer: δ 1.83, 2.18 (2 s, 2 × 3 H, CH_3CO), 3.68 (dd, 1 H, $J_{6,6'} =$ 12 Hz, $J_{6,5} = 3$ Hz, H-6*B*), 3.76 (m, 1 H, H-5*A*), 3.87−4.07 (m, 2 H, H-6*A* and H-5*B*), 4.12−4.32 (m, 4 H, H-6′*A*, H-2*B*, H-4*B* and H-6′*B*), 4.43 (d, 1 H, $J = 12$ Hz, OC*H*HCCl$_3$), 4.50 (dd, 1 H, $J_{2,1} = 8.5$ Hz, $J_{2,3} = 11$ Hz, H-2*A*), 4.64 (d, 1 H, OC*H*HCCl$_3$), 5.24 (d, 1 H, $J_{NH,2} = 9.5$ Hz, NH*B*), 5.54 (t, 1 H, $J_{4,3+4,5} = 19.5$ Hz, H-4*A*), 5.66 (dd, 1 H, $J_{3,2\ or\ 3,4} = 9$ Hz, $J_{3,2\ or\ 3,4} = 11$ Hz, H-3*B*), 5.77 (d, 1 H, H-1*A*), 6.11 (dd, 1 H, H-3*A*), 6.15 (d, 1 H, $J_{1,2} = 3$ Hz, H-1*B*), 7.20−8.20 (m, 24 H, aromatics).

1,6-Di-*O*-acetyl-4-*O*-(3,4,6-tri-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-deoxy-2-(((trichloroethoxy)carbonyl)amino)-α-D-glucopyranosyl Bromide (15). A solution of HBr in AcOH (33%, 800 μL) was added to a solution of the diacetate **14** (49 mg, 0.043 mmol) in anhydrous CH_2Cl_2 (2 mL). The reaction mixture was stirred under N_2 for 3 h at room temperature, and solvents were evaporated. Residual acid was coevaporated with toluene (4 × 8 mL), and the residue was dissolved in CH_2Cl_2 (8 mL); this solution was rapidly washed with aqueous saturated $NaHCO_3$

(8 mL) and brine (8 mL). The aqueous washings were reextracted with CH_2Cl_2 (2 × 8 mL) and the combined organic phases were dried and concentrated. Residual traces of water and solvents were coevaporated successively with anhydrous toluene and anhydrous CH_2Cl_2, and the bromide, whose purity was controlled by ^{1}H NMR (49 mg, 98%), was kept at −20 °C until use. ^{1}H NMR: δ 1.86 (s, 3 H, CH_3CO), 3.81 (dd, 1 H, $J_{6,6'}$ = 12.5 Hz, $J_{6,5}$ = 3 Hz, H-6*B*), 3.86 (m, 1 H, H-5*A*), 4.02 (dd, 1 H, $J_{6,6'}$ = 12.5 Hz, $J_{6,5}$ = 4.5 Hz, H-6*A*), 4.12−4.28 (m, 4 H, H-6′*A*, H-2*B*, H-4*B*, and H-5*B*), 4.42 (bd, 1 H, H-6′*B*), 4.47 (d, 1 H, *J* = 12 Hz, OC*H*HCCl$_3$), 4.51 (dd, 1 H, $J_{2,1}$ = 8 Hz, $J_{2,3}$ = 11 Hz, H-2*A*), 4.65 (d, 1 H, OC*H*HCCl$_3$), 5.40 (d, 1 H, $J_{NH,2}$ = 9 Hz, NH*B*), 5.54 (t, 1 H, $J_{4,3\,+\,4,5}$ = 19.5 Hz, H-4*A*), 5.74 (dd, 1 H, $J_{3,2\ or\ 3,4}$ = 8.5 Hz, $J_{3,2\ or\ 3,4}$ = 10 Hz, H-3*B*), 5.79 (d, 1 H, H-1*A*), 6.12 (dd, 1 H, H-3*A*), 6.42 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1*B*), 7.20−8.20 (m, 24 H, aromatics).

2-Acetamido-1,6-di-*O*-acetyl-3-*O*-benzoyl-2-deoxy-4-*S*-(6-*O*-acetyl-3-*O*-benzoyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-benzoyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl)-2-(((trichloroethoxy)carbonyl)amino)-*β*-D-glucopyranosyl)-2-(((trichloroethoxy)carbonyl)amino)-4-thio-α-D-glucopyranose (17). A cold solution (0 °C) of the known[1] thiol **16** (19.9 mg, 0.047 mmol) in anhydrous O_2-free THF (3.5 mL) was transferred under Ar at 0 °C to a stirred suspension of NaH (2 mg, 55% in oil, 0.046 mmol) in THF (0.6 mL). The flask that contained the thiol was rinsed with aliquots of THF (0.5 and 0.2 mL) which were added under Ar to the NaH suspension. The reaction mixture was stirred at 0 °C under Ar until gas evolution had ceased, then, it was warmed to room temperature and transferred under Ar to a flask containing the bromide **15** (45.7 mg, 0.039 mmol). The flask was rinsed with aliquots of THF (2 × 0.4 mL), which were added to the bromide solution. The reaction was left to proceed under Ar at room temperature for 2.5 h; it was quenched by addition of AcOH (30 μL), and solvents were removed in vacuo. Chromatography (3:7, toluene−EtOAc) of the residue gave the thiotrisaccharide **17** (29.2 mg, 49%) contaminated with 10% of an impurity that could not be removed even after repeated silica gel chromatography. The thiotrisaccharide was obtained pure upon LH20 gel permeation chromatography (18 mg, 30%) as a colorless glass. $[\alpha]^{20}_D$ = +12 (*c* 1.0, CH_2Cl_2). ^{1}H NMR: δ 1.81, 1.85, 2.06, 2.08 (4 s, 4 × 3 H, CH_3CO), 3.18 (t, 1H, $J_{4,3\,+\,4,5}$ = 22 Hz, H4*C*), 3.62 (d, 1 H, *J* = 12.5 Hz, OC*H*HCCl$_3$), 3.68 (m, 2H, H-5*B* and H-6*A*, or H-6*B*, or H-6*C*), 3.80−4.00 (m, 4H, H-5*A*, H-2*B*, H-4*B*, and H-6*A* or H-6*B* or H-6′*A* or H-6′*B* or H-6′*C*), 4.21−4.31 (m, 4H, H-5*C*, and H-6*A* or H-6*B* or H-6*C*, and H-6′*A* and H-6′*B*, or H-6′*A* and H-6′*C*, or H-6′*B* and H-6′*C*), 4.47−4.56 (m, 2H, H-5*A*, and H-6′*A* or H-6′*B* or H-6′*C*), 4.66 (d, 1 H, OC*H*HCCl$_3$),), 4.74 (dt, 1 H, $J_{2,1}$ = 3.5 Hz, $J_{2,3\,+\,2,NH}$ = 20.5 Hz, H-2*C*), 4.93 (d, 1 H, $J_{1,2}$ = 10 Hz, H-1*B*), 5.36 (d, 1 H, $J_{NH,2}$ = 10 Hz, NH*B*), 5.49 (t, 1 H, $J_{3,2\,+\,3,4}$ = 21.5 Hz, H-3*C*), 5.59 (t, 2 H, $J_{4,3\,+\,4,5} \approx J_{3,2\,+\,3,4}$ = 19.5 Hz, H-4*A* and H-3*B*), 5.67 (d, 1 H, $J_{NH,2}$ = 9.5 Hz, NH*C*), 5.76 (d, 1 H, $J_{1,2}$ = 8.5 Hz, H-1*A*), 6.10 (dd, 1 H, $J_{3,2\ or\ 3,4}$ = 9.5 Hz, $J_{3,2\ or\ 3,4}$ = 10.5 Hz, H-3*A*), 6.20 (d, 1 H, $J_{1,2}$ = 3 Hz, H-1*C*), 7.20−8.20 (m, 29 H, aromatics). ^{13}C NMR: δ_C 20.3, 20.8, and 20.9 (3 × *C*H$_3$COO), 23.0 (*C*H$_3$CON), 45.6 (C-4*C*), 51.4, 55.1, and 55.2 (C-2*A*, C-2*B*, and C-2*C*), 62.4 and 63.0 (C-6*A*, C-6*B*, and C-6*C*), 68.0, 69.5, 70.8, 71.1, 72.0, 74.2, 75.9, and 76.6 (C-3*A*, C-4*A*, C-5*A*, C-3*B*, C-4*B*, C-5*B*, C-3*C*, and C-5*C*), 82.0 (C-1*B*), 91.6 (C-1*C*), 97.8 (C-1*A*). FABMS: *m*/*z* 1512.4 [M$^+$ + 1]. Anal. Calcd for $C_{72}H_{66}Cl_3N_3O_{25}S$: C, 57.2; H, 4.4; N, 2.8. Found: C, 57.0; H, 4.6; N, 2.7.

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