Pergamon

Bioorganic & Medicinal Chemistry 8 (2000) 2709–2718

BIOORGANIC & MEDICINAL CHEMISTRY

The Synthesis of Biotinylated Carbohydrates as Probes for Carbohydrate-Recognizing Proteins

Donald I. Angus,[a] Milton J. Kiefel[a,b] and Mark von Itzstein[a,b,*]

[a]*Department of Medicinal Chemistry, Monash University (Parkville Campus), 381 Royal Parade, Parkville, Victoria 3052, Australia*
[b]*Centre for Biomolecular Science and Drug Discovery, Griffith University (Gold Coast Campus), PMB 50 Gold Coast Mail Centre, Queensland 9726, Australia*

Received 6 June 2000; accepted 18 July 2000

Abstract—The intimate involvement of carbohydrate–protein interactions in a number of important biological processes has prompted several research efforts towards developing new methods of investigating these glycobiological interactions. Biotinylated oligosaccharides are emerging as a new and powerful tool in this area of research, primarily due to their high affinity towards streptavidin and their ease of immobilization on matrices. Here we describe a novel synthetic approach towards biotinylated saccharides which incorporate a UV absorbing group into the final compounds. The synthetic strategy described is applicable to a variety of saccharides, with examples of biotinylated mono-, di-, and trisaccharides being prepared with overall high efficiency.

Introduction

Carbohydrate-recognizing proteins are involved in a number of important biological processes, including cell–cell interactions,[1–3] bacterial and viral infections,[4–6] inflammation,[7–10] and metastasis.[11–13] Consequently, investigations into carbohydrate–protein interactions are becoming increasingly important in the search for a better understanding of these glycobiological processes.

The number of established techniques available for investigating carbohydrate–protein interactions, including affinity chromatography, electrophoresis, and NMR spectroscopy, is continually growing.[14–17] An important aspect of these investigations is the requirement for studies to be carried out with structurally-defined oligosaccharide sequences such that specific interactions can be analysed. Common techniques employed for carrying out these studies include the linking of saccharides to proteins, the linking of oligosaccharides to aminophospholipids, the incorporation of oligosaccharides into polymers, and the use of fluorescently-labelled oligosaccharides.[18–21]

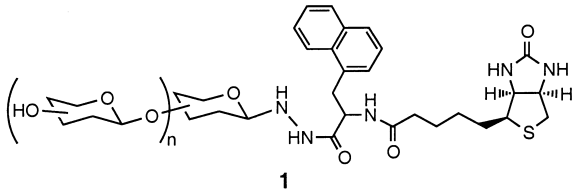
Recently it has been shown that biotinylated carbohydrates have great potential as probes for carbohydrate-recognizing proteins,[15,22–28] since they exploit the strong affinity of avidin and streptavidin for biotin.[29] Since each streptavidin molecule has four biotin binding sites, the complex resulting from interaction between streptavidin and biotinylated carbohydrate has the potential to behave as a multivalent ligand.[30] Thus far, applications of biotinylated carbohydrates have included the immobilization onto the surface of a sensor chip to facilitate kinetic measurements,[15,25] the production of IgG antibodies directed against oligosaccharide chains,[29] and immobilization on streptavidin-coated microwells for lectin binding studies.[30,31] Generally, oligosaccharides biotinylated at the anomeric centre of the reducing sugar have been prepared under either reducing or non-reducing conditions.[15,22–25] The reducing methods typically involve coupling of oligosaccharides with 2-amino-6-amidobiotinyl pyridine (BAP), whilst coupling under non-reducing conditions generally requires biotinylated hydrazine reagents. Unfortunately, many of these methods result in poor yields of the oligosaccharide being tagged.

A potential limitation of some of the reported biotinylated oligosaccharides is the lack of a chromophore, meaning, for example, that their use in the purification of oligosaccharides from natural sources is limited because of low sensitivity of detection. Recent efforts to improve sensitivity have focussed on the incorporation

*Corresponding author. Tel.: +617-5594-8232; fax: +617-5594-8098; e-mail: m.vonitzstein@mailbox.gu.edu.au

0968-0896/00/$ - see front matter
PII: S0968-0896(00)00201-7

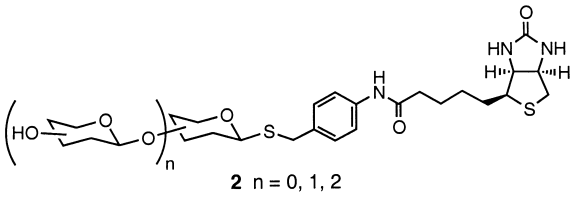
of a UV tag in addition to the biotinyl group. The resulting bifunctionally-labelled oligosaccharides, e.g., **1**, [22] have both the ability to be immobilized through the biotin portion and detected through the strong UV absorbing group. Such compounds have the advantage in that UV detection can be utilized during assays or purification procedures in the low picomolar range.[15,22–24]



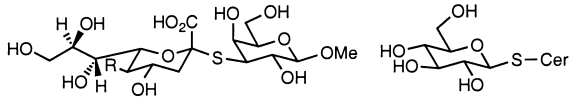
1

The potential applications of tagged biotinylated oligosaccharides (e.g., affinity chromatography, purification of complex oligosaccharides, binding assays with carbohydrate-recognizing proteins) prompted us to investigate the development of a versatile and efficient synthesis of appropriately tagged (oligo)saccharides. Studies of carbohydrate interactions with glycohydrolases and other carbohydrate-recognizing proteins, such as adhesins, are of importance for several disease states.[1,2,8,13] We therefore felt it appropriate to prepare tagged (oligo) saccharides of the general structure **2**, in which the thioglycosidically linked bifunctional aglycon unit would be stable to enzymatic hydrolysis. We have previously demonstrated, using ^{1}H NMR spectroscopy, that the thioglycosidic linkage in thiosialosides such as **3** is resistant to hydrolysis by sialidases.[32] Others[33–36] have also shown that thioglycosides (e.g., **4**[36]) are resistant to hydrolysis by glycohydrolases, and are therefore extremely useful compounds in studies with such enzymes.

Here we describe a novel approach to the synthesis of biotinylated carbohydrates that incorporate a UV active chromophore. This method utilizes our previous expertise in the preparation of thioglycosides,[37–40] and is flexible enough to facilitate the synthesis of tagged mono-, di- and trisaccharides in high yield.



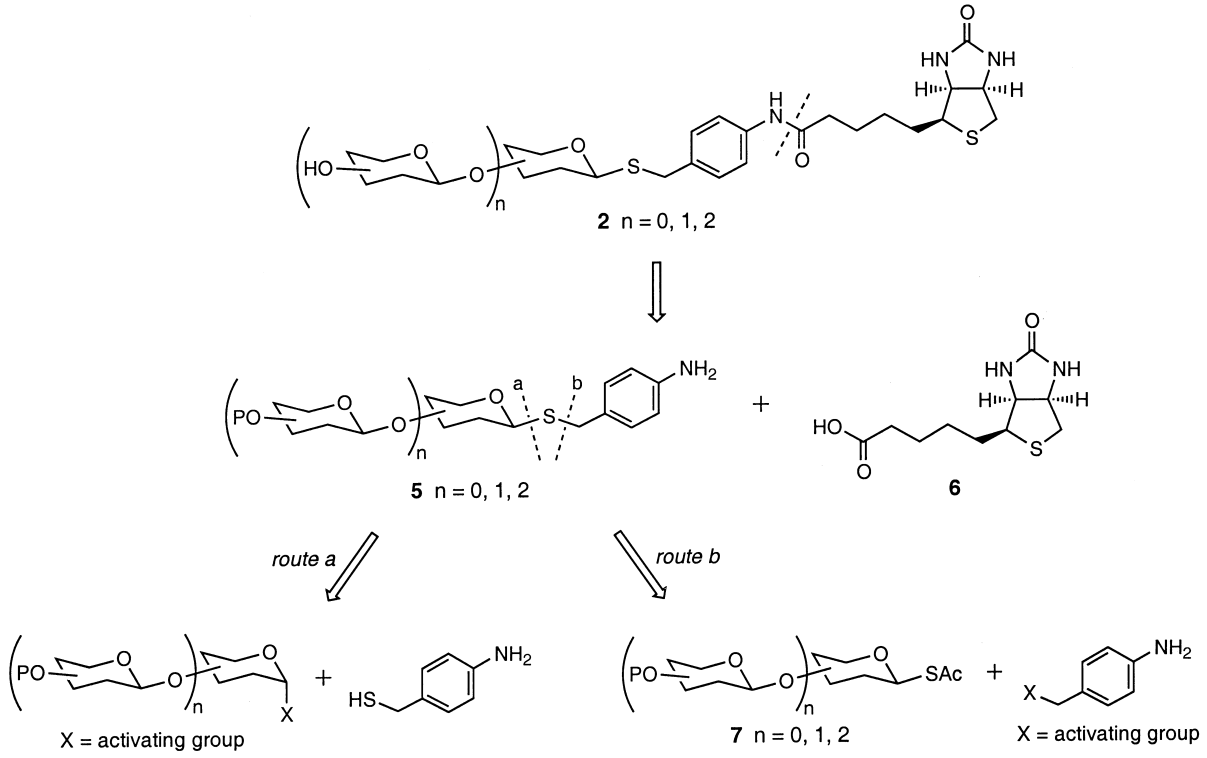
2 n = 0, 1, 2



3 R = NHAc **4**

Results and Discussion

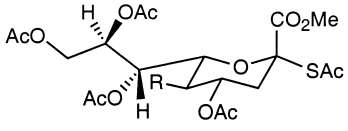
Our general strategy towards the synthesis of appropriately tagged (oligo)saccharides like **2** is shown in retrosynthetic terms in Scheme 1. We felt the most flexible and convergent approach to biotinylated (oligo)-saccharides like **2** would involve a final coupling between 4-aminobenzyl thioglycosides such as **5** and biotin (**6**), using well established peptide coupling techniques.[41]



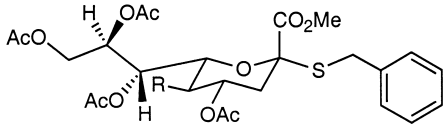
Scheme 1. Retrosynthetic approach towards biotinylated oligosaccharides.

An efficient synthesis of appropriate 4-aminobenzyl thioglycosides like **5** can be approached from either '*route a*' or '*route b*' (Scheme 1). Whilst both approaches have their merits, we felt the most efficient avenue into 4-aminobenzyl thioglycosides like **5** would be via '*route b*', since we have previously described the use of anomeric thiolacetyl compounds like **7** as precursors in the synthesis of thioglycosides.[37–40,42] In addition, we knew that appropriately activated 4-substituted benzyl derivatives would be commercially available, and some of the requisite anomeric thiolacetyl derivatives **7** were already available in our laboratories. Importantly, this approach also has the potential to allow maximum flexibility in terms of incorporating different carbohydrate units and various UV-tags whilst maintaining the same overall strategy.

In an initial investigation of the applicability of the approach shown in Scheme 1 we attempted coupling between the 2-thiolacetyl-Neu5Ac derivative **8**[43] and benzyl bromide. Interestingly, conditions we had previously used for the successful synthesis of thiosialosides, using Et_2NH promoted coupling between **8** and primary alkyl halides,[37,39] failed to furnish any of the desired benzyl thiosialoside derivative **9**. As the only components isolated from this reaction were unreacted benzyl bromide and the thiol corresponding to **8**, we can only assume that the thiolate derived in this way is not reactive enough to displace a benzylic bromide.



8 R = NHAc

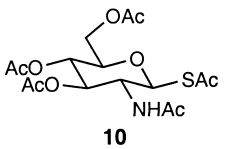


9 R = NHAc

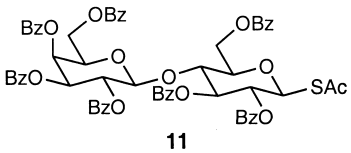
Fortunately, de-*S*-acetylation of **8** using hydrazinium acetate[44] proved more successful. Thus, treatment of the 2-thiolacetyl-Neu5Ac derivative **8** with hydrazinium acetate in the presence of benzyl bromide furnished the benzyl thiosialoside **9** in 73% yield. Having established the methodology for preparing benzyl thioglycosides like **9** in high yield, we sought an appropriate glycosyl acceptor for access towards compounds of the general structure **5**. Whilst it would have been useful to employ 4-aminobenzyl bromide as the glycosyl acceptor, preliminary investigations into the preparation of such an acceptor suggested it would be more efficient to use the corresponding nitro derivative and subsequently reduce the nitro group. Accordingly, hydrazinium acetate-mediated coupling between anomeric thiolacetyl derivatives **8**,[43] **10**,[45] and **11**, and 4-nitrobenzyl bromide proceeded smoothly to give the 4-nitrobenzyl thioglycoside derivatives **12**,[44] **13** and **14**, respectively, in high yield.

With the 4-nitrobenzyl thioglycosides in hand, our attention turned to reduction of the nitro group. Although many methods for the direct reduction of aromatic nitro groups to amines are available, the presence of the sulfur in compounds **12**, **13** and **14** prevents the use of metal-promoted catalytic hydrogenation.[46] It has been shown that the use of tin(II) chloride dihydrate is compatible with sulfur,[47] and has been successfully employed for the reduction of a number of 4-nitrobenzyl thioglycosides.[48] Accordingly, reduction of the nitro group in the thioglycosides **12**, **13** and **14**, with $SnCl_2{\cdot}2H_2O$ in ethanol, gave the 4-aminobenzyl thioglycosides **15**, **16** and **17**, respectively, in good yield.

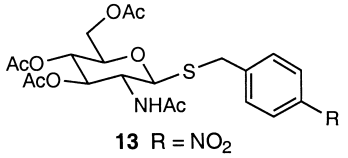
The coupling of the carboxylic acid group in biotin (**6**) to the amine residues in the substituted benzyl thioglycosides **15**, **16** and **17** could be achieved using a number of well known peptide coupling techniques.[41] Whilst the field of carboxylic acid activation for peptide coupling has been traditionally dominated by the use of carbodiimide reagents such as DCC and EDC, these reagents often result in the formation of side products.[49] The use of uronium salts (e.g., HBTU, TBTU) in peptide coupling reactions has been shown to be fast and with limited side reactions.[50] Accordingly, treatment of an *N*,*N*-DMF solution of the 4-aminobenzyl thiosialoside derivative **15** with D-biotin (**6**) in the presence of TBTU [*O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate][51] afforded the desired UV-tagged biotinylated thiosialoside **18** in 70% yield. Similarly, the



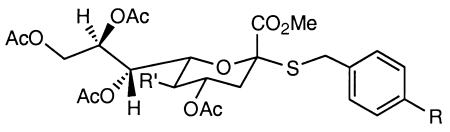
10



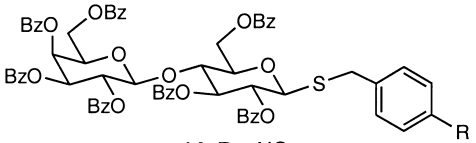
11



13 R = NO_2
16 R = NH_2



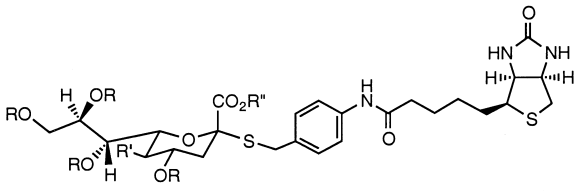
12 R = NO_2, R' = NHAc
15 R = NH_2, R' = NHAc



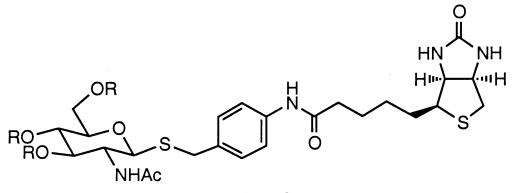
14 R = NO_2
17 R = NH_2

biotinylated glucosamine derivative **19** and the biotinylated lactose derivative **20** were obtained in 92 and 86% yields, respectively.

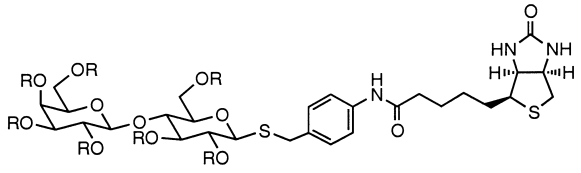
The overall yields obtained for the introduction of the UV-tag and subsequent biotinylation of the three thiolacetyl glycosides **8**, **10** and **11** are 26, 43, and 43%, respectively. The protected saccharides **18**, **19** and **20** were readily deprotected using standard procedures (NaOMe or NaOH, see Experimental for details). In this way the biotinylated saccharides **21**, **22** and **23** were obtained in good yield after chromatography.



18 R = Ac, R' = NHAc, R" = Me
21 R = R" = H, R' = NHAc



19 R = Ac
22 R = H



20 R = Bz
23 R = H

In an attempt to further demonstrate the general applicability of this approach to the synthesis of biotinylated (oligo)saccharides as probes for carbohydrate-recognizing proteins we sought to prepare the tagged sialyllactoside **24**.

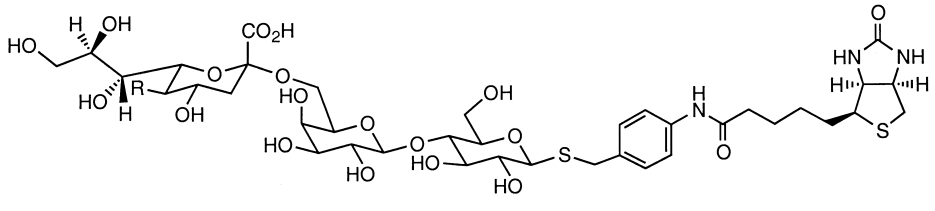
Of the many possible routes to a compound like **24**, we felt the most convergent would involve sialylation of a 4-nitrobenzyl thiolactoside, followed by reduction of the nitro group and subsequent biotinylation. Accordingly, the lactoside **14** was deprotected (NaOMe in MeOH) to give the lactoside **25** in high yield (Scheme 2). For selective sialylation on the C-6 hydroxyl of the galactose unit of a lactoside derivative, previous work within the group had shown this could most efficiently be achieved if the other hydroxyl groups in the lactoside were protected.[32] Towards this end, the lactoside **25** was treated to a sequence of 4,6-*O*-benzylidenation, acetylation, and subsequent de-*O*-benzylidenation to give the selectively protected lactoside **26** in 68% yield from **25**. Sialylation of the lactoside **26**, using a minor modification (see Experimental) of the method developed by Schmidt and co-workers[52] involving TMSOTf-mediated coupling with the sialosyl phosphite **27**, resulted in the formation of the sialyl α(2,6)-lactoside **28** in 57% yield. Reduction of the nitro group in **28** ($SnCl_2 \cdot 2H_2O$) gave the amine **29** (63%) and subsequent biotinylation in the same way as described above gave the desired biotinylated sialyllactoside **30** in 85% yield. The overall yield of **30** is 31% from the lactoside **26**.

In conclusion, we have developed a new method for the preparation of tagged biotinylated carbohydrates. Importantly, our strategy is sufficiently flexible, utilizes mild reagents and reaction conditions, and is efficient enough to provide ready access to a range of suitably functionalized biologically relevant carbohydrates. We feel certain that compounds such as those described herein will prove extremely useful in investigations involving carbohydrate-recognizing proteins. We are currently investigating the use of these compounds in an extension of our earlier efforts[40,53] in developing highly specific affinity chromatography supports.

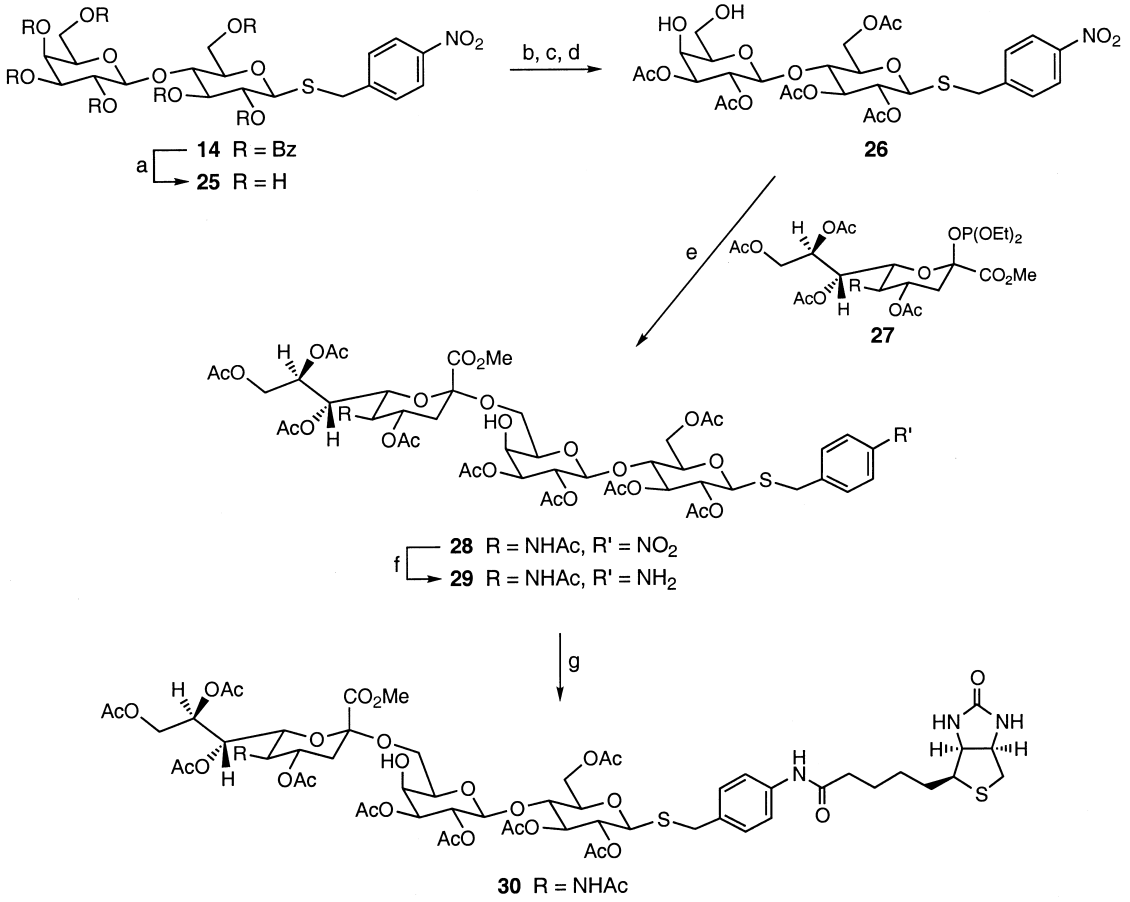
Experimental

General procedures

^1H and ^{13}C spectra were recorded using a Brüker DRX-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_3OD: 3.31 for ^1H; 49.0 for ^{13}C) or relative to external Me_4Si for D_2O spectra. Two-dimensional DQF-COSY and HMQC experiments were recorded in order to assist with spectral assignment. Typically, the following parameters were used: DQF-COSY — 16 scans, 512 slices, relaxation delay 2.0 s; ^1H–^{13}C HMQC — 48 scans, 256 slices, relaxation delay 2.5 s. 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. Reactions were monitored by TLC (Merck silica gel plates GF_{254},



24 R = NHAc



Scheme 2. Conditions: (a) NaOMe, MeOH, 0 °C to rt, 2 h, 83%; (b) PhCHO, HCO_2H, rt, 2 h; (c) Ac_2O, pyridine, rt, 16 h, 86%; (d) 90% aq CF_3CO_2H, CH_2Cl_2, 0 °C, 2 h, 79%; (e) **27**, THF, TMSOTf, 4 Å sieves, −40 to 0 °C, 3 h, 57%; (f) $SnCl_2{\cdot}2H_2O$, EtOH, 70 °C, 15 h, 63%; (g) **6**, TBTU, DMF, Et_3N, rt, 16 h, 85%.

cat. #1.05554) and products were generally purified by flash chromatography using Merck silica gel 60 (0.040–0.063 mm, cat. #1.09385). Microanalyses were performed at the Department of Chemistry, University of Queensland, Australia. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-*S*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate (**8**),[43] 2-acetamido-3,4,6-tri-*O*-acetyl-1-*S*-acetyl-2-deoxy-1-thio-β-D-glucopyranose (**10**),[45] and 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1,4)-*O*-1-*S*-acetyl-2,3,6-tri-*O*-benzoyl-1-thio-β-D-glucopyranose (**11**) were all prepared by reacting the corresponding glycosyl halides with potassium thioacetate using published procedures. All solvents were distilled prior to use or were of analytical grade. Degassed solutions were obtained using the technique described in Vogel[54] involving the bubbling of dry N_2 through the solution for 20 min.

Synthesis of thio-4-nitrobenzyl glycosides

Methyl (*S*-4-nitrobenzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (12). To a solution of **8** (600 mg, 1.09 mmol) in dry DMF (10 mL) was added hydrazinium acetate (147 mg, 1.60 mmol) and the solution degassed. After stirring for 30 min at rt, 4-nitrobenzyl bromide (471 mg, 2.18 mmol) and Et_3N (223 μL, 1.60 mmol) were added and the solution stirred for 2 h. The reaction mixture was diluted with EtOAc (20 mL), washed with HCl (0.1 M, 10 mL), H_2O (10 mL), satd NaCl (aq) (15 mL), dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography (EtOAc:Et_2O, 3:2; R_f 0.3) to give **12** (473 mg, 67%) as a pale yellow amorphous solid: ^{1}H NMR ($CDCl_3$): **Neu5Ac unit**: δ 1.87 (3H, s, AcN), 2.05, 2.11, 2.18, 2.20 (4×H, 4×s, 4×AcO), 2.70 (1H, dd, $J_{3e,3a}$ 12.7, $J_{3e,4}$ 4.5 Hz, H-3e), 3.51 (3H, s, CO_2Me), 3.84 (1H, d, $J_{6,5}$ 10.2 Hz, H-6), 4.05–4.15 (2H, m, H-5/H-9), 4.32 (1H, d, $J_{9',9}$ 11.8, $J_{9',8}$ 2.5 Hz, H-9′), 4.86 (1H, ddd, $J_{4,3a} = J_{4,5} = 10.5$, $J_{4,3e}$ 4.5 Hz, H-4), 5.11 (1H, d, $J_{NH,5}$ 9.9 Hz, NH), 5.37 (1H, d, $J_{7,8}$ 9.1 Hz, H-7), 5.46 (1H, m, H-8); **4-NO_2-Ar unit**: δ 3.99 (2H, s, S-CH_2), 7.54 (2H, d, ArH-2/6), 8.20 (2H, d, ArH-3/5); ^{13}C NMR ($CDCl_3$): **Neu5Ac unit**: δ 21.1, 21.5 (4×OC(O)*Me*), 23.4 (NC(O)*Me*), 38.1 (C-3), 47.8 (C-5), 53.1 (CO_2*Me*), 67.1 (C-9), 67.6 (C-8), 68.5 (C-7), 69.7 (C-4), 74.4 (C-6), 83.1 (C-2), 168.5 (C-1), 170.3, 170.5, 170.8, 171.0 (4×O*C*(O)Me/N*C*(O)Me); **4-NO_2-Ar unit**: δ 32.7 (S-CH_2), 124.1 (C-3/5), 130.3 (C-2/6), 145.2 (C-4); ESIMS: 665 [M + Na] (100%), 643 [M + H] (70); found: 643.17888; $C_{27}H_{35}N_2O_{14}S$ requires: 643.18086.

In a similar manner were prepared

***S*-(4-Nitrobenzyl) 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranoside (13).** Prepared in 77% yield by reaction between the thiolacetyl GlcNAc derivative **10**

and 4-nitrobenzyl bromide (chromatography: EtOAc: hexane, 3:2; R_f 0.4). ^{1}H NMR ($CDCl_3$): **GlcNAc unit**: δ 1.96 (3H, s, AcN), 2.02, 2.11 (2×s, 9H, 3×AcO), 3.59–3.67 (1H, m, H-5), 4.12 (1H, dd, $J_{6,6'}$ 12.3, $J_{6,5}$ 2.4 Hz, H–6), 4.20–4.26 (2H, m, H-2/H-6′), 4.32 (1H, d, $J_{1,2}$ 10.2 Hz, H-1), 5.05–5.09 (2H, m, H-3/H-4), 5.48 (1H, d, $J_{NH,2}$ 9.0 Hz, NH); **4-NO_2-Ar unit**: δ 3.90 (1H, d, J_{gem} 13.0 Hz, S-CH_aH$_b$), 4.06 (1H, d, J_{gem} 13.0 Hz, S-CH$_a$$H_b$), 7.42–7.50 (2H, m, ArH-2/6), 8.15–8.20 (2H, m, ArH-3/5), assignments confirmed by COSY; ^{13}C NMR ($CDCl_3$): **GlcNAc unit**: δ 20.4, 20.5, 20.6 (3×OC(O)*Me*), 23.1 (NC(O)*Me*), 52.8 (C-2), 62.2 (C-6), 68.2 (C-4), 72.1 (C-3), 76.0 (C-5), 82.8 (C-1), 169.1, 170.0, 170.3, 171.0 (3×O*C*(O)Me/N*C*(O)Me); **4-NO_2-Ar unit**: δ 32.7 (S-CH_2), 123.6 (C-3/5), 129.9 (C-2/6), 145.2 (C-1), 147.0 (C-4); ESIMS: 521 [M + Na] (28%), 516 [M + NH_4] (100), 499 [M + H] (80).

***S*-(4-Nitrobenzyl) 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside (14).** Prepared in 74% yield by reaction between the thiolacetyl derivative **11** and 4-nitrobenzyl bromide (chromatography: EtOAc:hexane, 2:3; R_f 0.4). ^{1}H NMR ($CDCl_3$): **Gal unit**: δ 3.71–3.99 (3H, m, H-5/H-6/6′), 4.53 (1H, d, $J_{1,2}$ 9.6 Hz, H-1), 5.38 (1H, dd, $J_{3,2}$ 10.2, $J_{3,4}$ 3.6 Hz, H-3), 5.68–5.75 (2H, m, H-2/H-4); **Glc unit**: δ 3.71–3.99 (1H, m, H-5), 4.22 (1H, dd, $J_{4,3} = J_{4,5}$ = 9.6 Hz, H-4), 4.46 (1H, dd, $J_{6,6'}$ 12.3, $J_{6,5}$ 4.8 Hz, H-6), 4.53 (1H, d, $J_{1,2}$ 8.1 Hz, H-1), 4.61 (1H, d, $J_{6',6}$ 12.3 Hz, H-6′), 5.51 (1H, dd, $J_{2,3}$ 9.6, $J_{2,1}$ 8.1 Hz, H-2), 5.77 (1H, dd, $J_{3,2} = J_{3,4}$ = 9.6 Hz, H-3); **other**: δ 3.71–3.99 (2H, m, S-CH_2), 7.15–8.19 (39H, m, Ar); ^{13}C NMR ($CDCl_3$): **Gal unit**: δ 61.0 (C-6), 67.4 (C-2), 69.8 (C-4), 71.7 (C-3), 77.5 (C-3), 100.8 (C-1); **Glc unit**: δ 62.4 (C-6), 71.3 (C-5), 73.6 (C-3), 74.0 (C-2), 75.6 (C-4), 78.8 (C-1); **other**: δ 32.7 (S-CH_2), 124.2, 127.1, 127.4, 129.5, 129.8, 133.0, 133.4, (8×Ph), 144.8 (C-4 ArNH_2), 164.6, 164.8, 165.2, 165.4, 165.5, 165.9 (7×O*C*(O)Ph); ESIMS: 1245 [M + Na] (100%). Anal. calcd for $C_{68}H_{55}NO_{19}S$: C, 66.8; H, 4.5; N, 1.15; found: C, 66.7; H, 4.5; N, 0.95.

Synthesis of sialosyllactoside 28

***S*-(4-Nitrobenzyl) β-D-galactopyranosyl-(1,4)-1-thio-β-D-glucopyranoside (25).** To a solution of **14** (2.0 g, 1.64 mmol) in dry MeOH (40 mL) at 0 °C under N_2 was added sodium metal (~0.2 g) in portions over 10 min. After 30 min at 0°C the mixture was allowed to warm to rt, and stirred for a further 2 h before being neutralized (IR-120H$^+$ resin). The resin was removed by filtration, washed with MeOH (3×40 mL) and the solvent removed under reduced pressure. Chromatography (EtOAc:MeOH, 4:1; R_f 0.4) gave **25** (0.67 g, 83%) as a pale yellow solid: ^{1}H NMR (CD_3OD): **Gal unit**: δ 3.29–3.37 (2H, m, H-2/H-5), 3.43 (1H, dd, $J_{3,2}$ 9.3, $J_{3,4}$ 3.3 Hz, H-3), 3.48 (1H, d, $J_{6,5}$ 6.3 Hz, H-6), 3.50 (1H, d, $J_{6',5}$ 5.7 Hz, H-6′), 3.89 (1H, d, $J_{4,3}$ 3.3 Hz, H-4), 4.17 (1H, d, $J_{1,2}$ 9.6 Hz, H-1); **Glc unit**: δ 3.29–3.37 (2H, m, H-2/H-5), 3.59 (1H, t, $J_{3,2} = J_{3,4}$ = 9.6 Hz, H-3), 3.69 (1H, dd, $J_{6,6'}$ 11.4, $J_{6,5}$ 5.1 Hz, H-6), 3.70–3.76 (1H, m, H-4), 3.72 (1H, d, $J_{1,2}$ 8.1 Hz, H-1), 3.75 (1H, dd, $J_{6',6}$ 11.4, $J_{6',5}$ 7.2 Hz, H-6′); **4-NO_2-Ar unit**: δ 3.97 (1H, d, J_{gem} 13.2 Hz, S-CH_aH$_b$), 4.15 (1H, d, J_{gem} 13.2 Hz, S-CH$_a$$H_b$), 7.63 (2H, d, ArH-2/6), 8.18 (2H, d, ArH-3/5).

***S*-(4-Nitrobenzyl) 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside.** Benzaldehyde (5 mL) was added to a solution of the lactoside **25** (0.7 g, 1.42 mmol) in HCO_2H (5 mL) at rt under N_2.[55] After stirring for 2 h at rt the mixture was concentrated and quickly chromatographed (EtOAc:MeOH, 20:1; R_f 0.5). Without characterization, the 4,6-*O*-benzylidenated product was dissolved in pyridine (10 mL), Ac_2O (7 mL) added, and the mixture stirred for 16 h at rt before being concentrated. Chromatography (EtOAc; R_f 0.5) gave the title compound (0.97 g, 86%) as a pale yellow amorphous mass: ^{1}H NMR ($CDCl_3$): **Gal unit**: δ 3.57 (1H, brs, H-5), 4.02 (1H, d, $J_{6,6'}$ 12.0 Hz, H-6), 4.31 (1H, d, $J_{6',6}$ 12.0 Hz, H-6′), 4.41 (1H, d, $J_{1,2}$ 9.6 Hz, H-1), 4.44 (1H, d, $J_{4,3}$ 3.3 Hz, H-4), 4.98 (1H, dd, $J_{3,2}$ 9.9, $J_{3,4}$ 3.3 Hz, H-3), 5.48–5.57 (1H, m, H-2); **Glc unit**: δ 3.65–3.77 (1H, m, H-5), 3.99 (1H, dd, $J_{4,3} = J_{4,5}$ = 9.6 Hz, H-4), 4.06 (1H, dd, $J_{6,6'}$ 12.6, $J_{6,5}$ 1.8 Hz, H-6), 4.08 (1H, d, $J_{1,2}$ 8.1 Hz, H-1), 4.30 (1H, dd, $J_{6',6}$ 12.6, $J_{6',5}$ 1.5 Hz, H-6′), 4.43 (1H, dd, $J_{2,3}$ 9.6, $J_{2,1}$ 8.1 Hz, H-2), 5.48–5.57 (1H, m, H-3); **other**: δ 2.05, 2.08 (2×), 2.09 (2×) (15H, 3×s, 5×AcO), 3.96 (1H, d, J_{gem} 12.9 Hz, S-CH_aH$_b$), 4.11 (1H, d, J_{gem} 12.9 Hz, S-CH$_a$$H_b$), 5.48–5.57 (1H, m, PhCH), 7.34–7.41 (3H, m, ArH), 7.47–7.52 (4H, m, ArH), 8.07 (2H, d, ArH-3/5).

***S*-(4-Nitrobenzyl) 2,3-di-*O*-acetyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (26).** To a solution of *S*-(4-nitrobenzyl) 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (0.9 g, 1.14 mmol) in CH_2Cl_2 (20 mL) at 0 °C under N_2 was added CF_3CO_2H (90% aq. 2.5 mL) and the mixture stirred for 2 h at 0 °C.[55] The mixture was diluted with CH_2Cl_2 (50 mL), washed with H_2O (25 mL), satd aq $NaHCO_3$ (25 mL), H_2O (2×25 mL), dried ($MgSO_4$) and concentrated. Chromatography (EtOAc:hexanes, 5:1; R_f 0.3) gave **26** (0.63 g, 79%) as an off-white solid: ^{1}H NMR ($CDCl_3$): **Gal unit**: δ 3.54–3.60 (1H, m, H-5), 3.83 (1H, dd, $J_{6,6'}$ 12.0, $J_{6,5}$ 4.5 Hz, H-6), 3.85–3.97 (1H, m, H-6′), 4.21 (1H, d, $J_{4,3}$ 2.7 Hz, H-4), 4.37 (1H, d, $J_{1,2}$ 9.6 Hz, H-1), 4.95 (1H, dd, $J_{3,2}$ 9.6, $J_{3,4}$ 2.7 Hz, H-3), 5.43 (1H, dd, $J_{2,3} = J_{2,1}$ = 9.6 Hz, H-2); **Glc unit**: δ 3.54–3.60 (1H, m, H-5), 3.85–3.97 (3H, m, H-4/H-6/H-6′), 4.36 (1H, d, $J_{1,2}$ 9.0 Hz, H-1), 4.98 (1H, dd, $J_{2,3}$ 9.6, $J_{2,1}$ 9.0 Hz, H-2), 5.44 (1H, dd, $J_{3,4} = J_{3,2}$ = 9.6 Hz, H-3); **other**: δ 2.05 (6H, s, 2×AcO), 2.10 (9H, s, 3×AcO), 3.93 (1H, d, J_{gem} 13.2 Hz, S-CH_aH$_b$), 4.08 (1H, d, J_{gem} 13.2 Hz, S-CH$_a$$H_b$), 7.51 (2H, d, ArH-2/6), 8.17 (2H, d, ArH-3/5).

***S*-(4-Nitrobenzyl) methyl [5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate-(2,6)-2,3-di-*O*-acetyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (28).** To a solution of methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosylonate (196 mg, 0.40 mmol) in dry CH_3CN (10 mL) at rt under N_2 was added EtN′Pr_2 (165 μL, 0.95 mmol) and then $ClP(OEt)_2$ (115 μL, 0.80 mmol). After stirring for 1 h at rt the mixture was concentrated

under reduced pressure, and the residue chromatographed through a short (5×1 cm) column of silica (toluene:acetone, 3:2; R_f 0.6) to give the unstable sialosyl phosphite **27** in >90% yield. To a solution of **27** in dry THF (10 mL) at −40 °C under N_2 containing 4 Å sieves (~250 mg) was added the lactoside **26** (350 mg, 0.50 mmol) and then TMSOTf (10 μL). After stirring for 30 min at −40 °C the mixture was allowed to warm to 0 °C and stirred for a further 2 h. The mixture was neutralized with Et_3N, allowed to warm to rt, the sieves removed by filtration, washed with THF (3×10 mL), and the solution concentrated. Chromatography (toluene: acetone, 3:2, R_f 0.33; followed by $CHCl_3$:MeOH, 25:1, R_f 0.27) gave **28** (268 mg, 57%) as an off-white amorphous mass: ν_{max} 3420 (br), 1746, 1666, 1522, 1370, 1346, 1224, 1034 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): **Neu5Ac unit**: δ 1.88 (3H, s, AcN), 1.97 (1H, dd, $J_{3a,3e}$ 13.0, $J_{3a,4}$ 12.0 Hz, H-3a), 2.59 (1H, dd, $J_{3e,3a}$ 13.0, $J_{3e,4}$ 4.5 Hz, H-3e), 3.84 (3H, s, CO_2Me), 4.04–4.13 (3H, m, H-5/H-6/H-9), 4.41 (1H, dd, $J_{9',9}$ 12.5, $J_{9',8}$ 2.0 Hz, H-9′), 4.88 (1H, ddd, $J_{4,3a}$ 12.0, $J_{4,5}$ 10.0, $J_{4,3e}$ 4.5 Hz, H-4), 5.12 (1H, d, $J_{NH,5}$ 9.5 Hz, NH), 5.32-5.41 (2H, m, H-7/H-8); **Gal unit**: δ 3.65–3.71 (2H, m, H-5/H-6), 3.79–3.84 (1H, m, H-6′), 4.15 (1H, d, $J_{4,3}$ 3.0 Hz, H-4), 4.30 (1H, d, $J_{1,2}$ 10.0 Hz, H-1), 4.91 (1H, dd, $J_{3,2}$ 9.5, $J_{3,4}$ 3.0 Hz, H-3), 5.32–5.41 (1H, m, H-2); **Glc unit**: δ 3.65–3.71 (1H, m, H-4), 3.79–3.88 (2H, m, H-5/H-6), 4.04–4.13 (2H, m, H-1/H-6′), 5.32–5.41 (2H, m, H-2/H-3); **other**: δ 2.03 (3H), 2.04 (9H), 2.10 (3H), 2.14 (6H), 2.15 (6H) (all s, 9×AcO), 3.79–3.88 (2H, m, SCH_2), 7.52 (2H, d, ArH-2/6), 8.16 (2H, d, ArH-3/5), assignments confirmed by COSY.

Reduction of nitro group

Methyl (*S*-4-aminobenzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (15). Compound **12** (266 mg, 0.41 mmol) was dissolved in EtOH (20 mL) and $SnCl_2{\cdot}2H_2O$ (466 mg, 2.07 mmol) was added.[47] The mixture was heated at 70 °C for 15 h, before being allowed to cool to rt. The mixture was then poured onto ice/H_2O (20 mL), the pH adjusted to 8 with solid $NaHCO_3$, extracted with EtOAc (3×50 mL) and dried ($MgSO_4$). Chromatography (CH_2Cl_2:EtOH, 100:4; R_f 0.3) gave **15** (138 mg, 55%) as a light yellow solid: ^1H NMR ($CDCl_3$): **Neu5Ac unit**: δ 1.85 (3H, s, AcN), 2.00, 2.02, 2.13, 2.16 (4×3H, 4×s, 4 ×AcO), 2.70 (1H, dd, $J_{3e,3a}$ 12.7, $J_{3e,4}$ 4.6 Hz, H-3e), 3.62 (3H, s, CO_2Me), 3.68 (1H, dd, $J_{6,5}$ 9.7, $J_{6,7}$ 2.4 Hz, H-6), 4.07 (1H, d, $J_{9,9'}$ 12.4, $J_{9,8}$ 5.5 Hz, H-9), 4.08–4.13 (1H, m, H-5), 4.34 (1H, dd, $J_{9',9}$ 12.4, $J_{9',8}$ 2.6 Hz, H-9′), 4.86 (1H, ddd, $J_{4,3a}=J_{4,5}=10.5$, $J_{4,3e}$ 4.5 Hz, H-4), 5.11 (1H, d, $J_{NH,5}$ 9.2 Hz, NH), 5.34 (1H, dd, $J_{7,8}$ 7.8, $J_{7,6}$ 2.2 Hz, H-7), 5.46 (ddd, 1H, $J_{8,7}$ 7.8, $J_{8,9}$ 5.5, $J_{8,9'}$ 2.6 Hz, H-8); **4-NH_2-Ar unit**: δ 3.88 (2H, s, S-CH_2), 6.58 (2H, d, ArH-3/5), 7.07 (2H, d, ArH-2/6); ^{13}C NMR ($CDCl_3$): **Neu5Ac unit**: δ 20.7, 20.8, 21.2 (4×OC(O)*Me*), 23.2 (NC(O)*Me*), 37.8 (C-3), 49.5 (C-5), 52.8 (CO_2*Me*), 62.3 (C-9), 67.8 (C-8), 68.6 (C-7), 69.7 (C-4), 76.5 (C-6), 83.2 (C-2), 168.3 (C-1), 170.1, 170.9 (4×O*C*(O)Me/N*C*(O)Me); **4-NH_2-Ar unit**: δ 32.7 (S-CH_2), 117.5 (C-3/5), 130.3 (C-2/6), 133.4 (C-1), 145.0 (C-4); ESIMS: 635 [M + Na] (23%), 324 (52), 301 (33).

In a similar manner were prepared:

***S*-(4-Aminobenzyl) 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranoside (16).** Prepared in 60% yield by reduction of **13** (chromatography: EtOAc:hexane, 8:1; R_f 0.2). ^1H NMR ($CDCl_3$): **GlcNAc unit**: δ 1.75 (3H, s, AcN), 1.92, 2.02 (9H, 2×s, 3×AcO), 3.45–3.48 (1H, m, H-5), 4.05–4.17 (4H, m, H-1/H-2/H-6/H-6′), 4.87 (1H, dd, $J_{3,2}=J_{3,4}=9.6$ Hz, H-3), 4.99 (1H, dd, $J_{4,5}=J_{4,3}$ 9.6 Hz, H-4), 5.15 (1H, d, $J_{NH,2}$ 8.4 Hz, NH); **4-NH_2-Ar unit**: δ 3.62 (1H, d, J_{gem} 13.2 Hz, S-CH_aH$_b$), 3.74 (1H, d, J_{gem} 13.2 Hz, S-CH$_a$$H_b$), 6.54 (2H, d, ArH-3/5), 6.99 (2H, d, ArH-2/6), assignments confirmed by COSY; ^{13}C NMR ($CDCl_3$): **GlcNAc unit**: δ 20.6, 20.7, 20.8 (3×OC(O)*Me*), 23.2 (NC(O)*Me*), 52.7 (C-2), 62.5 (C-6), 68.4 (C-4), 74.0 (C-3), 75.8 (C-5), 82.7 (C-1), 169.9, 171.0, 171.1 (3×O*C*(O)Me/N*C*(O)Me); **4-NH_2-Ar unit**: δ 33.4 (S-CH_2), 115.3 (C-3/5), 130.1 (C-2/6), 132.5 (C-1) 145.3 (C-4); ESIMS 491 [M + Na] (11%), 469 [M + H] (7), 106.2 (100).

***S*-(4-Aminobenzyl) 2,3,4,6-tetra-*O*-benzoyl-β-D-galacto-pyranosyl-(1,4)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-glucopyr-anoside (17).** Prepared in 67% yield by reduction of **14** (chromatography: Et_2O; R_f 0.35). ^1H NMR ($CDCl_3$): **Gal unit**: δ 3.75–3.83 (2H, m, H-6/6′), 3.91 (1H, t, $J_{5,6/6'}$ 6.6 Hz, H-5), 4.91 (1H, d, $J_{1,2}$ 8.1 Hz, H-1), 5.40 (1H, dd, $J_{3,2}$ 10.5, $J_{3,4}$ 3.3 Hz, H-3), 5.71 (1H, d, $J_{2,1}$ 8.1 Hz, H-2), 5.74 (1H, d, $J_{4,3}$ 3.3 Hz, H-4); **Glc unit**: δ 3.75–3.83 (1H, m, H-5), 4.24 (1H, dd, $J_{4,3}=J_{4,5}=9.6$ Hz, H-4), 4.43 (1H, dd, $J_{6,6'}$ 12.3, $J_{6,5}$ 5.1 Hz, H-6), 4.49 (1H, d, $J_{1,2}$ 9.6 Hz, H-1), 4.60 (1H, d, $J_{6,6'}$ 12.3 Hz, H-6′), 5.52 (1H, dd, $J_{2,3}=J_{2,1}=9.6$ Hz, H-2), 5.77 (1H, dd, $J_{3,2}=J_{3,4}=9.6$ Hz, H-3); **4-NH_2-Ar unit**: δ 3.75–3.83 (2H, m, S-CH_2), 6.54 (2H, d, ArH-3/5), 6.97 (2H, d, ArH-2/6); **other**: δ 7.11–8.15 (35H, m, 7×Ph); ^{13}C NMR ($CDCl_3$): **Gal unit**: δ 60.3 (C-6), 67.5 (C-2), 70.5 (C-4), 71.8 (C-3), 76.9 (C-5), 100.8 (C-1); **Glc unit**: δ 62.7 (C-6), 69.9 (C-5), 71.3 (C-3), 74.0 (C-2), 76.0 (C-4), 82.2 (C-1); **other**: δ 28.1 (S-CH_2), 115.2, 126.2, 128.1, 129.1, 129.8, 129.9, 133.2, 133.4, (8×Ph), 145.4 (C-4 ArNH_2), 164.7, 165.1, 165.2, 165.3, 165.5, 165.7 (7×O*C*(O)Ph); ESIMS: 1193 [M + H] (15%), 932 (71). Anal. calcd for $C_{68}H_{57}NO_{17}S$: C, 68.5; H, 4.8; N, 1.2; found: C, 68.5; H, 4.8; N, 1.0.

***S*-(4-Aminobenzyl) methyl [5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate-(2,6)-2,3-di-*O*-acetyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (29).** Prepared in 63% yield by reduction of **28** (chromatography: $CHCl_3$:MeOH, 20:1; R_f 0.35). ν_{max} 3400 (br), 1746, 1516, 1370, 1226, 1038 cm^{-1}; ^1H NMR ($CDCl_3$): **Neu5Ac unit**: δ 1.88 (3H, s, AcN), 2.61 (1H, dd, $J_{3e,3a}$ 12.9, $J_{3e,4}$ 4.5 Hz, H-3e), 3.84 (3H, s, CO_2Me), 4.03–4.15 (3H, m, H-5/H-6/H-9), 4.42 (1H, dd, $J_{9',9}$ 12.4, $J_{9',8}$ 2.5 Hz, H-9′), 4.89 (1H, ddd, $J_{4,3a}$ 11.8, $J_{4,5}$ 9.8, $J_{4,3e}$ 4.5 Hz, H-4), 5.17 (1H, d, $J_{NH,5}$ 9.8 Hz, NH), 5.31–5.43 (2H, m, H-7/H-8); **Gal unit**: δ 3.62 (1H, t, *J* 6.3 Hz, H-5), 3.72 (1H, dd, $J_{6,6'}$ 11.2, $J_{6,5}$ 6.3 Hz, H-6), 3.85–3.93 (1H, m, H-6′), 4.03–4.15 (1H, m, H-4), 4.26 (1H, d, $J_{1,2}$ 10.0 Hz, H-1), 4.87 (1H, dd, $J_{3,2}$ 9.8, $J_{3,4}$ 3.0 Hz, H-3), 5.31–5.43 (1H, m, H-2); **Glc unit**: δ 3.68–3.76 (1H, m, H-4), 3.85–3.93 (2H, m, H-5/H-6), 4.03–4.15 (2H,

m, H-1/H-6′), 4.99 (1H, dd, $J_{2,3}$ 9.6, $J_{2,1}$ 8.5 Hz, H-2), 5.31–5.43 (1H, m, H-3); **other**: δ 2.01, 2.03, 2.04 (2×), 2.05, 2.08, 2.14 (2×), 2.15 (27H, 7×s, 9×AcO), 3.74 (1H, d, J_{gem} 12.6 Hz, SCH_aH$_b$), 3.94 (1H, d, J_{gem} 12.6 Hz, SCH$_a$$H_b$), 6.68 (2H, d, ArH-3/5), 7.15 (2H, d, ArH-2/6).

Coupling with biotin

Methyl (*S*-D-biotinoyl-4-aminobenzyl 5-acetamido-4,7,8, 9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (18). A mixture of **15** (100 mg, 0.16 mmol), TBTU (58 mg, 0.18 mmol) and D-biotin (**6**) (43 mg, 0.18 mmol) was dissolved in dry DMF (5 mL) under N_2 at rt. After stirring for 20 min Et_3N (34 μL, 0.25 mmol) was added and the mixture was left to stir for 16 h. Solvents were removed in vacuo and the residue was chromatographed (EtOAc:hexane, 10:1; R_f 0.3) to give **18** (97 mg, 71%) as a light yellow amorphous mass: ^{1}H NMR (500 MHz, $CDCl_3$) **Neu5Ac unit**: δ 1.86 (3H, s, AcN), 2.00, 2.02, 2.14, 2.16 (4×3H, 4×s, 4×AcO), 2.67 (1H, dd, $J_{3e,3a}$ 12.2, $J_{3e,4}$ 4.2 Hz, H-3e), 3.53 (3H, s, CO_2Me), 3.90 (1H, dd, $J_{6,5}$ 10.1, $J_{6,7}$ 1.5 Hz, H-6), 4.06–4.09 (1H, m, H-5), 4.11 (1H, dd, $J_{9,9'}$ 7.3, $J_{9,8}$ 3.2 Hz, H-9), 4.33 (1H, dd, $J_{9',9}$ 7.3, $J_{9',8}$ 1.5 Hz, H-9′), 4.83 (1H, ddd, $J_{4,3a}$ 10.6, $J_{4,5}$ 9.6, $J_{4,3e}$ 4.2 Hz, H-4), 5.23 (1H, dd, $J_{7,8}$ 8.3, $J_{7,6}$ 1.9 Hz, H-7), 5.42–5.47 (1H, m, H-8), 5.49 (1H, d, $J_{NH,5}$ 10 Hz, NH); **Benzyl unit**: δ 3.75 (1H, d, J_{gem} 13.1 Hz, S-CH_aH$_b$), 3.87 (1H, d, J_{gem} 13.1 Hz, S-CH$_a$$H_b$), 7.22 (2H, d, ArH-2/6), 7.51 (2H, d, ArH-3/5); **Biotin unit**: δ 1.43 (2H, m, H-1′), 1.63 (2H, m, H-2′), 1.73 (2H, m, H-3′), 2.33 (2H, m, H-4′), 2.71 (1H, d, $J_{5a,5b}$ 12.8 Hz, H-5a), 2.87 (1H, dd, $J_{5b,5a}$ 12.8, $J_{5b,4}$ 4.6 Hz, H-5b), 3.09–3.12 (1H, m, H-2), 4.27 (1H, dd, $J_{3,4}$ 7.1, $J_{3,2}$ 4.5 Hz, H-3), 4.47 (1H, dd, $J_{4,3}$ 7.1, $J_{4,5b}$ 5.0 Hz, H-4), 5.70 (1H, bs, NH), assignments confirmed by COSY; ^{13}C NMR ($CDCl_3$) **Neu5Ac unit**: δ 20.7, 20.8, 21.2, (4×OC(O)*Me*), 23.0 (NC(O)*Me*), 37.7 (C-3), 49.1 (C-5), 52.8 (CO_2*Me*), 62.3 (C-9), 67.4 (C-7), 68.7 (C-8), 69.6 (C-4), 74.0 (C-6), 83.1 (C-2), 170.1, 170.2, 170.3, 170.7, 170.8 (4×O*C*(O)Me/N*C*(O)Me), 171.9 (C-1); **Benzyl unit**: δ 32.6 (S-CH_2), 120.1 (C-3/5), 129.5 (C-2/6), 131.7 (C-1), 137.4 (C-4); **Biotin unit**: δ 25.5 (C-3′), 27.9 (C-1′), 28.1 (C-2′), 36.4 (C-4′), 40.4 (C-5), 55.5 (C-2), 60.2 (C-4), 62.1 (C-3), 164.1 (C-2″), 168.2 (C-5′), assignments confirmed by HMQC; ESIMS: 861 [M + Na] (100%); found: 839.2847; $C_{37}H_{51}N_4O_{14}S_2$ requires: 839.2842.

In a similar manner were prepared:

***S*-D-Biotinoyl-4-aminobenzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranoside (19).** Prepared in 92% yield by reaction between **16**, TBTU and D-biotin (chromatography: CH_2Cl_2:MeOH, 10:1; R_f 0.3). ^{1}H NMR ($CDCl_3$): **GlcNAc unit**: δ 1.71 (3H, s, AcN), 1.81, 1.84, 1.94 (3×3H, 3×s, 3 ×AcO), 3.41–3.45 (1H, m, H-5), 3.91–3.99 (2H, m, H-2/H-6), 4.05 (1H, dd, $J_{6',6}$ 12.3, $J_{6',5}$ 5.1 Hz, H-6′), 4.12 (1H, d, $J_{1,2}$ 10.8 Hz, H-1), 4.84–4.87 (2H, m, H-3/H-4); **Benzyl unit**: δ 3.58 (1H, d, J_{gem} 12.9 Hz, S-CH_aH$_b$), 3.71 (1H, d, J_{gem} 12.9 Hz, S-CH$_a$$H_b$), 7.08 (2H, d, ArH-2/6), 7.35 (2H, d, ArH-3/5); **Biotin unit**: δ 1.31–1.61 (6H, m, H-1′/H-2′/H-3′), 2.21 (2H, t, $J_{4',3'}$ 7.2 Hz, H-4′), 2.54 (1H, d, $J_{5a,5b}$ 12.9 Hz, H-5a), 2.73 (1H, dd, $J_{5b,5a}$ 12.9, $J_{5b,4}$ 4.8 Hz, H-5b), 2.98 (1H, m, H-2), 4.14 (1H, d, $J_{3,4}$ 7.6 Hz, H-3), 4.32 (1H, dd, $J_{4,3}$ 7.6, $J_{4,5b}$ 4.8 Hz, H-4), assignments confirmed by COSY; ^{13}C NMR ($CDCl_3$): **GlcNAc unit**: δ 20.0, 20.2 (3×OC(O)*Me*), 22.0 (NC(O)*Me*), 52.2 (C-2), 62.3 (C-6), 68.6 (C-4), 73.8 (C-3), 75.2 (C-5), 82.1 (C-1), 170.7, 170.8, 171.2, 172.5 (3×O*C*(O)Me/N*C*(O)Me); **Benzyl unit**: δ 32.9 (S-CH_2), 120.1 (C-2/6), 129.2 (C-3/5), 132.6 (C-1), 137.1 (C-4); **Biotin unit**: δ 25.1 (C-1′), 27.8, 28.0 (C-2′/C-3′), 36.2 (C-4′), 39.9 (C-5), 55.3 (C-2), 55.9 (C-4), 61.5 (C-3), 163.8 (C-2″), assignments confirmed by HMQC; ESIMS: 717 [M + Na] (11%), 695 [M + H] (100); found: 695.24712; $C_{31}H_{43}N_4O_{10}S_2$ requires: 695.24201.

***S*-D-Biotinoyl-4-aminobenzyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside (20).** Prepared in 87% yield by reaction between **17**, TBTU and D-biotin (chromatography: CH_2Cl_2:MeOH, 10:1; R_f 0.4). ^{1}H NMR (600 MHz, $CDCl_3$): **Gal unit**: δ 3.79–3.94 (2H, m, H-6/6′), 4.02 (1H, t, $J_{5,6/6'}$ 6.6 Hz, H-5), 5.00 (1H, d, $J_{1,2}$ 9.0 Hz, H-1), 5.48 (1H, dd, $J_{3,2}$ 9.9 Hz, $J_{3,4}$ 3.0 Hz, H-3), 5.77 (1H, dd, $J_{2,3}$ 9.9, $J_{2,1}$ 9.0 Hz, H-2), 5.83 (1H, m, H-4); **Glc unit**: δ 3.79–3.94 (1H, m, H-5), 4.30 (1H, dd, $J_{4,5} = J_{4,3} =$ 9.6 Hz, H-4), 4.58 (1H, d, $J_{6,6'}$ 11.4, $J_{6,5}$ 4.8 Hz, H-6), 4.58 (1H, d, $J_{1,2}$ 8.7 Hz, H-1), 4.72 (1H, d, $J_{6',6}$ 11.4 Hz, H-6′), 5.57 (1H, dd, $J_{2,3}$ 9.6, $J_{2,1}$ 8.7 Hz, H-2), 5.83 (1H, m, H-3); **Biotin unit**: δ 1.15–1.19 (6H, m, H-1′/H-2′/H-3′), 2.48 (2H, t, $J_{4',3'}$ 7.2 Hz, H-4′), 2.83 (1H, d, $J_{5a,5b}$ 13.2 Hz, H-5a), 2.95 (1H, m, H-5b), 3.21 (1H, m, H-2), 4.14 (1H, d, $J_{3,2}$ 6.6 Hz, H-3), 4.56 (1H, dd, $J_{4,5a}$ 4.8 Hz, H-4); **other**: δ 3.79–3.94 (2H, m, S-CH_2), 7.17–8.14 (39H, m, Ar), assignments confirmed by COSY; ^{13}C NMR ($CDCl_3$): **Gal unit**: δ 60.9 (C-6), 67.4 (C-2), 70.5 (C-4), 71.2 (C-3), 76.5 (C-5), 100.2 (C-1); **Glc unit**: δ 60.9 (C-6), 69.8 (C-2), 71.2 (C-5), 73.8 (C-3), 76.0 (C-4), 82.4 (C-1); **Biotin unit**: δ 25.7 (C-3′), 27.5 (C-2′), 29.3 (C-1′), 36.9 (C-4′), 41.2 (C-5), 54.9 (C-2), 60.3 (C-4), 62.0 (C-3), 164.2 (C-2″), 168.2 (C-5′); **other**: δ 32.6 (S-CH_2), 128.1, 128.3, 128.5, 128.6, 129.4, 129.5, 129.8, 130.9, 133.2, 133.3, 133.4 (8×Ph), 137.5 (C_{ipso}), 164.5, 165.1, 165.2, 165.3, 165.5, 165.8 (7×O*C*(O)Ph). ESIMS 1419 [M + H] (37%), 840 (42), 676 (100); found: 1418.4224; $C_{78}H_{72}N_3O_{19}S_2$ requires: 1418.4201.

***S*-D-Biotinoyl-4-aminobenzyl methyl [5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate-(2,6)-2,3-di-*O*-acetyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (30).** Prepared in 85% yield by reaction between **29**, TBTU and D-biotin (chromatography: $CHCl_3$:MeOH, 10:1; R_f 0.25). ν_{max} 3412 (br), 1746, 1692, 1532, 1370, 1230, 1044 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): **Neu5Ac unit**: δ 1.87 (3H, s, AcN), 2.59 (1H, dd, $J_{3e,3a}$ 12.8, $J_{3e,4}$ 4.4 Hz, H-3e), 3.81 (3H, s, CO_2Me), 4.01–4.13 (3H, m, H-5/H-6/H-9), 4.39–4.44 (1H, m, H-9′), 4.92 (1H, ddd, $J_{4,3a}$ 11.2, $J_{4,5}$ 9.9, $J_{4,3e}$ 4.4 Hz, H-4), 5.27–5.39 (2H, m, H-7/H-8), 5.75 (1H, d, $J_{NH,5}$ 9.5 Hz, NH); **Gal unit:** δ 3.62 (1H, dd, $J_{5,6} = J_{5,6'} =$ 5.9 Hz, H-5), 3.75 (1H, dd, $J_{6,6'}$ 12.1, $J_{6,5}$ 5.9 Hz, H-6), 3.98 (1H, dd, $J_{6',6}$ 12.2, $J_{6',5}$ 5.9 Hz, H-6′), 4.01–4.13 (1H, m, H-4), 4.28 (1H, d, $J_{1,2}$ 8.9 Hz, H-1), 4.87 (1H, dd, $J_{3,2}$ 9.5, $J_{3,4}$ 2.9 Hz, H-3), 5.27–5.39 (1H, m, H-2); **Glc unit**: δ 3.67 (1H, dd, $J_{4,3} = J_{4,5} =$ 9.5 Hz, H-4), 3.86–3.92 (1H, m, H-5), 4.04–4.13 (2H, m, H-1/H-6), 4.39–4.44 (1H, m, H-6),

4.84–4.98 (1H, m, H-3), 5.27–5.39 (1H, m, H-2); **Biotin unit**: δ 1.46–1.49 (2H, m, H-2′), 1.63–1.80 (4H, m, H-1′/H-3′), 2.38 (2H, t, $J_{4',3'}$ 7.1 Hz, H-4′), 2.71 (1H, d, $J_{5a,5b}$ 12.5 Hz, H-5a), 2.89 (1H, dd, $J_{5b,5a}$ 12.5, $J_{5b,4}$ 4.2 Hz, H-5b), 3.12–3.17 (1H, m, H-2), 4.31–4.33 (1H, m, H-3), 4.48–4.52 (1H, m, H-4); **other**: δ 2.01, 2.02, 2.03 (3×6H, 3×s, 6×AcO), 2.07 (3H, s, AcO), 2.13 (6H, s, 2×AcO), 3.85–3.95 (2H, m, SCH_2), 7.28 (2H, d, ArH-2/6), 7.53 (2H, d, ArH-3/5), assignments confirmed by COSY; ESIMS: 1103 [M−270] (100%).

Deprotection of biotinylated saccharides

***S*-D-Biotinoyl-4-aminobenzyl 5-acetamido-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosidonic acid (21).** Compound **18** (67 mg, 0.08 mmol) was dissolved in dry MeOH (2 mL) and a solution of NaOMe [1 mL, made from Na (20 mg) in dry MeOH (3 mL)] was added at 0 °C under N_2. The mixture was allowed to warm to rt and stirred for 5 h before being concentrated under reduced pressure. The residue was dissolved in H_2O (3 mL), the pH adjusted to 12 with NaOH (0.1 M), and the solution was stirred for a further 16 h before being neutralized with Dowex 50W-H^+ resin and filtered. The solvent was removed in vacuo and the residue chromatographed (EtOAc:MeOH:H_2O, 7:2:1; R_f 0.1) to give **21** (30 mg, 58%) as a white powder: ^{1}H NMR (600 MHz, CD_3OD): **Neu5Ac unit**: δ 1.75 (1H, dd, $J_{3a,3e}$ 12.0, $J_{3a,4}$ 10.8 Hz, H-3a), 1.96 (3H, s, AcN), 2.80 (1H, dd, $J_{3e,3a}$ 12.0, $J_{3e,4}$ 5.1 Hz, H-3e), 3.62–3.67 (2H, m, H-6/H-7), 3.71–3.80 (2H, m, H-5/H-9), 3.84–3.96 (3H, m, H-4/H-8/H-9′); **Benzyl unit**: δ 4.03 (1H, d, J_{gem} 12.1 Hz, S-CH_aH$_b$), 4.18 (1H, d, J_{gem} 12.1 Hz, S-CH$_a$$H_b$), 7.42 (d, 2H, H-2/6), 7.60 (d, 2H, H-3/5); **Biotin unit**: δ 1.59–1.89 (6H, m, H-1′/H-2′/H-3′), 2.52 (2H, t, $J_{4',3'}$ 7.2 Hz, H-4′), 2.82 (1H, d, $J_{5a,5b}$ 12.6 Hz, H-5a), 3.04 (1H, dd, $J_{5b,5a}$ 12.6, $J_{5b,4}$ 4.8 Hz, H-5b), 3.33–3.40 (1H, m, H-2), 4.46 (1H, dd, $J_{3,4}$ 7.5, $J_{3,2}$ 4.5 Hz, H-3), 4.61 (1H, dd, $J_{4,3}$ 7.5, $J_{4,5b}$ 4.8 Hz, H-4), assignments confirmed by COSY; ^{13}C NMR (500 MHz, CD_3OD): **Neu5Ac unit**: δ 22.7 (NC(O)*Me*), 43.1 (C-3), 54.2 (C-5), 64.7 (C-9), 69.9 (C-4), 70.6 (C-7), 73.1 (C-8), 76.8 (C-6), 87.4 (C-2), 168.3 (C-1); **Benzyl unit**: δ 34.7 (S-CH_2), 121.3 (C-3/5), 130.9 (C-2/6), 135.1 (C-1), 138.2 (C-4); **Biotin unit**: δ 26.9 (C-2′), 29.6 (C-1′), 29.9 (C-3′), 37.8 (C-4′), 41.2 (C-5), 57.1 (C-2), 61.8 (C-4), 63.5 (C-3), assignments confirmed by HMQC; found: 657.22585; $C_{28}H_{41}N_4O_{10}S_2$ requires: 657.22636.

***S*-D-Biotinoyl-4-aminobenzyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside (22).** To a solution of **19** (98 mg, 0.14 mmol) in MeOH (4 mL) at 0 °C was added freshly prepared NaOMe (1 mL, prepared as above). The mixture was allowed to warm to rt and then stirred for 16 h, before being neutralized with Amberlite IR 120H^+ resin. After filtration and concentration, the residue was chromatographed (EtOAc:MeOH:H_2O; 7:2:1; R_f 0.3) to give **22** (45 mg, 56%) as a colourless powder: ^{1}H NMR (D_2O): **GlcNAc unit**: δ 1.87 (3H, s, AcN), 3.22–3.26 (1H, m, H-3), 3.32–3.35 (2H, m, H-4/H-5), 3.60 (1H, dd, $J_{6,6'}$ 12.6, $J_{6,5}$ 5.7 Hz, H-6), 3.64–3.67 (1H, m, H-2), 3.76 (1H, dd, $J_{6',6}$ 12.6, $J_{6',5}$ 5.1 Hz, H6′), 4.20 (1H, d, $J_{1,2}$ 10.5 Hz, H-1); **Benzyl unit**: δ 3.80 (1H, d, *J* 13.2 Hz, S-CH_aH$_b$), 3.86 (1H, d, *J* 13.2 Hz, S-CH$_a$$H_b$), 7.20 (2H, d, H-2/6), 7.31 (d, 2H, H-3/5); **Biotin unit**: δ 1.37–1.69 (6H, m, H-1′/H-2′/H-3′), 2.21 (2H, t, $J_{4',3'}$ 7.5 Hz, H-4′), 2.67 (1H, d, $J_{5a,5b}$ 13.2 Hz, H-5a), 2.88 (1H, dd, $J_{5b,5a}$ 12.9, $J_{5b,4}$ 4.8 Hz, H-5b), 3.22–3.26 (1H, m, H-2), 4.31 (1H, dd, $J_{3,4}$ 7.8, $J_{3,2}$ 4.2 Hz, H-3), 4.95 (1H, dd, $J_{4,3}$ 7.7, $J_{4,5b}$ 4.8 HZ, H-4), assignments confirmed by COSY; ^{13}C NMR (D_2O): **GlcNAc unit**: δ 21.5 (NC(O)*Me*), 53.9 (C-2), 60.5 (C-6), 69.3 (C-4), 74.7 (C-5), 79.5 (C-3), 82.3 (C-1), 171.1 (N*C*(O)Me); **Benzyl unit**: δ 33.0 (S-CH_2), 121.8 (C-2/6), 129.3 (C-3/5), 133.7 (C-1), 137.1 (C-4); **Biotin unit**: δ 24.5 (C-1′), 27.2, 27.4 (C-2′/C-3′), 35.6 (C-4′), 39.1 (C-5), 54.8 (C-2), 59.8 (C-4), 61.6 (C-3), assignments confirmed by HMQC; ESIMS: 569.4 [M+H] (100%); found: 569.21065; $C_{25}H_{37}N_4O_7S_2$ requires: 569.21033.

***S*-D-Biotinoyl-4-aminobenzyl β-D-galactopyranosyl-(1,4)-1-thio-β-D-glucopyranoside (23).** To a solution of **20** (57 mg, 0.04 mmol) in MeOH (2 mL) was added NaOH (0.1 M) to pH 13 and the mixture left to stir at rt for 16 h. Evaporation to dryness followed by column chromatography (EtOAc/MeOH/H_2O; R_f 0.2) afforded **23** (25 mg, 90%). ^{1}H NMR (D_2O): **Gal unit**: δ 3.66–3.72 (1H, m, H-5), 3.80–3.83 (1H, m, H-4), 3.91–4.01 (3H, m, H-3/H-6/H-6′), 4.34 (1H, d, $J_{1,2}$ 9.9 Hz, H-1), 4.47 (1H, dd, $J_{2,1}$ 9.9, $J_{2,3}$ 7.5 Hz, H-2); **Glc unit**: δ 3.36–3.50 (3H, m, H-3/H-4/H-5), 3.50 (2H, d, $J_{6,6'}$ 12 Hz, H-6/6′), 3.66–3.72 (1H, m, H-2), 3.80–3.83 (2H, m, $J_{1,2}$ 8.1 Hz, H-1); **Biotin unit**: δ 1.51–1.83 (6H, m, H-1′/H-2′/H-3′), 2.49 (2H, t, $J_{4',3'}$ 7.2 Hz, H-4′), 2.80 (1H, d, $J_{5a,5b}$ 13.2 Hz, H-5a), 3.03 (1H, d, $J_{5b,5a}$ 13.2 Hz, H-5b), 3.36–3.50 (1H, m, H-2), 4.71 (1H, dd, $J_{3,4}$ 7.8, $J_{3,2}$ 4.4 Hz, H-3), 4.64 (1H, dd, $J_{4,3}$ 7.8, $J_{4,5b}$ 4.8 Hz, H-4); **Benzyl unit**: δ 3.99–4.05 (2H, m, S-CH_2), 7.59–7.94 (4H, m, ArH); ESIMS: 712 [M+Na] (64%), 690.5 [M+H] (57); found: 690.23241; $C_{29}H_{44}N_3O_{12}S_2$ requires: 690.23659.

Acknowledgements

Dr Robin Thomson is thanked for helpful discussions and comments. We also thank Monash University for a Monash Graduate Scholarship (to DIA), and the NIH (Grant number PO1 A1 38084) and the ARC for financial support.

References

1. Schauer, R.; Kamerling, J. P. In *Glycoproteins II*; Montreuil, J.; Vliengenthart, J. F. G.; Schachter, H., Eds.; Elsevier: Amsterdam, 1997, pp. 243–402.
2. Varki, A. *Glycobiology* **1993**, *3*, 97–130.
3. Crocker, P. R.; Feizi, T. *Curr. Opin. Struct. Biol.* **1996**, *6*, 679.
4. Lis, H.; Sharon, N. *Eur. J. Biochem.* **1993**, *218*, 1.
5. Karlsson, K.-A. *Curr. Opin. Struct. Biol.* **1995**, *5*, 622.
6. *Bacterial Adhesion to Cells and Tissues*, Ofek, I.; Doyle, R. J.; Eds.; Chapman Hall: New York, 1994.
7. Simanek, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong, C.-H. *Chem. Rev.* **1998**, *98*, 833.
8. Sears, P.; Wong, C.-H. *Cell. Mol. Life Sci.* **1998**, *54*, 223.
9. Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683.

10. Rosen, S. D.; Bertozzi, C. R. *Curr. Biol.* **1996**, *6*, 261.
11. Hakomori, S. *Adv. Cancer Res.* **1989**, *52*, 257.
12. Feizi, T. *Nature* **1985**, *314*, 53.
13. Sato, M.; Narita, T.; Kimura, N.; Zenita, K.; Hashimoto, T.; Manabe, T.; Kannagi, R. *Anticancer Res.* **1997**, *17*, 3505.
14. Lee, Y. C. *FASEB J.* **1992**, *6*, 3193.
15. Shinohara, Y.; Sota, H.; Gotoh, M.; Hasebe, M.; Tosu, M.; Nakao, J.; Hasegawa, Y. *Anal. Chem.* **1996**, *68*, 2573.
16. Honda, S.; Taga, A.; Suzuki, K.; Suzuki, S.; Kakehi, K. *J. Chromatogr.* **1992**, *597*, 377.
17. Galanina, O. E.; Tuzikov, A. B.; Rapoport, E.; Le Pendu, J.; Bovin, N. V. *Anal. Biochem.* **1998**, *265*, 282 and references therein.
18. Feizi, T.; Stoll, M. S.; Yuen, C.-T.; Chai, W.; Lawson, A. M. *Methods Enzymol.* **1994**, *230*, 484.
19. Stoll, M. S.; Feizi, T.; Loveless, R. W.; Chai, W.; Lawson, A. M.; Yuen, C.-T. *Eur. J. Biochem.* **2000**, *267*, 1795.
20. Kremsky, J. N.; Pluskal, M.; Casey, S.; Perry-O'Keefe, H.; Kates, S. A.; Sinha, N. D. *Tetrahedron Lett.* **1996**, *37*, 4313.
21. Dohi, H.; Nishida, Y.; Mizuno, M.; Shinkai, M.; Kobayashi, T.; Takeda, T.; Uzawa, H.; Kobayashi, K. *Bioorg. Med. Chem. Lett.* **1999**, *7*, 2053.
22. Leteux, C.; Childs, R. A.; Chai, W.; Stoll, M. S.; Kogelberg, H.; Feizi, T. *Glycobiology* **1998**, *8*, 227.
23. Rothenberg, B. E.; Hayes, B. K.; Toomre, D.; Manzi, A. E.; Varki, A. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 11939.
24. Toomre, D. K.; Varki, A. *Glycobiology* **1994**, *4*, 653.
25. Shinohara, Y.; Sota, H.; Kim, F.; Shimizu, M.; Gotoh, M.; Tosu, M.; Hasegawa, Y. *J. Biochem.* **1995**, *117*, 1076.
26. Sakai, T.; Naidenko, O. V.; Iijima, H.; Kronenberg, M.; Koezuka, Y. *J. Med. Chem.* **1999**, *42*, 1836.
27. McReynolds, K. D.; Hadd, M. J.; Gervay-Hague, J. *Bioconjugate Chem.* **1999**, *10*, 1021.
28. Tamura, J.-i.; Miura, Y.; Freeze, H. H. *J. Carbohydr. Chem.* **1999**, *18*, 1.
29. Wilchek, M.; Bayer, E. A. *Anal. Biochem.* **1988**, *171*, 1.
30. Shao, M.-C.; Chen, L.-M.; Wold, F. *Methods Enzymol.* **1990**, *184*, 653.
31. Shao, M.-C. *Anal. Biochem.* **1992**, *205*, 77.
32. Wilson, J. C.; Kiefel, M. J.; Angus, D. I.; von Itzstein, M. *Org. Lett.* **1999**, *1*, 443.
33. Suzuki, Y.; Sato, K.; Kiso, M.; Hasegawa, A. *Glycoconjugate J.* **1990**, *7*, 349.
34. Sabesan, S.; Neira, S.; Davidson, F.; Duus, J. Ø.; Bock, K. *J. Am. Chem. Soc.* **1994**, *116*, 1616.
35. Defaye, J; Gelas, J. *Studies in Natural Products Chemistry*, vol 8; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 1991, pp. 315–357
36. Albrecht, B.; Pütz, U.; Schwarzmann, G. *Carbohydr. Res.* **1995**, *276*, 289.
37. Bennett, S.; von Itzstein, M.; Kiefel, M. J. *Carbohydr. Res.* **1994**, *259*, 293.
38. Kiefel, M. J.; Beisner, B.; Bennett, S.; Holmes, I. D.; von Itzstein, M. *J. Med. Chem.* **1996**, *39*, 1314.
39. Angus, D. I.; von Itzstein, M. *Carbohydr. Res.* **1995**, *274*, 279.
40. Ciccotosto, S.; Kiefel, M. J.; Abo, S.; Stewart, W.; Quelch, K.; von Itzstein, M. *Glycoconjugate J.* **1998**, *15*, 663.
41. Bodansky, M. In *Principles of Peptide Synthesis*, 2nd Ed.; Springer-Verlag: Berlin, 1993.
42. Kiefel, M. J.; Thomson, R. J.; Radovanovic, M.; von Itzstein, M. *J. Carbohydr. Chem.* **1999**, *18*, 937.
43. Hasegawa, A.; Nakamura, J.; Kiso, M. *J. Carbohydr. Chem.* **1986**, *5*, 11.
44. Park, W. K. C.; Meunier, S. J.; Zanini, D.; Roy, R. *Carbohydr. Lett.* **1995**, *1*, 179.
45. Horton, D.; Wolfrom, M. L. *J. Org. Chem.* **1962**, *27*, 1794.
46. Hudlicky, M. *Reductions in Organic Chemistry*, 2nd Ed.; ACS Publishing: Washington, 1996.
47. Cao, S. D.; Meunier, S. J.; Andersson, F. O.; Letellier, M.; Roy, R. *Tetrahedron: Asymmetry* **1994**, *5*, 2303.
48. For a recent example see: Cao, S.; Hernández-Matéo, F.; Roy, R. *J. Carbohydr. Chem.* **1998**, *17*, 609.
49. Humphrey, J. M.; Chamberlin, A. R. *Chem. Rev.* **1997**, *97*, 2243.
50. See for example Bailén, M. A.; Chinchilla, R.; Dodsworth, D. J.; Nájera, C. *J. Org. Chem.* **1999**, *64*, 8936, and references therein
51. Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927.
52. Martin, T. J.; Brescello, R.; Toepfer, A.; Schmidt, R. R. *Glycoconjugate J.* **1993**, *10*, 16.
53. Abo, S.; Ciccotosto, S.; Alafaci, A.; von Itzstein, M. *Carbohydr. Res.* **1999**, *322*, 201.
54. *Vogel's Textbook of Practical Organic Chemistry*, 5th Ed.; Longman Scientific & Technical, UK, 1989, p.122.
55. Jansson, K.; Ahlfors, S.; Frejd, T.; Kihlberg, J.; Magnusson, G. *J. Org. Chem.* **1988**, *53*, 5629.