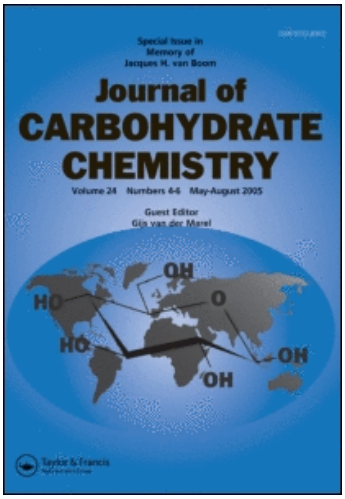


Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Synthesis of Carbohydrates with an Anomeric Thiol Moiety for Elaboration into Metabolically Stable Thioglycosides

Milton J. Kiefel; Robin J. Thomson; Milica Radovanovic; Mark von Itzstein

To cite this Article Kiefel, Milton J. , Thomson, Robin J. , Radovanovic, Milica and von Itzstein, Mark(1999) 'Synthesis of Carbohydrates with an Anomeric Thiol Moiety for Elaboration into Metabolically Stable Thioglycosides', Journal of Carbohydrate Chemistry, 18: 8, 937 — 959

To link to this Article: DOI: 10.1080/07328309908544045

URL: http://dx.doi.org/10.1080/07328309908544045

J. CARBOHYDRATE CHEMISTRY, 18(8), 937–959 (1999)

SYNTHESIS OF CARBOHYDRATES WITH AN ANOMERIC THIOL MOIETY FOR ELABORATION INTO METABOLICALLY STABLE THIOGLYCOSIDES

Milton J. Kiefel, Robin J. Thomson, Milica Radovanovic and Mark von Itzstein*

Department of Medicinal Chemistry, Monash University (Parkville Campus), 381 Royal Parade, Parkville, Victoria, 3052, Australia

March 3, 1999 - Final Form August 5, 1999

ABSTRACT

The synthesis of thioglycosides for use as metabolically stable biological probes is an area of continued interest. This paper describes the synthesis of functionalised carbohydrates which contain an anomeric thio group. During the course of this work we have examined the most viable route into compounds such as the specifically functionalised carbohydrates **36** and **37**, and have also investigated the usefulness of disulfides as protecting groups for anomeric thiols.

INTRODUCTION

Carbohydrate-protein interactions are increasingly being recognised as crucial in a number of biological processes,[1,2] including cell-cell communication,[1,3,4] microbial adhesion,[5,6] certain aspects of cancer metastasis,[7-9] and pathological modification of normal cell behaviour.[1,10] Fundamental to an understanding of the significance of carbohydrate-protein interactions is the ability to analyse the interactions of carbohydrate recognising proteins with either their natural substrates or synthetic analogues. Such

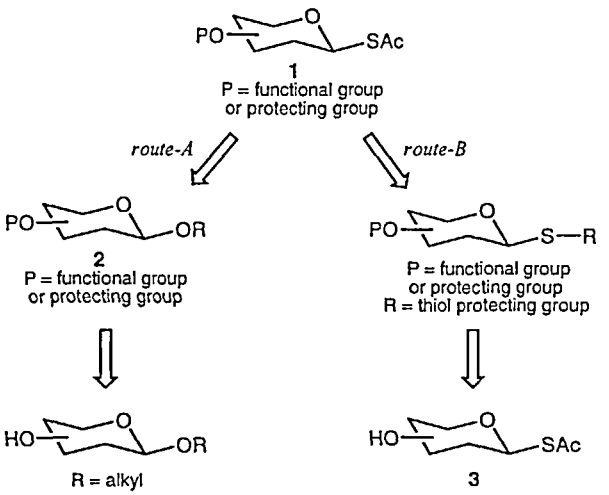
studies with carbohydrate recognising proteins result in an improved knowledge of their functions, the nature of their carbohydrate binding sites, and their modes of action.

The synthetic compounds used as biological probes for investigations into carbohydrate-protein interactions, especially those involving glycohydrolases, are often metabolically-stable glycosides in order to prevent unwanted hydrolysis of glycosidic linkages. Several groups,[11-14] including ourselves,[15-17] have an interest in the development of new and more efficient syntheses of thioglycosides for use as biological probes,[11,12,18-20] potential protein or enzyme inhibitors,[15,18,20] or as metabolically stable affinity chromatography ligands.[21]

As part of our continued interest in the synthesis of metabolically stable glycosides we had cause to prepare specifically functionalised carbohydrates in which the anomeric thio group was suitably protected such that it could be selectively unmasked and coupled to a variety of aglycon acceptors. It has been shown by several groups[11-21] that the anomeric thioacetyl group is ideal for such purposes, since it can be selectively unmasked in the presence of several functional groups, including *O*-acetates. During the course of this investigation we have explored two different avenues towards the synthesis of carbohydrate derivatives containing an anomeric thio functionality. The results of this study, including the use of disulfides as protecting groups for anomeric thiols during functional group manipulations, are presented here.

RESULTS AND DISCUSSION

The two possible approaches we envisaged towards the efficient synthesis of functionalised carbohydrate derivatives containing an anomeric thio group (e.g. **1**) are shown in Scheme 1. The first option, *route-A*, involves the introduction of the requisite thio functionality at a late stage in the synthetic sequence. Such an approach is attractive since the synthesis of specifically functionalised simple β-alkyl glycosides like **2** is well established. However, we harboured some concern about the sensitivity of other functionality in **2** to the conditions necessary to transform **2** to **1**. The second approach (*route-B*) introduces the thio group at the beginning of the synthesis, to give compounds such as **3**. The question remaining to be answered in *route-B*, however, is the nature of any thiol protection necessary, its stability during subsequent transformations, and how it can be unmasked to ultimately give compounds like **1**.

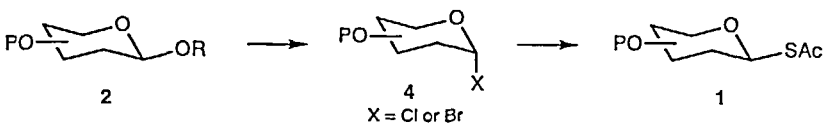


Scheme 1. Approaches to the synthesis of anomeric thio functionalised carbohydrates

Transformation of Alkyl Glycosides to Anomeric Thioacetates (*Route-A*)

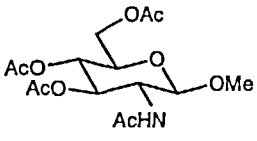
The direct transformation of alkyl glycosides into anomeric thioacetates is attractive, since it allows introduction of the requisite thio functionality late in the synthetic sequence. An example of such a conversion has been reported for 1,6-anhydro sugars which were converted, under a variety of thioacetolysis conditions (Ac_2S, AcSH, H_2SO_4 or Ac_2S, Lewis acid), to the corresponding sugars with an anomeric thioacetate group.[22] However, the products obtained from these thioacetolysis reactions were always anomeric mixtures (albeit with sometimes high selectivity for one anomer),[22] whereas we sought a route to compounds like **1** specifically as the β-thioglycosides.

An alternative strategy involves the conversion of alkyl glycosides to glycosyl halides, followed by displacement with thioacetate to give compounds like **1**. The transformation of simple alkyl glycosides (e.g., **2**, R = Me, TMSE) to glycosyl halides can be accomplished by a number of methods.[23-25] In our case (Scheme 2), the conversion of **2** to the glycosyl halide **4** would provide a two-step sequence to the target compounds **1**.

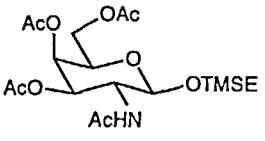


Scheme 2. Glycosyl halide route towards thioglycosides

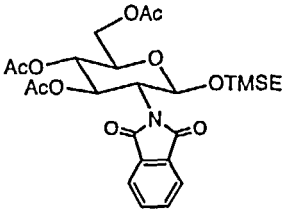
Of the many possible options available for such a transformation,[23-25] the use of a dihalomethyl methyl ether in the presence of zinc(II) halide is particularly attractive, since it has been shown that several functional groups, including acid sensitive substituents such as acetals, are stable to the reaction conditions.[23] The β-methyl glycosides of fully *O*-acetylated sugars can be converted to the corresponding α-glycosyl halides in reasonable yield using this method. However, we observed that treatment of the GlcNAc derivative **5** with dichloromethyl methyl ether (DCMME) in the presence of $ZnCl_2$ resulted in a complex mixture of products. This observation is comparable to the results obtained by Jansson, *et al.*[24] with the corresponding GalNAc TMSE glycoside **6**. Interestingly, the 2-phthalamido glucose derivative **7** can be smoothly transformed under the same conditions into predominantly the corresponding β-glycosyl chloride.[23,24]



5

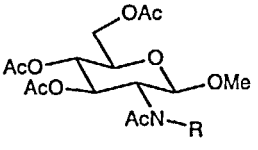


6

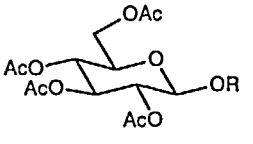


7

Since we were interested in employing 2-amino sugars in our work it was decided to investigate this transformation further. Accordingly, the protected GlcNAc derivatives **8** and **9** were treated with DCMME and $ZnCl_2$ under conditions analogous to those successfully employed with fully *O*-acetylated sugars such as **10**.[23] Disappointingly all attempts at generating the glycosyl chlorides of the GlcNAc derivatives **5**, **8** and **9** resulted in complex reaction products (by TLC analysis), with the desired glycosyl chlorides being present in only trace amounts. In the case of the Boc protected GlcNAc derivative **8**, the Boc group was rapidly hydrolysed under the reaction conditions to give **5** which then behaved in accordance with the results obtained with **5** itself.



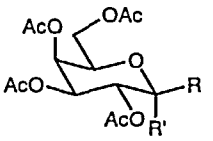
8 R = Boc
9 R = Ac



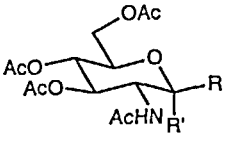
10 R = alkyl, aryl

Manipulations of Simple Thioglycosides

Our second approach towards the synthesis of functionalised carbohydrate derivatives containing an anomeric thio group (*route-B*, Scheme 1) required the introduction of the thio group at the beginning of our synthetic sequence. Accordingly, the glycosyl halides **11**[26] and **12**[27] of Gal and GlcNAc, respectively, were treated with potassium thioacetate in acetone to give the known thioacetate derivatives **13**[28] and **14**.[27]

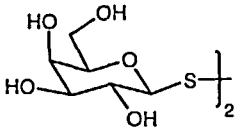


11 R = H, R' = Br
13 R = SAc, R' = H

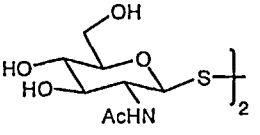


12 R = H, R' = Cl
14 R = SAc, R' = H

Since it was our intention to perform various manipulations of the hydroxyl groups in compounds **13** and **14**, we needed to ascertain the best protection strategy for the anomeric thio group. Deprotection of the acetates (NaOMe in MeOH) in the Gal derivative **13** resulted in the formation of two products. Oxidation (I_2, aq MeOH)[29] of the total reaction product gave a single component (89% isolated yield) which was identified as the Gal disulfide derivative **15**.[30] Interestingly, treatment of the protected GlcNAc derivative **14** with NH_4OH in MeOH directly gave the corresponding disulfide derivative **16**[31] in 72% yield.



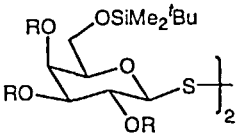
15



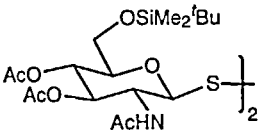
16

With the ready formation of the disulfide derivatives **15** and **16** we decided to investigate the feasibility of using anomeric disulfides as a thiol protecting group[32] during synthetic manipulations. Initially, we sought to selectively protect the C-6 position of compounds like **15** and **16**, since this would ultimately provide access to a range of selectively C-6 functionalised sugars. Accordingly, silylation of **15** under standard conditions (TBDMSCl, pyridine) gave the 6-*O*-silylated disulfide derivative **17**, which was then acetylated (Ac_2O, pyridine) to furnish the selectively C-6 protected Gal disulfide

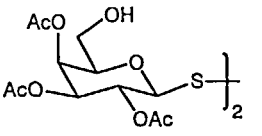
derivative **18** in 77% yield from **15**. Similarly, the GlcNAc disulfide **16** could be selectively 6-*O*-silylated, and subsequently acetylated, to give **19** (75% yield from **16**). Deprotection of the 6-*O*-silyl ether in **18** (80% aq HOAc) gave the expected Gal disulfide **20** in 84% yield after chromatography.



17 R = H
18 R = Ac

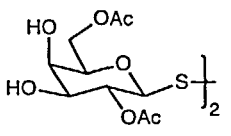


19

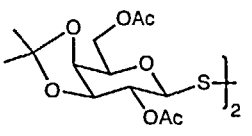


20

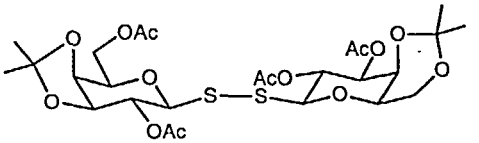
It can be seen from these studies that simple C-6 manipulation of anomeric disulfides is comparable to the results obtained with the corresponding monomeric alkyl glycosides. Unfortunately, the same conclusion cannot be made with attempts at selective acetal protection of diols in the disulfides **15** and **16**. In work aimed at preparing anomeric thio functionalised precursors suitable for use as glycosyl acceptors we sought to prepare compounds like **21**. In an initial experiment, the Gal disulfide **15** was treated under conditions [$Me_2C(OMe)_2$, *p*-TsOH·H_2O, *N,N*-DMF, 80 °C] known[33] to give 3,4-*O*-isopropylidenated derivatives of simple galactosides. Interestingly, TLC analysis of the reaction mixture after acetylation revealed several components. The two major compounds isolated from this reaction were identified as the expected 3,4-*O*-isopropylidenated product **22** (43% yield) and the unsymmetrical 3,4-4',6'-bis-*O*-isopropylidenated compound **23** (39% yield). That the less moblie product is indeed the unsymmetrical disulfide **23** was evident from examination of the ^1H NMR spectrum, which shows two sets of resonances (δ_H 1.33, 1.39, 1.50, 1.56) for the isopropylidene groups. The location of one of the isopropylidene groups on the C-4 and C-6 hydroxyls is clear from the chemical shifts of the corresponding H-6 protons (δ_H 3.93 and 4.02, cf. ~4.3 when C-6 is acetylated) together with the presence of a doublet of doublets ($J_{3,2}$ = 9.6, $J_{3,4}$ = 3.6 Hz) for H-3 at δ 4.92 which is consistent with C-3 bearing an acetyl group.



21

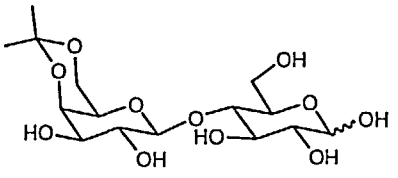


22

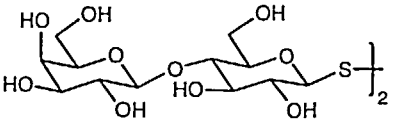


23

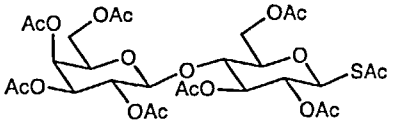
The isolation of the unsymmetrical galactose derivative **23** from this reaction is unusual, since 4,6-*O*-isopropylidenated products are not observed when the reaction is performed on simple galactosides.[33] However, it has been shown[34] that exposure of anhydrous lactose to $Me_2C(OMe)_2$ and *p*-TsOH in *N,N*-DMF at 25 °C results in the smooth formation of the 4',6'-*O*-isopropylidenated lactose derivative **24**. Interestingly, treatment of the lactose disulfide **25**, prepared from the known[35] lactoside **26** in a similar manner to that described for **15**, with 2,2-dimethoxypropane/*p*-TsOH followed by acetylation gave the 3',4'-*O*-isopropylidenated derivative **27** in 73% yield. The reasons for the differences in behaviour between the galactose disulfide **15** and the lactose disulfide **25** under isopropylidenation conditions are yet to be determined.



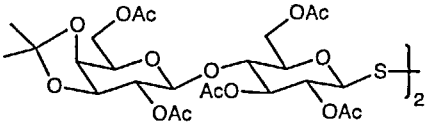
24



25

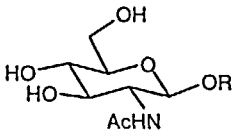


26

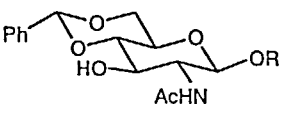


27

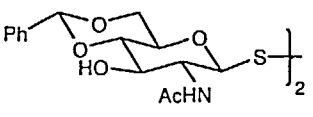
A further anomaly found in performing diol protection with anomeric disulfides when compared with monomeric carbohydrates was observed with the GlcNAc disulfide **16**. Whilst 4,6-*O*-benzylidenation [$PhCH(OMe)_2$, *p*-TsOH][33] of simple glucosamines (e.g., **28**) proceeds as expected to give **29**, the analogous reaction performed on the disulfide **16** resulted in the formation of several highly insoluble compounds from which the desired 4,6-*O*-benzylidenated disulfide **30** could not be isolated.



28



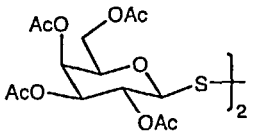
29



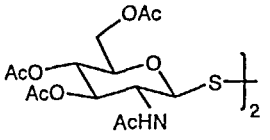
30

Disulfide Cleavage

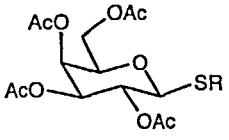
Having prepared some selectively derivatised anomeric disulfides, our attention turned to unmasking the desired anomeric thio functionality. There are a number of methods available for the reductive cleavage of disulfide linkages.[32,36-40] Of particular interest to our work was the stability of various functional/protecting groups, the stability of interglycosidic linkages, and the form of the anomeric sulfur after cleavage. By way of preliminary examination of these methods, reductive cleavage (PBu_3 in aq dioxane)[40] of the disulfide linkages in the fully acetylated derivatives **31** and **32** proceeded smoothly to give the corresponding thiols, which were then benzoylated to check for acetate migration. The *S*-benzoylated derivatives **33** and **34**, respectively, were prepared in moderate to high yield with no *S*-acetyl group (δ_H ~2.36 ppm) apparent in the ^1H NMR spectra of the crude reaction mixtures. The fully acetylated lactose disulfide was also cleanly transformed to the *S*-benzoylated derivative **35**. This reductive cleavage methodology therefore directly provides the corresponding anomeric thiols, which could be used in subsequent transformations as required.



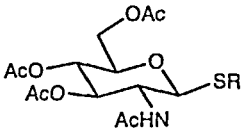
31



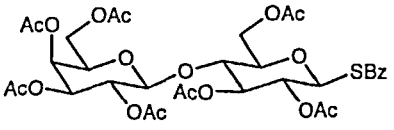
32



33 R = Bz
13 R = Ac



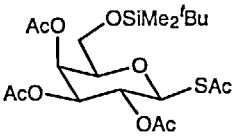
34 R = Bz
14 R = Ac



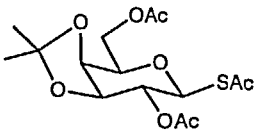
35

Alternatively, reductive cleavage of disulfide linkages with Zn in Ac_2O at elevated temperature[36,38] offers direct access to the anomeric thioacetates, which could subsequently serve as versatile intermediates. In an initial experiment towards this goal, treatment of disulfides **31** and **32** with Zn in Ac_2O[36,38] gave the corresponding thioacetates **13** and **14**, respectively, in high yield.

Although delighted with this preliminary result, consideration had to be given to the tolerance of acid sensitive functional groups (e.g., the primary silyl ethers in compounds **18** and **19**, and the 3,4-*O*-isopropylidene groups in compounds **22** and **27**) to the reaction conditions. After some experimentation it was found that treatment of the 6-*O*-silylated disulfide **18** with Zn powder (80% by mass) in Ac_2O at 65°C for 30 min gave the thioacetate derivative **36** in 85% yield after purification. Variation of these conditions, for example by using less Zn and allowing the reaction to run over longer periods or at higher temperatures, resulted in significant levels of desilylation and/or decomposition. Reductive acylation of the 3,4-*O*-isopropylidenated disulfide **22** resulted in the formation of the desired thioacetate derivative **37**, in 76% yield after purification.

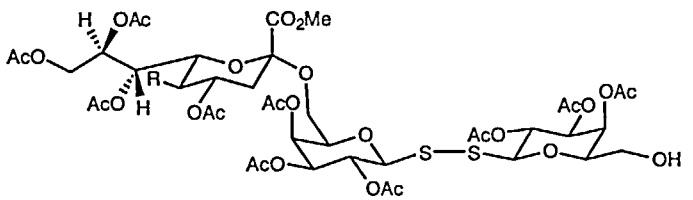


36

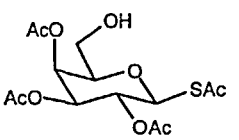


37

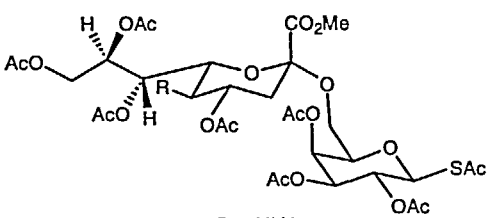
We were also interested in the stability of interglycosidic linkages under these reductive acylation conditions, and therefore carried out the following transformations. Treatment of the unsymmetrical disulfide **38**[41] with Zn in Ac_2O at 65°C resulted in a total reaction product which contained, by TLC comparison with authentic samples, the expected sialoside **39** and the galactoside **40**. Similarly, exposure of the lactose disulfide **41** to Zn and Ac_2O at 65 °C gave the expected lactoside **26**.



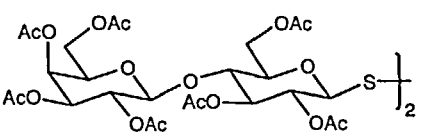
38 R = NHAc



40

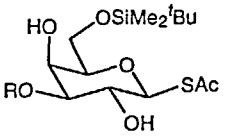


39 R = NHAc

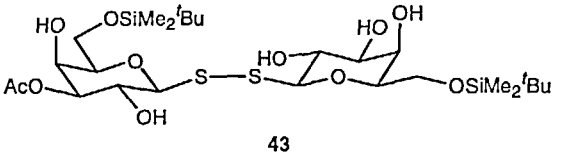


41

In a further experiment aimed at exploring the scope of this reductive acylation method, the 6-*O*-silylated Gal disulfide **17** was subjected to Zn in Ac_2O at 65 °C for 30 min. Interestingly, the two major compounds isolated from this reaction were identified as the 3-*O*-acetyl galactose thioacetate derivative **42** (37%) and the unsymmetrical 3-*O*-acetyl disulfide derivative **43** (34%). None of the expected thioacetate **44** was isolated from this reaction. The isolation of the partially *O*-acetylated disulfide **43** from this transformation is significant, since it shows that under the reaction conditions 3-*O*-acetylation is more facile than reduction of the disulfide bond.



42 R = Ac
44 R = H



43

CONCLUSION

The results presented here show that it is indeed feasible to introduce an anomeric thio functionality early in a synthetic sequence. The disulfide linkage serves as a versatile protecting group, which can be readily unmasked to give either an anomeric thiol or thioacetate functionality. In particular, the results obtained with disulfide cleavage in the presence of primary silyl ethers (e.g., **18** → **36**) or acid labile acetals (e.g., **22** → **37**) demonstrate the flexibility of this strategy. It can be envisaged that desilylation of **36**, or deisopropylidenation of **37**, would lead to compounds which could act as glycosyl acceptors. The products of such glycosidations would then contain an anomeric thio functionality which could be selectively unmasked and coupled to a variety of acceptors. Our efforts in this regard will be published shortly.

EXPERIMENTAL

General methods. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. ^1H and ^{13}C spectra were recorded using a Brüker AM-300 spectrometer unless indicated otherwise. Chemical shifts are given in ppm relative to the solvent used [$CDCl_3$: 7.26 for ^1H; 77.0 for ^{13}C; $(CD_3)_2SO$: 2.50 for ^1H; 39.5 for ^{13}C] or

relative to external Me_4Si for D_2O spectra. Two-dimensional ^1H-^1H-COSY spectra were obtained where necessary, in order to assist with spectral assignment. ESI mass spectra were obtained using a Micromass Platform II electrospray spectrometer. Infrared spectra were recorded as KBr discs using a Hitachi 270-30 spectrophotometer. Optical rotations were measured using a Jasco DIP-370 digital polarimeter. Reactions were monitored by TLC (Merck silica gel plates GF_{254}, cat. # 1.05554) and products were generally purified by flash chromatography using Merck silica gel 60 (0.040-0.063mm, cat. # 1.09385). 2,3,4,6-Tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-galactopyranose (**13**),[28] 2-acetamido-3,4,6-tri-*O*-acetyl-1-*S*-acetyl-2-deoxy-1-thio-β-D-glucopyranose (**14**)[27] and 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1,4)-*O*-2,3,6-tri-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-glucopyranose (**26**)[35] were all prepared by reacting the corresponding α-glycosyl halides with potassium thioacetate using published procedures.[27,28,35,42] Freshly fused $ZnCl_2$ was prepared according to the method described by Kovác.[23]

Methyl 2-(*N*-acetyl-*N*-*tert*-butoxycarbonylamino)-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (8). According to the procedure of Flynn *et al.*,[43] methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**5**) (500 mg, 1.38 mmol) was dissolved in anhydrous CH_2Cl_2 (3 mL) under N_2, Et_3N (192 μL, 1.38 mmol), di-*tert*-butyl dicarbonate (602 mg, 2.76 mmol), and 4-dimethylaminopyridine (169 mg, 1.38 mmol) were added, and the solution stirred at rt (~ 20 °C). After 20 h, TLC (hex:acetone; 2:1, **5** R_f 0.05, **8** R_f 0.40) showed ~75% conversion. A further portion of di-*tert*-butyl dicarbonate (602 mg, 2.76 mmol) in anhydrous CH_2Cl_2 (1 mL) was added and the reaction heated at ca. 45 °C for 2 h. The reaction mixture was concentrated and the residue chromatographed (silica, hex:acetone 3:1) to give a pale yellow oil (388 mg, 61%); IR 1750, 1694, 1370, 1332, 1228, 1146, 1042 cm^{-1}; The ^1H NMR (300 MHz, $CDCl_3$) spectrum for **8** at 30 °C showed 2 species in a ratio of ~ 1.3:1, possibly rotamers about one of the amide bonds, leading to a fairly broad spectrum. The ^1H NMR [300 MHz, $(CD_3)_2SO$] spectrum for **8** at 80 °C was resolved into a single species for all protons except for H-2 which was just appearing from the baseline. ^1H NMR (300 MHz, $CDCl_3$, 30 °C) δ 1.53 & 1.57 (9 H, 2 × br.s, ^{t}Bu), 1.96 (3 H, br.s, AcO), 2.01 (3 H, s, AcO), 2.09 (3 H, s, AcO), 2.36 & 2.41 (3 H, 2 x br.s, AcN), 3.46 (3 H, s, OMe), 3.73 (1 H, br.m, H-5), 4.13 (1 H, br.d, $J_{6,6'}$ 12.3 Hz, H-6), 4.14 (~ 0.5 H, br.m, part H-2), 4.29 (1 H, dd, $J_{6',5}$ 4.2, $J_{6',6}$ 12.3 Hz, H-6'), 4.88 (~ 0.5 H, br.m, part H-2), 5.08 (~ 1.5 H, br.m,

H-4* and part H-1), 5.27 (~ 0.5 H, br.m, part H-1), 5.60-5.82 (1 H, 2 x br.m, H-3*); ^{1}H NMR [300 MHz, $(CD_3)_2SO$, 80 °C] δ 1.53 (9 H, s, ^{t}Bu), 1.91 (3 H, s, AcO), 1.99 (3 H, s, AcO), 2.03 (3 H, s, AcO), 2.31 (3 H, br.s, AcN), 3.38 (3 H, s, OMe), 3.83 (1 H, ddd, $J_{5,4}$ 10.2, $J_{5,6}$ 2.7, $J_{5,6'}$ 4.8 Hz, H-5), 4.10 (1 H, dd, $J_{6,5}$ 2.7, $J_{6,6'}$ 12.3 Hz, H-6), 4.21 (1 H, dd, $J_{6',5}$ 4.8, $J_{6',6}$ 12.3 Hz, H-6'), 4.30-4.60 (1 H, br, H-2), 4.87 (1 H, dd, $J_{4,3}$ 9.0, $J_{4,5}$ 10.2 Hz, H-4*), 5.05 (1 H, br.d, $J_{1,2}$ 7.5 Hz, H-1), 5.58 (1 H, dd, $J_{3,2}$ 10.5, $J_{3,4}$ 9.0 Hz, H-3*) (*assignments interchageable); ^{13}C NMR (75.5 MHz, $CDCl_3$, 30 °C) δ 20.4, 20.5, 20.6, 26.6, 27.1, 27.8 (C*Me*$_3$), 56.3, 56.9 (br.), 61.3, 62.0 (C-6), 69.5, 70.6, 71.4 (br.), 100.3 & 101.0 (C-1), 169.5, 170.0, 170.5; *m/z* (ESI) 500 [$(M+K)^+$, 4%], 484 [$(M+Na)^+$, 48], 479 [$(M+NH_4)^+$, 51], 462 [$(M+H)^+$, 10].

Methyl 3,4,6-tri-*O*-acetyl-2-(*N*,*N*-diacetylamino)-2-deoxy-β-D-glucopyranoside (9). According to literature methodology,[44] methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (5) (200 mg, 0.55 mmol) was dissolved in anhydrous acetonitrile (3 mL) under N_2, ^{i}Pr$_2$NEt (106 μL, 0.61 mmol), and acetyl chloride (43 μL, 0.61 mmol) were added, and the solution stirred at RT. After 20 h, TLC (hex:acetone; 2:1, 5 R_f 0.05, 9 R_f 0.33) showed ~50% conversion. Further portions of ^{i}Pr$_2$NEt (106 μL, 0.61 mmol), and acetyl chloride (43 μL, 0.61 mmol) were added, and the reaction continued for a further 20 h. The reaction mixture was concentrated with toluene, the residue was taken up in EtOAc, and the mixture filtered through celite. The filtrate was concentrated, and the residue chromatographed (silica, hex:acetone; 10:3) to give 9 (200 mg, 90%) as an amorphous mass; IR 1754, 1708, 1368, 1280, 1224, 1052 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.98, 2.01, 2.08 (3 × 3 H, 3 × s, 3 × AcO), 2.33 (3 H, br.s, AcN), 2.36 (3 H, br.s, AcN), 3.47 (3 H, s, MeO), 3.69 (1 H, dd, $J_{2,1}$ 7.8, $J_{2,3}$ 10.5 Hz, H-2), 3.79 (1 H, ddd, $J_{5,4}$ 10.2, $J_{5,6}$ 2.4, $J_{5,6'}$ 4.5 Hz, H-5), 4.11 (1 H, dd, $J_{6,5}$ 2.4, $J_{6,6'}$ 12.3 Hz, H-6), 4.33 (1 H, dd, $J_{6',5}$ 4.5, $J_{6',6}$ 12.3 Hz, H-6'), 5.07 (1 H, dd, $J_{4,3}$ 8.7, $J_{4,5}$ 10.2 Hz, H-4), 5.26 (1 H, d, $J_{1,2}$ 7.8 Hz, H-1), 5.81 (1 H, dd, $J_{3,4}$ 8.7, $J_{3,2}$ 10.5 Hz, H-3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 20.3, 20.5, 20.6 (3 × AcO), 24.9, 27.6 (2 × AcN), 57.2 (MeO), 61.9 (C-6), 62.2 (C-2), 69.3, 70.5, 71.3 (C-3/C-4/C-5), 100.2 (C-1), 169.6 (2 × Ac), 170.5, 173.8, 174.6 (3 × Ac); *m/z* (ESI) 426 [$(M+Na)^+$, 100%].

General procedure for the reaction of β-methyl-glycosides with α,α-dichloromethyl methyl ether and $ZnCl_2$.[23] Under anhydrous conditions, a solution of carbohydrate glycoside (0.2 mmol) in dry 1,2-dichloroethane (1 mL) was treated with

α,α-dichloromethyl methyl ether (DCMME) (200 μL) and a catalytic amount of freshly fused $ZnCl_2$ (~ 4 mg), and the mixture heated at 55 °C. Reactions were monitored by TLC every hour, and further portions of DCMME and $ZnCl_2$ were added after 2 h.

Thio [1-thio-β-D-galactopyranose]-(1,1)-*S*-1-thio-β-D-galactopyranoside (15). To a solution of 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-galactopyranoside (**13**)[28] (1.5 g, 3.7 mmol) in dry MeOH (20 mL) at 0 °C under N_2 was added Na metal (~200 mg). The mixture was allowed to warm to rt over ~ 30 min. and stirred for a further 2 h at rt before being concentrated. The pale yellow residue was dissolved in 10% aq MeOH (30 mL) and I_2 crystals added until a permanent red colour remained. The mixture was stirred for 24 h at RT before being concentrated and chromatographed (silica, EtOAc: MeOH:H_2O; 4:2:0.2; R_f 0.2) to give **15** (643 mg, 89%) as colourless clusters from MeOH; mp 114-118 °C; IR 3400 (br), 1132, 1078, 1054, 858 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 3.65 (1 H, dd, $J_{3,2}$ 9.3, $J_{3,4}$ 3.0 Hz, H-3), 3.68-3.83 (4 H, m, H-2/H-5/H-6/H-6'), 3.94 (1 H, d, $J_{4,3}$ 3.0 Hz, H-4), 4.51 (1 H, d, $J_{1,2}$ 9.6 Hz, H-1); ^{13}C NMR (75.5 MHz, D_2O) δ 61.1 (C-6), 68.7, 68.8, 73.8, 79.5 (C-2/C-3/C-4/C-5), 89.9 (C-1).

Thio [2-acetamido-2-deoxy-1-thio-β-D-glucopyranose]-(1,1)-*S*-2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside (16). To a solution of 2-acetamido-3,4,6-tri-*O*-acetyl-1-*S*-acetyl-2-deoxy-1-thio-β-D-glucopyranoside (**14**)[27] (385 mg, 0.95 mmol) in MeOH (10 mL) was added ammonia solution (28%, 2 mL). The mixture was stirred at rt for 24 h before being concentrated to give **16** (162 mg, 72%; R_f 0.14, EtOAc:MeOH: H_2O; 7:2:1) as an off-white powder, which was reprecipitated from MeOH; mp 196 °C [lit[31]: mp 212-213 °C (MeOH)]; $[\alpha]_D$ -236° (*c* 0.9, H_2O, 28 °C) [lit[31]: $[\alpha]_D$ -223° (*c* 0.9, H_2O, 16 °C)]; *m/z* (ESI) 511 [$(M+K)^+$, 18%], 495 [$(M+Na)^+$, 100], 473 [$(M+H)^+$, 9]. The disulfide **16** could be more readily purified through conversion to the peracetylated derivative **32**.

Thio [2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranose]-(1,1)-*S*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranoside (32). The disulfide **16** (100 mg, 0.21 mmol) was dissolved in pyridine (2 mL) and Ac_2O (1 mL) and the reaction stirred for 16 h at rt before being concentrated. Column chromatography (silica, EtOAc:MeOH; 30:1; R_f 0.20) gave **32** (146 mg, 95%) as an amorphous mass: IR 1744, 1660, 1552, 1432, 1370, 1228, 1042, 908, 600 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$/ CD_3OD) δ 1.93 (3 H, s, AcN), 1.98 (6 H, s, 2 × AcO), 2.08 (3 H, s, AcO), 3.73 (1 H, m,

H-5), 3.93 (1 H, dd, $J_{2,1}$ 10.5, $J_{2,3}$ 9.9 Hz, H-2), 4.06 (1 H, br.d, $J_{6,6'}$ 12.3 Hz, H-6), 4.34 (1 H, dd, $J_{6',5}$ 5.1, $J_{6',6}$ 12.3 Hz, H-6'), 4.79 (1 H, d, $J_{1,2}$ 10.5 Hz, H-1), 4.93 (1 H, dd, $J_{4,3}$ 9.6, $J_{4,5}$ 9.9 Hz, H-4*), 5.30 (1 H, dd, $J_{3,4}$ 9.6, $J_{3,2}$ 9.9 Hz, H-3*) (*assignments interchangeable); ^{13}C NMR (75.5 MHz, $CDCl_3/CD_3OD$) δ 20.0 (2 × Ac), 20.2, 22.2 (2 × Ac), 52.7 (C-2), 61.8 (C-6), 68.4, 73.2, 75.6 (C-3/C-4/C-5), 88.1 (C-1), 169.6, 170.5, 171.0, 171.4 (4 × Ac); *m/z* (ESI) 725 [$(M+H)^+$, 100].

Anal. Calcd for $C_{28}H_{40}N_2O_{16}S_2$·H: 725.18975. Found: 725.18883.

Thio {2,3,4-tri-*O*-acetyl-6-*O*-[(*tert*-butyldimethylsilyl)oxy]-1-thio-β-D-galacto-pyranose}-(1,1)-*S*-2,3,4-tri-*O*-acetyl-6-*O*-[(*tert*-butyldimethylsilyl)oxy]-1- thio-β-D-galactopyranoside (18). To a solution of the disulfide **15** (500 mg, 1.28 mmol) in pyridine (5 mL) at 0 °C under N_2 was added TBDMSCl (425 mg, 2.82 mmol). The mixture was allowed to warm to rt and stirred for 16 h before being concentrated. The residue was dissolved in pyridine (10 mL) and Ac_2O (20 mL) and stirred for 16 h at rt before being concentrated. Column chromatography (silica, EtOAc:hex; 1:2; R_f 0.4) gave **18** (858 mg, 77%) as an amorphous mass: IR 1749, 1668, 1545, 1371, 1224, 1122, 1032, 834 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.02, 0.04 (2 × 3 H, 2 × s, 2 × SiMe), 0.86 (9 H, s, Si^{t}Bu), 1.96, 2.07, 2.13 (3 × 3 H, 3 × s, 3 × AcO), 3.54-3.60 (1 H, m, H-5), 3.72-3.82 (2 H, m, H-6/H-6'), 4.57 (1 H, d, $J_{1,2}$ 9.9 Hz, H-1), 5.08 (1 H, dd, $J_{3,2}$ 9.9, $J_{3,4}$ 3.4 Hz, H-3), 5.31 (1 H, dd, $J_{2,1} = J_{2,3}$ 9.9 Hz, H-2), 5.51 (1 H, d, $J_{4,3}$ 3.4 Hz, H-4); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ -5.6 ($SiMe_2$), 18.0 ($SiCMe_3$), 20.5, 20.6, 20.7 (3 × C(O)CH_3), 25.6 ($SiCMe_3$), 60.2 (C-6), 66.9, 67.7, 72.1, 77.6 (C-2/C-3/C-4/C-5), 88.9 (C-1), 169.2, 169.7, 169.9 (3 × C(O)CH_3); *m/z* (ESI) 894 [$(M+Na)^+$, 55%], 403 (95), 241 (100).

Thio {2-acetamido-3,4-di-*O*-acetyl-6-*O*-[(*tert*-butyldimethylsilyl)oxy]-2-deoxy-1-thio-β-D-glucopyranose}-(1,1)-S-2-acetamido-3,4-di-*O*-acetyl-6-*O*-[(*tert*-butyl-dimethylsilyl)oxy]-2-deoxy-1-thio-β-D-glucopyranoside (19). To a solution of the disulfide **16** (167 mg, 0.35 mmol) in anhydrous pyridine (4 mL) under N_2, was added TBDMSCl (132 mg, 0.85 mmol). The mixture was stirred for 24 h at rt before being concentrated with anhydrous toluene to give a residual colourless solid (R_f 0.54, EtOAc:MeOH:H_2O; 7:2:1). The residue was dissolved in pyridine (10 mL) and Ac_2O (20 mL) and stirred for 16h at rt before being concentrated. Column chromatography (silica, hex:acetone; 2:1) gave **19** (230 mg, 75%; R_f 0.40, hex:acetone; 1:1) as an amorphous mass: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.07, 0.09 (2 × 3 H, 2 × s, 2 × SiMe),

0.91 (9 H, s, Si^{t}Bu), 2.01 (9 H, br.s, 3 × Ac), 3.54 (1 H, ddd, $J_{5,4}$ 9.9, $J_{5,6}$ 4.5, $J_{5,6'}$ 2.1 Hz, H-5), 3.70 (1 H, dd, $J_{6,5}$ 4.5, $J_{6,6'}$ 11.4 Hz, H-6), 3.79 (1 H, dd, $J_{6',5}$ 2.1, $J_{6',6}$ 11.4 Hz, H-6'), 3.94 (1H, ddd, $J_{2,1}$ 10.5, $J_{2,3}$ 9.9, $J_{2,NH}$ 8.4 Hz, H-2), 4.92 (1 H, d, $J_{1,2}$ 10.5 Hz, H-1), 5.03 (1 H, dd, $J_{4,3}$ 9.3, $J_{4,5}$ 9.9 Hz, H-4*), 5.31 (1 H, dd, $J_{3,2}$ 9.9, $J_{3,4}$ 9.3 Hz, H-3*), 5.86 (1 H, br.d, $J_{NH,2}$ 8.4 Hz, NH) (*assignments interchangeable); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ -5.5, -5.2 (2 × SiMe), 18.3 (Si*C*Me$_3$), 20.6 (2 × Ac), 23.3 (Ac), 25.8 (SiC*Me*$_3$), 53.5 (C-2), 62.5 (C-6), 68.7, 73.7, 78.9 (C-3/C-4/C-5), 89.0 (C-1), 169.2 (Ac), 170.5 (2 × Ac); *m/z* (ESI) 886 [(M+NH$_4$)$^+$, 100%], 869 [(M+H)$^+$, 52].

Anal. Calcd for $C_{36}H_{64}N_2O_{14}S_2Si_2$·H: 869.34158. Found: 869.34186.

Thio [2,3,4-tri-*O*-acetyl-1-thio-β-D-galactopyranose]-(1,1)-*S*-2,3,4-tri-*O*-acetyl-1-thio-β-D-galactopyranoside (20). A solution of the disulfide **18** (400 mg, 0.46 mmol) in 80% aq AcOH (10 mL) was stirred at 50 °C for 1 h. The solution was allowed to cool, concentrated and chromatographed (silica, EtOAc:hex; 2:1; R_f 0.2) to give **20** (248 mg, 84%) as an amorphous mass: IR 3500 (br), 1746, 1370, 1220, 1078, 1050 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.00, 2.08, 2.17 (3 × 3 H, 3 × s, 3 × AcO), 2.72 (1 H, brs, OH), 3.52-3.59 (1 H, m, H-5), 3.70-3.77 (1 H, m, H-6), 3.84-3.88 (1 H, m, H-6'), 4.61 (1 H, d, $J_{1,2}$ 8.9 Hz, H-1), 5.08 (1 H, dd, $J_{3,2}$ 10.2, $J_{3,4}$ 3.3 Hz, H-3), 5.35 (1 H, dd, $J_{2,3}$ 10.2, $J_{2,1}$ 8.9 Hz, H-2), 5.39 (1 H, d, $J_{4,3}$ 3.3 Hz, H-4); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 20.4, 20.5, 20.6 (3 × C(O)*C*H$_3$), 61.2 (C-6), 67.9, 68.0, 71.8, 78.3 (C-2/C-3/C-4/C-5), 88.8 (C-1), 169.5, 169.8, 170.6 (3 × C(O)CH3); *m/z* (ESI) 665 [(M+Na)$^+$, 47%], 660 [(M+NH$_4$)$^+$, 72%], 643 [(M+1)$^+$, 10%].

Anal. Calcd for $C_{24}H_{34}O_{16}S_2$·H_2O: C 43.63; H 5.49. Found: C 43.54; H 5.14.

Thio [2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranose]-(1,1)-*S*-2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside (22) and Thio [2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranose]-(1,1)-*S*-2,3-di-*O*-acetyl-4,6-*O*-isopropylidene-1-thio-β-D-galactopyranoside (23). A solution of the galactose disulfide **15** (700 mg, 1.8 mmol), 2,2-dimethoxypropane (1.1 mL, 8.9 mmol), and *p*-TsOH·H_2O (20 mg) were heated at 80 °C in DMF (10 mL) for 30 min. The mixture was allowed to cool, neutralised with Et_3N and concentrated. The residue was dissolved in pyridine (10 mL) and Ac_2O (5 mL) added, and the mixture stirred for 16 h at rt before being concentrated. The residue was chromatographed (silica, EtOAc:hex; 3:2) to give **22** (490 mg, 43%, R_f 0.5) and **23** (446 mg, 39%, R_f 0.4).

Compound 22: IR 1750, 1372, 1220, 1082, 1044, 844 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.34, 1.57 (2 × 3 H, 2 × s, 2 × O_2CMe), 2.09, 2.10 (2 × 3 H, 2 × s, 2 × AcO), 4.01-4.05 (1 H, m, H-5), 4.19-4.25 (2 H, m, H-3/H-4), 4.29 (1 H, dd, $J_{6,6'}$ 11.7, $J_{6,5}$ 6.0 Hz, H-6), 4.39 (1 H, dd, $J_{6',6}$ 11.7, $J_{6',5}$ 7.2 Hz, H-6'), 4.53 (1 H, d, $J_{1,2}$ 9.0 Hz, H-1), 5.12 (1 H, dd, $J_{2,1}$ 9.0, $J_{2,3}$ 6.6 Hz, H-2); *m/z* (ESI) 656 [$(M+NH_4)^+$, 100%].

Anal. Calcd for $C_{26}H_{38}O_{14}S_2$: C 48.89; H 6.00. Found: C 48.90; H 5.90.

Compound 23: IR 1748, 1374, 1222, 1084, 1042, 866 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.33, 1.39, 1.50, 1.56 (4 × 3 H, 4 × s, 4 × O_2CMe), 2.05, 2.07, 2.10, 2.16 (4 × 3 H, 4 × s, 4 × AcO), 3.44 (1 H, brs, H-5†), 3.93 (1 H, dd, $J_{6,6'}$ 12.9, $J_{6,5}$ 1.5 Hz, H-6†), 4.02 (1 H, dd, $J_{6',6}$ 12.9, $J_{6',5}$ 1.8 Hz, H-6'†), 4.02-4.05 (1 H, m, H-5), 4.19-4.24 (2 H, m, H-3/H-4), 4.30-4.43 (3 H, m, H-6/H-6'/H-4†), 4.55 (1 H, d, $J_{1,2}$ 9.9 Hz, H-1†), 4.76 (1 H, dd, $J_{1,2}$ 9.6 Hz, H-1), 4.92 (1 H, dd, $J_{3,2}$ 9.6, $J_{3,4}$ 3.6 Hz, H-3†), 5.07 (1 H, dd, $J_{2,1}$ 9.6, $J_{2,3}$ 6.6 Hz, H-2), 5.59 (1 H, dd, $J_{2,1}$ 9.9, $J_{2,3}$ 9.6 Hz, H-2†), †indicates those resonances due to protons on the 4,6-*O*-isopropylidenated galactose unit; *m/z* (ESI) 656 [$(M+NH_4)^+$, 100%].

Anal. Calcd for $C_{26}H_{38}O_{14}S_2 \cdot NH_4$: 656.20467. Found: 656.20394.

Thio [(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1,4)-*O*-2,3,6,-tri-*O*-acetyl-1-thio-β-D-glucopyranose]-(1,1)-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1,4)-*O*-2,3,6,-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (41). A solution of the known[35] lactoside **26** (500 mg, 0.72 mmol) in MeOH (20 mL) was treated with aqueous NaOH solution (1 M, to pH 10) at rt for 20 h before being concentrated. The pale yellow residue was dissolved in 10% aq. MeOH (30 mL) and I_2 crystals added until a permanent red colour remained. The mixture was stirred for 24 h at rt before being concentrated with toluene. The residue (TLC EtOAc:MeOH:H_2O; 4:2:1; R_f 0.12) was dissolved in pyridine (5 mL) and Ac_2O (4 mL) and stirred at rt for 16 h before being concentrated. Column chromatography (silica, hex:acetone; 4:3; R_f 0.12) gave **41** (310 mg, 66%) as an amorphous solid: IR 1750, 1432, 1370, 1226, 1166, 1134, 1050, 948, 906, 598 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.97, 2.04 (2 × 3 H, 2 × s, 2 × AcO), 2.05 (6 H, s, 2 × AcO), 2.06, 2.15, 2.17 (3 × 3 H, 3 × s, 3 × AcO), 3.69 (1 H, m, GlcH-5), 3.84 (1 H, dd, $J_{4,3}$ 9.3, $J_{4,5}$ 9.6 Hz, GlcH-4), 3.90 (1 H, br.dd, $J_{5,6}$ 6.9, $J_{5,6'}$ 7.2 Hz, GalH-5), 4.04-4.20 (3 H, m, GlcH-6/GalH-6/GalH-6'), 4.55 (1 H, d, $J_{1,2}$ 9.6 Hz, GlcH-1), 4.58 (1 H, d, $J_{1,2}$ 7.8 Hz, GalH-1), 4.63 (1 H, br.d, $J_{6,6'}$ 12.3 Hz, GlcH-6'), 4.98 (1 H, dd, $J_{3,2}$ 10.5, $J_{3,4}$ 3.3 Hz, GalH-3), 5.06 (1 H, dd, $J_{2,1}$ 9.6, $J_{2,3}$ 9.0 Hz, GlcH-2), 5.12 (1 H, dd, $J_{2,1}$ 7.8, $J_{2,3}$ 10.5 Hz,

GalH-2), 5.24 (1 H, dd, $J_{3,2}$ 9.0, $J_{3,4}$ 9.3 Hz, GlcH-3), 5.35 (1 H, d, $J_{4,3}$ 3.3 Hz, GalH-4); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 20.4 (Ac), 20.5 (4 × Ac), 20.6, 20.8 (2 × Ac), 60.6, 61.5 (GlcC-6/GalC-6), 66.5, 69.0, 70.1, 70.5, 70.9, 73.8, 75.6, 76.9 (Glc and Gal C-2/C-3/C-4/C-5), 86.7 (GlcC-1), 100.8 (GalC-1), 168.9, 169.2, 169.6, 169.9, 170.0, 170.1, 170.3 (7 × Ac); *m/z* (ESI) 1320 [$(M+NH_4)^+$, 100%].

Anal. Calcd for $C_{52}H_{70}O_{34}S_2 \cdot NH_4$: 1320.35337. Found: 1320.35188.

Thio [(2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1,4)-*O*-2,3,6,-tri-*O*-acetyl-1-thio-β-D-glucopyranose]-(1,1)-*S*-(2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1,4)-*O*-2,3,6,-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (27). A solution of the peracetylated lactose disulfide **41** (184 mg, 0.14 mmol) in MeOH (5 mL) was treated with aqueous NaOH solution (1 M, to pH 10) at rt for 20 h, at which point TLC analysis (EtOAc:MeOH:H_2O; 4:2:1) showed a single component (R_f 0.12) corresponding to the disulfide **25**. The solution was neutralised by addition of Dowex 50W×8 (H^+) resin, filtered, and the residue washed with 4:1 MeOH/H_2O. The filtrate was evaporated repeatedly with toluene to give an off-white amorphous solid (96 mg, 0.13 mmol, 95%). The crude disulfide **25** was dissolved in DMF (1 mL), 2,2-dimethoxypropane (76 μL, 0.62 mmol) and *p*-TsOH·H_2O (2 mg) were added, and the mixture heated at 80 °C for 30 min. A further portion of 2,2-dimethoxypropane (76 μL) was added and heating continued for 40 min. before the mixture was cooled, neutralised with Et_3N and concentrated. The residue was dissolved in pyridine (1 mL) and Ac_2O (0.5 mL) and stirred at rt for 16 h before being concentrated. Column chromatography (silica, hex:acetone; 4:3; R_f 0.19) gave **27** (119 mg, 73%) as an amorphous mass: IR 1756, 1432, 1372, 1226, 1154, 1130, 1048, 898, 866, 602 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32, 1.53 (2 × 3 H, 2 × s, 2 × O_2CMe), 2.03, 2.07, 2.08, 2.11, 2.15 (5 × 3 H, 5 × s, 5 × AcO), 3.70 (1 H, m, GlcH-5), 3.75 (1 H, dd, $J_{4,3}$ 9.0 Hz, GlcH-4), 3.95 (1 H, br.dd, GalH-5), 4.15 (2 H, m, GalH-3/GalH-4), 4.21-4.34 (3 H, m, GlcH-6/GalH-6/GalH-6'), 4.42 (1 H, d, $J_{1,2}$ 7.5 Hz, GalH-1), 4.56 (1 H, br.d, GlcH-6'), 4.57 (1 H, d, $J_{1,2}$ 9.9 Hz, GlcH-1), 4.83 (1 H, m, GalH-2), 5.06 (1 H, dd, $J_{2,1}$ 9.9, $J_{2,3}$ 9.3 Hz, GlcH-2), 5.22 (1 H, dd, $J_{3,4}$ 9.0 Hz, GlcH-3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 20.6 (2 × Ac), 20.7 (2 × Ac), 20.8 (Ac), 26.0, 27.2 (2 × $O_2C\mathit{Me}$), 62.0, 63.0 (GlcC-6/GalC-6), 70.0, 70.7, 72.5, 73.0, 73.5, 75.5, 76.7, 77.2 (Glc and Gal C-2/C-3/C-4/C-5), 86.9 (GlcC-1), 100.2 (GalC-1), 110.7 ($O_2\mathit{C}Me_2$), 169.0, 169.2, 169.8, 170.3, 170.5 (5 × Ac); *m/z* (ESI) 1232 [$(M+NH_4)^+$, 100%].

Anal. Calcd for $C_{50}H_{70}O_{30}S_2 \cdot NH_4$: 1232.37371. Found: 1232.37199.

Attempted 4,6-*O*-benzylidenation of the GlcNAc disulfide (16). A solution of the GlcNAc disulfide **16** (187 mg, 0.39 mmol) and benzaldehyde dimethyl acetal (240 μL, 1.6 mmol) in *N,N*-DMF (3 mL) was treated with *p*-TsOH (20 mg) under standard conditions.[33] This resulted in the formation of several components by TLC examination, all of which were highly insoluble in a range of solvents (and only sparingly soluble in DMSO). None of these components were identified.

General procedure for the reductive cleavage of disulfide linkages using PBu_3 in aqueous dioxane.[40] The disulfide (0.05 mmol) and PBu_3 (0.075 mmol) were dissolved in aqueous dioxane solution (1 mL, [prepared by combining distilled dioxane (8 mL), degassed water (2 mL) and 1 drop of 50% HCl under N_2]) and the solution was heated at ca. 40 °C under N_2 for 1 h. The reaction was monitored by TLC and a further portion of PBu_3 (0.075 mmol) was added if cleavage (indicated by an increase in R_f) was incomplete. The solution was concentrated with toluene, and the residue benzoylated using pyridine (1 mL) and benzoic anhydride (0.15 mmol) for 16h at rt. The reaction was quenched with methanol, the solution was concentrated, and the residue chromatographed on silica to give the corresponding 1-*S*-benzoyl derivative.

2,3,4,6-Tetra-*O*-acetyl-1-*S*-benzoyl-1-thio-β-D-galactopyranose (33). Prepared from **31** following the above procedure in 81% yield as an amorphous mass (R_f 0.45, hex:EtOAc; 1:1): ^{1}H NMR (300MHz, $CDCl_3$) δ 2.01 (6 H, s, 2 × AcO), 2.04 (3 H, s, AcO), 2.18 (3 H, s, AcO), 4.11-4.23 (3 H, m, H-5/H-6/H-6'), 5.19 (1 H, dd, $J_{3,2}$ 8.4, $J_{3,4}$ 3.3 Hz, H-3), 5.43-5.53 (3 H, m, H-1/H-2/H-4), 7.46-7.51 (2 H, m, ArH), 7.62 (1 H, m, ArH), 7.94 (1 H, m, ArH), 8.12 (1 H, m, ArH).

2-Acetamido-3,4,6-tri-*O*-acetyl-1-*S*-benzoyl-2-deoxy-1-thio-β-D-glucopyranose (34). Prepared from **32** following the above procedure in 50% yield as an amorphous mass (R_f 0.48, EtOAc): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.89 (3 H, s, Ac), 2.05 (6 H, s, 2 × Ac), 2.08 (3 H, s, Ac), 3.86 (1 H, m, H-5), 4.13 (1 H, br.d, $J_{6,6'}$ 12.3 Hz, H-6), 4.28 (1 H, dd, $J_{6',5}$ 3.3, $J_{6',6}$ 12.3 Hz, H-6'), 4.51 (1 H, ddd, $J_{2,1}$ 10.5, $J_{2,NH}$ 9.9 Hz, H-2), 5.12-5.23 (2 H, m, H-3/H-4), 5.39 (1 H, d, $J_{1,2}$ 10.5 Hz, H-1), 5.59 (1 H, br.d, $J_{NH,2}$ 9.9 Hz, NH), 7.44-7.49 (2 H, m, ArH), 7.61 (1 H, m, ArH), 7.95 (2 H, m, ArH).

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1,4)-*O*-2,3,6-tri-*O*-acetyl-1-*S*-benzoyl-1-thio-β-D-glucopyranose (35). Prepared from **41** following the above

procedure in 82% yield as an amorphous mass (R_f 0.33, hex:acetone; 4:3): ^{1}H NMR (300MHz, $CDCl_3$) δ 1.97, 2.00 (2 × 3 H, 2 × s, 2 × AcO), 2.06 (6 H, br.s, 2 × AcO), 2.08, 2.10, 2.16 (3 × 3 H, 3 × s, 3 × AcO), 3.85-3.91 (3 H, m), 4.06-4.18 (3 H, m), 4.45-4.51 (2 H, m, H-6/GalH-1), 4.96 (1 H, dd, $J_{3,2}$ 10.8 Hz, $J_{3,4}$ 3.0 Hz, GalH-3), 5.10-5.47 (5 H, m, GlcH-1/GlcH-2/GlcH-3/GalH-2/GalH-4), 7.44-7.50 (2 H, m, ArH), 7.61 (1 H, m, ArH), 7.92 (1 H, d, *J* 7.5 Hz, ArH), 8.11 (1 H, d, *J* 7.5 Hz, ArH).

General procedure for the reductive cleavage of disulfide linkages using Zn and Ac_2O.[36,38] To a solution of the disulfide in Ac_2O (1 mL per mmol) was added Zn powder (at least 80% by mass) and the mixture heated at 65 °C for 30 to 40 min. The mixture was cooled, filtered through celite, concentrated and chromatographed on silica to give the corresponding thioacetate derivative.

2,3,4,6-Tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-galactopyranose (13).[28] Prepared from **31** following the above procedure in 82% yield as an amorphous mass, which was identical in all respects to that material prepared directly from the galactosyl bromide **11** according to the method of Holland, *et al.*[28]

2-Acetamido-3,4,6-tri-*O*-acetyl-1-*S*-acetyl-2-deoxy-1-thio-β-D-glucopyranose (14).[27] Prepared from **32** following the above procedure in 78% yield as an amorphous mass, which was identical in all respects to that material prepared directly from the glucosyl chloride **12** according to the method of Horton and Wolfrom.[27]

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1,4)-*O*-2,3,6-tri-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-glucopyranose (26).[35] Prepared from **41** following the above procedure (except reaction performed at 85 °C) in 80% yield as an amorphous mass, which was identical in all respects to that material prepared directly from 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1,4)-*O*-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide according to the method of Goodman *et al.*[35]

2,3,4-Tri-*O*-acetyl-1-*S*-acetyl-6-*O*-[(*tert*-butyldimethylsilyl)oxy]-1-thio-β-D-galactopyranose (36). Prepared by treating the disulfide **18** with Zn in Ac_2O according to the above procedure in 85% yield after purification (silica, EtOAc:hex; 1:2; R_f 0.5) as an amorphous mass: IR 1756, 1712, 1368, 1240, 1082, 834 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.01, 0.02 (2 × 3 H, 2 × s, 2 × SiMe), 0.85 (9 H, s, ^{t}Bu), 1.98, 2.08, 2.14 (3 × 3 H, 3 × s, 3 × AcO), 2.37 (3 H, s, AcS), 3.51-3.60 (1 H, m, H-5), 3.68-3.82 (2 H, m, H-6/H-6'), 5.03 (1 H, dd, $J_{3,2}$ 9.6, $J_{3,4}$ 3.0 Hz, H-3), 5.15 (1 H, d, $J_{1,2}$ 9.3 Hz, H-1), 5.26

(1 H, dd, $J_{2,3}$ 9.6, $J_{2,1}$ 9.3 Hz, H-2), 5.53 (1 H, d, $J_{4,3}$ 3.0 Hz, H-4); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ -5.7 ($SiMe_2$), 18.0 ($SiCMe_3$), 20.5, 20.6 (2 × AcO), 25.6 ($SiCMe_3$), 30.7 (AcS), 60.1 (C-6), 66.7, 67.0, 72.1, 77.5 (C-2/C-3/C-4/C-5), 80.5 (C-1), 169.5, 169.7 (2 × AcO), 192.0 (AcS).

2,6-Di-*O*-acetyl-3,4-*O*-isopropylidene-1-*S*-acetyl-1-thio-β-D-galactopyranose (37). Prepared by treating the disulfide **22** with Zn in Ac_2O according to the above procedure in 76% yield after purification (silica, EtOAc:hex; 2:3; R_f 0.4) as an amorphous mass: IR 1738, 1700, 1374, 1225, 1078, 1056 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34, 1.55 (2 × 3 H, 2 × s, 2 × O_2CMe), 2.08 (6 H, s, 2 × AcO), 2.37 (3 H, s, AcS), 4.10-4.15 (1 H, m, H-5), 4.20-4.27 (3 H, m, H-3/H-4/H-6), 4.36 (1 H, dd, $J_{6',6}$ 11.7, $J_{6',5}$ 4.2 Hz, H-6'), 5.12 (1 H, dd, $J_{2,1}$ 8.7, $J_{2,3}$ 5.7 Hz, H-2), 5.21 (1 H, d, $J_{1,2}$ 8.7 Hz, H-1); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 20.8 (AcO), 25.9, 27.2 (O_2CMe_2), 30.6 (AcS), 63.3 (C-6), 70.1, 73.2, 74.2, 75.9 (C-2/C-3/C-4/C-5), 79.1 (C-1), 169.4, 170.7 (2 × AcO), 192.6 (AcS); *m/z* (ESI) 380 [$(M+NH_4)^+$, 32%], 287 (100).

Anal. Calcd for $C_{15}H_{22}O_8S$: C 49.72; H 6.12. Found: C 49.86; H 6.19.

***S*-Acetyl *O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2,6)-*O*-2,3,4-tri-*O*-acetyl-1-thio-β-D-galactopyranose (39).** Prepared by treating the disulfide **38**[41] with Zn in Ac_2O according to the above procedure. TLC analysis of the reaction product showed two components which were identified as the thioacetate derivatives **39** (silica, $CHCl_3$:MeOH; 20:1; R_f 0.4) and **40** (silica, $CHCl_3$:MeOH; 20:1; R_f 0.6) by direct comparison with authentic samples.[41]

3-*O*-Acetyl-1-*S*-acetyl-6-*O*-[(*tert*-butyldimethylsilyl)oxy]-1-thio-β-D-galactopyranose (42) and thio {3-*O*-acetyl-1-thio-6-*O*-[(*tert*-butyldimethylsilyl)oxy]-β-D-galactopyranose}-(1,1)-*S*-6-*O*-[(*tert*-butyldimethylsilyl)oxy]-1-thio-β-D-galactopyranoside (43). Prepared by treating the C-6 silylated galactose disulfide **17** with Zn in Ac_2O according to the above procedure. After workup the residue was chromatographed (silica, $CHCl_3$:MeOH; 20:1) to give **42** (37%, R_f 0.5) and **43** (34%, R_f 0.2).

Compound 42: IR 3490 (br), 1716, 1252, 1076, 834 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.07 (6 H, s, $SiMe_2$), 0.88 (9 H, s, ^{t}Bu), 2.18 (3 H, s, AcO), 2.40 (3 H, s, AcS), 3.27 (1 H, brs, OH), 3.64 (1 H, brt, $J_{5,6} = J_{5,6'} = 4.2$ Hz, H-5), 3.84-3.91 (2 H, m, H-6/H-6'), 4.06 (1 H, dd, $J_{2,1}$ 10.2, $J_{2,3}$ 9.6 Hz, H-2), 4.27 (1 H, brs, H-4), 4.87 (1 H, d, $J_{3,2}$ 9.6 Hz, H-3),

5.12 (1 H, d, $J_{1,2}$ 10.2 Hz, H-1); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ –5.6 ($SiMe_2$), 18.1 ($SiCMe_3$), 21.0 (AcO), 25.7 ($SiCMe_3$), 30.8 (AcS), 63.1 (C-6), 67.5, 68.5 (C-2/C-4), 77.1, 77.5 (C-3/C-5), 83.1 (C-1), 171.0 (AcO), 193.0 (AcS); *m/z* (ESI) 417 [$(M+Na)^+$, 27%].

Anal. Calcd for $C_{16}H_{30}O_7SSi{\cdot}NH_4$: 412.18253. Found: 412.18123.

Compound 43: IR 3470 (br), 1725, 1252, 1098, 832 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.09 (12 H, s, 2 × $SiMe_2$), 0.89 (18 H, s, 2 × ^{t}Bu), 2.17 (3 H, s, AcO), 3.54 (4 H, m, H-5/H-5'/H-3/ OH), 3.81-3.87 (4 H, m, 2 × H-6/H-6'), 4.01-4.06 (2 H, m, H-4/H-2), 4.16 (1 H, brs, H-4), 4.24 (1 H, dd, $J_{2,3}$ 9.6, $J_{2,1}$ 9.3 Hz, H-2), 4.43 (1 H, $J_{1,2}$ 9.3 Hz, H-1), 4.52 (1 H, $J_{1',2'}$ 9.3 Hz, H-1'), 4.85 (1 H, d, $J_{3,2}$ 9.6 Hz, H-3); *m/z* (ESI) 683 [$(M+Na)^+$, 85%], 678 [$(M+NH_4)^+$, 34], 643 (85), 319 (50), 241 (61), 175 (100).

Anal. Calcd for $C_{26}H_{52}O_{11}S_2Si_2{\cdot}NH_4$: 678.28334. Found: 678.28219.

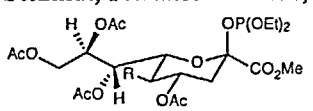
ACKNOWLEDGMENTS

Financial support of this work by Glaxo Wellcome, UK, is gratefully acknowledged, as is the Australian Research Council for a SPIRT grant.

REFERENCES AND NOTES

1. R. Schauer and J.P. Kamerling in *Glycoproteins II*, J. Montreuil, J.F.G. Vliengenthart, H. Schachter, Eds.; Elsevier: Amsterdam, 1997, pp 243-402.
2. A. Varki, *FASEB J.*, **11**, 248 (1997).
3. A. Varki, *Glycobiology*, **3**, 97 (1993).
4. P.R. Crocker and T. Feizi, *Curr. Opin. Struct. Biol.*, **6**, 679 (1996).
5. K.-A. Karlsson, *Curr. Opin. Struct. Biol.*, **5**, 622 (1995).
6. *Bacterial Adhesion to Cells and Tissues*, I. Ofek, R.J. Doyle, Eds.; Chapman Hall: New York, 1994.
7. S. Hakomori, *Adv. Cancer Res.*, **52**, 257 (1989).
8. T. Feizi, *Nature*, **314**, 53 (1985).
9. M. Sato, T. Narita, N. Kimura, K. Zenita, T. Hashimoto, T. Manabe and R. Kannagi, *Anticancer Res.*, **17**, 3505 (1997).
10. T. Feizi, *Curr. Opin. Struct. Biol.*, **3**, 701 (1993).
11. H. Driguez, *Top. Curr. Chem.*, **187**, 85 (1997).
12. A. Hasegawa and M. Kiso in *Carbohydrates: Synthetic Methods and Applications in Medicinal Chemistry*, H. Ogura, A. Hasegawa, T. Suami, Eds.; VCH: New York, 1992, pp 243-266.
13. R. Roy in *Modern Methods in Carbohydrate Synthesis*, S.H. Khan, R.A. O'Neill, Eds.; Harwood Academic: Amsterdam, 1996, pp 378-402.
14. T. Eisele, A. Toepfer, G. Kretzschmar and R.R. Schmidt, *Tetrahedron Lett.*, **37**, 1389 (1996).
15. M.J. Kiefel, B. Beisner, S. Bennett, I.D. Holmes and M. von Itzstein, *J. Med. Chem.*, **39**, 1314 (1996).

16. D.I. Angus and M. von Itzstein, *Carbohydr. Res.*, **274**, 279 (1995).
17. B. Smalec and M. von Itzstein, *Carbohydr. Res.*, **266**, 269 (1995).
18. M. von Itzstein and M.J. Kiefel, in *Carbohydrates in Drug Design*, Z.J. Witczak, K.A. Nieforth, Eds.; MDI: New York, 1997, pp 39-82.
19. M.J. Kiefel and M. von Itzstein, *Tetrahedron Lett.*, **37**, 7307 (1996).
20. R. Roy, in *Carbohydrates in Drug Design*, Z.J. Witczak, K.A. Nieforth, Eds.; MDI: New York, 1997, pp 83-135.
21. S. Ciccotosto, M.J. Kiefel, S. Abo, W. Stewart, K. Quelch and M. von Itzstein, *Glycoconjugate J.*, **15**, 663 (1998).
22. L-x. Wang, N. Sakairi and H. Kuzuhara, *J. Carbohydr. Chem.*, **9**, 441 (1990).
23. P. Kovác in *Modern Methods in Carbohydrate Synthesis*, S.H. Khan, R.A. O'Neill, Eds.; Harwood Academic: Amsterdam, 1996, pp 55-81.
24. K. Jansson, G. Noori and G. Magnusson, *J. Org. Chem.*, **55**, 3181 (1990).
25. See for example: (a) G.R. Perdomo and J.J. Krepinsky, *Tetrahedron Lett.*, **28**, 5595 (1987) and references therein; (b) P.M. Collins, P. Premarante, A. Manro and A. Hussain, *Tetrahedron Lett.*, **30**, 4721 (1989); (c) K. Higashi, K. Nakayama, E. Shioya and T. Kusama, *Chem. Pharm. Bull.*, **39**, 2502 (1991).
26. Prepared by treating peracetylated Gal with HBr in AcOH according to the method described in *Vogel's Textbook of Practical Organic Chemistry*, 5th Ed., Longman Scientific and Technical, UK, 1989, pp 648-649.
27. D. Horton and M.L. Wolfrom, *J. Org. Chem.*, **27**, 1794 (1962).
28. C.V. Holland, D. Horton and M.J. Miller, *J. Org. Chem.*, **32**, 3077 (1967).
29. (a) W. Schneider and A. Bansa, *Chem. Ber.*, **64**, 1321 (1931): (b) G. Capozzi and G. Modena in *The Chemistry of the Thiol Group, Part 2*, S. Patai, Ed.; John Wiley and Sons: London, 1974, pp 785-839.
30. The formation of a Gal disulfide derivative during NaOMe catalysed anomeric thioacetate deprotection has recently been mentioned in: U.J. Nilsson, E.J.-L. Fournier and O. Hindsgaul, *Bioorg. Med. Chem.*, **6**, 1563 (1998).
31. M. Akagi, S. Tejima and M. Haga, *Chem. Pharm. Bull.*, **9**, 360 (1961).
32. See for example: (a) J.L. Wardell in *The Chemistry of the Thiol Group, Part 1*, S. Patai, Ed.; John Wiley and Sons: London, 1974, pp 163-269; (b) D. Horton and J.D. Wander in *The Carbohydrates: Chemistry and Biochemistry*, W. Pigman, Ed.; Academic Press: New York, 1980, Vol. 1B, pp 799-842.
33. K. Jansson, S. Alfors, T. Frejd, J. Kihlberg and G. Magnusson, *J. Org. Chem.*, **53**, 5629 (1988).
34. H.H. Baer and S.A. Abbas, *Carbohydr. Res.*, **77**, 117 (1979).
35. I. Goodman, L. Salce and G.H. Hitchings, *J. Med. Chem.*, **11**, 516 (1968).
36. F. Wrede, *Z. Physiol. Chem.*, **119**, 46 (1922); *CA* **16**: 3637.
37. A. Fontana and C. Toniolo in *The Chemistry of the Thiol Group, Part 1*, S. Patai, Ed.; John Wiley and Sons: London, 1974, pp 271-324.
38. D. Horton, *Methods Carbohydr. Chem.*, **2**, 433 (1963).
39. R.E. Humphrey and J.L. Potter, *Anal. Chem.*, **37**, 164 (1965).
40. L.E. Overman, J. Smoot and J.D. Overman, *Synthesis*, 59 (1974).
41. Disulfide **41** was prepared by TMSOTf mediated coupling between the sialosyl phosphite **i** and the disulfide **21** using the method described by T.J. Martin and R.R. Schmidt, *Tetrahedron Lett.*, **33**, 6123 (1992).



I R = NHAc

42. For a recent example of the reaction of halides with KSAc in acetone see: T-C. Zheng, M. Burkart and D.E. Richardson, *Tetrahedron Lett.*, **40**, 603 (1999).
43. D.L. Flynn, R.E. Zelle, and P.A. Grieco, *J. Org. Chem.*, **48**, 2424 (1983).
44. (a) J.C. Castro-Palomino and R.R. Schmidt, *Tetrahedron Lett.*, **36**, 6871 (1995); (b) A.V. Demchenko and G.-J. Boons, *Tetrahedron Lett.*, **39**, 3065 (1998).