Synthesis of *S*-linked thiooligosaccharide analogues of Nod factors. Part 1: selectively *N*-protected 4-thiochitobiose precursors

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The synthesis of *S*-linked thio-analogues of chitobiose diversely substituted at both 2-acetamido-2-deoxy-D-glucopyranosyl units has been achieved successfully starting either from a 1,6-anhydro-4-*O*-triflyl galactosaminide derivative in reaction with a glucosamide 1-thiolate in DMF or a 1,6-anhydro-4-thiolate of a D-glucosaminide derivative with a Troc-protected 2-amino-2-deoxy-D-glucose derivative. Alternatively, reaction of an acylated 4-thiolate of 2-acetamido-2-deoxy glucose with an *N*-Troc-protected glucosaminyl bromide afforded the expected *S*-linked 4-thio-analogue of chitobiose. Acid catalysis involving reaction of compound **17** with an *N*-Troc-protected glucosaminyl imidate in the presence of trimethylsilyl triflate gave only poor yields of the expected 1,6-anhydro 4-thio-*S*-linked disaccharide.

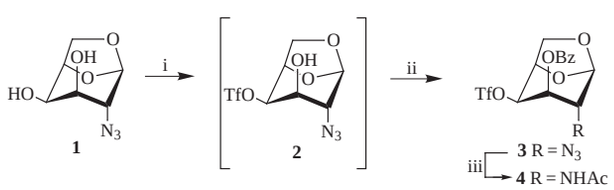
Introduction

Symbiotic association[1] of leguminous plants with soil bacteria of the genus *Rhizobium*, *Bradyrhizobium* or *Azorhizobium* is of considerable interest to agriculture since it could provide an alternative to the use of costly and environmentally hazardous fertilizers (*e.g.*, nitrates). Nodulation of the plant roots, a key step of the infection process, is induced by the interaction of specifically acylated chitooligosaccharides (nodulation factors, Nod factors) produced by the bacteria with specific plant receptors.[2] Although only very small concentrations (10^{-6}–10^{-12} mol l^{-1}) of Nod factors are required to initiate nodulation, the activity of these oligosaccharides is limited by the action of chitinases.[3] Therefore, we have developed a programme aimed at preparing thio-analogues of Nod factors in which a sulfur atom would replace one or more interglycosidic oxygen atoms with the prospect of increasing the overall resistance of the lipooligosaccharide to chitinases.[4]

Syntheses of thio-analogues of chitin with (DP) < 4 have recently been reported[5] involving sequential displacement of a 2-acetamido-2-deoxy-D-galactopyranosyl 4-*O*-triflate by a 2-acetamido-2-deoxy-D-glucopyranose-1-thiolate nucleophile. A main drawback in this synthetic Scheme was, however, the competitive base-catalysed elimination of the trifluoromethanesulfonyl (triflyl) group resulting in modest to low yields. Since this approach does not allow access to specifically acylated chitooligosaccharides, strategies involving either the glycosylation of 4-thio-analogues of acyl 2-acetamido-2-deoxyglucopyranose with 2,2,2-trichloroethoxycarbonylamino (*N*-Troc)-protected glucosaminyl donors or the reaction of a glucosaminide 1-thiolate with a 1,6-anhydro-4-*O*-triflate galactosaminosyl have been investigated.

Results and discussion

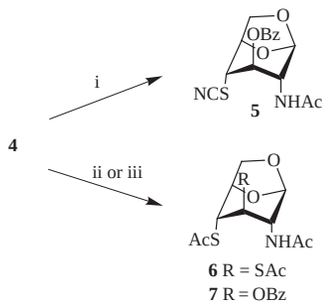
The 1,6-anhydro-4-*O*-triflate **3** was prepared in 78% overall yield from the known[6] 1,6-anhydro-2-azido diol **1** by treatment with one equivalent of triflic anhydride at −10 °C followed by *in situ* benzoylation of the remaining secondary alcohol (Scheme 1). Selectivity of the triflation was confirmed by the downfield shift of H-4 (δ 5.06) in the ^{1}H NMR spectrum of the intermediate **2**. The azido derivative **3** was, in turn, reduced in the presence of acetic anhydride to give the analogous *N*-acetamido derivative **4** (Scheme 1).



Scheme 1 *Reagents*: i, Tf_2O; ii, BzCl; iii, H_2–Pd/C–AcOEt–Ac_2O.

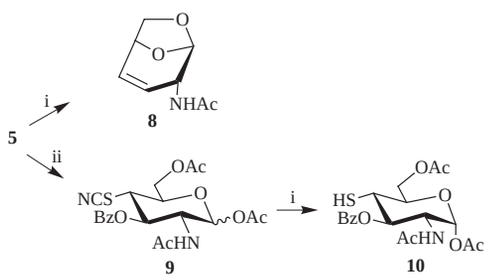
Reaction of the triflate **4** with potassium thiocyanate in HMPA at 80 °C gave the thiocyanate **5** (64%). The same conditions using potassium thioacetate as nucleophile gave the bis(thioacetate) **6** in 64% yield (Scheme 2). The structure of product **6** was confirmed by ^{1}H NMR spectroscopy and by fast-atom bombardment mass spectrometry (FABMS). The unexpected chemical shift of H-3 to high field (δ 3.73), and the absence of aromatic protons, together with the appearance of two signals (δ 2.35 and 2.41) ascribable to thioacetate groups, showed that compound **6** was substituted with two thioacetate groups, at C-4 and C-3. In addition, the *gluco* configuration of compound **6** was confirmed by the small coupling constants between H-2, H-3, H-4 and H-5 (each less than 1 Hz respectively). It is likely that formation of the bis(thioacetate) **6** with retention of configuration at C-3 took place through the formation of the thioacetate **7**. In fact, the *trans*-diaxial configuration of the 4-thioacetyl group relative to the 3-benzoyl group in structure **7** allows its departure *via* the formation of a cyclic thioacylsulfonium ion which can be subsequently opened by a thioacetate ion. Indeed, while the same reaction carried out in HMPA at rt led to a TLC-homogeneous mixture of compounds **6** and **7**, in a ratio 2:3 as determined by ^{1}H NMR

spectroscopy, the thioacetate **7** was finally isolated pure in 80% yield when the reaction was conducted in DMF (Scheme 2).



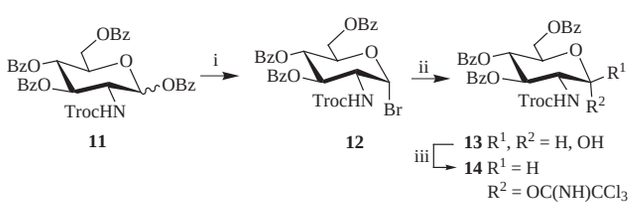
Scheme 2 *Reagent and conditions*: i, KSCN–HMPA, 80 °C; ii, KSAc–HMPA, 80 °C; iii, KSAc–DMF, 20 °C.

Attempted reduction of the thiocyanate **5** with zinc in acetic acid led to the unsaturated derivative **8** (Scheme 3) whose structure was determined by ^{1}H NMR spectroscopy and confirmed by FABMS. The absence of signals corresponding to aromatic protons together with the chemical shifts and coupling constants measured for H-3 (δ 5.62) and H-4 (δ 6.19, ^3J 10 Hz) were consistent with the presence of a double bond between C-3 and C-4. However, opening of the anhydro ring in substrate **5** led to an anomeric mixture of the diacetate **9** from which zinc reduction of the thiocyanate group in compound **9** gave the thiol **10** without concomitant elimination (Scheme 3).



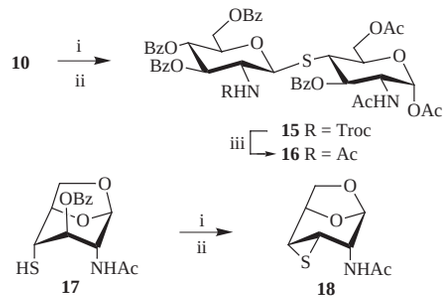
Scheme 3 *Reagents and conditions*: i, Zn–AcOH, 110 °C; ii, AcOH–TFA, 65 °C.

Glycosylation of 4-thiol derivatives of 2-acetamido-2-deoxy-D-glucose with *N*-Troc-protected glucopyranosylaminyl donors was first faced as a way to prepare selectively *N*-protected *S*-linked thiochitobiose analogues. Thus, the per(benzoate) **11** was prepared in two steps from 2-amino-2-deoxy-D-glucose hydrochloride and it was converted to the anomeric bromide **12**. The hemiacetal **13** obtained *via* the hydrolysis[7] of the bromide **12** was then converted into the trichloroacetimidate **14** by treatment with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (Scheme 4).



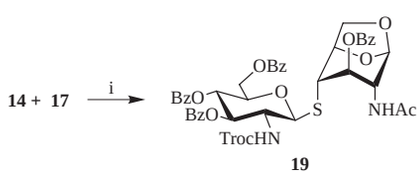
Scheme 4 *Reagents*: i, HBr–AcOH; ii, aq. MeCN; iii, DBU–Cl_3CCN. Troc = CCl_3CH_2OCO.

The thiol **10** was treated at 0 °C with sodium hydride in anhydrous, oxygen-free THF and the resulting 4-thiolate was added at rt to a solution of the bromide **12** in the same solvent, resulting in the selectively *N*-Troc-protected *S*-linked thiodisaccharide **15** in 64% yield. This was, in turn, converted to the *N*-acetyl analogue **16** *via* treatment with zinc in acetic anhydride thus showing the compatibility of the Troc group with our overall strategy to prepare specifically acylated *S*-linked thiochitooligosaccharides (Scheme 5).



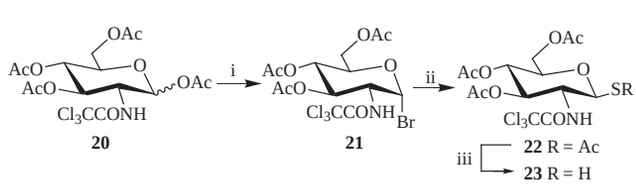
Scheme 5 *Reagents and conditions*: i, NaH; ii, **12**; iii, Zn–Ac_2O, 65 °C.

Similar coupling of the the anomeric bromide **12** with the 1,6-anhydro thiol **17**, easily prepared *via* aminolysis of the thioacetate **7**, failed to give the expected thiodisaccharide. Instead, the episulfide **18** was isolated in 97% yield (Scheme 5) and its structure was determined by ^{1}H NMR spectroscopy and confirmed by FABMS. The absence of signals corresponding to aromatic protons, together with the upfield shift of H-3 from δ 5.04 in thiol **17** to δ 3.39 in compound **18**, showed that C-3 was bearing a sulfur atom. In addition, the absence of an exchangeable signal corresponding to a C-4 thiol group, together with a change of configuration at C-3 assessed by the ^3J coupling constants measured between H-2 and H-3, and H-3 and H-4 of 6 and 6.5 Hz, respectively, pointed to the formation of the episulfide ring. Alternatively, coupling of the imidate **14** with the thiol **17** under catalysis with trimethylsilyl triflate[8] afforded the thiodisaccharide **19** in 33% yield (Scheme 6) while the unchanged thiol was recovered (47%).



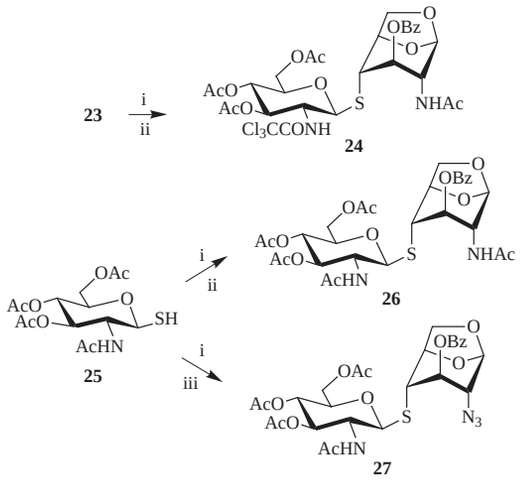
Scheme 6 *Reagent*: i, TMSOTf.

In view of these disappointing results, we then considered the nucleophilic displacement of the 1,6-anhydro triflates **3** and **4** by anomeric thiolates[4] as a way to prepare 1,6-anhydro *S*-linked disaccharides. Thus, the bromide **21** was prepared (HBr–AcOH) from the known[9] trichloroacetamido derivative **20** and was converted[10] into the thioacetate **22**. Aminolysis[11] of the thioacetyl group in **22** gave the thiol **23** (Scheme 7) which



Scheme 7 *Reagents*: i, HBr–AcOH; ii, AcSH–KOH; iii, $H_2N(CH_2)_2SH$.

was then treated (at 0 °C) with sodium hydride and coupled with the triflate **4** in anhydrous oxygen-free DMF (Scheme 8). Best results were obtained when the reaction was carried out overnight at 42 °C, resulting in the obtention of the thiodisaccharide **24** in 74% yield. Similarly, the known[12] thiol **25** was first deprotonated with sodium hydride and the resulting thiolate was coupled with the triflates **4** and **3** leading to the thiodisaccharides **26** (64%) and **27** (70%) respectively (Scheme 8). Interestingly, elemental analysis and NMR experiments performed in deuterated DMSO (δ 3.33 ppm)[13] showed that despite



Scheme 8 *Reagents*: i, NaH; ii, **4**; iii, **3**.

high-vacuum drying, the di-*N*-acetylated analogue **26** retained one molecule of water.

The results reported here show that the nucleophilic displacement of a 4-triflate derivative of 2-amino-1,6-anhydro-D-galactose is an efficient method to prepare 4-thio-analogues of 2-amino-2-deoxy-D-glucose. Both the base-promoted displacement of an anomeric bromide by a $^4C_1$ thiolate analogue of glucosamine, and the nucleophilic displacement of the 4-triflate in a 1,6-anhydrogalactosaminyl derivative by anomeric thiolates provided access to *S*-linked thiodisaccharides that can be used in further reactions to prepare thio-analogues of Nod factors.

Experimental

General methods

^{1}H NMR (400 MHz) and ^{13}C NMR (100.6 MHz) spectra were recorded with a Bruker AC-400 NMR for solutions in: $CDCl_3$ (internal standard, for ^{1}H: residual $CHCl_3$ δ 7.27; for ^{13}C: $CDCl_3$ δ_C 77.0). First-order chemical shifts and coupling constants (*J*/Hz) were obtained from one-dimensional spectra and assignments of protons resonances were based on chemical-shift correlation spectroscopy (COSY) experiments. Liquid secondary-ion mass spectra (LSIMS) were recorded on a Micromass Autospec instrument at an accelerating voltage of 8 keV using a caesium ion gun operating at 20 keV. A mixture of 1:1 *m*-nitrobenzyl alcohol–glycerol was the matrix used in positive detection mode. TLC was performed on precoated aluminium plates with Kieselgel silica gel 60 F_{254} (E. Merck) and spots were detected with UV light and/or charred with a 10% H_2SO_4 solution in EtOH. Compounds were purified by flash[14] or atmospheric pressure chromatography with Silica Gel 60, 230–400 mesh or 70–120 mesh, respectively. HMPA was purchased from E. Merck (Darmstadt, Germany) and used without further purification. Other solvents were distilled and dried according to standard procedures,[15] 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 necessary for analytical purposes gel permeation chromatography (GPC) of protected compounds was performed using a Sephadex LH20 column (1.5 × 105 cm) eluted with 1:1 $CHCl_3$–MeOH. Optical rotations were measured at rt with a JASCO DIP-370 digital polarimeter and are given in 10^{-1} deg cm^2 g^{-1}.

1,6-Anhydro-2-azido-3-*O*-benzoyl-2-deoxy-4-*O*-trifluoromethylsulfonyl-β-D-galactopyranose 3

A stirred solution of 1,6-anhydro-2-azido-2-deoxy-β-D-galactopyranose[6] **1** (1.47 g, 7.8 mmol) in freshly distilled anhydrous CH_2Cl_2 (130 cm^3) containing anhydrous pyridine (15 cm^3) was cooled under N_2 to −10 °C and triflic anhydride (1.3 cm^3, 7.8 mmol) was added dropwise. After 1 h at −7 °C, more anhydride (70 mm^3, 0.4 mmol) was added and the reaction mixture was stirred for 1 h at −5 °C. An aliquot (5 cm^3) of the reaction mixture was removed and washed successively with water, 2 M HCl, and saturated aq. $NaHCO_3$. The washings were re-extracted with CH_2Cl_2 (10 cm^3) and the combined organic phases were dried and concentrated. Chromatography of the residue (4:1 cyclohexane–EtOAc) gave the triflate **2** (50 mg, 68%) as assessed by its ^{1}H NMR spectrum, δ_H 3.82 (m, 2 H, H-6 and -2), 4.29 (m, 1 H, H-3), 4.52 (d, 1 H, $J_{6',6}$ 8, H′-6), 4.68 (br t, 1 H, $J_{5,4\,+\,5,6}$ 8, H-5), 5.06 (br t, 1 H, $J_{4,3\,+\,4,5}$ 9, H-4) and 5.55 (s, 1 H, H-1).

The remaining reaction mixture was treated with benzoyl chloride (1.37 cm^3, 11.8 mmol) and allowed to reach rt slowly overnight. MeOH (3.7 cm^3) was added to quench the reaction and the reaction mixture was diluted with CH_2Cl_2 (100 cm^3) and washed successively with water (100 cm^3), 1 M HCl (200 cm^3), saturated aq. $NaHCO_3$ (2 × 100 cm^3) and brine (100 cm^3). The washings were re-extracted with CH_2Cl_2 (100 cm^3) and the combined organic phases were dried and concentrated. Chromatography (1:1 cyclohexane–EtOAc) of the residue gave the title compound, which crystallized on storage (2.6 g, 78%), mp 90–91 °C (from hexane); $[a]_D$ +15 (*c* 1.2, CH_2Cl_2); δ_H 3.88 (br s, 1 H, H-2), 3.95 (dd, 1 H, $J_{6,6'}$ 8, $J_{6,5}$ 5, H-6), 4.60 (d, 1 H, H′-6), 4.75 (br t, 1 H, $J_{5,4}$ 4, H-5), 5.30 (br t, 1 H, $J_{4,3}$ 5.5, H-4), 5.55 (br s, 1 H, H-1), 5.61 (m, 1 H, H-3) and 7.52, 7.65 and 8.05 (3 m, 5 H, ArH) (Found: C, 39.9; H, 2.9; N, 10.0. $C_{14}H_{12}F_3N_3O_7S$ requires C, 39.72; H, 2.86; N, 9.93%).

2-Acetamido-1,6-anhydro-3-*O*-benzoyl-2-deoxy-4-*O*-trifluoromethylsulfonyl-β-D-galactopyranose 4

Acetic anhydride (2 cm^3) and palladium-on-carbon catalyst (10%-on-C, 50% in water; 800 mg) were added to a solution of the azide **3** (1.67 g, 3.95 mmol) in EtOAc (50 cm^3) and the reaction mixture was stirred under H_2 at rt for 20 h. The catalyst was filtered off, rinsed with EtOAc and the combined filtrate and washings were concentrated. Residual Ac_2O was co-evaporated with toluene and the acetamide **4** (1.49 g, 87%) crystallized on storage, mp 125–127 °C (from 9:1 hexane–EtOAc); $[a]_D$ −28 (*c* 1.3, CH_2Cl_2); δ_H 2.10 (s, 3 H, CH_3CO), 3.93 (dd, 1 H, $J_{6,6'}$ 8, $J_{6,5}$ 5, H-6), 4.49 (br d, 1 H, $J_{2,NH}$ 9.5, H-2), 4.59 (d, 1 H, H′-6), 4.69 (br t, 1 H, $J_{5,4}$ 3.5, H-5), 5.23 (br t, 1 H, $J_{4,3}$ 5.5, H-4), 5.44 (br s, 1 H, H-1), 5.60 (m, 1 H, H-3), 6.08 (d, 1 H, NH) and 7.50, 7.65 and 8.05 (3 m, 5 H, ArH); δ_C 23.1 (CH_3CO), 53.3 (C-2), 64.7 (C-6), 68.3 and 72.4 (C-5 and -3), 77.0 (C-4) and 100.9 (C-1). Purity of the triflate **4** was assessed by NMR spectroscopy and it was stored at 4 °C until further use. As a result of its instability, a satisfactory elemental analysis for the triflate **4** could not be obtained.

2-Acetamido-1,6-anhydro-3-*O*-benzoyl-4-*S*-cyano-2-deoxy-4-thio-β-D-glucopyranose 5

Potassium thiocyanate (155 mg, 1.595 mmol) was added to a solution of the triflate **4** (201 mg, 0.46 mmol) in HMPA (2 cm^3) and the mixture was stirred overnight at 80 °C before being cooled to rt, diluted with EtOAc (7 cm^3) and washed successively with water (10 cm^3), 10% aq. $KHSO_4$ (10 cm^3), and water (10 cm^3). The aq. phases were re-extracted with EtOAc (3 × 5 cm^3) and the combined organic solutions were dried and concentrated. Chromatography of the oily brownish residue (1:1 toluene–EtOAc) gave the pure thiocyanate **5** (102 mg, 64%), which crystallized on storage, mp 147–149 °C (from 9:1 hexane–EtOAc); $[a]_D$ −105 (*c* 1.2, CH_2Cl_2); δ_H 2.09 (s, 3 H, CH_3CO), 3.70 (s, 1 H, H-4), 4.00 (dd, 1 H, $J_{6,6'}$ 8, $J_{6,5}$ 5.5, H-6), 4.25 (d, 1 H, H′-6), 4.35 (br d, 1 H, $J_{2,NH}$ 9.5, H-2), 4.80 (br d, 1 H, H-5), 5.14 (m, 1 H, H-3), 5.55 (br s, 1 H, H-1), 6.14

(br d, 1 H, NH) and 7.50, 7.65 and 8.08 (3 m, 5 H, ArH); δ_C 23.2 (CH_3CO), 47.8 and 49.5 (C-2 and -4), 68.0 (C-6), 71.3 and 74.0 (C-5 and -3) and 101.3 (C-1) (Found: C, 55.2; H, 4.65; N, 7.9. $C_{16}H_{16}N_2O_5S$ requires C, 55.16; H, 4.63; N, 8.00%).

2-Acetamido-3,4-di-*S*-acetyl-1,6-anhydro-2-deoxy-3,4-dithio-β-D-glucopyranose 6

Potassium thioacetate (40 mg, 0.35 mmol) was added to a solution of the triflate **4** (20 mg, 0.046 mmol) in HMPA (1 cm^3) and the mixture was stirred overnight at 80 °C before being cooled to rt and worked up using the same conditions as described above for the synthesis of compound **5**. Chromatography of the brownish residue (1:1 toluene–EtOAc) gave the bis(thioacetate) **6** (9.1 mg, 64%), which crystallized on storage, mp 137–139 °C; $[\alpha]_D$ −39 (*c* 1.1, CH_2Cl_2); δ_H 2.05 (s, 3 H, CH_3CON), 2.35 and 2.41 (2 s, 2 × 3 H, CH_3COS), 3.70 (br s, 1 H, H-4), 3.73 (br s, 1 H, H-3), 3.82 (dd, 1 H, $J_{6,6'}$ 8, $J_{6,5}$ 5, H-6), 4.14 (br d, 1 H, $J_{2,NH}$ 9, H-2), 4.44 (d, 1 H, H′-6), 4.46 (br d, 1 H, H-5), 5.37 (br s, 1 H, H-1) and 5.97 (br d, 1 H, NH); δ_C 23.3 (CH_3CON), 30.0 and 30.5 (2 × CH_3COS), 42.1, 46.0 and 51.1 (C-2, -3 and -4), 68.1 (C-6), 76.3 (C-5) and 101.0 (C-1) [Found: FABMS MH^+, 320.0. $C_{12}H_{17}NO_5S_2$ (*M*H) requires *m*/*z*, 320.06].

2-Acetamido-4-*S*-acetyl-1,6-anhydro-3-*O*-benzoyl-2-deoxy-4-thio-β-D-glucopyranose 7

Potassium thioacetate (400 mg, 3.5 mmol) was added to a solution of the triflate **4** (403 mg, 0.92 mmol) in DMF (20 cm^3) and the mixture was stirred overnight at rt before being concentrated, and the residue, dissolved in CH_2Cl_2 (40 cm^3), was washed with water (2 × 30 cm^3). The aq. washings were re-extracted with CH_2Cl_2 (2 × 40 cm^3) and the combined organic solutions were dried and concentrated. Chromatography of the residue (1:1, then 3:7 toluene–EtOAc) gave the thioacetate **7** (268 mg, 80%), which crystallized on storage, mp 203–205 °C; $[\alpha]_D$ −60 (*c* 1.3, CH_2Cl_2); δ_H 2.07 (s, 3 H, CH_3CON), 2.43 (s, 3 H, CH_3COS), 3.82 (br s, 1 H, H-4), 3.93 (dd, 1 H, $J_{6,6'}$ 7.5, $J_{6,5}$ 5.5, H-6), 4.23 (m, 1 H, H-2), 4.37 (d, 1 H, H′-6), 4.53 (br d, 1 H, H-5), 5.03 (m, 1 H, H-3), 5.46 (br s, 1 H, H-1), 5.84 (br d, 1 H, $J_{NH,2}$ 9.5, NH) and 7.48, 7.61 and 8.08 (3 m, 5 H, ArH); δ_C 23.3 (CH_3CON), 30.5 (CH_3COS), 44.2 (C-4), 49.7 (C-2), 68.0 (C-6), 71.8 and 75.4 (C-3 and -5) and 101.0 (C-1) (Found: C, 55.8; H, 5.3; N, 3.7. $C_{17}H_{19}NO_6S$ requires C, 55.88; H, 5.24; N, 3.83%).

2-Acetamido-1,6-anhydro-2,3,4-trideoxy-β-D-*erythro*-hex-3-enopyranose 8

Activated zinc (186 mg) was added to a solution of the thiocyanate **5** (51 mg, 0.146 mmol) in AcOH (5 cm^3) and the suspension was stirred at 110 °C for 6 h. The reaction mixture was cooled to rt, more zinc (145 mg) was added and the reaction was allowed to proceed under stirring overnight at 110 °C. After cooling of the mixture, the zinc was removed by filtration and washed with CH_2Cl_2. The combined filtrate and washings were concentrated to dryness and residual AcOH was coevaporated with toluene. Chromatography (3:7 toluene–EtOAc) gave the oily title compound **8** (15 mg, 60%), δ_H 2.00 (s, 3 H, CH_3CON), 3.70 (dd, 1 H, $J_{6,6'}$ 7, $J_{6,5}$ 4.5, H-6), 3.81 (d, 1 H, H′-6), 4.25 (m, 1 H, H-2), 4.68 (br t, 1 H, $J_{5,4}$ 4.5, H-5), 5.43 (br s, 1 H, H-1), 5.62 (ddd, 1 H, $J_{3,2}$ 4.5, $J_{3,4}$ 10, $^4J_{3,2}$ 2, H-3), 5.67 (br d, 1 H, NH) and 6.19 (ddd, 1 H, $^4J_{4,2}$ 2, H-4) [Found: FABMS MH^+, 170.0. $C_8H_{12}NO_3$ (*M*H) requires *m*/*z*, 170.08].

2-Acetamido-1,6-di-*O*-acetyl-3-*O*-benzoyl-4-*S*-cyano-2-deoxy-4-thio-α,β-D-glucopyranose 9

The thiocyanate **5** (158 mg, 0.454 mmol) was dissolved in acetic anhydride–TFA (9:1; 30 cm^3) and the solution was stirred overnight at 65 °C. The solvents were evaporated off and residual traces of acid were coevaporated with toluene. The dry residue was adsorbed on silica gel and submitted to chromatography (1:1 toluene–EtOAc) to give an anomeric mixture of diacetate **9** (165 mg, 81%) as a powder. ^{1}H NMR spectroscopy showed that the α-anomer was in a large majority (>90%). δ_H for the α-anomer: 1.85, 2.16 and 2.25 (3 s, 3 × 3 H, 3 × CH_3CO), 3.44 (t, 1 H, $J_{4,3+4,5}$ 21.5, H-4), 4.29 (dt, 1 H, H-5), 4.46 (dd, 1 H, $J_{6',6}$ 12.5, $J_{6,5}$ 1.5, H-6), 4.55 (dd, 1 H, $J_{6,5}$ 3, H′-6), 4.70 (ddd, 1 H, $J_{2,1}$ 4, $J_{2,NH}$ 9, H-2), 5.67 (t, 1 H, $J_{3,2+3,4}$ 21.5, H-3), 5.96 (br d, 1 H, NH), 6.24 (d, 1 H, H-1) and 7.50, 7.65 and 8.05 (3 m, 5 H, ArH); δ_C for the α-anomer: 20.7, 20.9 and 22.9 (CH_3CO), 46.7 (C-4), 52.1 (C-2), 62.4 (C-6), 69.6 and 70.6 (C-3 and -5) and 90.8 (C-1).

2-Acetamido-1,6-di-*O*-acetyl-3-*O*-benzoyl-2-deoxy-4-thio-α-D-glucopyranose 10

The thiocyanate **9** (150 mg, 0.33 mmol) was dissolved in AcOH (15 cm^3), activated zinc (900 mg) was added and the reaction mixture was stirred at 110 °C for 1 h. Solids were filtered off, rinsed with CH_2Cl_2 and the pooled filtrate and washing were concentrated to dryness. The residue was dissolved in CH_2Cl_2 (20 cm^3) and the solution was washed with saturated aq. $NaHCO_3$ (20 cm^3) and saturated aq. NaCl (20 cm^3). The aq. washings were re-extracted with CH_2Cl_2 (5 × 20 cm^3) and the combined organic extracts were dried and concentrated. Chromatography of the residue (2:3 toluene–EtOAc) gave the anomerically pure α-thiol **10** (108.8 mg, 77%) as an amorphous solid, $[\alpha]_D$ +48 (*c* 1.2, CH_2Cl_2); δ_H 1.66 (d, 1 H, $J_{SH,4}$ 9, SH), 1.83, 2.14 and 2.22 (3 s, 3 × 3 H, 3 × CH_3CO), 3.26 (dt, 1 H, $J_{4,3+4,5}$ 21.5, H-4), 3.98 (ddd, 1 H, $J_{5,6}$ 2, $J_{5,6'}$ 5, H-5), 4.43 (dd, 1 H, $J_{6,6'}$ 12.5, H-6), 4.51 (dd, 1 H, H′-6), 4.58 (ddd, 1 H, $J_{2,1}$ 3, $J_{2,NH}$ 9, H-2), 5.32 (t, 1 H, $J_{3,2+3,4}$ 21.5, H-3), 5.74 (br d, 1 H, NH), 6.26 (d, 1 H, H-1) and 7.48, 7.64 and 8.08 (3 m, 5 H, ArH); δ_C 20.8, 21.0 and 23.7 (3 × CH_3CO), 40.1 (C-4), 51.9 (C-2), 63.2 (C-6), 73.5 and 73.6 (C-3 and -5) and 91.2 (C-1) (Found: C, 53.4; H, 5.45; N, 3.3. $C_{19}H_{23}NO_8S$ requires C, 53.64; H, 5.45; N, 3.29%).

1,3,4,6-Tetra-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α,β-D-glucopyranose 11

2-Amino-2-deoxy-D-glucose hydrochloride (1 g, 4.6 mmol) was suspended in freshly distilled anhydrous MeOH (50 cm^3). Triethylamine (4 cm^3) and 2,2,2-trichloroethyl chloroformate (1.3 cm^3, 9.4 mmol) were added to the suspension stirred under N_2 at 0 °C and the reaction mixture was stirred overnight at rt. Solvents were removed under reduced pressure and residual MeOH was coevaporated with toluene. The dry residue was dissolved in anhydrous pyridine (30 cm^3), and benzoyl chloride (4 cm^3) was added dropwise to the mixture stirred under N_2 in a water-bath. The reaction was left to proceed for 2 days at rt, excess of benzoyl chloride was destroyed by addition of water (4 cm^3), and solvents were evaporated. The residue was co-concentrated twice from toluene, dissolved in CH_2Cl_2 (100 cm^3) and the solution was washed successively with HCl (2 M; 100 cm^3), saturated aq. $NaHCO_3$ (100 cm^3) and saturated aq. NaCl (100 cm^3). The aq. washings were re-extracted with CH_2Cl_2 (2 × 50 cm^3) and the combined organic extracts were dried and concentrated. Flash chromatography of the residue (4:1 hexane–EtOAc) gave the benzoate **11** (2.07 g, 58%) as a 7:3 α/β mixture as determined from the ^{1}H NMR spectrum: δ_H 4.31 (ddd, 1 Hβ, $J_{5,4}$ 9.5, $J_{5,6}$ 4.5, $J_{5,4}$ 3, H-5β), 4.43–4.66 (m, 5 Hα and 4 Hβ, H-2α, H-5α, H-6α, H′-6α, OC*H*$HCCl_3$α, H-2β, H-6β, H′-6β, OC*H*$HCCl_3$β), 4.74 (d, 1 Hα,β, *J* 12, OC*H*$HCCl_3$), 5.43 (br d, 1 Hα,β, *J* 9.5, NH), 5.72 (br t, 1 Hβ, $J_{3,4+3,2}$ or $J_{4,3+4,5}$ 20, H-3β or -4β), 5.81 (br t, 1 Hβ, $J_{3,4+3,2}$ or $J_{4,3+4,5}$ 19, H-4β or -3β), 5.87 (t, 1 Hα, $J_{3,4+3,2}$ or $J_{4,3+4,5}$ 19, H-3α or -4α), 5.93 (t, 1 Hα, $J_{3,4+3,2}$ or $J_{4,3+4,5}$ 20.5, H-4α or -3α), 6.13 (d, 1 Hβ, $J_{1,2}$ 9, H-1β), 6.66 (d, 1 Hα, $J_{1,2}$ 3.5, H-1α) and 7.30–8.22 (m, 20 Hα,β, ArH).

3,4,6-Tri-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl bromide 12

The benzoate **11** (400 mg, 0.519 mmol) was dissolved in freshly distilled anhydrous CH_2Cl_2 (4 cm^3) and a solution of HBr in AcOH (33%; 1.6 cm^3) was added. The reaction mixture was stirred at rt for 2.5 h and solvents were evaporated. Residual traces of acid were coevaporated with toluene (2 × 10 cm^3) and a solution of the dry residue in CH_2Cl_2 (25 cm^3) was washed rapidly and successively with ice-cold saturated aq. $NaHCO_3$ (20 cm^3) and ice-cold saturated aq. NaCl (20 cm^3). The aq. washings were re-extracted with CH_2Cl_2 (2 × 15 cm^3) and the combined organic extracts were dried and concentrated. The dry residue was co-concentrated twice from anhydrous toluene and freshly distilled anhydrous CH_2Cl_2, and dried under high vacuum to give the bromide **12** as a glass (366 mg, 97%). The structure and purity of bromide **12** were confirmed by ^1H NMR spectroscopy and it was stored at −20 °C until use; δ_H 4.40 (dt, 1 H, $J_{2,1}$ 4, $J_{2,3\,+\,2,NH}$ 19.5, H-2), 4.49 (dd, 1 H, $J_{6,6'}$ 13, $J_{6,5}$ 4.5, H-6), 4.55 (d, 1 H, *J* 12, OC*H*$HCCl_3$), 4.65 (m, 2 H, H-5 and H′-6), 4.71 (d, 1 H, OC*H*$HCCl_3$), 5.53 (br d, 1 H, NH), 5.85 (m, 2 H, H-3 and -4), 6.65 (d, 1 H, H-1) and 7.34–8.12 (m, 15 H, ArH).

3,4,6-Tri-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α,β-D-glucopyranose 13

The benzoate **11** (936 mg, 1.2 mmol) was converted to the bromide **12** as described above. After co-concentration of the reaction mixture with toluene, the residue was dissolved in 4:1 CH_3CN–water (50 cm^3) and the solution was stirred overnight at rt. The solvents were partly evaporated off and the residual biphasic mixture was diluted with EtOAc (50 cm^3) and decanted. The organic phase was washed successively with water (25 cm^3), saturated aq. $NaHCO_3$ (25 cm^3) and saturated aq. NaCl (25 cm^3). The aq. washings were re-extracted with EtOAc (2 × 20 cm^3) and the combined organic extracts were dried and concentrated. Flash chromatography (7:3 hexane–EtOAc) gave the hemiacetal **13** (702 mg, 87%) as a glass. ^1H NMR spectroscopy in $CDCl_3$ showed the α anomer to be in a large majority: δ_H for the α anomer: 4.34 (br dt, 1 H, $J_{2,1}$ 3.5, $J_{2,3\,+\,2,NH}$ 21, H-2), 4.43 (dd, 1 H, $J_{6,6'}$ 12.5, $J_{6,5}$ 4.5, H-6), 4.48 (d, 1 H, *J* 12, OC*H*$HCCl_3$), 4.65 (m, 2 H, H-5 and H′-6), 4.70 (d, 1 H, OC*H*$HCCl_3$), 5.47 (br t, 1 H, $J_{1,2\,+\,1,OH}$ 7, H-1), 5.70 (m, 1 H, NH), 5.72 (t, 1 H, $J_{3,4\,+\,3,2}$ or $J_{4,3\,+\,4,5}$ 20, H-3 or -4), 5.89 (t, 1 H, $J_{3,4\,+\,3,2}$ or $J_{4,3\,+\,4,5}$ 20.5, H-4 or -3) and 7.25–8.10 (m, 15 H, ArH).

3,4,6-Tri-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl trichloroacetimidate 14

Trichloroacetonitrile (377 mm^3, 3.76 mmol) and DBU (35 mm^3, 0.23 mmol) were added to a solution of the hemiacetal **13** (623 mg, 0.93 mmol) in anhydrous CH_2Cl_2 (30 cm^3) and the reaction mixture was stirred overnight under N_2 at rt. Solvents were evaporated off and the residue, dissolved in 9:1 toluene–EtOAc, was poured over a bed of silica gel (4.5 × 3 cm) which was, in turn, eluted with the same solvent mixture. The imidate **14** was obtained (493 mg, 65%) pure as a glass and was stored at −20 °C until use, δ_H 4.47 (dd, 1 H, $J_{6,6'}$ 12, $J_{6,5}$ 4.5, H-6), 4.55 (m, 2 H, H-2 and -5), 4.60 (d, 1 H, *J* 12, OC*H*-$HCCl_3$), 4.61 (dd, 1 H, $J_{6',5}$ 2.5, H′-6), 4.66 (d, 1 H, OC*H*-$HCCl_3$), 5.42 (d, 1 H, $J_{NH,2}$ 9.5, N*H*Ac), 5.83 (m, 2 H, H-3 and -4), 6.58 (d, 1 H, $J_{1,2}$ 3.5, H-1), 7.35–8.10 (m, 15 H, ArH) and 8.85 (s, 1 H, C=N*H*).

2-Acetamido-1,6-di-*O*-acetyl-3-*O*-benzoyl-2-deoxy-4-thio-4-*S*-[3,4,6-tri-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-α-D-glucopyranose 15

A solution of the thiol **10** (11.4 mg, 0.0268 mmol) in anhydrous, O_2-free THF (2 cm^3) was transferred under Ar at 0 °C to a stirred suspension of NaH (55% in oil; 1.6 mg, 0.037 mmol) in THF (0.5 cm^3). The flask that contained the thiol was rinsed with aliquots of THF (2 × 250 mm^3) which were then added under Ar to the NaH suspension. The reaction mixture was stirred at 0 °C under Ar until evolution of gas had ceased, then was warmed to rt and transferred under Ar to a flask containing the bromide **12** (25.4 mg, 0.0348 mmol). The flask that had contained the thiolate was rinsed with aliquots of THF (0.5 and 0.2 cm^3) which were then added to the bromide solution. The reaction was left to proceed under Ar at rt for 2 h. It was quenched by addition of AcOH (25 mm^3) and solvents were removed under reduced pressure. Chromatography (1:1 toluene–EtOAc) of the residue gave the thiodisaccharide **15**, which crystallized on storage (18.5 mg, 64%), mp 128–130 °C; $[\alpha]_D$ −8 (*c* 1.3, CH_2Cl_2); δ_H 1.87, 2.06 and 2.09 (3 s, 3 × 3 H, 3 × CH_3CO), 3.30 (t, 1 H, $J_{4,3\,+\,4,5}$ 22, H-4A), 3.73 (d, 1 H, *J* 12, OC*H*$HCCl_3$), 4.07 (m, 2 H, H-2B and -5B), 4.33 (dd, 1 H, $J_{6,6'}$ 12.3, $J_{6,5}$ 7, H-6B), 4.39 (m, 2 H, H-5A and -6A), 4.63 (m, 2 H, H′-6A and -6B), 4.71 (d, 1 H, OC*H*$HCCl_3$), 4.75 (dt, 1 H, $J_{2,1}$ 3.5, $J_{2,3\,+\,2,NH}$ 20, H-2A), 5.11 (d, 1 H, $J_{1,2\ or\ NH,2}$ 10.5, H-1B or NHB), 5.52 (m, 4 H, H-3A, NHA, NHB or H-1B, H-4B), 5.71 (t, 1 H, $J_{3,2\,+\,3,4}$ 20, H-3B), 6.31 (d, 1 H, H-1A) and 7.30–8.15 (m, 20 H, ArH); δ_C 20.76 and 20.82 (2 × *C*H_3COO), 23.1 (*C*H_3CON), 45.6 (C-4A), 51.7 (C-2A), 55.1 (C-2B), 63.0 and 63.7 (C-6A and -6B), 68.5, 69.6, 71.7, 73.7 and 76.4 (C-3A, -5A, -3B, -4B and -5B), 73.8 (O*C*H_2CCl_3), 82.4 (C-1B) and 91.3 (C-1A) [Found: FABMS (MH^+), 1075.3. $C_{49}H_{48}Cl_3N_2O_{17}S$ (*M*H) requires *m*/*z*, 1075.17].

2-Acetamido-4-*S*-(2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-1,6-di-*O*-acetyl-3-*O*-benzoyl-2-deoxy-4-thio-α-D-glucopyranose 16

The thiodisaccharide **15** (64.0 mg, 0.060 mmol) was dissolved in Ac_2O (5 cm^3), activated powdered zinc (160 mg) was added, and the suspension was stirred at 65 °C for 5 h. The catalyst was filtered off, rinsed with EtOAc, and the combined filtrate and washings were concentrated. Residual Ac_2O was coevaporated with toluene, and chromatography (3:7, followed by 1:9 toluene–EtOAc) gave the title compound **16** (39.6 mg, 71%) as a glass, $[\alpha]_D$ −10 (*c* 0.9, CH_2Cl_2); δ_H 1.40, 1.84, 2.06 and 2.09 (4 s, 4 × 3 H, 4 × CH_3CO), 3.30 (t, 1 H, $J_{4,3\,+\,4,5}$ 22, H-4A), 4.06 (m, 1 H, H-5B), 4.29–4.44 (m, 4 H, H-5A, -6A, -2B and -6B), 4.65 (m, 2 H, H′-6A and -6B), 4.70 (m, 1 H, H-2A), 5.01 (d, 1 H, $J_{1,2\ or\ NH,2}$ 10.5, H-1B or NHB), 5.51 (t, 1 H, $J_{4,3\,+\,4,5}$ 19.5, H-4B), 5.54 (t, 1 H, $J_{3,2\,+\,3,4}$ 21.5, H-3A), 5.61 (t, 1 H, $J_{3,2\,+\,3,4}$ 20, H-3B), 5.66 (d, 1 H, $J_{NH,2}$ 9.5, NHA), 5.77 (d, 1 H, $J_{1,2\ or\ NH,2}$ 9, NHB or H-1B), 6.31 (d, 1 H, $J_{1,2}$ 3, H-1A) and 7.30–8.10 (m, 20 H, ArH); δ_C 20.74 and 20.79 (2 × *C*H_3COO), 22.5 and 23.0 (2 × *C*H_3CON), 45.5 (C-4A), 51.9 and 52.8 (C-2A and -2B), 63.0 and 63.5 (C-6A and -6B), 68.6, 69.4, 71.8, 74.0 and 76.2 (C-3A, -5A, -3B, -4B and -5B), 83.0 (C-1B) and 91.1 (C-1A) [Found: FABMS (MH^+), 941.5. $C_{48}H_{49}N_2O_{16}S$ (*M*H) requires *m*/*z* 941.28].

2-Acetamido-1,6-anhydro-3-*O*-benzoyl-2-deoxy-4-thio-β-D-glucopyranose 17

Cysteamine (42 mg, 0.54 mmol) was added to a solution of the thioacetate **7** (166 mg, 0.455 mmol) in CH_3CN (17 cm^3) and the reaction mixture was stirred at 65 °C for 20 min and cooled to rt. Acetic acid (50 mm^3) was added and the solution was concentrated to give a residue, which was submitted to chromatography (1:1 toluene–EtOAc) to yield the thiol **17**, which crystallized on storage (109 mg, 74%), mp 159–161 °C; $[\alpha]_D$ −60 (*c* 1.3, CH_2Cl_2); δ_H 2.07 (s, 3 H, CH_3CON), 2.40 (d, 1 H, $J_{SH,4}$ 6, SH), 3.32 (br d, 1 H, H-4), 3.90 (dd, 1 H, $J_{6,6'}$ 7.5, $J_{6,5}$ 5.5, H-6), 4.21 (d, 1 H, H′-6), 4.32 (br d, 1 H, $J_{NH,2}$ 9.5, H-2), 4.60 (br d, 1 H, H-5), 5.04 (m, 1 H, H-3), 5.49 (br s, 1 H, H-1), 6.42 (br d, 1 H, NH) and 7.46, 7.61 and 8.06 (3 m, 5 H, ArH); δ_C 23.4 (*C*H_3CON), 39.7 (C-4), 49.7 (C-2), 67.9 (C-6), 74.7 and 75.7

(C-3 and -5) and 101.2 (C-1) (Found: C, 56.0; H, 5.4; N, 4.3. $C_{15}H_{17}NO_5S$ requires C, 55.71; H, 5.30; N, 4.33%).

2-Acetamido-1,6-anhydro-2-deoxy-3,4-epithio-β-D-allopyranose 18

A solution of the thiol **17** (11.9 mg, 0.037 mmol) in anhydrous, O_2-free THF (2 cm^3) was transferred under Ar at 0 °C to a stirred suspension of NaH (55% in oil; 2.1 mg, 0.048 mmol) in THF (0.5 cm^3). The flask that contained the thiol was rinsed with aliquots of THF (2 × 250 mm^3) which were then added under Ar to the NaH suspension. The reaction mixture was stirred at 0 °C under Ar until evolution of gas had ceased, then the suspension was warmed to rt and transferred under Ar to a flask containing the bromide **12** (34.8 mg, 0.048 mmol). The flask was rinsed with aliquots of THF (4 × 0.5 cm^3) which were then added to the bromide solution. The reaction was left to proceed under Ar at rt for 2 h, was quenched by addition of AcOH (25 mm^3), and solvents were removed under reduced pressure. Chromatography (1:1 toluene–EtOAc) of the residue gave the title compound **18** as a glass (7.2 mg, 97%), $[\alpha]_D$ −88 (*c* 0.6, CH_2Cl_2); δ_H 2.05 (s, 3 H, CH_3CON), 3.20 (br d, 1 H, $J_{4,3}$ 6.5, H-4), 3.39 (br t, 1 H, $J_{3,2}$ 6, H-3), 3.90 (dd, 1 H, $J_{6,6'}$ 7.5, $J_{6,5}$ 4.5, H-6), 4.15 (d, 1 H, H′-6), 4.39 (dd, 1 H, $J_{2,NH}$ 8.5, H-2), 4.79 (br d, 1 H, H-5), 5.14 (br s, 1 H, H-1) and 5.90 (br d, 1 H, NH) [Found: FABMS (MH$^+$), 202.0. $C_8H_{12}NO_3S$ (*M*H) requires *m*/*z*, 202.05].

2-Acetamido-1,6-anhydro-3-*O*-benzoyl-2-deoxy-4-thio-4-*S*-[3,4,6-tri-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-β-D-glucopyranose 19

A mixture of the thiol **17** (30 mg, 0.093 mmol) and the trichloroacetimidate **14** (84 mg, 0.26 mmol) in anhydrous CH_2Cl_2 (5 cm^3) containing 4 Å activated molecular sieves (0.5 g) was stirred at rt under N_2 for 0.5 h and cooled to −75 °C. A solution of TMSOTf in anhydrous CH_2Cl_2 (0.28 M; 30 mm^3, 8.4 μmol) was added, and the mixture was stirred at −70 °C for 1 h, then at −35 °C for 0.5 h and at rt for 1 h. More TMSOTf (7.6 μmol) was added at rt portionwise over the following 8 h and the reaction was left to proceed overnight at rt under N_2. Triethylamine (10 mm^3, 72 μmol) was added, the molecular sieves were removed by decantation and washed with CH_2Cl_2 (10 cm^3), and the combined organic solutions were concentrated. Chromatography (7:3 toluene–EtOAc) gave the thiodisaccharide **19** (30 mg, 33%) contaminated with trichloroacetamide. Title compound **19** was obtained pure after GPC (23 mg, 25%). Further elution of the column (7:3 toluene–EtOAc) gave the unchanged thiol **17** (14 mg, 47%) as an amorphous solid. Characteristics for the pure thiodisaccharide **19** were as follows: $[\alpha]_D$ +46 (*c* 1.2, CH_2Cl_2); δ_H 2.08 (s, 3 H, CH_3CON), 3.32 (br s, 1 H, H-4A), 3.80 (dd, 1 H, $J_{6,6'}$ 7.5, $J_{6,5}$ 6, H-6A), 4.03 (d, 1 H, H′-6A), 4.15 (br d, 1 H, $J_{NH,2}$ 10, H-2A), 4.17 (m, 1 H, H-2B), 4.33 (dd, 1 H, $J_{6,6'}$ 12, $J_{6,5}$ 7.5, H-6B), 4.44 (m, 1 H, H-5B), 4.52 (br d, 1 H, H-5A), 4.57 (d, 1 H, *J* 12, OC*H*HCCl$_3$), 4.67 (dd, 1 H, $J_{6',5}$ 1.5, H′-6B), 4.71 (d, 1 H, OC*H*HCCl$_3$), 5.38 (d, 1 H, $J_{NH,2}$ 11, NHA), 5.48 (br s, 1 H, H-1A), 5.55 (br s, 1 H, H-3A), 5.59 (t, 1 H, $J_{4,3+4,5}$ 19.5, H-4B), 5.66 (d, 1 H, $J_{1,2}$ 9.5, H-1B), 5.76 (t, 1 H, $J_{3,2+3,4}$ 20, H-3B), 6.47 (br d, 1 H, NHB) and 7.05–8.00 (m, 20 H, ArH); δ_C 23.3 (*C*H$_3$CO), 48.3 and 49.8 (C-4A and -2A), 55.8 (C-2B), 63.2 (C-6B), 67.7 (C-6A), 69.1, 71.4, 73.8, 74.2 and 76.2 (C-3A, -5A, -3B, -4B and -5B), 74.4 (O*C*H$_2$CCl$_3$), 86.7 (C-1B) and 100.7 (C-1A) [Found: C, 55.4; H, 4.4; N, 2.9; FABMS (MH$^+$), 973.3. $C_{45}H_{41}Cl_3N_2O_{14}S$ requires C, 55.59; H, 4.25; N, 2.88%; (*M*H) *m*/*z*, 973.14].

3,4,6-Tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-α-D-glucopyranosyl bromide 21

The peracetate **20**[9] (506 mg, 1.03 mmol) was dissolved in anhydrous CH_2Cl_2 (10 cm^3) and a solution of HBr in AcOH (45%; 2.5 cm^3) was added. The reaction mixture was stirred for 3 h under N_2 at rt, concentrated to dryness, and residual acid was coevaporated with anhydrous toluene. The oily residue was dissolved in CH_2Cl_2 (50 cm^3) and the solution was washed successively with ice-cold, saturated aq. $NaHCO_3$ (50 cm^3). The aq. solution was re-extracted with CH_2Cl_2 (2 × 20 cm^3) and the combined organic phases were dried and concentrated. The dry residue was co-concentrated twice from anhydrous toluene and freshly distilled anhydrous CH_2Cl_2, and dried under high vaccum to give the bromide **21** as a glass (545 mg, quant). The purity of bromide **21** was confirmed by its ^{1}H NMR spectrum and it was stored at −20 °C until use, δ_H 2.05, 2.08 and 2.11 (3 s, 3 × 3 H, 3 × CH_3CO), 4.14 (dd, 1 H, $J_{6,6'}$ 12.5, $J_{6,5}$ 2, H-6), 4.27 (m, 2 H, H-2 and -5), 4.35 (dd, 1 H, $J_{6,5}$ 4.5, H′-6), 5.29 (t, 1 H, $J_{3,2+3,4}$ or $J_{4,3+4,5}$ 20, H-3 or -4), 5.44 (t, 1 H, $J_{3,2+3,4}$ or $J_{4,3+4,5}$ 20, H-4 or -3), 6.58 (d, 1 H, $J_{1,2}$ 4, H-1) and 7.06 (br d, 1 H, $J_{NH,2}$ 8, NH); δ_C 20.6 and 20.8 (3 × *C*H$_3$CO$_2$), 55.4 (C-2), 60.8 (C-6), 66.2, 70.5 and 72.8 (C-3, -4 and -5), 89.2 (C-1), 91.5 (CCl$_3$), 161.9 (O*C*OCCl$_3$) and 169.0, 170.4 and 171.4 (3 × O*C*OCH$_3$).

3,4,6-Tri-*O*-acetyl-1-*S*-acetyl-2-deoxy-1-thio-2-trichloroacetamido-β-D-glucopyranose 22

Thioacetic acid (145 mm^3, 2.06 mmol) and a solution of KOH in 95% EtOH (2.02 M; 920 mm^3, 1.85 mmol) were added to a solution of the crude bromide **21** (545 mg, 1.03 mmol) in acetone (10 cm^3). The reaction mixture was stirred at rt for 1 h, diluted with CH_2Cl_2 (50 cm^3) and washed successively with saturated aq. $NaHCO_3$ (40 cm^3) and brine (40 cm^3). The washings were re-extracted with CH_2Cl_2 (2 × 30 cm^3) and the combined organic phases were dried and concentrated. Flash chromatography (7:3, 300 cm^3; then 3:2, 100 cm^3, hexane–EtOAc) of the residue gave the thioacetate **22**, which crystallized on storage (438 mg, 83%), mp 158–160 °C; $[\alpha]_D$ +19 (*c* 0.9, CH_2Cl_2); δ_H 2.05, 2.08 and 2.10 (3 s, 3 × 3 H, 3 × CH_3CO_2), 2.38 (s, 3 H, CH_3COS), 3.85 (ddd, 1 H, $J_{5,4}$ 9.5, $J_{5,6}$ 2, $J_{5,6'}$ 5, H-5), 4.13 (dd, 1 H, $J_{6,6'}$ 12.5, H-6), 4.28 (dd, 1 H, H′-6), 4.31 (m, 1 H, H-2), 5.19 (t, 1 H, $J_{4,3+4,5}$ 19.5, H-4), 5.30 (d, 1 H, $J_{1,2}$ 10.5, H-1), 5.30 (t, 1 H, $J_{3,2+3,4}$ 20, H-3) and 6.90 (br d, 1 H, $J_{2,NH}$ 9.5, NH); δ_C 20.6 and 20.8 (3 × *C*H$_3$CO$_2$), 30.9 (*C*H$_3$COS), 54.5 (C-2), 61.9 (C-6), 67.7, 73.3 and 77.1 (C-3, -4 and -5), 81.1 (C-1), 92.2 (CCl$_3$), 162.1 (O*C*OCCl$_3$), 169.3, 170.7 and 171.3 (3 × O*C*OCH$_3$) and 193.4 (S*C*OCH$_3$) (Found: C, 37.8; H, 4.0; N, 2.8. $C_{16}H_{20}Cl_3NO_9S$ requires C, 37.77; H, 3.96; N, 2.75%).

3,4,6-Tri-*O*-acetyl-2-deoxy-1-thio-2-trichloroacetamido-β-D-glucopyranose 23

Cysteamine (33 mg, 0.43 mmol) was added to a solution of the thioacetate **22** (200 mg, 0.393 mmol) in CH_3CN (20 cm^3) and the reaction mixture was stirred at 65 °C for 25 min. The stirring bar was removed and rinsed with EtOAc (3 cm^3) and the combined washings and solution were concentrated. The residue was dissolved in EtOAc (20 cm^3) and the solution was washed successively with 1 M HCl (20 cm^3), saturated aq. $NaHCO_3$ (20 cm^3) and water (20 cm^3). The washings were re-extracted with EtOAc (2 × 20 cm^3) and the combined organic phases were dried and concentrated. Chromatography (3:2 hexane–EtOAc) of the residue gave the thiol **23** as a glass (137 mg, 75%), $[\alpha]_D$ −7 (*c* 1.0, CH_2Cl_2); δ_H 2.05, 2.055 and 2.12 (3 s, 3 × 3 H, 3 × CH_3CO_2), 2.52 (d, 1 H, $J_{SH,1}$ 10, SH), 3.75 (ddd, 1 H, $J_{5,4}$ 9.5, $J_{5,6}$ 2, $J_{5,6'}$ 5, H-5), 4.10 (q, 1 H, $J_{2,3+2,4+2,NH}$ 31, H-2), 4.15 (dd, 1 H, $J_{6,6'}$ 12.5, H-6), 4.28 (dd, 1 H, H′-6), 4.67 (t, 1 H, $J_{1,2+1,SH}$ 20.5, H-1), 5.17 (t, 1 H, $J_{4,3+4,5}$ 18, H-4), 5.23 (t, 1 H, $J_{3,2+3,4}$ 19.5, H-3) and 6.90 (br d, 1 H, $J_{2,NH}$ 9.1, NH); δ_C 20.6 and 20.8 (3 × *C*H$_3$CO$_2$), 58.5 (C-2), 62.1 (C-6), 68.0, 72.7 and 76.5 (C-3, -4 and -5), 79.6 (C-1), 162.3 (O*C*OCCl$_3$) and 169.2, 170.7 and 171.1 (3 × O*C*OCH$_3$) (Found: C, 36.2; H, 3.95; N, 3.1. $C_{14}H_{18}Cl_3NO_8S$ requires C, 36.02; H, 3.89; N, 3.00%).

2-Acetamido-1,6-anhydro-3-*O*-benzoyl-2-deoxy-4-thio-4-*S*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-β-D-glucopyranose 24

A solution of the thiol **23** (20 mg, 0.043 mmol) in anhydrous, O_2-free DMF (3 cm^3) was cooled to 0 °C under Ar, transferred with stirring to a flask containing NaH (55% in oil; 1.9 mg, 0.043 mmol) and kept at 0 °C. The flask that had contained the thiol was rinsed with aliquots of DMF (2 × 150 mm^3) which were then added under Ar to the NaH suspension. The reaction mixture was stirred at 0 °C under Ar until evolution of gas had ceased, then was warmed to rt and transferred under Ar to a flask containing the triflate **4** (19 mg, 0.043 mmol). The flask that contained the thiolate was rinsed with aliquots of DMF (3 × 150 mm^3) which were then added to the reaction mixture. The reaction was left to proceed under Ar at rt for 2 h, then at 45 °C for 17 h, and was finally quenched by the addition of AcOH (24 mm^3) and solvents were removed under reduced pressure. Chromatography (1:4, then 1:2, and finally 1:1 EtOAc–cyclohexane) of the residue gave the thiodisaccharide **24** (24 mg, 74%), which crystallized on storage and was submitted for analytical purposes to GPC, mp 268–269 °C (decomp.); $[\alpha]_D$ −53 (*c* 1.0, CH_2Cl_2); δ_H 1.96, 2.05, 2.07 and 2.08 (4 s, 4 × 3 H, CH_3CO), 3.31 (br s, 1 H, H-4A), 3.88 (dd, 1 H, $J_{6,6'}$ 7.5, $J_{6,5}$ 5.5, H-6A), 4.04 (ddd, 1 H, $J_{5,4}$ 10, $J_{5,6}$ 2, $J_{5,6'}$ 6, H-5B), 4.14 (m, 3 H, H-2B, -6B, H′-6A), 4.26 (dd, 1 H, $J_{6,6'}$ 12.5, H′-6B), 4.35 (br d, 1 H, $J_{NH,2}$ 9, H-2A), 4.57 (br d, 1 H, H-5A), 5.16 (t, 1 H, $J_{4,3+4,5}$ 19.5, H-4B), 5.28 (d, 1 H, $J_{1,2}$ 10.5, H-1B), 5.37 (t, 1 H, $J_{3,2+3,4}$ 20, H-3B), 5.52 (br s, 1 H, H-1A), 5.56 (br s, 1 H, H-3A), 6.30 (d, 1 H, $J_{NH,2}$ 9.5, NHA), 6.94 (br d, 1 H, NHB) and 7.46–8.10 (m, 5 H, ArH) [Found: C, 46.3; H, 4.6; N, 3.8%; FABMS (MH^+), 757.1. $C_{29}H_{33}Cl_3N_2O_{13}S$ requires C, 46.07; H, 4.40; N, 3.71%; (*M*H) *m*/*z*, 757.08].

2-Acetamido-4-*S*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-1,6-anhydro-3-*O*-benzoyl-2-deoxy-4-thio-β-D-glucopyranose 26

The thiol **25**[12] (100 mg, 0.275 mmol) was treated with NaH (55% in oil; 12.2 mg, 0.279 mmol) and the resulting thiolate was coupled to the triflate **4** (101 mg, 0.229 mmol) using the same reaction conditions as those described for the synthesis of compound **24**. After quenching and work-up of the reaction mixture as described for compound **24** (AcOH, 120 mm^3), chromatography (100:1, 200 cm^3; 50:1, 70 cm^3; and 97:3, 100 cm^3 $CHCl_3$–MeOH) of the residue gave the thiodisaccharide **26** (96.1 mg, 64%) pure as observed by NMR spectroscopy and which was, nevertheless, submitted to GPC on Sephadex LH20. Even though ^{1}H NMR analysis did not show any impurity and despite the GPC and an intensive drying at 77 °C under high vacuum, a good elemental analysis could not be obtained for the thiodisaccharide **26**. Since the analysis corresponded to the thiodisaccharide hydrated with one molecule of water which gave a signal at δ 3.33 when the ^{1}H NMR experiments were carried out in deuterated Me_2SO, we propose that a molecule of water is trapped by the title compound through hydrogen bonding; $[\alpha]_D$ −39 (*c* 1.05, CH_2Cl_2); δ_H ($CDCl_3$) 1.93, 1.97, 2.04, 2.05 and 2.06 (5 s, 5 × 3 H, CH_3CO), 3.31 (br s, 1 H, H-4A), 3.88 (dd, 1 H, $J_{6,6'}$ 7.5, $J_{6,5}$ 5.5, H-6A), 3.99 (ddd, 1 H, H-5B), 4.12–4.24 (m, 4 H, H′-6A, H-2B, -6B, H′-6B), 4.33 (br d, 1 H, $J_{NH,2}$ 9, H-2A), 4.59 (br d, 1 H, H-5A), 5.10 (t, 1 H, $J_{4,3+4,5}$ 19.5, H-4B), 5.16 (d, 1 H, $J_{1,2}$ 10.5, H-1B), 5.24 (t, 1 H, $J_{3,2+3,4}$ 19.5, H-3B), 5.51 (br s, 1 H, H-1A), 5.60 (br s, 1 H, H-3A), 5.73 (d, 1 H, $J_{NH,2}$ 9.0, NHB), 6.38 (d, 1 H, NHA) and 7.46–8.10 (m, 5 H, ArH); δ_H [$(CD_3)_2SO$] 1.74, 1.85, 1.89, 1.92 and 1.98 (5 s, 5 × 3 H, CH_3CO), 3.33 (s, 2 H, H_2O), 3.39 (br s, 1 H, H-4A), 3.75 (dd, 1 H, H-6A), 3.80 (br d, 1 H, H-2A), 3.84–3.95 (m, 2 H, H-2B, -5B), 4.00 (dd, 1 H, H-6B), 4.10–4.17 (m, 2 H, H′-6A, -6B), 4.67 (br d, 1 H, H-5A), 4.91 (t, 1 H, H-4B), 5.05 (d, 1 H, H-1B), 5.10 (t, 1 H, H-3B), 5.33 (br s, 1 H, H-3A), 5.43 (br s, 1 H, H-1A), 7.58 (t, 2 H, ArH), 7.70 (m, 2 H, NHA, ArH), 7.99 (d, 2 H, ArH) and 8.07 (d, 1 H, NHB); δ_C ($CDCl_3$) 20.6, 20.7 and 20.8 (3 × CH_3CO_2), 23.3 and 23.4 (2 × CH_3CON), 48.1, 49.8 and 53.6 (C-2A, -4A and -2B), 62.3 and 67.9 (C-6A and -6B), 68.0, 71.4, 73.9, 74.4 and 76.0 (C-3A, -5A, -3B, -4B and -5B), 86.5 (C-1B), 101.0 (C-1A), 166.0 (O*C*OPh) and 169.5, 169.7, 170.3, 170.6 and 171.4 (5 × O*C*OCH$_3$) (Found: C, 52.2; H, 5.7; N, 4.2. $C_{29}H_{36}N_2O_{13}S\cdot H_2O$ requires C, 51.93; H, 5.71; N, 4.18%).

4-*S*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-1,6-anhydro-2-azido-3-*O*-benzoyl-2-deoxy-4-thio-β-D-glucopyranose 27

A solution of the thiol **25** (100 mg, 0.275 mmol) was treated with NaH (55% in oil; 12.2 mg, 0.279 mmol) and the resulting thiolate was coupled to the triflate **3** (97 mg, 0.229 mmol) using the same reaction conditions as those described for the synthesis of compound **24**. After quenching and work-up of the reaction mixture as described for compound **24** (AcOH, 120 mm^3), chromatography (1:1, 100 cm^3; 1:4, 70 cm^3, cyclohexane–EtOAc) of the residue gave the thiodisaccharide **27** contaminated with residual DMF. The product was submitted to GPC on Sephadex LH20 and the title compound **27** was obtained pure (101 mg, 70%) as a glass, $[\alpha]_D$ −33 (*c* 1.08, CH_2Cl_2); δ_H 1.88, 1.99, 2.04 and 2.05 (4 s, 4 × 3 H, CH_3CO), 3.16 (br s, 1 H, H-4A), 3.67 (br s, 1 H, H-2A), 3.86 (dd, 1 H, $J_{6,6'}$ 7.5, $J_{6,5}$ 5, H-6A), 3.89 (m, 1 H, H-5B), 4.04 (q, 1 H, $J_{2,1+2,3+2,NH}$ 30, H-2B), 4.11–4.25 (m, 3 H, H′-6A, -6B and H′-6B), 4.90 (br d, 1 H, H-5A), 5.12 (t, 1 H, $J_{4,3+4,5}$ 19.5, H-4B), 5.17 (d, 1 H, $J_{1,2}$ 10.5, H-1B), 5.32 (t, 1 H, $J_{3,2+3,4}$ 19, H-3B), 5.50 (br s, 1 H, H-1A), 5.55 (m, 1 H, H-3A), 5.80 (d, 1 H, $J_{NH,2}$ 9.5, NHB) and 7.47–8.08 (m, 5 H, ArH) (Found: C, 50.9; H, 5.2; N, 8.7. $C_{27}H_{32}N_4O_{12}S$ requires C, 50.94; H, 5.07; N, 8.80%).

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