Stereoselective synthesis of *N*-acetyl thiochitooligosaccharides. Different behaviours of methyl *N*-acetyl-α- and -β-thiochitobiosides during acetolysis

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A stereoselective synthesis of *N*-acetyl-thiochito-di-, -tri- and -tetra-saccharides is described. Coupling of methyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-4-*O*-triflyl-β- 4 or -α-D-galactopyranoside 19 with 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranose 5 in the presence of cysteamine in DMF gave, after de-*O*-acylation, methyl *N*,*N*′-diacetyl-β- 9 and -α-thiochitobioside 21, respectively. Different behaviours of peracetylated methyl β- 10 and α-thiochitobioside 22 towards acetolysis with Ac_2O–AcOH–H_2SO_4 solution were observed, with the β-isomer giving acyclic sugar species together with the desired thiochitobiose peracetate 11, while the α-isomer gave exclusively the thiochitobiose peracetate 11. This remarkable difference between α- and β-glycosides was further demonstrated by comparative acetolysis of methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α- 23 and -β-D-glucopyranoside 24. Methyl *N*,*N*′,*N*″-triacetylthiochitotriosides 29 and 30 were synthesized through conversion of *N*,*N*′-diacetylthiochitobiose peracetate 11 into *N*,*N*′-diacetyl-1,4-dithiochitobiose derivative 28, followed by its coupling with triflates 4 and 19 in the presence of cysteamine. Similarly, extension of the sugar chain to a higher homologue was achieved by converting methyl *N*,*N*′,*N*″-triacetylthiochitotrioside 30 into the *N*,*N*′,*N*″-triacetyl-1,4,4′-trithiochitotriose derivative 33, the coupling of which with triflate 19 in the presence of cysteamine provided the methyl *N*,*N*′,*N*″,*N*‴-tetraacetylthiochitotetraoside 34 after de-*O*-acylation.

Introduction

Beta-1,4-linked oligosaccharides of *N*-acetyl glucosamine (*N*-acetylchitooligosaccharides) are widely distributed in nature. For example, the second most abundant natural organic compound, chitin, is solely composed of β-1,4-linked *N*-acetylglucosamine residues;[1] the inner core structure of *N*-glycosides of most glycoproteins consists of the *N*,*N*′-diacetylchitobiose structure.[2] Oligosaccharides derived from chitin and chitosan are shown to function as regulatory molecules which elicit defence-related responses in various plants.[3] In addition, *N*-acetylchitooligosaccharides are also known as inducers of various chitinases.[4] More recently, novel *N*-acylated lipo-tetra- and -penta-oligosaccharides of β-1,4-linked D-glucosamine were found to be nodulation signal molecules which determine the host specificity of bacteria in *Rhizobium*–legume symbiosis.[5] In nature, the structure and biological functions of these molecules are regulated by various enzymes such as exo- and endo-*N*-acetylglucosaminidases and chitinases.[6]

In order to study these enzymes and related biological systems, we set out to prepare the thioglycosidic analogues of *N*-acetylchitooligosaccharides in which the glycosidic oxygen atoms are replaced by sulfur atoms so that they would be resistant to enzymic hydrolysis. Although some thiooligosaccharides have been prepared and utilized for biological studies,[7] *N*-acetylthiochitooligosaccharides have never been reported. The chemical synthesis of *N*-acetylchitooligosaccharides is also a challenge in contemporary carbohydrate chemistry.[8]

There are two general strategies for construction of interthioglycosidic bonds: (a) S_N2 displacement of a leaving group in one sugar moiety by a 1-thioglycose or the displacement of a glycosyl halide by a sugar thiolate[9] and (b) Lewis acid-catalysed condensation between a glycosyl acceptor containing an SH group and a suitable glycosyl donor.[10] We chose the first strategy because of its well defined stereochemical outcome. In addition, we decided to make use of D-glucosamine or D-galactosamine in their common *N*-acetyl form throughout our syntheses instead of other N-protected forms to minimize protection–deprotection manipulations in a multi-step synthesis. Here we describe a stereoselective synthesis of *N*-acetylthiochito-di-, -tri- and -tetra-saccharides.[11]

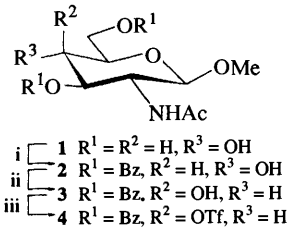
Results and Discussion

Synthesis of methyl *N*,*N*′-diacetyl-β-thiochitobioside and acetolysis of its peracetylated † derivative

Treatment of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[12] with sodium methoxide (3 mol equiv.) in MeOH accomplished the formation of the methyl β-glycoside (93% yield) and de-O-acetylation simultaneously. Neither formation of the possible oxazoline nor other side reactions were observed under these conditions. Selective benzoylation of compound **1** with 2.2 mol equiv. of benzoyl chloride in pyridine at −60 °C gave the benzoate **2** (83%) with a free 4-OH group. Inversion of the C-4 configuration was carried out in a one-pot manner as reported.[13] In brief, compound **2** was esterified with trifluoromethanesulfonic anhydride (triflic anhydride, Tf_2O) in CH_2Cl_2 and pyridine, and the 4-trifloxy group was subsequently displaced with nitrite ion in *N*,*N*-dimethylformamide (DMF), followed by *in situ* hydrolysis of the nitrite ester, to afford the *galacto*-derivative **3** in 82% overall yield. Compound **3** was finally converted into the triflate **4** by triflation with Tf_2O in CH_2Cl_2 and pyridine (Scheme 1).

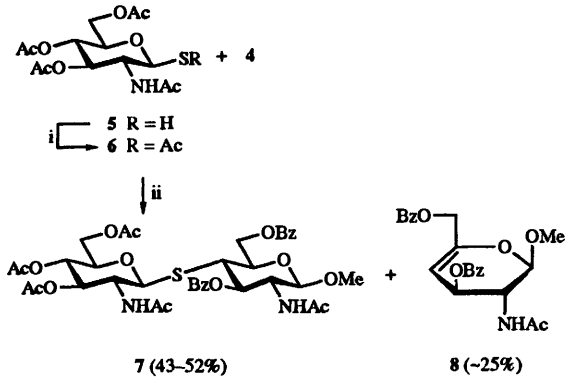
To construct a β-1,4-thioglycosidic linkage between two *N*-acetylglucosamine moieties, the coupling of the triflate **4** with the sodium salt of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranose **5**, prepared by mild alkaline hydrolysis of *S*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)pseudothiourea hydrochloride,[14] was first attempted. Thus, the reaction of the triflate **4** with the sodium salt of thiol **5**, generated *in situ* by addition of an equivalent amount of sodium

† Throughout this paper, the terms peracetate/peracetylated refer to fully acetylated polyol compounds, and not derivatives of peracetic acid.



Scheme 1 *Reagents and solvents:* i, BzCl (2.2 mol equiv.), pyridine (83%); ii, (a) Tf_2O, pyridine, CH_2Cl_2; (b) sodium nitrite, DMF; iii, Tf_2O, pyridine, CH_2Cl_2 (82%)

hydride in DMF at 0 °C, led to a 45% yield of methyl 2-acetamido-*S*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzoyl-2-deoxy-4-thio-β-D-glucopyranoside **7** after chromatographic purification. In the ^{1}H NMR [$(CD_3)_2SO$] spectrum of compound **7**, a doublet at δ 4.851 (J 10.6 Hz) attributable to 1′-H‡ and a triplet at δ 3.356 (J 10.5 Hz) for 4-H clearly indicated that the product was the desired β-1,4-linked thiodisaccharide. In addition to the disaccharide **7**, a monosaccharide by-product was also isolated and identified as methyl 2-acetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-α-L-*threo*-hex-4-enopyranoside **8**, which apparently was derived from the triflate **4** by elimination of triflic acid (Scheme 2).

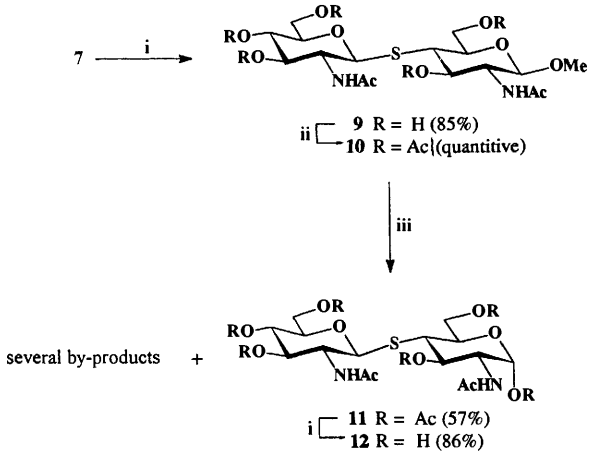


Scheme 2 *Reagents and solvents:* i, Ac_2O, pyridine (93%); ii, sodium hydride, DMF or cysteamine, dithioerythritol (DTE), DMF

In an attempt to reduce formation of the by-product and to raise the yield of disaccharide **7**, the procedure of Blanc-Muesser and Driguez[15] *via in situ* de-S-acetylation and activation of 1-*S*-acetyl-1-thioglucose derivative by 2-sulfanylethylamine (cysteamine) was used. By this method, the coupling of 2-acetamido-3,4,6-tri-*O*-acetyl-1-*S*-acetyl-2-deoxy-1-thio-β-D-glucopyranose **6**[14] with the triflate **4** in DMF indeed provided the thiodisaccharide **7** in 43% yield. The direct cysteamine-promoted reaction of the thiol **5** with the triflate **4** in DMF was also attempted, which gave a somewhat higher yield (52%) of compound **7**. In both cases, however, a significant amount (20–25%) of elimination by-product **8** was again formed (Scheme 2). Since the use of hexamethylphosphoramide (HMPA) as the solvent for the coupling of substrate **5** or **6** with triflate **4** did not seem to improve the yield of thiodisaccharide **7**, and still led to the elimination by-product **8**, DMF was chosen hereafter, because of its ease of removal by evaporation under reduced pressure. De-O-acylation of compound **7** with MeONa–MeOH gave methyl *N*,*N*′-diacetyl-β-thiochitobioside **9** (85%), which was then converted into its peracetylated derivative **10** by O-acetylation with acetic anhydride in pyridine.

To prepare the reducing oligosaccharide, acetolysis was used to remove the anomeric methoxy group. When the peracetylated methyl *N*,*N*′-diacetyl-β-thiochitobioside **10** was treated with an acetolysis solution [acetic anhydride–acetic acid–sulfuric acid (8:2:0.1, v/v)] at room temperature, the reaction gave a mixture of several relatively fast moving compounds compared with substrate **10**, from which the desired thiochitobiose peracetate **11** was isolated only in a low to moderate yield (the best yield in several runs was 57%) with considerable formation of by-products (which will be described in the next section). All attempts to improve the yield of peracetate **11** by using milder acetolysis conditions, such as a lower reaction temperature (0–4 °C) and a lower sulfuric acid concentration were not successful, and still caused significant side reactions.

Interestingly, when the thiochitobiose peracetate **11** was treated with the same acetolysis solution at room temperature for 24 h, the starting material was completely recovered. Therefore, it was clear that side reactions occurred during the cleavage of the anomeric methoxy group rather than after the acetolytic removal of the methoxy group. Conversion of the peracetate **11** into *N*,*N*′-diacetylthiochitobiose **12** was straightforward without difficulty *via* de-O-acetylation with MeONa–MeOH (Scheme 3).



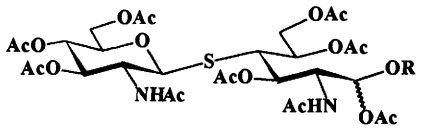
Scheme 3 *Reagents and conditions:* i, MeONa (cat.), MeOH, 20 °C; ii, Ac_2O, pyridine, 20 °C; iii, Ac_2O–AcOH–H_2SO_4 (8:2:0.1, v/v), 20 °C, 7 h

Analysis of the by-products from the acetolysis of compound 10

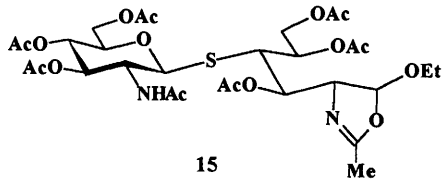
The by-products from the acetolysate were further separated into two fractions by silica gel column chromatography. The fraction emerging slightly later contained at least three acyclic sugar species:§ compounds **13a**, **13b** and **14**, together with minor UV-absorbing compounds. In this mixture, the ^{1}H NMR signal for 5-H in compound **10** at δ 3.92 (a doublet of triplets) moved to a lower field (δ > 4.50) as evidenced by the result of irradiating 4-H at δ 3.05–3.30, indicating O-acetylation of the 5-hydroxy group in these components. Moreover, the mass spectrum (FAB, positive mode) revealed the molecular ions 795 (MH^+ for **14**) and 767 (MH^+ for **13a** and **13b**) in support of the above contention. Finally, de-O-acetylation of these species with MeONa–MeOH gave a single product, *N*,*N*′-diacetylthiochitobiose **12** (Scheme 4). These facts support the formation of the acyclic products during acetolysis, for which there is a precedent.[16]

‡ Primed numbers refer to the second sugar moiety, and double-primed and triple-primed numbers refer to the third and the fourth sugar moieties, respectively.

§ Attempts at further chromatography failed to provide pure compounds.



13a, 13b R = Me (diastereoisomers)
14 R = Ac



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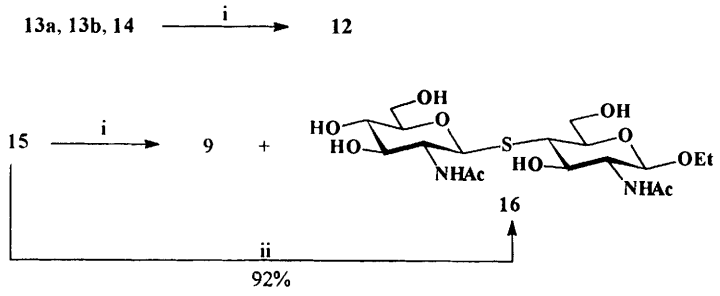
Putative structures of by-products from the acetolysate of *N,N'*-diactetyl β-thiochitobioside **10**



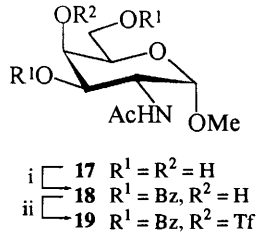
Scheme 4 *Reagents and conditions:* i, (a) MeONa–MeOH, 20 °C; (b) Dowex 50W-X8 (H^+ form); ii, (a) EtONa–EtOH, 20 °C; (b) Dowex 50W-X8 (H^+ form)

Also isolated from the second fraction was a pure compound, the structure of which was assigned on the basis of NMR, MS and elemental analyses (see Experimental section) to be the oxazoline derivative **15**.¶ Interestingly, de-O-acetylation with MeONa–MeOH, followed by neutralization of the base with Dowex 50W-X8 (H^+ form), did not give the expected, corresponding de-O-acetylated oxazoline species, but instead led to the formation of methyl *N,N'*-diacetyl-β-thiochitobioside **9** (39%) and ethyl *N,N'*-diacetyl-β-thiochitobioside **16** (45%) (Scheme 4). On the other hand, treatment of compound **15** with EtONa–EtOH instead of MeONa–MeOH, followed by decationization with Dowex 50W-X8 (H^+ form), provided the ethyl *N,N'*-diacetyl-β-thiochitobioside **16** in excellent yield (Scheme 4). Although the mechanism of the reaction is yet to be clarified, this novel, high-yield transformation may become useful in organic synthesis.

Synthesis of methyl *N,N'*-diacetyl-α-thiochitobioside and acetolysis of its peracetylated derivative

Methyl α- rather than β-glycosides are usually used for 1,4-linked thiooligosaccharide syntheses, and the acetolysis of the resultant methyl α-glycosides of thiooligosaccharides does not seem to cause serious side reactions.[7a,7b,9,17] To improve the acetolysis yield and the yield of thioglycoside, we also turned to the corresponding methyl α-glycosides in our synthesis.

Methyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-galactopyranoside **18** was prepared by selective benzoylation of methyl 2-acetamido-2-deoxy-α-D-galactopyranoside **17** in essentially the same way as described for compound **2**. Triflation of compound **18** with Tf_2O in CH_2Cl_2–pyridine gave the corresponding triflate **19** in quantitative yield (Scheme 5). The coupling of the triflate **19** with the thiol **5** in DMF in the presence of cysteamine gave a 63% yield of thiodisaccharide **20**, substantially better than that from the coupling of β-glycoside **4** with the thiol **5**. Although formation of the elimination product (not isolated for further identification) was also detected by TLC, it occurred to a lesser extent as compared with that of β-glycoside **4**. Again, the β-1,4-thioglycosidic linkage in compound **20** was confirmed by NMR spectroscopy. De-O-acylation of compound **20** with MeONa–MeOH gave methyl *N,N'*-diacetyl-α-thiochitobioside **21**, which was converted into



17 $R^1 = R^2 = H$
18 R^1 = Bz, $R^2 = H$
19 R^1 = Bz, R^2 = Tf

Scheme 5 *Reagents and solvents:* i, BzCl (2.2 mol equiv.), pyridine (85%); ii, Tf_2O, pyridine, CH_2Cl_2 (quantitative)

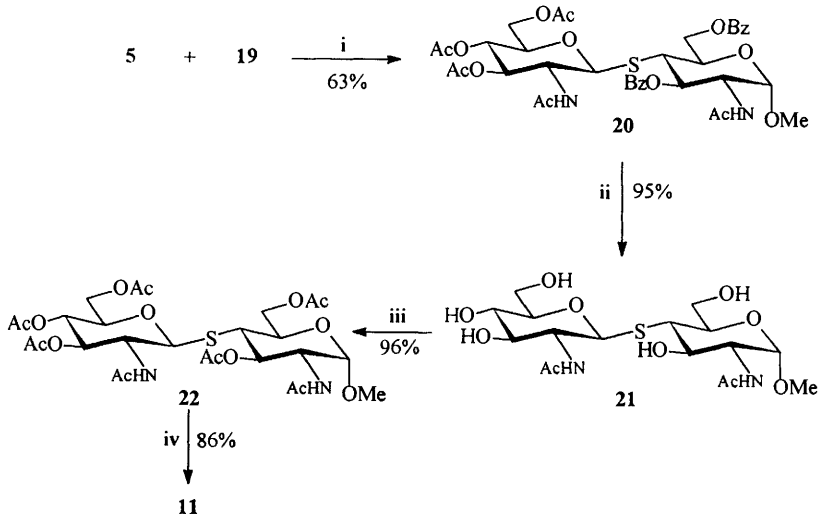
its peracetylated derivative **22**. Acetolysis of the peracetylated methyl α-thiochitobioside **22** with Ac_2O–AcOH–H_2SO_4 (8:2:0.1, v/v) at room temperature proceeded very smoothly to give the desired thiochitobiose peracetate **11** in excellent yield (Scheme 6). These results clearly demonstrated the different behaviour of peracetylated methyl α- and β-thiochitobiosides towards acetolysis.

Comparative acetolysis of methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α- and -β-D-glucopyranoside

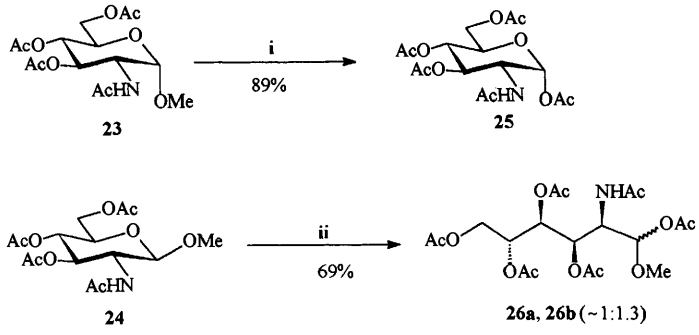
The different behaviours manifested by methyl α- and β-thiochitobiosides during acetolysis prompted us to examine the reaction of methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α- (**23**) and -β-D-glucopyranoside (**24**) with an Ac_2O–AcOH–H_2SO_4 mixture to examine whether those observations described above are a general phenomenon. It was found that acetolysis of α-glycoside **23** led to 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose **25** in good yield without any detectable side reactions, whereas reaction of the β-glycoside **24** with the same acetolysis solution gave a mixture of several products, among which only a trace of pyranose derivative **25** could be detected by NMR spectroscopy. TLC [15:1, v/v ($CHCl_3$–EtOH)] of the reaction mixture showed the formation of two major components which moved at almost the same position. The two compounds were carefully separated by silica gel column chromatography and identified on the basis of NMR and MS analyses to be *R* and *S* isomers of acyclic acetals, (1*R* and 1*S*)-2-acetamido-1,3,4,5,6-penta-*O*-acetyl-2-deoxy-1-methoxy-D-glucitol (**26a** and **26b**, ~1:1.3),‖ in a combined

¶ The pathway for formation of this novel compound, especially the introduction of the ethyl group, is not clear as yet. It may be derived from the acyclic sugar species during the work-up and chromatography with a mixture of $CHCl_3$ and EtOH.

‖ The absolute configuration of C-1 in compounds **26a** and **26b** is yet to be determined.



Scheme 6 *Reagents and conditions:* i, cysteamine, DTE, DMF, 20 °C; ii, MeONa (cat.), MeOH, 20 °C; iii, Ac_2O, pyridine, 20 °C; iv, Ac_2O–AcOH–H_2SO_4 (8:2:0.1, v/v), 20 °C, 7 h



Scheme 7 *Reagents and conditions:* i, Ac_2O–AcOH–H_2SO_4 (8:2:0.1, v/v), 20 °C, 6 h; ii; Ac_2O–AcOH–H_2SO_4 (8:2:0.1, v/v), 20 °C, 2 h

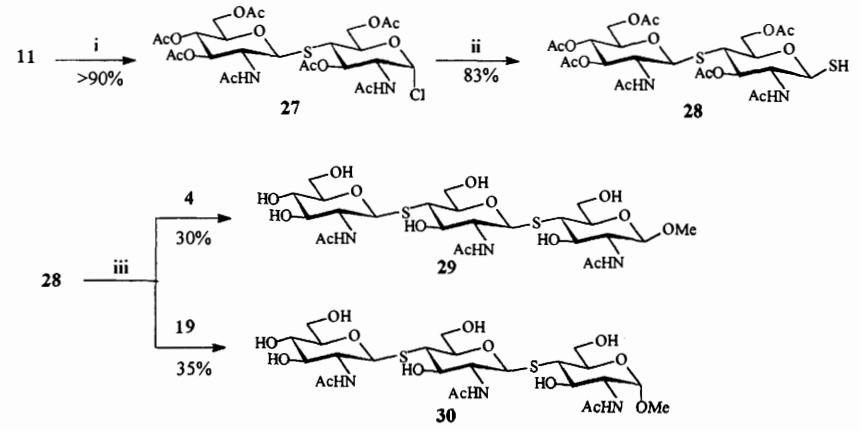
yield of 69% (Scheme 7). It was noteworthy that the acetolysis of β-glycoside **24** was much faster than that of the corresponding α-isomer **23** under the same reaction conditions. Angibeaud and Utille[18] reported a similar result in the reaction of methyl α- and β-D-gluco- or -D-manno-pyranosides with acetic anhydride in the presence of trimethylsilyl trifluoromethanesulfonate, which resulted in different products, with the α-isomer giving the 1-*O*-acetylpyranose product while the β-isomer gave the acyclic sugars resulting from ring opening. Our results showed that the same stereoselective reactions also occur with 2-acetamido-2-deoxysugars, although the acetolysis conditions used are different. Although these results might be due to some stereoelectronic effects that α- and β-anomeric methoxy groups differentially exert, the fact that peracetylated methyl *N*,*N*′-diacetyl-β-thiochitobioside **10** still yielded a significant amount of the pyranose product **11**, while the peracetylated methyl β-glucosaminide **24** gave almost exclusively the acyclic sugars, suggests that the substituents (*e.g.*, the 4-*S*-substituent and the equatorial 2-acetamido group in compound **10**) may also control the acetolytic outcome. This contention is also enhanced by the observation of Nishimura and Kuzuhara[19] on the acetolysis of peracetylated methyl *N*,*N*′-diphthaloyl-β-chitobioside, which gave exclusively the corresponding chitobiose peracetate instead of acyclic products.

Extension to higher thiochitooligosaccharides

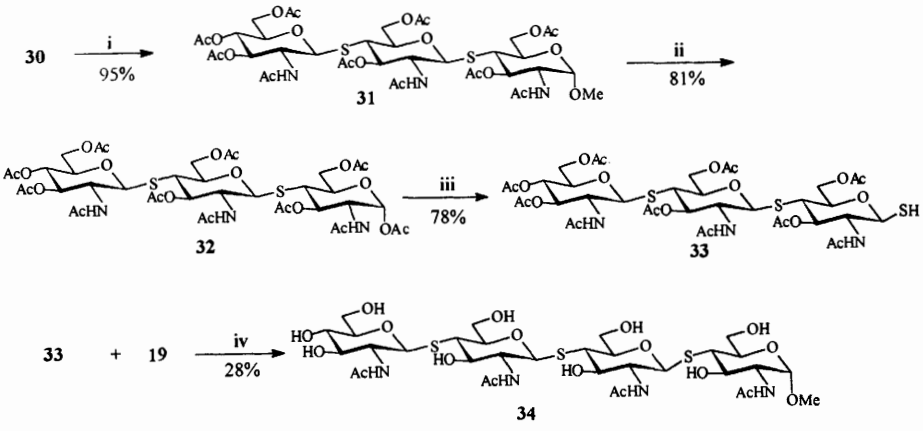
To extend the thiochitobiose to higher thiooligosaccharides, thiochitobiose peracetate **11** was converted into its 1-thiol derivative **28** *via* glycosyl chloride **27**. Treatment of compound **11** with acetyl chloride saturated with hydrogen chloride gave glycosyl chloride **27**, which was refluxed with thiourea in dry acetone to give the thiol **28** (83% in two steps). Coupling of the thiol **28** with the triflate **4** in DMF in the presence of cysteamine, followed by de-O-acylation and purification, afforded methyl *N*,*N*′,*N*″-triacetyl-β-thiochitotrioside **29** in 30% yield. Similarly, reaction of thiol **28** with the triflate **19** under the same condition yielded the methyl *N*,*N*′,*N*″-triacetyl-α-thiochitotrioside **30** (35%) (Scheme 8).

A synthesis of thiochitotetraoside was carried out in the same manner. First, trisaccharide **30** was O-acetylated with acetic anhydride–pyridine to give **31**. The 1-methoxy group in compound **31** was removed by acetolysis with Ac_2O–AcOH–H_2SO_4, providing thiochitotriose peracetate **32** in 81% yield. Conversion of 1-acetate **32** into the 1-thiol derivative **33** was successfully achieved in the same way as that described for the preparation of the thiol **28** *via* the glycosyl chloride (see above). The overall yield from these two steps was 78%. Finally, coupling of the thiol **33** with the triflate **19** was carried out in DMF in the presence of cysteamine to give, after de-O-acylation and chromatographic purification, the target methyl *N*,*N*′,*N*″,*N*‴-tetraacetyl-α-thiochitotetraoside **34** in 28% yield (from compound **33**) (Scheme 9). The structure of compound **34** was confirmed by NMR spectroscopy. The ^1H NMR spectrum of compound **34** showed that its 1-H resonated at δ 4.754 as a doublet with a small *J*-value (3.3 Hz), indicating the presence of an α-D-glycosidic linkage; the three other anomeric protons, 1′-H, 1″-H and 1‴-H, resonated at δ 4.701–4.672, each as a doublet with a relatively large coupling constant (*J* 10 Hz), clearly showing that all three thio-glycosidic linkages in compound **34** are of β-D-configuration. Other spectral features are in agreement with the structure.

In summary, *N*-acetylthiochitooligosaccharides up to tetraose were stereoselectively synthesized for the first time, *via* an S_N2-type reaction. The stereoselective reaction of peracetylated methyl α- and β-thiochitobiosides with acetic anhydride and acetic acid in the presence of sulfuric acid (acetolysis) was



Scheme 8 *Reagents and conditions:* i, acetyl chloride, CH_2Cl_2, HCl (gas), 0–20 °C; ii, (a) thiourea, acetone, reflux; (b) aq. sodium sulfite, 20 °C; (c) 5% hydrochloric acid; iii, (a) cysteamine, DTE, DMF, 20 °C; (b) MeONa, MeOH, 20 °C; (c) purification by Sephadex G-10 column chromatography



Scheme 9 *Reagents and conditions:* i, Ac_2O, pyridine, 20 °C; ii, Ac_2O–AcOH–H_2SO_4 (8:2:0.1, v/v), 20 °C, 7 h; iii, (a) acetyl chloride, CH_2Cl_2, HCl (gas), 0–20 °C; (b) thiourea, acetone, reflux; (c) aq. sodium sulfite, 20 °C; (d) 5% hydrochloric acid; iv, (a) cysteamine, DTE, DMF, 20 °C; (b) MeONa, MeOH, 20 °C; (c) purification by Sephadex G-10 column chromatography

observed. The difference between the α- and β-isomers was further demonstrated by comparative acetolysis of methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α- and -β-D-glucopyranoside. Biological applications of the synthetic *N*-acetylthiochitooligosaccharides are currently under way.

Experimental

General procedures

Mps were determined with a Fisher-Johns apparatus and are not corrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. ^{1}H NMR spectra were recorded at 25 °C with a Bruker AMX-300 spectrometer (300 MHz) for solutions in $CDCl_3$ (internal standard: Me_4Si, δ 0), D_2O (internal standard: HDO, δ 4.778) or [$(CD_3)_2SO$] (internal standard: Me_2SO, δ 2.500), as specified. Assignment of the sugar ring-protons was made by first-order analysis and confirmed by the homonuclear decoupling technique. *J* Values are given in Hz. Mass spectra were recorded with a VG 70-S mass spectrometer in a chemical ionization (CI) mode (reagent gas, NH_3) or a fast-atom bombardment (FAB) positive mode (matrix: 3-nitrobenzyl alcohol, NBA). TLC was carried out on precoated plates of silica gel (E. Merck, $60F_{254}$, layer thickness 0.25 mm), and the carbohydrate components were detected by charring at 140 °C after spraying of the plates with a solution of 15% H_2SO_4 in 50% EtOH, or by UV absorption. Column chromatography was performed on silica gel (E. Merck). Ratios of solvents for TLC and column chromatography were expressed in volumes. All evaporations and concentrations were carried out below 40 °C under reduced pressure using a water aspirator. Light petroleum refers to the fraction with distillation range 60–90 °C.

Methyl 2-acetamido-2-deoxy-β-D-glucopyranoside 1

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride [12] (14.65 g, 40 mmol) was dissolved in CH_2Cl_2 (10 cm^3)–MeOH (70 cm^3); the solution was treated with a solution of sodium methoxide in MeOH (4.37 mol dm^{-3}; 27.5 cm^3), and stirred at room temperature for 30 min. The suspension was neutralized with Dowex 50W-X8 (H^+ form) resin as judged by pH test paper, filtered, and the resin was washed with methanol (2 × 50 cm^3). The filtrate and washings were combined and concentrated to give the methyl β-glycoside **1** (8.8 g, 93%) as a solid. Crystallization from ethanol gave crystalline **1** (7.15 g, 76%), mp 198–200 °C (lit.,[20] 204 °C); R_f 0.31 (65:25:4 $CHCl_3$–MeOH–water); $\delta_H(D_2O)$ 4.413 (1 H, d, *J* 8.4, 1-H), 3.926–3.629 (3 H, m, 3-H and 6-H_2), 3.475 (3 H, s, OMe), 3.525–3.366 (3 H, m, 2-, 4- and 5-H) and 2.005 (3 H, s, NAc).

Methyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranoside 2

To a solution of compound **1** (2.35 g, 10 mmol) in dry pyridine (25 cm^3) at −60 °C was added dropwise benzoyl chloride (2.56 cm^3, 22 mmol). The resultant suspension was stirred at that temperature for 2 h, then was warmed gradually to room temperature. Upon addition of methanol (1.5 cm^3), the reaction mixture became a clear solution. Pyridine was removed by evaporation. The residue was dissolved in chloroform (100 cm^3), and the solution was washed successively with 5% hydrochloric acid, cold saturated aq. $NaHCO_3$ and water, dried (Na_2SO_4), filtered and evaporated to give a solid residue. Crystallization from EtOH gave crystalline dibenzoate **2** (2.20 g). The mother liquor was concentrated and the residue was subjected to column chromatography with (25:1) $CHCl_3$–

EtOH to obtain additional material (1.50 g). The total yield of dibenzoate **2** was 3.70 g (83%) (Found: C, 61.0; H, 5.7; N, 2.95. $C_{23}H_{25}NO_8 \cdot 0.5H_2O$ requires C, 61.05; H, 5.8; N, 3.1%); mp 97–100 °C (from EtOH); R_f 0.58 (9:1 $CHCl_3$–EtOH); $\delta_H(CDCl_3)$ 8.102–7.410 (10 H, m, 2 × Bz), 5.602 (1 H, br d, *J* 8.8, NH), 5.334 (1 H, t, *J* 9.1, 3-H), 4.795 (1 H, dd, *J* 3.5 and 12.1, 6-H^a), 4.602 (1 H, dd, *J* 2.4, 6-H^b), 4.568 (1 H, d, *J* 8.4, 1-H), 4.135 (1 H, q, *J* 8.7, 2-H), 3.801 (1 H, t, *J* 9.0, 4-H), 3.743 (1 H, m, 5-H), 3.529 (3 H, s, OMe), 3.160 (1 H, br s, OH) and 1.877 (3 H, s, NAc).

Methyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-galacto-pyranoside 3

To a solution of triflic anhydride (0.76 cm^3, 4.5 mmol) in CH_2Cl_2 (15 cm^3) at −15 °C was added dropwise a solution of pyridine (0.73 cm^3, 9.0 mmol) in CH_2Cl_2 (2 cm^3). A solid appeared during the initial phase of the reaction, but dissolved after the complete addition of pyridine. Then a solution of compound **2** (1.33 g, 3.0 mmol) in CH_2Cl_2 (5 cm^3) was added, and the resulting mixture was stirred at −15 °C for 1 h. The reaction mixture was diluted with CH_2Cl_2 (50 cm^3), washed successively with cold 5% hydrochloric acid, aq. $NaHCO_3$ and water, dried (Na_2SO_4) and filtered. The filtrate was evaporated to obtain methyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-4-*O*-trifluoromethanesulfonyl-β-D-glucopyranoside (1.90 g, quantitative) as a yellow-brown syrup, which was used immediately in the next step without purification.

The syrupy triflate thus obtained was dissolved in DMF (6 cm^3). Sodium nitrite (2.10 g, 30 mmol) was added, and the mixture was stirred at room temperature for 16 h. After dilution with $CHCl_3$ (100 cm^3), the reaction mixture was washed thoroughly and successively with brine and water, dried (Na_2SO_4), filtered and evaporated. Crystallization of the solid residue from ethanol gave compound **3** (720 mg). The mother liquor was evaporated and the residue was subjected to column chromatography with (25:1) $CHCl_3$–EtOH as eluent, which gave additional material (370 mg). The total yield of *compound* **3** was 1.09 g (82% from **2**) (Found: C, 62.1; H, 5.8; N, 3.0. $C_{23}H_{25}NO_8$ requires C, 62.3; H, 5.7; N, 3.15%); mp 237–242 °C; R_f 0.55 (9:1 $CHCl_3$–EtOH); $\delta_H[(CD_3)_2SO]$ 8.014–7.503 (10 H, m, 2 × Bz), 7.862 (1 H, d, *J* 9.3, NH), 5.463 (1 H, d, *J* 5.86, OH), 4.922 (1 H, dd, *J* 3.0 and 9.5, 3-H), 4.462 (1 H, d, *J* 8.5, 1-H), 4.440 (2 H, t, *J* 6.4, 6-H_2), 4.310 (1 H, q, 2-H), 4.135 (1 H, m, 4-H), 4.007 (1 H, t, *J* 6.4, 5-H), 3.353 (3 H, s, OMe) and 1.699 (3 H, s, NAc).

Methyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-4-*O*-trifluoro-methanesulfonyl-β-D-galactopyranoside 4

To a cold solution of triflic anhydride (0.672 cm^3, 4.0 mmol) in CH_2Cl_2 (15 cm^3) were added pyridine (0.65 cm^3, 8.0 mmol), and then compound **3** (887 mg, 2.0 mmol) in portions, and the mixture was stirred at 0 °C for 2 h. The reaction mixture was poured into ice–water and extracted with CH_2Cl_2 (2 × 20 cm^3). The extracts were combined, washed successively with cold 5% hydrochloric acid, cold saturated aq. $NaHCO_3$ and water, dried (Na_2SO_4), filtered and washed. The filtrate and washings were combined and evaporated to give the triflate **4** (1.25 g, quantitative) as a pale yellow foam; R_f 0.72 (9:1 $CHCl_3$–EtOH); $\delta_H(CDCl_3)$ 8.022–7.398 (10 H, m, 2 × Bz), 5.917 (1 H, dd, *J* 2.8 and 10.5, 3-H), 5.879 (1 H, d, *J* 8.4, NH), 5.428 (1 H, d, *J* 2.8, 4-H), 4.961 (1 H, d, *J* 8.3, 1-H), 4.716–4.628 (1 H, m, 6-H^a), 4.281–4.193 (2 H, m, 5-H and 6-H^b), 3.897 (1 H, dt, 2-H), 3.485 (3 H, s, OMe) and 1.838 (3 H, s, NAc). The pale-yellow foam was used in the next step without purification.

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-gluco-pyranose 5 and 2-acetamido-3,4,6-tri-*O*-acetyl-1-*S*-acetyl-2-deoxy-1-thio-β-D-glucopyranose 6

The reaction of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (7.32 g, 20 mmol) and powdered thiourea (3.05 g, 40 mmol) in dry acetone (100 cm^3) was performed according to the procedure of Horton and Wolfrom[14] to give *S*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl) pseudothiourea hydrochloride (7.1 g, 80%) as crystals, mp 178–180 °C (lit.,[14] 179–181 °C).

The pseudothiourea derivative (7.0 g, 15.8 mmol) thus obtained was dissolved in a mixture of water (60 cm^3) and acetone (10 cm^3), powdered sodium sulfite (4.03 g, 32 mmol) was added, and the resultant mixture was stirred for 20 min at room temperature under nitrogen. The solution was then adjusted to pH 5 (judged with pH test paper) by dropwise addition of 10% hydrochloric acid, and extracted with chloroform (3 × 50 cm^3). The organic layer was washed successively with brine and water, dried (Na_2SO_4) and filtered. The filtrate was evaporated to afford the thiol **5** (5.07 g, 88%) as a solid which was sufficiently pure for the next reaction. An analytical sample was obtained by crystallization from ethanol; mp 150–152 °C (from EtOH); R_f 0.46 (9:1 $CHCl_3$–EtOH); $\delta_H(CDCl_3)$ 5.603 (1 H, d, *J* 9.3, NH), 5.135 (1 H, t, *J* 9.3, 3-H), 5.071 (1 H, t, *J* 9.4, 4-H), 4.569 (1 H, t, *J* 9.9, 1-H), 4.245 (1 H, dd, *J* 4.8 and 12.5, 6-H^a), 4.180–4.082 (2 H, m, 2-H and 6-H^b), 3.682 (1 H, m, 5-H), 2.577 (1 H, d, *J* 9.6, SH), 2.104, 2.047, 2.033 and 1.991 (each 3 H, s, together 3 × OAc and NAc).

S-Acetylation of the thiol **5** (1.1 g, 3.0 mmol) with acetic anhydride (3 cm^3) and pyridine (5 cm^3) at room temperature for 3 h afforded, after the usual work-up, the 1-*S*-acetyl derivative **6**[14] (1.13 g, 93%) as needles; mp 194–196 °C (from EtOH) [lit.,[14] 199–200 °C (from MeOH–diethyl ether]; R_f 0.60 (9:1 $CHCl_3$–EtOH); $\delta_H(CDCl_3)$ 5.589 (1 H, d, *J* 9.7, NH), 5.48 (1 H, d, *J* 10.7, 1-H), 5.141 (1 H, t, *J* 9.8, 3-H), 5.089 (1 H, t, *J* 9.5, 4-H), 4.356 (1 H, q, *J* 10.1, 2-H), 4.242 (1 H, dd, *J* 4.4 and 12.5, 6-H^a), 4.089 (1 H, dd, *J* 1.9 and 12.5, 6-H^b), 3.768 (1 H, m, 5-H), 2.375 (3 H, s, SAc), 2.082, 2.037 (2) and 1.924 (each 3 H, s, together 3 × OAc and NAc).

Methyl 2-acetamido-*S*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzoyl-2-deoxy-4-thio-β-D-glucopyranoside 7 and methyl 2-acetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-α-L-*threo*-hex-4-enopyranoside 8

(a) Coupling of the thiol 5 with the triflate 4 by sodium hydride. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranose **5** (400 mg, 1.1 mmol) was dissolved in DMF (4 cm^3), the solution was cooled to 0 °C, and sodium hydride (60% dispersion in mineral oil; 48 mg, 1.2 mmol) was added. The mixture was stirred for 10 min, then the triflate **4** (590 mg, ~1.0 mmol) was added, and the resultant mixture was stirred at 0–20 °C for 4 h, when TLC (9:1 $CHCl_3$–EtOH) showed complete disappearance of triflate **4**. The reaction mixture was poured into ice–water containing $NaHCO_3$, and a pale yellow precipitate was formed, which was collected by filtration. The precipitate was dissolved in $CHCl_3$ (80 cm^3) containing EtOH (8 cm^3) which facilitated the dissolution. The solution was washed successively with aq. $NaHCO_3$ and water, dried, filtered and evaporated. The residue was dissolved in (5:1) $CHCl_3$–EtOH and applied to a silica gel column, which was first eluted with (30:1) $CHCl_3$–EtOH to give compound **8** (98 mg, 23%). Further elution of the column with (15:1) $CHCl_3$–EtOH as eluent provided the thiodisaccharide **7** (355 mg, 45%).

Compound **7** (Found: C, 56.1; H, 5.7; N, 3.4; S, 4.0. $C_{27}H_{44}N_2O_{15}S$ requires C, 56.3; H, 5.6; N, 3.55; S, 4.1%); mp 165 °C (decomp.); R_f 0.50 (9:1 $CHCl_3$–EtOH); $\delta_H[(CD_3)_2SO]$ 8.061–7.465 (12 H, m, 2 × Bz and 2 × NH), 5.187 (1 H, t, *J* 10.5, 3-H), 5.022 (1 H, t, *J* 9.5, 3′-H), 4.870 (1 H, dd, *J* 1.9 and 12.3, 6-H^a), 4.851 (1 H, d, *J* 10.6, 1′-H), 4.671 (1 H, t, *J* 9.6, 4′-H), 4.608 (1 H, dd, *J* 4.8 and 12.2, 6-H^b), 4.544 (1 H, d, *J* 8.5, 1-H), 4.072–3.610 (6 H, m, 2-, 2′-, 5- and 5′-H and 6′-H_2), 3.356 (1 H, t, *J* 10.5, 4-H), 3.297 (3 H, s, OMe), 1.997, 1.932 and 1.872 (each 3 H, s, OAc) and 1.629 and 1.592 (each 3 H, s, NAc); *m/z*

(FAB$^+$) 789 [(M + H)$^+$, 10%], 330 (32), 154 (65) and 105 (100).

Compound **8** (Found: C, 64.6; H, 5.6; N, 3.2. $C_{23}H_{23}NO_7$ requires C, 64.9; H, 5.4; N, 3.2%); mp 258 °C (decomp.); R_f 0.63 (15:1, $CHCl_3$–EtOH); $\delta_H(CDCl_3)$ 8.080–7.425 (10 H, m, 2 × Bz), 5.814 (1 H, d, *J* 9.1, NH), 5.404 (1 H, dd, *J* 0.85 and 3.91, 4-H), 5.246 (1 H, ddd, *J* 0.80, 2.1 and 3.5, 3-H), 5.069 (1 H, dd, *J* 0.72 and 2.9, 1-H), 4.869 and 4.778 (each 1 H, d, *J* 13.6, together 6-H_2), 4.608 (1 H, m, 2-H), 3.519 (3 H, s, OMe) and 2.026 (3 H, s, NAc); *m/z* (CI$^+$) 443 [(M + NH_4)$^+$, 0.3%], 426 [(M + H)$^+$, 1] and 304 (100).

(b) Coupling of the *S*-acetate 6 with the triflate 4 in the presence of cysteamine. To a solution of *S*-acetate **6** (487 mg, 1.2 mmol) and dithioerythritol (DTE) (154 mg, 1.0 mmol) in DMF (8 cm^3) at 0 °C were sequentially added cysteamine (116 mg, 1.5 mmol) and the triflate **4** (580 mg, 1.0 mmol). The mixture was then stirred under nitrogen at room temperature overnight. The reaction mixture was poured onto crushed ice and the pale yellow precipitate generated was collected and subjected to chromatography, as described in procedure (**a**), to give thiodisaccharide **7** (339 mg, 43%) and compound **8** (76.6 mg, 18%).

(c) Coupling of the thiol 5 with the triflate 4 in the presence of cysteamine. To a solution of thiol **5** (799 mg, 2.2 mmol) in DMF (10 cm^3) at 0 °C were added cysteamine (193 mg, 2.5 mmol), DTE (231 mg, 1.5 mmol), and then the triflate **4** (1.25 g, ~2.0 mmol) in portions. The mixture was stirred at room temperature for 3 h, when TLC showed complete disappearance of substrate **4**. Processing of the reaction mixture in the same way as described in procedure (**b**) gave the thiodisaccharide **7** (820 mg, 52%) and compound **8** (187 mg, 22%).

Methyl 2-acetamido-*S*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-deoxy-4-thio-β-D-glucopyranoside 9

Compound **7** (513 mg, 0.65 mmol) was added to a solution of MeONa–MeOH (10 mmol dm^{-3}; 20 cm^3). The mixture was stirred at room temperature overnight. The precipitate in the reaction mixture was dissolved by addition of water, and the solution was neutralized with Dowex 50W-X8 (H$^+$ form) as judged by pH test paper and filtered. The filtrate was evaporated to dryness and the residue was dissolved in water (40 cm^3). After extraction with diethyl ether (3 × 20 cm^3), the water layer was evaporated to give a precipitate, which was crystallized from EtOH to furnish crystalline *compound* **9** (252 mg, 85%) (Found: C, 44.7; H, 6.8; N, 6.2; S, 7.0. $C_{17}H_{30}N_2O_{10}S$ requires C, 44.9; H, 6.65; N, 6.2; S, 7.05%); mp 200 °C (decomp.); R_f 0.44 (55:40:5 $CHCl_3$–MeOH–water); $\delta_H(D_2O)$ 4.639 (1 H, d, *J* 10.4, 1′-H), 4.336 (1 H, d, *J* 8.33, 1-H), 4.041–3.346 (11 H, m, sugar ring protons), 3.418 (3 H, s, OMe), 2.797 (1 H, t, *J* 10.6, 4-H) and 1.970 and 1.956 (each 3 H, s, Ac); *m/z* (CI$^+$) 455 [(M + H)$^+$, 10%], 252 (71) and 204 (100).

Methyl 2-acetamido-*S*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-deoxy-4-thio-β-D-glucopyranoside 10

A solution of compound **9** (600 mg, 1.32 mmol) in pyridine (10 cm^3) and acetic anhydride (10 cm^3) was stirred at room temperature overnight. Ice–water (10 cm^3) was added to the mixture, which was then stirred at room temperature for 3 h, then evaporated to dryness. Column chromatography of the residue with (20:1) $CHCl_3$–EtOH as eluent afforded compound **10** (880 mg, quantitative) as crystals, mp 248–250 °C; R_f 0.40 (9:1 $CHCl_3$–EtOH); $\delta_H(CDCl_3)$ 5.700 (1 H, d, *J* 8.8, NH), 5.647 (1 H, t, *J* 10.3, 3-H), 5.610 (1 H, d, *J* 8.0, NH), 5.065 (1 H, t, *J* 9.3, 3′-H), 5.010 (1 H, t, *J* 9.3, 4′-H), 4.763 (1 H, d, *J* 8.4, 1-H), 4.731 (1 H, d, *J* 9.5, 1′-H), 4.532 (2 H, br d, *J* 3.2, 6-H_2), 4.218–4.155 (2 H, m, 2′-H and 6′-H^a), 4.057 (1 H, dd, *J* 6.3 and 12.2, 6′-H^b), 3.918 (1 H, dt, *J* 3.2 and 10.5, 5-H), 3.729 (1 H, m, 5′-H), 3.479 (3 H, s, OMe), 3.454 (1 H, m, 2-H), 2.834 (1 H, t, *J* 10.5, 4-H), 2.104, 2.096, 2.071, 2.034, 2.012, 1.996 and 1.910 (each 3 H, s, together 5 × OAc and 2 × NAc); *m/z* (CI$^+$) 665 [(M + H)$^+$, 53%], 633 (M$^+$ − OMe, 12) and 330 (100).

2-Acetamido-*S*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-1,3,6-tri-*O*-acetyl-2-deoxy-4-thio-α-D-glucopyranose 11. Acetolysis of peracetylated methyl β-D-thiochitobioside 10

Compound **10** (680 mg, 1.02 mmol) was dissolved in a freshly prepared solution of Ac_2O–AcOH–H_2SO_4 (8:2:0.1, 12 cm^3), and the mixture was stirred at room temperature for 7 h, when TLC (9:1 $CHCl_3$–EtOH) showed complete disappearance of the starting material. After addition of sodium acetate (100 mg), the reaction mixture was poured into ice–water (10 cm^3) and stirred for 3 h at room temperature to decompose the excess of acetic anhydride. The solution was then evaporated to dryness. The residue was dissolved in $CHCl_3$, and the solution was washed successively with aq. $NaHCO_3$ and water, dried (Na_2SO_4) and filtered. The filtrate was evaporated to give a residue, which was subjected to column chromatography. Elution of the column first with (35:1) $CHCl_3$–EtOH gave a mixture of by-products (100 mg), which was collected and subjected to further analysis. Subsequent elution of the column with (15:1) $CHCl_3$–EtOH gave the peracetylated *N,N′*-diacetyl-4-thio-α-D-chitobiose **11** (404 mg, 57%), mp 215 °C (decomp.) (from EtOH); R_f 0.45 (9:1 $CHCl_3$–EtOH); $\delta_H(CDCl_3)$ 6.161 (1 H, d, *J* 3.4, 1-H), 5.814 and 5.476 (each 1 H, d, *J* 9.5, NH), 5.173 (1 H, t, *J* 10.7, 3-H), 5.084 (1 H, t, *J* 9.6, 3′-H), 4.991 (1 H, t, *J* 9.6, 4′-H), 4.630 (1 H, dd, *J* 3.2 and 12.1, 6-H^a), 4.575 (1 H, d, *J* 10.5, 1′-H), 4.503 (1 H, dt, *J* 3.5 and 9.6, 2-H), 4.304 (1 H, dd, *J* 1.8 and 12.1, 6-H^b), 4.185–4.086 (4 H, m, 2′- and 5-H and 6′-H_2), 3.636–3.604 (1 H, m, 5′-H), 3.007 (1 H, t, *J* 11.2, 4-H), 2.203, 2.110, 2.106, 2.051, 2.035, 2.025, 1.975 and 1.935 (each 3 H, s, together 6 × OAc and 2 × NAc); *m/z* (FAB$^+$) 693 [(M + H)$^+$, 16%], 633 (8) and 330 (100).

2-Acetamido-*S*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-deoxy-4-thio-D-glucopyranose 12

To a solution of methanol (20 cm^3) containing a catalytic amount of sodium methoxide was added compound **11** (510 mg, 0.736 mmol). After the mixture had been stirred at room temperature overnight, a precipitate appeared, which was dissolved by addition of water (5 cm^3). The mixture was neutralized with Dowex 50W-X8 (H$^+$ form) as judged by pH test paper, and the resin was filtered off and washed with water. The filtrate was evaporated to give a solid, which was crystallized from EtOH to provide crystalline *compound* **12** (279 mg, 86%) (Found: C, 40.2; H, 6.9; N, 5.9; S, 6.8. $C_{16}H_{28}N_2O_{10}S{\cdot}2H_2O$ requires C, 40.3; H, 6.8; N, 5.9; S, 6.7%); mp 230–232 °C (from EtOH); R_f 0.30–0.38 (55:40:5 $CHCl_3$–MeOH–water); $\delta_H(D_2O)$ 5.176 (0.57 H, d, *J* 3.3, 1-H in α-anomer), 4.682 (0.57 H, d, *J* 10.4, 1′-H in α-anomer), 4.661 (0.43 H, d, *J* 10.4, 1′-H in β-anomer), 4.621 (0.43 H, d, *J* 8.2, 1-H in β-anomer), 4.120–3.401 (11 H, m, sugar ring protons), 2.869 and 2.833 (1 H in total, each t, *J* 10.3, together 4-H), 1.986 (6 H, br s, 2 × NAc); *m/z* (FAB$^+$) 463 [(M + Na)$^+$, 58%], 441 [(M + H)$^+$, 6], 307 (30) and 176 (100).

Separation and analysis of by-products from the acetolysate of peracetylated methyl β-thiochitobioside 10

A mixture (~100 mg) of the by-products resulting from the acetolysis of compound **10** was further chromatographed with (40:1) $CHCl_3$–EtOH as eluent to give two closely eluting major fractions. The slightly later eluting fraction of the two was actually a mixture of several components including compounds **13a**, **13b** and **14**, together with a minor unidentified UV-absorbing material. This fraction was evaporated to provide a syrupy oil (15 mg); $\delta_H(CDCl_3)$ 6.952 (d, *J* 4.6, 1-H from **14**), 5.685–5.722 (overlapping, 1-H from **13a** and **13b**), 3.450 and 3.458 (s, OMe from **13a** and **13b**), 3.300–3.053 (overlapping,

4-H in **13a**, **13b** and **14**); *m/z* (FAB$^+$) 795 [(M + H)$^+$, 0.6%, from **14**), 767 [(M + H)$^+$, 1.3%, from **13a** and **13b**) and 330 (100).

Treatment of the syrupy oil (10 mg) with MeONa–MeOH (50 mmol dm^{-3}; 1 cm^3) for 2 h, followed by neutralization (judged with pH test paper) with Dowex 50W-X8 (H$^+$ form) and evaporation, gave a solid, which was fractionated on a Sephadex G-10 column (1.5 × 95 cm) pre-equilibrated and eluted with water to provide *N,N'*-diacetylthiochitobiose **12** (3.2 mg), which was identical with the known standard.

The second fraction (~50 mg), which contained a major component, was purified by repeated column chromatography with (45:1) $CHCl_3$–EtOH as eluent to give a syrupy residue, which was triturated in acetone–diethyl ether to provide *compound* **15** (32 mg) as a solid (Found: C, 50.2; H, 6.5; N, 3.5; S, 4.4. $C_{30}H_{44}N_2O_{16}S$ requires C, 50.0; H, 6.2; N, 3.9; S, 4.4%); mp 170–171 °C (from acetone–diethyl ether); R_f 0.62 (9:1 $CHCl_3$–EtOH); $\delta_H(CDCl_3)$ 7.454 (1 H, d, *J* 9.7, disappears on deuteriation, NH), 5.486 (1 H, dt, *J* 3.0 and 10.8, 5-H), 5.424 (1 H, dd, *J* 3.6 and 9.9, 3-H), 5.341 (1 H, d, *J* 10.6, 1'-H), 5.110 (1 H, dd, *J* 3.0 and 9.8, 2-H), 5.029 (1 H, t, *J* 9.6, 3'-H), 4.956 (1 H, t, *J* 9.6, 4'-H), 4.564 (2 H, d, *J* 2.9, 6-H_2), 4.398 (1 H, d, *J* 3.1, 1-H), 4.237 (1 H, q, *J* 9.8, 2'-H), 4.172 (1 H, dd, *J* 1.9 and 12.3, 6'-H^a), 4.061 (1 H, dd, *J* 6.8 and 12.3, 6'-H^b), 3.710 (1 H, m, 1/2 C*H*$_2$Me), 3.615 (1 H, m, 5'-H), 3.523 (1 H, m, 1/2 C*H*$_2$Me), 3.345 (1 H, dd, *J* 3.6 and 11.0, 4-H), 2.115, 2.023 (2), 2.013 (2) and 1.979 (each 3 H, s, together 6 × OAc and NAc), 1.920 (3 H, s, Me on oxazoline) and 1.207 (3 H, t, *J* 7.1, CH$_2$*Me*); *m/z* (FAB$^+$) 721 [(M + H)$^+$, 60%] and 330 (100).

Conversion of compound 15 into ethyl 2-acetamido-*S*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-deoxy-4-thio-β-D-glucopyranoside 16

Compound **15** (6 mg, 8.3 μmol) was treated with EtONa–EtOH (60 mmol dm^{-3}; 1 cm^3) at room temperature overnight, decationized with Dowex 50W-X8 (H$^+$ form) and filtered. The filtrate was evaporated to give ethyl *N,N'*-diacetyl-β-thiochitobioside **16** (3.55 mg, 91%); R_f 0.51 (55:40:5 $CHCl_3$–MeOH–water); $\delta_H(D_2O)$ 4.661 (1 H, d, *J* 10.4, 1'-H), 4.359 (1 H, d, *J* 8.3, 1-H), 4.056–3.401 (13 H, overlapping, 11 sugar ring protons and OC*H*$_2$Me), 2.818 (1 H, t, *J* 10.6, 4-H), 1.991 and 1.979 (each 3 H, s, NAc) and 1.105 (3 H, t, *J* 7.1, OCH$_2$*Me*).

Acetylation of compound **16** (2 mg, 4.27 μmol) with Ac_2O–pyridine (1:1; 0.4 cm^3) gave, after the usual work-up and column chromatography (25:1 $CHCl_3$–EtOH), ethyl-2-acetamido-*S*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-3,4,6-tri-*O*-acetyl-2-deoxy-4-thio-β-D-glucopyranoside (2.69 mg, 93%); R_f 0.43 (9:1 $CHCl_3$–EtOH); $\delta_H(CDCl_3)$ 5.674 (1 H, d, *J* 8.4, NH), 5.727 (1 H, t, *J* 10.4, 3-H), 5.640 (1 H, d, *J* 8.1, NH), 5.058 (1 H, t, *J* 8.7, 3'-H), 5.007 (1 H, t, *J* 8.7, 4'-H), 4.908 (1 H, d, *J* 8.3, 1-H), 4.734 (1 H, d, *J* 10.5, 1'-H), 4.512 (2 H, d, *J* 3.2, 6-H_2), 4.230–4.115 (2 H, m, 2'-H and 6'-H^a), 4.045 (1 H, dd, *J* 6.3 and 12.3, 6'-H^b), 3.940–3.845 (2 H, m, 5-H and 1/2 OC*H*$_2$Me), 3.746 (1 H, m, 5'-H), 3.550 (1 H, m, 1/2 OC*H*$_2$Me), 3.372 (1 H, m, 2-H), 2.810 (1 H, t, *J* 10.9, 4-H), 2.102, 2.087, 2.061, 2.030, 2.008, 1.990 and 1.906 (each 3 H, s, together 5 × OAc and 2 × NAc) and 1.203 (3 H, t, *J* 7.1, OCH$_2$*Me*).

De-O-acetylation of compound 15 with MeONa–MeOH

Compound **15** (12 mg, 16.7 μmol) was treated with MeONa–MeOH (50 mmol dm^{-3}; 1 cm^3) at room temperature for 5 h, and the solution was then treated with Dowex 50W-X8 (H$^+$ form). The resin was filtered off, and washed with water. The filtrate and washings were combined and evaporated. The residue was subjected to column chromatography (70:30:1 $CHCl_3$–MeOH–water) to give the methyl glycoside **9** (3.0 mg, 39%) and ethyl glycoside **16** (3.52 mg, 45%), which were identical with the known standards.

Methyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-galactopyranoside 18

To a suspension of *N*-acetyl-D-galactosamine (6.30 g, 28.3 mmol) was added Dowex 50W-X8 (H$^+$ form) (3.0 g) and the mixture was stirred under reflux. The solid dissolved within 1 h, and the clear solution was continuously stirred under reflux. TLC (65:25:4 $CHCl_3$–MeOH–water) showed that methyl galactofuranoside (R_f 0.62) was first formed during the reaction, and was gradually converted into methyl pyranoside derivative (R_f 0.55). After being refluxed for 24 h, the reaction mixture was cooled to room temperature and the Dowex resin was filtered off and washed with MeOH. The filtrate and washings were combined and evaporated to give a solid residue, which was crystallized from EtOH to provide methyl 2-acetamido-2-deoxy-α-D-galactopyranoside **17**[21] (5.90 g, 88%) as crystals, mp 200–202 °C (from EtOH) [lit.,[21] 217–218 °C (from alcohol–light petroleum)]; R_f 0.55 (65:25:4 $CHCl_3$–MeOH–water); $\delta_H(D_2O)$ 4.736 (1 H, d, *J* 3.7, 1-H), 4.108 (1 H, dd, *J* 3.6 and 10.6, 3-H), 3.923 (1 H, br d, *J* 2.2, 4-H), 3.890–3.808 (2 H, m, 6-H_2), 3.764–3.703 (2 H, m, 2- and 5-H), 3.331 (3 H, s, OMe) and 1.989 (3 H, s, NAc).

Selective benzoylation of compound **17** (5.69 g, 24.17 mmol) with benzoyl chloride (6.46 cm^3, 55.6 mmol) in pyridine (60 cm^3) was performed in the same way as described for the preparation of compound **2**, and gave compound **18** (9.12 g, 85%) as crystals (Found: C, 61.2; H, 5.9; N, 3.0. $C_{23}H_{25}NO_8 \cdot 0.5H_2O$ requires C, 61.05; H, 5.8; N, 3.1%); mp 188–189 °C (from EtOH); R_f 0.40 (15:1 $CHCl_3$–EtOH); $\delta_H(CDCl_3)$ 8.061–7.400 (10 H, m, 2 × Bz), 5.809 (1 H, d, *J* 9.6, NH), 5.333 (1 H, dd, *J* 2.95 and 10.0, 3-H), 4.890 (1 H, dt, *J* 3.7 and 10.5, 2-H), 4.834 (1 H, d, *J* 3.7, 1-H), 4.633 (1 H, dd, *J* 5.7 and 11.5, 6-H^a), 4.550 (1 H, d, *J* 6.0 and 11.5, 6-H^b), 4.256 (1 H, br d, *J* 2.7, 4-H), 4.231 (1 H, t, *J* 5.8, 5-H), 3.434 (3 H, s, OMe) and 1.877 (3 H, s, NAc).

Methyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-4-*O*-trifluoromethanesulfonyl-β-D-galactopyranoside 19

Compound **18** (3.99 g, 9.0 mmol) was triflated with Tf_2O (3.04 cm^3, 18.0 mmol) in CH_2Cl_2 (60 cm^3) containing pyridine (3.64 cm^3, 45 mmol) as described for the triflation of compound **4**, and gave compound **19** (5.30 g, quantitative) as a pale yellow foam, which was used immediately in the next step without purification: R_f 0.59 (15:1 $CHCl_3$–EtOH); $\delta_H(CDCl_3)$ 8.100–7.434 (10 H, m, 2 × Bz), 5.676 (1 H, d, *J* 9.8, NH), 5.495 (1 H, dd, *J* 2.7 and 10.1, 3-H), 5.478 (1 H, br d, *J* 2.0, 4-H), 4.898 (1 H, d, *J* 3.4, 1-H), 4.860 (1 H, dt, *J* 3.5 and 10.0, 2-H), 4.657 (1 H, dd, *J* 6.4 and 11.0, 6-H^a), 4.461 (1 H, br t, *J* 6.7, 5-H), 4.331 (1 H, dd, *J* 7.0 and 11.1, 6-H^b), 3.463 (3 H, s, OMe) and 1.898 (3 H, s, NAc).

Methyl 2-acetamido-*S*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzoyl-2-deoxy-4-thio-α-D-glucopyranoside 20

To a cold solution of the thiol **5** (2.54 g, 7.0 mmol) in DMF (30 cm^3) were added cysteamine (578 mg, 7.5 mmol), DTE (1.08 g, 7 mmol), and then the triflate **19** (3.50 g, 6.0 mmol) in portions. The mixture was stirred under nitrogen for 20 h, and DMF was evaporated off under reduced pressure. The residue was dissolved in $CHCl_3$ (150 cm^3), and the solution was washed successively with 5% hydrochloric acid, aq. $NaHCO_3$ and water, dried (Na_2SO_4) and evaporated. Column chromatography of the residue with (30:1) $CHCl_3$–EtOH as eluent gave the *thiodisaccharide* **20** (2.98 g, 63%) (Found: C, 56.2; H, 5.7; N, 3.4; S, 4.0. $C_{27}H_{44}N_2O_{15}S$ requires C, 56.3; H, 5.6; N, 3.55; S, 4.1%); mp 280–283 °C; R_f 0.65 (9:1 $CHCl_3$–EtOH); $\delta_H(CDCl_3)$ 8.081–7.421 (10 H, m, 2 × Bz), 5.730 (1 H, d, *J* 9.7, NH), 5.592 (1 H, d, *J* 9.6, NH), 5.491 (1 H, t, *J* 10.6, 3-H), 5.067 (1 H, t, *J* 9.3, 3'-H), 5.015 (1 H, t, *J* 9.3, 4'-H), 4.903 (1 H, dd, *J* 3.75 and 12.1, 6-H^a), 4.830 (1 H, d, *J* 3.4, 1-H), 4.810 (1 H, d, *J* 10.2, 1'-H), 4.737 (1 H, dd, *J* 2.6 and 12.1, 6-H^b), 4.577 (1 H, dt, *J* 3.5 and 10.1, 2-H),

4.288 (1 H, m, 5'-H), 4.164–4.092 (3 H, m, 2'-H and 6'-H_2), 3.623 (1 H, m, 5-H), 3.481 (3 H, s, OMe), 3.201 (1 H, t, *J* 11.0, 4-H) and 2.059, 2.022, 1.998, 1.860 and 1.507 (each 3 H, s, together 3 × OAc and 2 × NAc); *m/z* (FAB$^+$) 789 [(M + H)$^+$, 7%], 330 (35) and 105 (100).

Methyl 2-acetamido-*S*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-deoxy-4-thio-α-D-glucopyranoside 21

Compound **20** (2.95 g, 3.74 mmol) was de-O-acylated with MeONa–MeOH (10 mmol dm^{-3}; 80 cm^3) as described for the de-O-acylation of **7** to provide *compound* **21** (1.61 g, 95%) (Found: C, 44.5; H, 6.7; N, 5.9; S, 7.1. $C_{17}H_{30}N_2O_{10}S$ requires C, 44.9; H, 6.65; N, 6.2; S, 7.05%); mp 270 °C (decomp.); $\delta_H(D_2O)$ 4.750 (1 H, d, *J* 3.39, 1-H), 4.678 (1 H, d, *J* 10.4, 1'-H), 3.945–3.416 (11 H, m, sugar ring protons), 3.315 (3 H, s, OMe), 2.867 (1 H, t, *J* 10.5, 4-H) and 1.996 and 1.986 (each 3 H, s, NAc); *m/z* (CI$^+$) 455 [(M + H)$^+$, 9%], 252 (100) and 204 (82).

Methyl 2-acetamido-*S*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-deoxy-4-thio-α-D-glucopyranoside 22

Acetylation of compound **21** (1.66 g, 3.65 mmol) with acetic anhydride–pyridine (1:1; 30 cm^3) was performed in the same way as described for the preparation of compound **10** to provide compound **22** (2.33 g, 96%), which was crystallized from ethanol to form crystals, mp 250–252 °C (from EtOH); R_f 0.54 (9:1 $CHCl_3$–EtOH); $\delta_H(CDCl_3)$ 5.765 and 5.660 (each 1 H, each d, *J* 9.8, 2 × NH), 5.169 (1 H, t, *J* 10.6, 3-H), 5.092–5.005 (2 H, m, 3'- and 4'-H), 4.731 (1 H, d, *J* 3.5, 1-H), 4.633 (1 H, d, *J* 10.5, 1'-H), 4.583 (1 H, dd, *J* 3.7 and 12.1, 6-H^a), 4.445 (1 H, dd, *J* 1.98 and 12.1, 6-H^b), 5.358 (1 H, dt, *J* 3.5 and 10.0, 2-H), 4.177–4.113 (3 H, m, 2'-H and 6'-H_2), 4.069–4.012 (1 H, m, 5-H), 3.680–3.598 (1 H, m, 5'-H), 3.418 (3 H, s, OMe), 2.934 (1 H, t, *J* 1.0, 4-H) and 2.092 (2), 2.078, 2.024, 2.014, 1.990 and 1.914 (each 3 H, s, together 5 × OAc and 2 × NAc); *m/z* (CI$^+$) 665 [(M + H)$^+$, 45%], 633 (M$^+$ − OMe, 8) and 330 (100).

Acetolysis of peracetylated methyl α-D-thiochitobioside 22

To an acetolysis mixture of Ac_2O–AcOH–H_2SO_4 (8:2:0.1, v/v; 30 cm^3) was added compound **22** (1.88 g, 2.83 mmol). The mixture was stirred at room temperature for 7 h, when TLC (9:1 $CHCl_3$–EtOH) showed the complete conversion of compound **22** into a single product. The reaction mixture was then poured into ice–water containing NaOAc (500 mg), and this mixture was stirred at room temperature for 3 h and evaporated to dryness. The residue was suspended in $CHCl_3$ (120 cm^3), washed successively with cold aq. $NaHCO_3$, brine and water, dried (Na_2SO_4) and filtered. The filtrate was evaporated to give a solid, which was crystallized from ethanol to provide crystalline α-D-thiochitobiose peracetate **11** (1.68 g, 86%).

Acetolysis of methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside 23

Compound **23** (140 mg, 0.387 mmol), obtained by O-acetylation of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside,[22] was treated with (8:2:0.1) Ac_2O–AcOH–H_2SO_4 (3 cm^3) at room temperature for 6 h. The reaction mixture was then poured into ice–water containing sodium acetate and was stirred for 3 h. After the solution was evaporated to dryness, the residue was subjected to column chromatography using (35:1) $CHCl_3$–EtOH as eluent to give 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose **25**[22] (135 mg, 89%), mp 136–138 °C [lit.,[22] 138 °C (from diethyl ether)]; R_f 0.38 (15:1 $CHCl_3$–EtOH); $\delta_H(CDCl_3)$ 6.168 (1 H, d, *J* 3.5, 1-H), 5.910 (1 H, d, *J* 9.1, NH), 5.259 (1 H, t, *J* 9.3, 3-H), 5.208 (1 H, t, *J* 9.3, 4-H), 4.494 (1 H, dt, *J* 3.5 and 9.2, 2-H), 4.254 (1 H, dd, *J* 3.7 and 12.3, 6-H^a), 4.086–4.010 (2 H, m, 5-H and 6-H^b) and 2.201, 2.095, 2.053 (2) and 1.945 (each 3 H, s, together 4 × OAc and NAc).

(1*R* and 1*S*)-2-Acetamido-1,3,4,5,6-penta-*O*-acetyl-2-deoxy-1-methoxy-D-glucitol 26a and 26b. Acetolysis of methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside 24

Compound **24**[20] (150 mg, 0.415 mmol) was treated with (8:2:0.1) Ac_2O–AcOH–H_2SO_4 (3.5 cm^3) at room temperature. TLC (15:1 $CHCl_3$–EtOH) showed complete disappearance of the starting material **24** within 2 h to form two major components. The reaction mixture was processed in the same way as described for the acetolysate of compound **23**, followed by column chromatography using (50:1) $CHCl_3$–EtOH as eluent, to give, first, compound **26a** (58.5 mg, 30%), and then epimer **26b** (75 mg, 39%).

Compound **26a**: R_f 0.41 (15:1 $CHCl_3$–EtOH); $\delta_H(CDCl_3)$ 5.787 (1 H, d, *J* 10.0, NH), 5.610 (1 H, d, *J* 2.5, 1-H), 5.368 (1 H, dd, *J* 3.76 and 7.47, 4-H), 5.323 (1 H, dd, *J* 3.76 and 6.88, 3-H), 5.171–5.116 (1 H, ddd, *J* 3.53, 5.57 and 7.47, 5-H), 4.591–4.526 (1 H, ddd, *J* 2.5, 6.88 and 10.0, 2-H), 4.227 (1 H, dd, *J* 3.53 and 12.3, 6-H^a), 4.088 (1 H, dd, *J* 5.57 and 12.3, 6-H^b), 3.442 (3 H, s, OMe) and 2.176, 2.077, 2.057, 2.052, 2.042 and 2.008 (each 3 H, s, together 5 × OAc and NAc); *m/z* (FAB$^+$) 464 [(M + H)$^+$, 32%], 404 (100), 344 (5), 330 (10) and 284 (5).

Compound **26b**: R_f 0.38 (15:1 $CHCl_3$–EtOH); $\delta_H(CDCl_3)$ 5.816 (1 H, d, *J* 10.1, NH), 5.684 (1 H, d, *J* 5.26, 1-H), 5.425 (1 H, dd, *J* 3.83 and 5.20, 3-H), 5.412 (1 H, dd, *J* 1.99 and 3.80, 4-H), 5.142 (1 H, m, 5-H), 4.470 (1 H, dt, *J* 5.2 and 10.2, 2-H), 4.264 (1 H, dd, *J* 4.0 and 12.2, 6-H^a), 4.126 (1 H, dd, *J* 5.69 and 12.2, 6-H^b), 3.402 (3 H, s, OMe) and 2.130, 2.110, 2.091, 2.084, 2.040 and 2.031 (each 3 H, s, together 5 × OAc and NAc); *m/z* (FAB$^+$) 464 [(M + H)$^+$, 5%], 404 (100), 344 (5), 330 (8) and 284 (20).

2-Acetamido-*S*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-deoxy-1,4-dithio-β-D-glucopyranose 28

Compound **11** (1.40 g, 2.02 mmol) was suspended in a mixture of acetyl chloride (10 cm^3)–CH_2Cl_2 (6 cm^3), and the mixture was cooled to 0 °C. The mixture became a clear solution when dry hydrogen chloride gas was being bubbled into the suspension. The bubbling of hydrogen chloride was continued for 30 min, and the flask was sealed and kept at room temperature for 24 h. Volatile components were removed by evaporation and co-evaporation with CH_2Cl_2 (2 × 10 cm^3) using a rotary evaporator to give peracetylated α-thiochitobiosyl chloride **27** (1.40 g) as a powder (R_f 0.55; 9:1 $CHCl_3$–EtOH). The glycosyl chloride thus obtained was dissolved in dry acetone (30 cm^3), thiourea (760 mg, 10 mmol) was added, and the resulting mixture was stirred under reflux for 30 min. The reaction solution was evaporated to 10 cm^3, to which was added aq. Na_2SO_3 (504 mg, 4.0 mmol in 35 cm^3). The mixture was stirred at room temperature for 30 min, adjusted to pH 5 (as judged by pH test paper) with 5% hydrochloric acid, and extracted with CH_2Cl_2 (3 × 40 cm^3). The extracts were combined, washed successively with brine and water, dried (Na_2SO_4) and filtered. The filtrate was evaporated to give compound **28** (1.12 g, 83%) as a fine solid, which was pure enough for use in the next step; mp 185–190 °C (decomp.); R_f 0.41 (9:1 $CHCl_3$–EtOH); $\delta_H(CDCl_3)$ 5.797 (1 H, d, *J* 9.5, NH), 5.742 (1 H, d, *J* 8.7, NH), 5.275 (1 H, t, *J* 10.3, 3-H), 5.084 (1 H, t, *J* 9.4, 3'-H), 5.011 (1 H, t, *J* 9.4, 4'-H), 4.743 (1 H, t, *J* 9.6, 1-H), 4.691 (1 H, d, *J* 10.6, 1'-H), 4.497 (2 H, br d, *J* 3.1, 6-H_2), 4.140–4.113 (3 H, m, 2'-H and 6'-H_2), 3.916–3.835 (2 H, m, 2- and 5-H), 3.711 (1 H, m, 5'-H), 2.903 (1 H, t, *J* 10.9, 4-H), 2.430 (1 H, d, *J* 9.3, SH) and 2.106, 2.095 (2), 2.036, 2.020 (2) and 1.920 (each 3 H, s, together 5 × OAc and 2 × NAc); *m/z* (FAB$^+$) 667 [(M + H)$^+$, 30%], 633 (M$^+$ − SH, 15), 607 (5) and 330 (100).

Methyl 2-acetamido-*S*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-*S*-(2-acetamido-2-deoxy-4-thio-β-D-glucopyranosyl)-(1→4)-2-deoxy-4-thio-β-D-glucopyranoside 29

To a solution of the thiol **28** (25 mg, 37.5 μmol) in DMF (1.5 cm^3) were successively added cysteamine (8.7 mg, 113 μmol), DTE (8.7 mg, 56.5 μmol) and the triflate **4** (44 mg, 75 μmol). The mixture was stirred at room temperature under nitrogen for 20 h. After evaporation off of DMF with a high-vacuum pump, the solid residue was dissolved in a MeONa–MeOH solution (20 mmol dm^{-3}; 10 cm^3) and the solution was stirred at room temperature overnight. After decationization with Dowex 50W-X8 (H^+ form), water was added to dissolve the precipitate, and the resin was filtered off. Evaporation of the filtrate led to a residue, which was dissolved in a minimum amount of water and applied to a Sephadex G-10 column (1.5 × 95 cm). The column was eluted with water. Combination and freeze-drying of the fractions containing pure thiotrisaccharide **29** yielded a solid (7.6 mg, 30% from thiol **28**), mp 215 °C; R_f 0.28 (55:40:5 $CHCl_3$–MeOH–water); $\delta_H(D_2O)$ 4.671 and 4.668 (each 1 H, each d, *J* 10.4, 1′- and 1″-H), 4.354 (1 H, d, *J* 8.4, 1-H), 4.054–3.399 (16 H, m, sugar ring protons), 3.437 (3 H, s, OMe), 2.878 and 2.809 (each 1 H, t, *J* 10.4, 4- and 4′-H) and 1.994, 1.989 and 1.976 (each 3 H, s, NAc); *m/z* (FAB^+) 674 [$(M + H)^+$, 3%], 460 (12), 307 (100) and 289 (59).

Methyl 2-acetamido-*S*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-*S*-(2-acetamido-2-deoxy-4-thio-β-D-glucopyranosyl)-(1→4)-2-deoxy-4-thio-α-D-glucopyranoside 30

Cysteamine (278 mg, 3.60 mmol) and DTE (277 mg, 1.8 mmol) were added to a solution of the thiol **28** (1.20 g, 1.80 mmol) in DMF (15 cm^3). The triflate **19** (2.28 g, 3.96 mmol) was then added to the solution in portions. The resulting mixture was stirred at room temperature under nitrogen for 24 h. Evaporation off of the DMF gave a solid, which was dissolved in MeONa–MeOH (0.3 mol dm^{-3}; 50 cm^3), and the solution was stirred at room temperature overnight. The precipitate formed was collected by filtration and crystallized from ethanol to provide the thiotrisaccharide **30** (225 mg). The mother liquor was decationized with Dowex 50W-X8 (H^+ form), filtered and evaporated to dryness. The solid residue was dissolved in a minimum amount of water and fractionated on a column (1.5 × 95 cm) of Sephadex G-10 equilibrated and eluted with water to obtain additional material (199 mg). Total yield of the *methyl thiochitotrioside* **30** was 424 mg (35% from thiol **28**) (Found: C, 44.5; H, 6.7; N, 6.1; S, 9.3. $C_{25}H_{43}N_3O_{14}S_2$ requires C, 44.6; H, 6.4; N, 6.2; S, 9.5%), mp 260 °C; R_f 0.38 (55:40:5 $CHCl_3$–MeOH–water); $\delta_H(D_2O)$ 4.738 (1 H, d, *J* 3.43, 1-H), 4.672 (1 H, d, *J* 10.4, 1′-H), 4.670 (1 H, d, *J* 10.4, 1″-H), 3.998–3.393 (16 H, m, sugar ring protons), 3.303 (3 H, s, OMe), 2.868 (1 H, t, *J* 10.6, 4-H), 2.847 (1 H, t, *J* 10.7, 4′-H) and 1.986 (2) and 1.976 (each 3 H, s, NAc); *m/z* (FAB^+) 674 [$(M + H)^+$, 2%] and 154 (100).

Methyl 2-acetamido-*S*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-*S*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-thio-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-deoxy-4-thio-α-D-glucopyranoside 31

Compound **30** (250 mg, 371 μmol) was O-acetylated with acetic anhydride and pyridine (1:1; 8 cm^3) in the same way as described for the preparation of pentaacetate **22** to provide compound **31** (341 mg, 95%), mp 290 °C (decomp.); R_f 0.40 (9:1 $CHCl_3$–EtOH); $\delta_H[(CD_3)_2SO]$ 8.017, 7.859 and 7.781 (each 1 H, d, *J* 9.4, NH), 5.089 (1 H, t, *J* 9.7, 3-H), 4.898 and 4.836 (each 1 H, each t, *J* 9.5, 3″- and 4″-H), 4.755 and 4.657 (each 1 H, each d, *J* 10.4, 1′- and 1″-H), 4.617 (1 H, d, *J* 3.4, 1-H), 4.561–4.468 (2 H, m, 3′-H and 6-H[a]), 4.276–3.627 (11 H, m, sugar ring protons), 3.314 (3 H, s, OMe), 3.013 and 2.957 (each 1 H, t, *J* 11.2, together 4- and 4′-H), 2.099, 2.071, 2.032, 1.976, 1.954, 1.933 and 1.916 (each 3 H, s, OAc) and 1.794, 1.746 and 1.730 (each 3 H, s, NAc); *m/z* (FAB^+) 968 [$(M + H)^+$, 3%], 936 (5), 633 (8) and 330 (100).

2-Acetamido-*S*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-*S*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-thio-β-D-glucopyranosyl)-(1→4)-1,3,6-tri-*O*-acetyl-2-deoxy-4-thio-α-D-glucopyranose 32

Acetolysis of the methyl α-glycoside **31** (365 mg, 377 μmol) with (8:2:0.1) Ac_2O–AcOH–H_2SO_4 (8 cm^3) was performed in the same way as described for the acetolysis of compound **22** to provide the thiochitotriose peracetate **32** (305 mg, 81%), mp 235 °C (decomp.); R_f 0.37 (9:1 $CHCl_3$–EtOH); $\delta_H[(CD_3)_2SO]$ 8.035, 7.862 and 7.760 (each 1 H, d, *J* 9.4, 3 NH), 5.876 (1 H, d, *J* 3.3, 1-H), 5.087 and 4.982 (each 1 H, t, *J* 10.2, together 3- and 3′-H), 4.877 and 4.843 (each 1 H, t, *J* 9.4, together 3″- and 4″-H), 4.754 and 4.663 (each 1 H, d, *J* 10.8, together 1′- and 1″-H), 4.506–3.620 (12 H, m, sugar ring protons), 3.068 and 3.019 (each 1 H, t, *J* 11.0, together 4- and 4′-H), 2.157, 2.074, 2.062, 2.028, 1.983, 1.975, 1.956 and 1.916 (each 3 H, s, OAc) and 1.792, 1.747 and 1.733 (each 3 H, s, NAc); *m/z* (FAB^+) 996 [$(M + H)^+$, 2%], 936 (48), 633 (57) and 330 (100).

2-Acetamido-*S*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-*S*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-thio-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-deoxy-1,4-dithio-β-D-glucopyranose 33

Compound **32** (185 mg, 186 μmol) was suspended in a mixture of acetyl chloride (8 cm^3) and dichloromethane (2 cm^3). After hydrogen chloride gas had been bubbled into the suspension at 0 °C for 30 min, the reaction flask was sealed and kept at room temperature for 48 h. Removal of volatile components by rotary evaporation and co-evaporation with dichloromethane (3 × 8 cm^3) gave the expected glycosyl chloride (188 mg) (R_f 0.48; 9:1 $CHCl_3$–EtOH) as a pale yellow powder, which was used immediately for the next step.

The glycosyl chloride thus obtained was suspended in dry acetone (6 cm^3), to which powdered thiourea (71 mg, 930 μmol) was added, and the mixture was heated under reflux for 30 min. TLC (9:1 $CHCl_3$–EtOH) showed complete conversion of the glycosyl chloride into the expected glycosylpseudothiourea hydrochloride. After the reaction mixture had cooled to room temperature, aq. sodium sulfite (120 mg, 930 μmol in 10 cm^3) was added, and the mixture was stirred at room temperature for 20 min under nitrogen. The solution was adjusted to pH 5 (as judged by pH test paper) with 5% hydrochloric acid and extracted with CH_2Cl_2 (3 × 15 cm^3). The extracts were combined, and washed successively with 5% hydrochloric acid, brine and water, dried (Na_2SO_4) and filtered. The filtrate was evaporated to provide the thiol **33** (141 mg, 78% from **32**) as a powder, mp 189–192 °C (decomp.); R_f 0.34 (9:1 $CHCl_3$–EtOH); $\delta_H[(CD_3)_2SO]$ 8.034, 7.940 and 7.804 (each 1 H, d, *J* 9.4, NH), 5.089 (1 H, t, *J* 9.8, 3-H), 4.902–4.645 (5 H, m, 1-, 1′-, 1″-, 3″- and 4″-H), 4.586–4.450 (2 H, m, 3′-H and 6-H[a]), 4.345–3.600 (11 H, overlapping, sugar ring protons), 3.285 (1 H, d, *J* 8.0, SH), 3.014 and 2.926 (each 1 H, each t, *J* 10.5, 4- and 4′-H), 2.102, 2.067, 2.032, 1.977, 1.954 (2) and 1.918 (each 3 H, s, OAc) and 1.768, 1.750 and 1.726 (each 3 H, s, NAc); *m/z* (FAB^+) 970 [$(M + H)^+$, 24%], 633 (28) and 330 (100).

Methyl 2-acetamido-*S*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-*S*-(2-acetamido-2-deoxy-4-thio-β-D-glucopyranosyl)-(1→4)-*S*-(2-acetamido-2-deoxy-4-thio-β-D-glucopyranosyl)-(1→4)-2-deoxy-4-thio-α-D-glucopyranoside 34

To a solution of the thiol **33** (135 mg, 139 μmol), cysteamine (27 mg, 348 μmol) and DTE (32 mg, 208 μmol) in DMF (4 cm^3) at 0 °C was added triflate **19** (320 mg, 556 μmol) in portions. The mixture was then stirred at room temperature under nitrogen for 15 h, and DMF was removed by evaporation under reduced pressure. The residue was dissolved in an MeONa–MeOH solution (0.2 mol dm^{-3}; 10 cm^3) and the solution was stirred at room temperature for 5 h. The precipitate formed was collected by filtration and further purified with gel filtration using a Sephadex G-10 column (1.5 × 95 cm) which was eluted with

water to give pure thiotetrasaccharide **34** (26 mg). The mother liquor was decationized with Dowex 50W-X8 (H^+ form), filtered and evaporated. The residue was fractionated with a Sephadex G-10 column (1.5 × 95 cm) and eluted with water to provide addtional material (9 mg). Total yield of the thiotetrasaccharide **34** was 35 mg (28% from thiol **33**), mp 271 °C (decomp.); R_f 0.25 (55:40:5 $CHCl_3$–MeOH–water); $\delta_H(D_2O)$ 4.754 (1 H, d, *J* 3.3, 1-H), 4.701–4.672 (3 H, 3 d, *J* 10.2, 1′-, 1″- and 1‴-H), 4.025–3.413 (21 H, m, sugar ring protons), 3.319 (3 H, s, OMe), 2.886, 2.875 and 2.861 (each 1 H, t, *J* 10.5, together 4-, 4′- and 4″-H), 2.003 (9 H, s, 3 × NAc) and 1.993 (3 H, s, NAc); *m/z* (FAB^+) 893 [$(M + H)^+$, 1%], 307 (18) and 154 (100).

Acknowledgements

We thank Drs S. Roseman and N. Keyhani (Department of Biology) for helpful discussions and cooperation; and Dr J. L. Kachinski, Jr. (Department of Chemistry) for recording the mass spectra.

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Paper 5/03230J
Received 19*th May* 1995
Accepted 12*th September* 1995