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Syntheses of the 3- and 4-thio analogues of 4-nitrophenyl 2-acetamido-2-deoxy-β-D-gluco- and galactopyranoside

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Abstract—The syntheses of 4-nitrophenyl β-glycosides of the 3-thio and 4-thio analogues of the two principal 2-acetamido-2-deoxy-hexoses found in living systems, GlcNAc and GalNAc, are described. While synthesis of the 4-thio analogues could be achieved via nucleophilic displacements of sulfonate derivatives with thioacetate, problems with neighbouring group acetamido participation necessitated the use of sulfamidate intermediates for the 3-thio analogues. These 3- and 4-thio analogues are employed in the chemo-enzymatic synthesis of thio-oligosaccharide analogues of structures present in glycosaminoglycans, glycoproteins and glycolipids.

Keywords: 4-ThioGlcNAc; 4-Thio-GalNAc; 3-ThioGlcNAc; 3-ThioGalNAc; Sulfamidate; Nucleophilic displacement; Thioglycosides; Glycosidase inhibitors

1. Introduction

Oligosaccharide analogues that are resistant towards enzymatic hydrolysis have proved to be very useful as glycosidase inhibitors and have found application in studies of enzyme mechanisms especially in the crystallographic analysis of complexes of glycosidases with substrate analogues. Particularly useful in this regard have been thioglycosides: such sulfur analogues are otherwise tolerated by most biological systems and are also less susceptible to acid-catalysed hydrolysis.[1,2] They therefore have potential as therapeutics, especially in the form of stable antigens for use as glycoconjugate vaccines, or as more metabolically stable versions of therapeutic glycoproteins.[3–6] The synthesis of such thioglycosides typically requires, as a first step, the synthesis of the appropriate thio acceptor sugar, which is then coupled with the appropriate glycosyl donor.[1] Since such chemical couplings are not compatible with the assembly of thioglycosides on protein surfaces, there has been an increasing interest in the use of enzymatic coupling approaches. However, regular glycosyl transferases are only rarely found to be capable of forming thioglycosides and then only slowly.[7] Likewise glycosidases and glycosynthases are also unhelpful. However a new class of mutant glycosidases that selectively assemble thioglycosides was recently unveiled: the thioglycoligases.[8–13] In addition, efforts to evolve glycosyl transferases to synthesise thioglycosides are also bearing fruit.[14] Thus the way is now open to the assembly of thioglycosides on the surface of complex biomolecules.

Such strategies still, however, require the chemical synthesis of thiosugar acceptors. In order to apply this approach to the synthesis of thioglycoside analogues of glycosaminoglycans, gangliosides and glycoproteins, the 3- and 4-thio analogues of *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine were required. More specifically, analogues containing an aryl substituent at the anomeric centre were needed so that the binding of these thiosugar acceptors to the thioglycoligases was optimised, thus facilitating the monitoring of reactions and purifications by TLC and HPLC. The choice of a 4-nitrophenyl derivative in particular was dictated by its proven value in thioglycoligase and glycosynthase

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couplings[15] and by the fact that the 4-nitrophenyl deoxythio-*N*-acetyl-D-hexosaminides generated could also serve as substrates for the assay and screening of hexosaminidases capable of tolerating a sulfur substituent at their 3- or 4-positions. In addition, the disaccharide thioglycosides obtained after enzymatic coupling might then serve as valuable substrates for the assay or screening of *endo*-hexosaminidases.

The replacement of a sugar hydroxyl by a thiol is typically achieved by nucleophilic displacement of activated alcohols of the opposite stereochemistry to that required in the thiosugar product, usually via the intermediacy of sulfonate derivatives.[1,16,17] Such an approach proved suitable, in our hands, for the synthesis of the 4-substituted derivatives. However, it was considerably complicated by neighbouring group participation in the case of substitution at the 3-position of *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine, resulting in the incorrect products. The use of cyclic sulfamidates as temporary protecting/activating groups nicely obviates these problems and provided good access to the desired analogues.[18–20]

2. Results and discussion

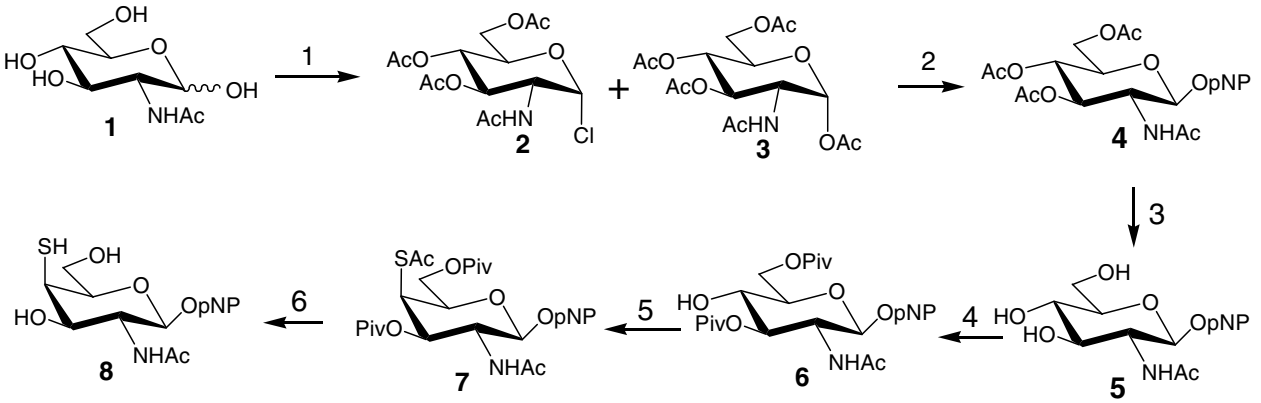
Due to the perceived limitations in reagents and approaches imposed by the presence of a 4-nitrophenyl group at the anomeric centre prior to installation of the thiol group, initial attempts to synthesise the 3- and 4-thio analogues of *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine were based upon the use of an allyl protecting group at the anomeric centre during thiol substitution, with subsequent replacement of the allyl by the desired 4-nitrophenyl group. Unfortunately this led to considerable complications, particularly that of elimination chemistry for the 3-thiosugars in their hemiacetal forms. In fact the strategy that was successful indeed involved incorporation of the 4-nitrophenyl group at the beginning and then use of synthetic routes compatible with this entity. The 4-nitrophenyl glycosides of *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine were both synthesised via the initial formation of the acylated glycosyl chloride using acetyl chloride, then direct glycosylation with 4-nitrophenol and anhydrous K_2CO_3 in acetone, followed by deprotection under Zemplen conditions. This approach[21] is quite straightforward and scalable and gives reasonable yields of product, though somewhat diminished for the *galacto*-derivative by the surprising water solubility of the protected galactosyl chloride intermediate.

2.1. Syntheses of 4-thio-*N*-acetylhexosaminides

Syntheses of the two derivatives in which the 4-position was substituted by sulfur were achieved from the 4-nitrophenyl glycoside of opposite configuration at C-4 via differential protection of the 3- and 6-hydroxyls with ester protecting groups, activation as the triflate and displacement using potassium thioacetate. Final deprotection was performed under Zemplen conditions with the inclusion of dithiothreitol (DTT) during workup while working under reasonably oxygen-free conditions to avoid the otherwise rapid oxidation to disulfide derivatives.

2.2. Synthesis of 4-nitrophenyl 2-acetamido-2-deoxy-4-thio-β-D-galactopyranoside (8)

N-Acetyl-D-glucosamine was reacted with acetyl chloride at room temperature to give a mixture of the fully acetylated α-chloride (**2**) and α-per-*O*-acetate (**3**) of GlcNAc in a ratio of about 3:2 (^{1}H NMR) (Scheme 1). This mixture was used directly for the glycosylation reaction with 4-nitrophenol and anhydrous K_2CO_3 in HPLC-grade acetone, and subsequently deacetylated to give **5**



Scheme 1. Reagents and conditions: (1) AcCl, rt. (2) K_2CO_3, acetone, 4-nitrophenol, 55 °C; 44% (two steps). (3) $CHCl_3$, CH_3OH, $NaOCH_3$, 97%. (4) CH_2Cl_2, pyridine, pivaloyl chloride, 0 °C, 91%. (5) CH_2Cl_2, pyridine, Tf_2O, 0 °C; DMPU, KSAc, rt; 77%; (6) CH_3OH, $NaOCH_3$; DTT, H_2O; 37%.

in 43% overall yield.[21] Selective protection of the hydroxyl groups at C-3 and C-6 was achieved using trimethylacetyl (pivaloyl) chloride at 0 °C to yield the required partially protected intermediate **6**.[8] The introduction of sulfur at C-4 was performed via a nucleophilic displacement on the triflate derivative of compound **6** (without its isolation) using potassium thioacetate. The ester protecting groups were removed using sodium methoxide in methanol and the disulfide formed between 2 equiv of product was reduced with DTT to give the desired product, which was fully characterised. The overall yield of the final steps was unfortunately compromised due to difficulties in the removal of the pivaloyl groups.

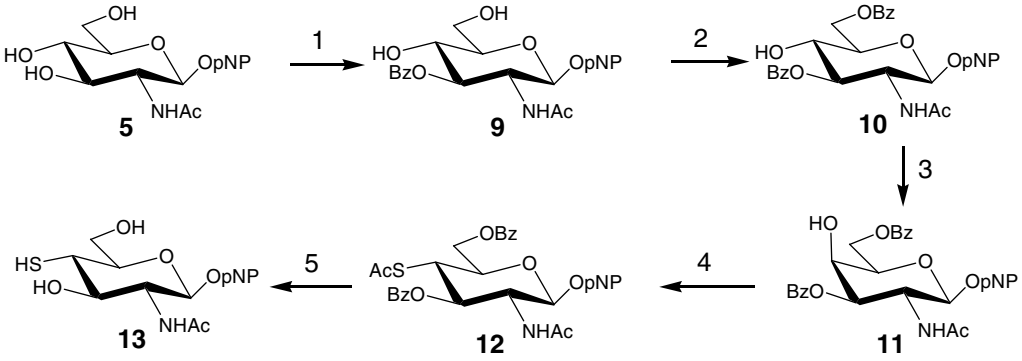
The equivalent reaction sequence, but starting from *N*-acetyl-D-galactosamine, was attempted as the logical route to the synthesis of 4-nitrophenyl 2-acetamido-2-deoxy-4-thio-β-D-glucopyranoside. While the thioacetate was successfully introduced at C-4, all attempts at deprotection, sadly, were unsuccessful, hence the following route.

2.3. Synthesis of 4-nitrophenyl 2-acetamido-2-deoxy-4-thio-β-D-glucopyranoside (13)

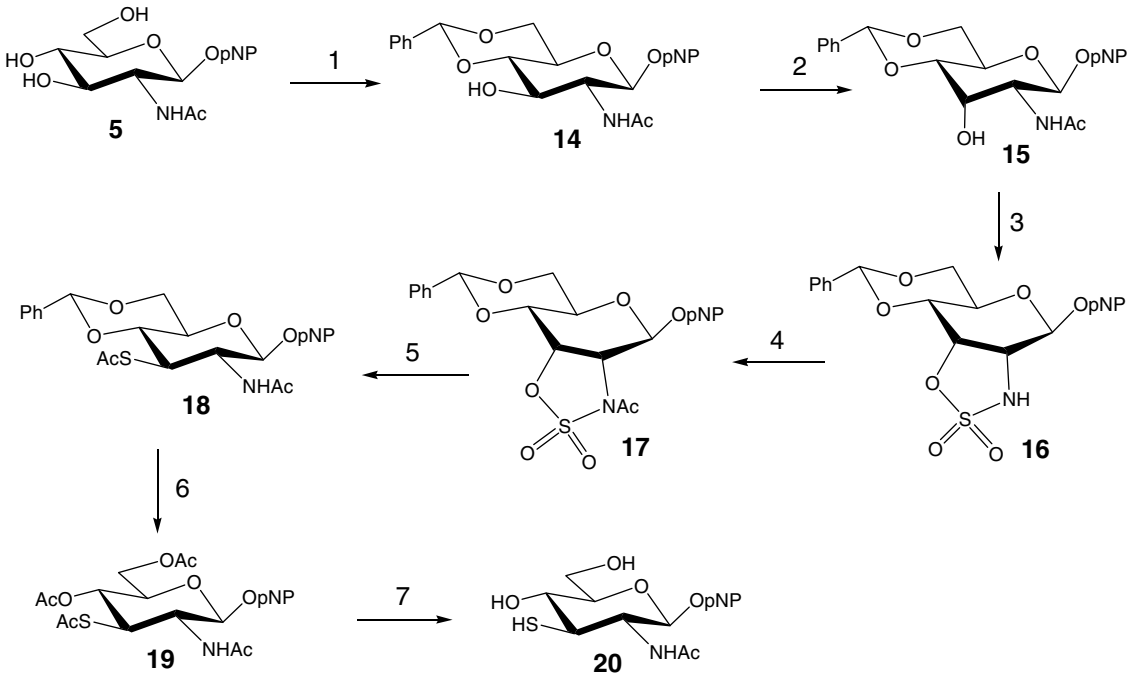
Due to the aforementioned difficulty in removal of pivaloyl protecting groups, an alternative strategy to the synthesis of thiosugar **13** that avoids the use of pivaloyl groups was developed (Scheme 2). 4-Nitrophenyl *N*-acetyl-D-glucosaminide **5** was temporarily protected at the 4- and 6-positions using an isopropylidene group, the free 3-OH, was benzoylated and then the isopropylidene hydrolysed to give the mono-*O*-benzoyl derivative **9**.[22] Selective benzoylation of the primary hydroxyl of **9** was performed by reaction with benzoyl cyanide in methylene chloride and pyridine to give the di-*O*-benzoyl derivative **10**.[23] Inversion of stereochemistry at the 4-hydroxyl was achieved by treatment of the triflate of **10** with $NaNO_2$ in DMF to give the dibenzoyl *galacto*-derivative **11**.[24] Introduction of the thiol at C-4 and subsequent deprotection were accomplished using the same procedures as those used for the preparation of the 4-thio-GalNAc derivative.

2.4. Synthesis of 4-nitrophenyl 2-acetamido-2-deoxy-3-thio-β-D-glucopyranoside (20)

As noted in Section 1, due to severe problems with neighbouring group participation from the 2-acetamide, resulting in the formation of a 2,3-oxazoline, a completely different synthetic strategy was used for the synthesis of 4-nitrophenyl 2-acetamido-2-deoxy-3-thio-β-D-glucopyranoside **20**. This strategy used a cyclic sulfamidate as a temporary protecting/activating group, allowing the introduction of the thioacetate at C-3 via a nucleophilic displacement.[18–20] The main problems encountered with this approach related to the solubilities of compounds **14**–**18**, which were found to be poor in any organic solvents except Me_2SO, DMF and pyridine. Fortunately, most of the intermediates could be precipitated by the addition of other organic solvents, and the impurities remained in the mother liquor, making this, ultimately, an advantage. Protection of the 4- and 6-hydroxyls of 4-nitrophenyl *N*-acetyl-β-D-glucosaminide **5** with a benzylidene gave **14** in which only the 3-hydroxyl was unprotected.[25] Reaction of the mesylate of **14** with sodium acetate in refluxing methoxyethanol and water gave the *allo*-derivative **15**,[18,26] which was reacted with 1,1′-sulfonyldiimidazole and sodium hydride in dry THF, followed by acetylation to afford the acylated sulfamidate **17**. Introduction of the thioacetate at C-3 by nucleophilic displacement gave compound **18**, which was precipitated by the addition of acetone and petroleum ether. This product contained a trace of impurity that was very difficult to separate from the desired compound **18**. In order to further purify compound **18** the benzylidene protecting group was hydrolysed in acetic acid and water, and the resulting residue was acetylated to give **19**. Compound **19** was soluble in most polar solvents, and easily purified by flash



Scheme 2. Reagents and conditions: (1) DMF, 2,2-dimethoxypropane, 4-TsOH, rt; pyridine, benzoyl chloride, 0 °C; AcOH, H_2O, 70 °C; 84% (three steps). (2) CH_2Cl_2, pyridine, benzoyl cyanide, rt, 86%. (3) CH_2Cl_2, pyridine, Tf_2O, 0 °C; DMF, $NaNO_2$, rt; 79%. (4) CH_2Cl_2, pyridine, Tf_2O, 0 °C; DMPU, KSAc, rt; 50%. (5) CH_3OH, $NaOCH_3$; DTT, H_2O; 76%.



Scheme 3. Reagents and conditions: (1) DMF, benzaldehyde dimethyl acetal, 4-TsOH, 50 °C, 96%. (2) CH_2Cl_2, pyridine, MsCl; 2-methoxyethanol, H_2O, NaOAc, refluxed; 63%. (3) THF, NaH, 1,1′-sulfonyldiimidazole, 82%. (4) CH_2Cl_2, pyridine, AcCl, 80%. (5) DMF, KSAc; THF, H_2O, H_2SO_4; 84%. (6) AcOH, H_2O, 60 °C; pyridine, Ac_2O, rt; 81%. (7) CH_3OH, $NaOCH_3$, rt, 100%.

column chromatography. Deprotection of the fully acetylated thiosugar **19** was easily performed under Zemplen conditions, with no complication from disulfide formation (Schemes 3 and 4).

2.5. Synthesis of 4-nitrophenyl 2-acetamido-2-deoxy-3-thio-β-D-galactopyranoside (33)

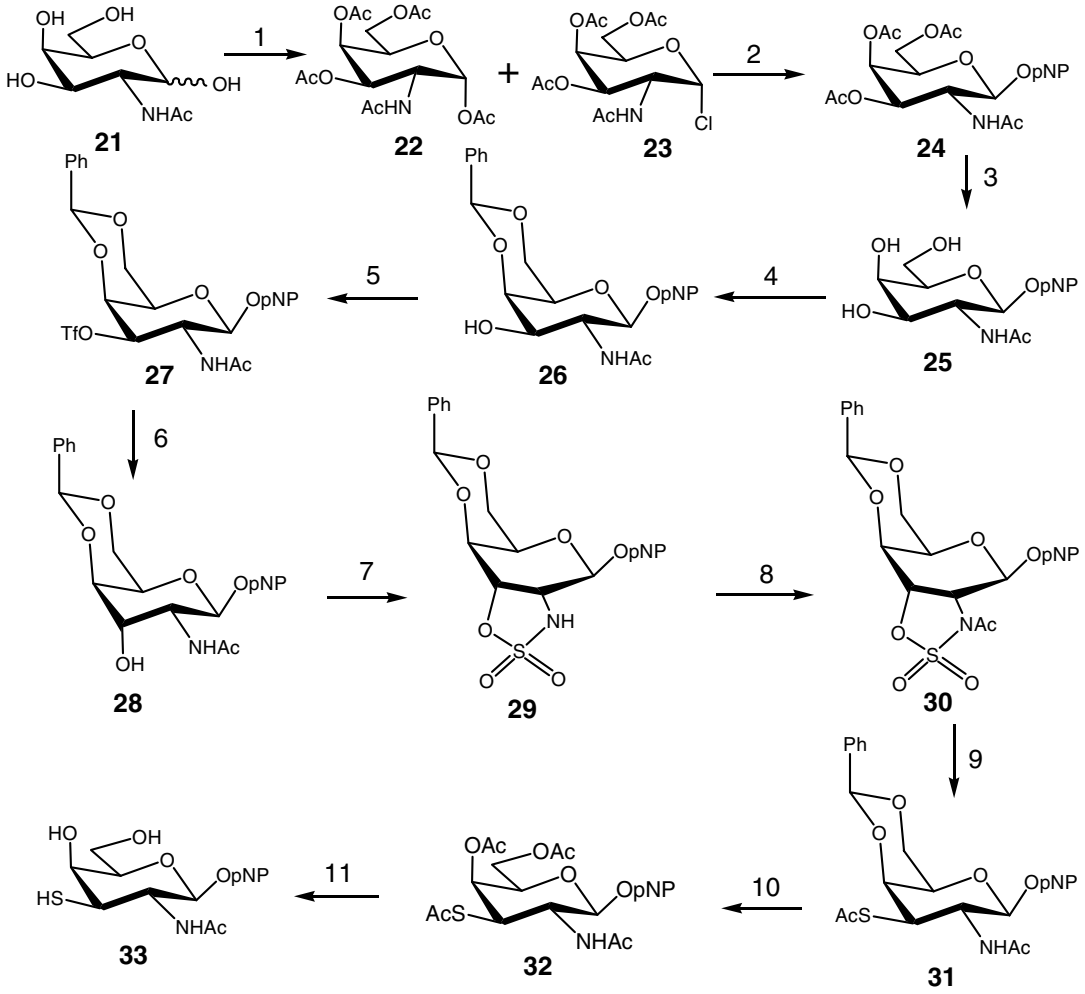
The same overall strategy as that used for the 3-thio-GlcNAc derivative, involving a sulfamidate intermediate, was applied in this sequence. Apart from the previously mentioned surprising water solubility of the acylated GalNAc chloride **23**, the only surprise was in the unreactive nature of the mesylate and tosylate derivatives of **26**. They were found to be so stable that no reaction occurred, even upon reaction with sodium acetate in methoxyethanol under reflux for two days. Consequently triflate activation was employed, the triflate derivative **27** proving to be surprisingly stable. Indeed it was purified by flash column chromatography and then only slowly reacted with $NaNO_2$ in DMF over 4 days to give compound **28**. There is clearly a large difference of reactivity of these triflates since, in the case of the *gluco*-derivative, the triflate had to be used directly in the next step. However, the origin of this reactivity difference is unclear.

Compound **30** was prepared from **28** in a similar manner to that described for **16**, but the reactivity of these two compounds was again very different. While the nucleophilic displacement reaction of **16** was complete in a matter of hours at room temperature, when the suspension of **30** and potassium thioacetate was stirred in DMF overnight at room temperature, no product was detected. Raising the reaction temperature above 100 °C resulted, instead, in displacement of the 4-nitrophenyl substituent from the anomeric centre to give the 1-thioacetate. The best temperature discovered for this reaction was around 65 °C, though this still yielded some by-products, which were difficult to separate from **31**. Consequently, crude **31** was hydrolysed and acetylated to give compound **32** and the per-*O*-acetate of 1,3-dithio-GalNAc, which were readily purified by flash column chromatography. The desired product **33** was then obtained by standard Zemplen deacetylation without significant disulfide formation.

3. Experimental

3.1. General

All chemicals were obtained from the Sigma Chemical Co. unless otherwise noted. Methylene chloride and pyridine were dried over CaH_2 and distilled prior to use. DMF was dried over 4 Å molecular sieves. MeOH was dried over magnesium and distilled prior to use. Analytical thin-layer chromatography (TLC) was performed on aluminium-backed sheets of Silica Gel $60F_{254}$ (E.



Scheme 4. Reagents and conditions: (1) AcCl, rt. (2) Acetone, K_2CO_3, 4-nitrophenol, 50 °C; 29% (two steps). (3) CH_3OH, $NaOCH_3$, rt, 100%. (4) DMF, 4-TsOH, benzaldehyde dimethyl acetal, 50 °C, 92%. (5) CH_2Cl_2, pyridine, Tf_2O, 0 °C. (6) DMF, $NaNO_2$, rt, 4 days; 50% (two steps). (7) THF, NaH, 1,2′-sulfonyldiimidazole, rt, 79%. (8) CH_2Cl_2, pyridine, AcCl, rt, 98%. (9) DMF, KSAc, 65 °C, 85%. (10) AcOH, H_2O, 70 °C, 67%. (11) CH_3OH, $NaOCH_3$, 98%.

Merck) of thickness 0.2 mm. The plates were visualised using UV light (254 nm) and or/ by exposure to 10% ammonium molybdate in 2 M H_2SO_4 followed by charring. Flash column chromatography was performed using silicycle Silicagel 60. The NMR spectra were recorded at Bruker AV-400 MHz or AV-300 MHz spectrometers. The optical rotations were recorded on a JASCO P-1010 Polarimeter. Elemental analysis was carried out at the University of British Columbia microanalytical laboratory. Mass spectra were carried out by using a PE-Sciex API 300 triple quadrupole mass spectrometer equipped with an electrospray ionisation (ESI) ion source.

3.2. Preparation of 4-nitrophenyl 2-acetamido-2-deoxy-4-thio-β-D-galactopyranoside (8)

3.2.1. 4-Nitrophenyl 2-acetamido-2-deoxy-3, 6-di-*O*-pivaloyl-β-D-glucopyranoside (6). To a soln of **5**[21] (0.95 g, 2.76 mmol) in dry CH_2Cl_2 (4.7 mL) and dry pyridine (7.5 mL) was added dropwise trimethylacetyl chloride (1.0 mL, 8.12 mmol, 2.9 equiv) at 0 °C with stirring. The reaction mixture was stirred for a further period of 2 h at 0 °C, quenched with MeOH, the solvent evaporated under diminished pressure and the residue redissolved in CH_2Cl_2 (100 mL). The soln was washed with satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The resulting residue was purified by flash column chromatography (4:1 and 3:1 petroleum ether–acetone) to give **6** as white crystals in 92% yield (1.3 g). $[\alpha]_D^{22}$ −33.7 (*c* 1.0, acetone). ^1H NMR (400 MHz, $CDCl_3$): δ 8.11 (m, 2H, Ar–H), 7.09 (m, 2H, Ar–H), 6.34 (d, 1H, *J* 4.4 Hz, N*H*Ac), 5.45 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1), 5.40 (dd, 1H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.5 Hz, H-3), 4.44 (dd, 1H, $J_{5,6a}$ 1.9, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.27 (dd, 1H, $J_{5,6b}$ 6.5 Hz, H-6b), 4.14 (dd, 1H, H-2), 3.93 (m, 1H, H-5), 3.58 (dd, 1H, $J_{4,5}$ 9.3 Hz, H-4), 1.88 (s, 3H, CH_3CO), 1.20 [s, 9H, $(CH_3)_3CCO$], 1.18 [s, 9H, $(CH_3)_3CCO$]. ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.6, 179.1, 170.5 (C=O); 161.7, 142.8, 125.6, 116.5

(Ar–C); 97.8, 74.3, 74.1, 69.4, 63.5, 54.1; 39.0, 38.9 [$(CH_3)_3CCO$]; 27.1, 27.0 [$(CH_3)_3CCO$]; 23.2 (CH_3CO). HRESIMS: calcd for $[C_{24}H_{34}N_2O_{10}+Na]^+$: 533.2111. Found *m*/*z*: 533.2103.

3.2.2. 4-Nitrophenyl 2-acetamido-4-*S*-acetyl-2-deoxy-3,6-di-*O*-pivaloyl-4-thio-β-D-galactopyranoside (7). A soln of **6** (8.45 g, 16.57 mmol) in dry CH_2Cl_2 (100 mL) and dry pyridine (27 mL) was stirred and cooled to 0 °C under argon, to which was added dropwise trifluoromethanesulfonic anhydride (6.0 mL, 35.68 mmol, 2.2 equiv). The reaction mixture was stirred for 1 h at 0 °C, diluted with CH_2Cl_2 (100 mL), washed with icy 1 M HCl, satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, concentrated and dried under diminished pressure to give a brown foam. To the brown foam were added DMPU (40 mL) and KSAc (5.6 g, 49.12 mmol, 3 equiv), and the mixture was stirred for 1.5 h under argon at rt, diluted with mixed solvents (350 mL, 1:1 EtOAc–Et_2O). The organic phase was washed with water (5 × 200 mL), dried over $MgSO_4$, filtered and concentrated. The resulting residue was purified by flash column chromatography (4:1 petroleum ether–acetone) to afford a brown solid, which was recrystallised from ethanol to give yellowish product **7** in 77% yield (7.2 g). $[\alpha]_D^{22}$ −31.4 (*c* 1.0, acetone). ^1H NMR (400 MHz, $CDCl_3$): δ 8.07 (m, 2H, Ar–H), 7.04 (m, 2H, Ar–H), 6.10 (d, 1H, *J* 4.4 Hz, N*H*Ac), 5.54 (dd, 1H, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 4.3 Hz, H-3), 5.32 (d, 1H, $J_{1,2}$ 8.3 Hz, H-1), 4.38–4.05 (m, 5H, H-2, H-4, H-5, H-6a and H-6b), 2.36 (s, 3H, CH_3CO), 1.90 (s, 3H, CH_3CO), 1.17 [s, 9H, $(CH_3)_3CCO$], 1.09 [s, 9H, $(CH_3)_3CCO$]. ^{13}C NMR (100 MHz, $CDCl_3$): δ 192.9, 177.9, 177.7, 170.4 (C=O); 161.6, 142.8, 125.6, 116.5 (Ar–C); 98.8, 72.3, 69.5, 63.8, 52.9, 45.8; 38.9, 38.7 [$(CH_3)_3CCO$]; 30.7 (CH_3CO); 27.1, 26.8 [$(CH_3)_3CCO$]; 23.2 (CH_3CO). HRESIMS: calcd for $[C_{26}H_{36}N_2O_{10}S+Na]^+$: 591.1988. Found *m*/*z*: 591.2003.

3.2.3. 4-Nitrophenyl 2-acetamido-2-deoxy-4-thio-β-D-galactopyranoside (8). To a suspension of **7** (0.9 g, 1.59 mmol) in dry MeOH (50 mL) was bubbled argon for 0.5 h at rt, and then sodium methylate soln (5.4 M, 0.8 mL, 4.32 mmol, 2.7 equiv) was added. The reaction mixture was stirred under argon for 48 h at rt, neutralised with Amberlite IR-120H ion exchange resin, filtered, washed with MeOH and concentrated to 10 mL. To this soln was added DTT (1.2 g in 50 mL of distilled water) with stirring, under argon for another 0.5 h and the mixture was stirred overnight at rt. The solvents were evaporated to give a residue that was dissolved in DMF, loaded onto silica gel and purified by flash column chromatography (15:1 and 9:1 $CHCl_3$–CH_3OH) to give **8** as a white powder in 37% yield (210 mg). $[\alpha]_D^{22}$ −27.9 (*c* 0.5, MeOH). ^1H NMR (400 MHz, CD_3OD): δ 8.19 (m, 2H, Ar–H), 7.18 (m, 2H, Ar–H), 5.16 (d, 1H, $J_{1,2}$ 8.3 Hz, H-1), 4.26 (dd, 1H, $J_{2,3}$ 10.3 Hz, H-2), 4.00–3.70 (m, 4H, H-3, H-5, H-6a and H-6b), 3.48 (dd, 1H, $J_{3,4}$ 4.1 Hz, $J_{4,5}$ 1.3 Hz, H-4), 1.97 (s, 3H, CH_3CO). ^{13}C NMR (100 MHz, CD_3OD): δ 174.2 (C=O), 163.6, 144.1, 126.7, 117.7 (Ar–C); 100.9, 76.8, 71.9, 63.4, 53.9, 45.4 (C-4); 23.0 (CH_3CO). HRESIMS: calcd for $[C_{14}H_{18}N_2O_7S+Na]^+$: 381.0732. Found *m*/*z*: 381.0721. Anal. Calcd for $C_{14}H_{18}N_2O_7S$: C, 46.92; H, 5.06; N, 7.82. Found: C, 46.84; H, 5.07; N, 8.22.

3.3. Preparation of 4-nitrophenyl 2-acetamido-2-deoxy-4-thio-β-D-glucopyranoside (13)

3.3.1. 4-Nitrophenyl 2-acetamido-3-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (9). To a soln of compound **5** (5.65 g, 16.52 mmol) in dry DMF (65 mL) were added 4-toluenesulfonic acid monohydrate (60 mg) and dimethoxypropane (8 mL, 65.15 mmol, 3.9 equiv). The reaction mixture was stirred overnight at rt, neutralised with Amberlite IR-45 (OH) ion exchange resin, filtered, evaporated and dried under diminished pressure to give a residue. To the residue were added dry pyridine (60 mL) and benzoyl chloride (6 mL, 51.66 mmol, 3.1 equiv) at 0 °C, and the mixture was stirred for 1.5 h at 0 °C. The mixture was poured into icy water, stirred for 0.5 h, and extracted with $CHCl_3$ (3 × 200 mL). The organic phase was washed with icy 1 M HCl, satd aq $NaHCO_3$ and brine, then evaporated. To the resultant syrup were added acetic acid (70 mL) and water (70 mL), and the mixture stirred for 3 h at 70 °C and concentrated. The product was purified by flash column chromatography (2:1 petroleum ether–acetone) to give **9** as a white foam in 84% overall yield (6.19 g). $[\alpha]_D^{22}$ +29.3 (*c* 0.5, acetone). ^1H NMR (400 MHz, Me_2CO-d_6): δ 8.20 (m, 2H, Ar–H), 8.01 (m, 2H, Ar–H), 7.60 (m, 1H, Ar–H), 7.49 (m, 2H, Ar–H), 7.26 (m, 2H, Ar–H), 5.63 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1), 5.45 (dd, 1H, $J_{2,3}$ 10.6 Hz, $J_{3,4}$ 9.0 Hz, H-3), 4.92 (d, 1H, *J* 5.3 Hz, N*H*Ac), 4.29 (ddd, 1H, H-2), 3.89–3.77 (m, 5H, H-4, H-5, H-6a, H-6b and OH), 2.06 (s, 3H, CH_3CO). ^{13}C NMR (75 MHz, DMSO-d_6): δ 169.4, 165.6 (C=O); 162.0, 142.1, 133.3, 130.0, 129.4, 128.6, 125.9, 116.7 (Ar–C); 97.6, 77.1, 76.4, 67.5, 60.1, 53.2; 22.7 (CH_3CO). HRESIMS: calcd for $[C_{21}H_{22}N_2O_9+Na]^+$: 469.1223. Found *m*/*z*: 469.1235.

3.3.2. 4-Nitrophenyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (10). To a soln of compound **9** (6.54 g, 14.66 mmol) in dry CH_2Cl_2 (250 mL) and dry pyridine (25 mL) was added benzoyl cyanide (3.27 g, 24.96 mmol, 1.7 equiv) at rt under argon, and the reaction mixture was stirred overnight at rt and quenched with MeOH. The solvents were evaporated to give a white solid, and the solid was recrystallised from chloroform and MeOH (1:1) to give compound **10** (6.05 g). The mother liquor was concentrated and

purified by flash column chromatography (2:1 petroleum ether–acetone) to give a second portion of product (0.88 g). The yield of the reaction was 86% (6.93 g). $[\alpha]_D^{22}$ +13.7 (*c* 0.5, acetone). ^{1}H NMR (400 MHz, Me_2SO-d_6): δ 8.14 (d, 1H, OH), 8.07 (m, 2H, Ar–H), 7.99 (m, 4H, Ar–H), 7.63 (m, 2H, Ar–H), 7.53 (m, 4H, Ar–H), 7.23 (m, 2H, Ar–H), 5.89 (d, 1H, *J* 5.9 Hz, N*H*Ac), 5.56 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 5.31 (dd, 1H, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 9.5 Hz, H-3), 4.64 (br d, 1H, H-6a), 4.44 (dd, 1H, $J_{5,6b}$ 6.7 Hz, $J_{6a,6b}$ 11.8 Hz, H-6b), 4.17 (m, 2H, H-2 and H-4), 3.79 (m, 1H, H-5), 1.66 (s, 3H, CH_3CO). ^{13}C NMR (100 MHz, Me_2SO-d_6): δ 169.4, 165.5, 165.4 (C=O); 161.5, 142.0, 133.5, 133.3, 129.8, 129.5, 129.4, 129.3, 128.7, 128.6, 125.6, 116.7 (Ar–C); 97.1, 76.0, 73.8, 68.2, 63.6, 53.1; 22.6 (*C*H_3CO). HRESIMS: calcd for $[C_{28}H_{26}N_2O_{10}+Na]^+$: 573.1485. Found *m*/*z*: 573.1484.

3.3.3. 4-Nitrophenyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-galactopyranoside (11). A soln of compound **10** (1.1 g, 2.00 mmol) in dry CH_2Cl_2 (14 mL) and dry pyridine (3 mL) was stirred under argon and cooled to 0 °C, to which was added dropwise Tf_2O (0.7 mL, 4.16 mmol, 2.1 equiv). The mixture was stirred for 1 h at 0 °C, diluted with CH_2Cl_2, washed with icy 1 M HCl, satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, evaporated and dried under diminished pressure to give a brown foam. To the brown foam were added dry DMF (20 mL) and $NaNO_2$ (1.4 g), and the mixture was stirred for 1.5 h at rt. The mixture was diluted with ethyl acetate (200 mL), washed with 1 M HCl, satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The resulting residue was purified by flash column chromatography (2:1, 3:2 and 1:1 petroleum ether–acetone) to give **11** in 79% yield (0.87 g). $[\alpha]_D^{22}$ +39.4 (*c* 0.5, acetone). ^{1}H NMR (400 MHz, Me_2SO-d_6): δ 8.10–7.90 (m, 8H, Ar–H, N*H*Ac, OH), 7.63 (m, 2H, Ar–H), 7.55 (m, 4H, Ar–H), 7.22 (m, 2H, Ar–H), 5.47 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 5.09 (dd, 1H, $J_{2,3}$ 11.0 Hz, $J_{3,4}$ 3.0 Hz, H-3), 4.70–4.30 (m, 4H, H-2, H-5, H-6a and H-6b), 4.23 (br d, 1H, H-4), 1.73 (s, 3H, CH_3CO). ^{13}C NMR (100 MHz, Me_2SO-d_6): δ 169.7, 165.5, 165.4 (C=O); 161.7, 141.9, 133.5, 133.4, 129.7, 129.6, 129.4, 129.3, 128.7, 128.6, 125.5, 116.6 (Ar–C); 97.6, 74.4, 72.7, 64.9, 63.7, 48.7, 22.8 (*C*H_3CO). HRESIMS: calcd for $[C_{28}H_{26}N_2O_{10}+Na]^+$: 573.1485. Found *m*/*z*: 573.1479.

3.3.4. 4-Nitrophenyl 2-acetamido-4-*S*-acetyl-3,6-di-*O*-benzoyl-2-deoxy-4-thio-β-D-glucopyranoside (12). The triflate of **11** (0.55 g, 1.00 mmol) was prepared as described above. The brown foam was treated with DMPU (4 mL) and KSAc (0.34 g, 2.98 mmol, 3.0 equiv), and the mixture was stirred for 1 h at rt. The mixture was diluted with mixed solvents (100 mL, 1:1 EtOAc–Et_2O), washed with water (5 × 100 mL), dried over $MgSO_4$, filtered and evaporated. The resulting residue was purified by flash column chromatography (2:1 petroleum ether–EtOAc) to give **12** (300 mg, 50%). $[\alpha]_D^{22}$ +38.8 (*c* 0.5, acetone). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05–7.90 (m, 6H, Ar–H), 7.60 (m, 2H, Ar–H), 7.44 (m, 4H, Ar–H), 7.06 (m, 2H, Ar–H), 6.06 (d, 1H, *J* 8.4 Hz, N*H*Ac), 5.75 (dd, 1H, $J_{2,3} = J_{3,4}$ 10.5 Hz, H-3), 5.60 (d, 1H, $J_{1,2}$ 8.1 Hz, H-1), 4.76 (br d, 1H, H-6a), 4.46 (dd, 1 H, $J_{5,6b}$ 7.3 Hz, $J_{6a,6b}$ 11.8 Hz, H-6b), 4.30 (m, 2H, H-2 and H-5), 3.99 (dd, 1H, $J_{4,5}$ 11.0 Hz, H-4), 2.24 (s, 3H, CH_3CO), 1.81 (s, 3H, CH_3CO). ^{13}C NMR (100 MHz, $CDCl_3$): δ 192.7, 170.7, 166.4, 165.9 (C=O); 161.4, 142.8, 133.8, 133.6, 129.9, 129.7, 129.5, 128.7, 128.6, 128.5, 125.6, 116.6 (Ar–C); 97.7, 73.4, 71.4, 63.8, 56.1, 44.5, 30.8, 23.3 (CH_3CO). HRESIMS: calcd for $[C_{30}H_{28}N_2O_{10}S+Na]^+$: 631.1362. Found *m*/*z*: 631.1350.

3.3.5. 4-Nitrophenyl 2-acetamido-2-deoxy-4-thio-β-D-glucopyranoside (13). The deprotection of **12** (1.25 g, 1.86 mmol) in dry MeOH (60 mL) as described for **8** gave a residue, which was purified by flash column chromatography ($CHCl_3$–CH_3OH = 12:1) to give **13** as a white powder in 76% yield (450 mg). $[\alpha]_D^{22}$ −9.1 (*c* 0.5, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 8.20 (m, 2H, Ar–H), 7.16 (m, 2H, Ar–H), 5.26 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 3.95 (dd, 1H, $J_{5,6a}$ 2.0 Hz, $J_{6a,6b}$ 12.3 Hz, H-6a), 3.90 (dd, 1H, $J_{2,3}$ 10.0 Hz, H-2), 3.84 (dd, 1H, $J_{5,6b}$ 4.9 Hz, H-6b), 3.61 (ddd, 1H, H-5), 3.53 (dd, 1H, H-3), 2.88 (dd, 1H, $J_{3,4} = J_{4,5}$ 10.3 Hz, H-4), 1.97 (s, 3H, CH_3CO). ^{13}C NMR (100 MHz, CD_3OD): δ 173.9 (C=O), 163.6, 144.0, 126.7, 117.7 (Ar–C); 99.8, 79.8, 76.6, 63.0, 58.4, 43.6, 22.9 (*C*H_3CO). HRESIMS: calcd for $[C_{14}H_{18}N_2O_7S+Na]^+$: 381.0732. Found *m*/*z*: 381.0728. Anal. Calcd for $C_{14}H_{18}N_2O_7S$: C, 46.92; H, 5.06; N, 7.82. Found: C, 47.14; H, 5.18; N, 8.13.

3.4. Preparation of 4-nitrophenyl 2-acetamido-2-deoxy-3-thio-β-D-glucopyranoside (20)

3.4.1. 4-Nitrophenyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (14). Compound **5** (2.2 g, 6.43 mmol), anhydrous DMF (30 mL), benzaldehyde dimethyl acetal (1.16 mL, 1.2 equiv) and 4-toluenesulfonic acid monohydrate (90 mg) were placed in a 100 mL round bottom flask. This was attached to a Büchi evaporator under diminished pressure, then rotated in a water bath at 50 °C, so that DMF refluxed in the vapour duct. The suspension was rotated for 3 h until a clear soln formed, then progress of the reaction was checked by TLC. The mixture was poured into satd aq $NaHCO_3$ (200 mL), and stirred for 0.5 h at rt. The white precipitate was filtered and washed with water and diethyl ether, then dried under diminished pressure to give compound **14** in 96% yield (2.67 g). ^{1}H NMR (400 MHz, Me_2CO-d_6): δ 8.22 (m, 2H, Ar–H), 7.51 (m, 2H, Ar–H), 7.38 (m, 3H, Ar–H), 7.28 (m, 2H, Ar–H), 5.67

(s, 1H, C*H*Ph), 5.63 (d, 1H, $J_{1,2}$ 8.3 Hz, H-1), 4.32 (dd, 1H, $J_{5,6a}$ 4.1 Hz, $J_{6a,6b}$ 9.4 Hz, H-6a), 4.10 (m, 1H, H-5), 3.98 (dd, 1H, $J_{5,6b}$ 8.6 Hz, H-6b), 3.83 (dd, 1H, $J_{4,5}$ 9.9 Hz, H-4), 3.77 (ddd, 1H, H-2), 3.67 (dd, 1H, $J_{2,3} = J_{3,4}$ 9.0 Hz, H-3), 1.89 (s, 3H, CH_3CO). ^{13}C NMR (75 MHz, Me_2SO-d_6): δ 170.0 (C=O); 162.4, 142.6, 138.2, 129.5, 128.6, 126.9, 126.4, 117.2 (Ar–C); 101.3 (*C*HPh); 99.0, 81.3, 70.7, 68.2, 66.7, 56.4, 23.6 (*C*H_3CO). HRESIMS: calcd for $[C_{21}H_{22}N_2O_8+Na]^+$: 453.1274. Found *m*/*z*: 453.1269.

3.4.2. 4-Nitrophenyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-allopyranoside (15). To a suspension of **14** (2.17 g, 5.05 mmol) in CH_2Cl_2 (20 mL) at rt was added dry pyridine (20 mL) under argon. The suspension was stirred for 5 min, and MsCl (1.2 mL, 15.5 mmol, 3 equiv) was added dropwise. The reaction mixture was stirred for 19 h at rt, quenched with MeOH and evaporated to give a yellow residue. To the yellow residue was added MeOH (40 mL), and the suspension was stirred for 0.5 h at rt. The precipitate was filtered and washed with MeOH to give the mesylate as a solid (1.90 g), which was suspended in 10:1 2-methoxyethanol–water (60 mL), and reacted with sodium acetate (2.5 g) at 130 °C for 8 h. The mixture was cooled to rt, and the solvents were evaporated to give a residue, to which was added distilled water (300 mL), and the suspension stirred for 0.5 h at rt. The white precipitate was filtered and washed with water and diethyl ether to give **15** in 63% yield (1.37 g). ^1H NMR (400 MHz, Me_2CO-d_6): δ 8.23 (m, 2H, Ar–H), 7.50 (m, 2H, Ar–H), 7.38 (m, 3H, Ar–H), 7.29 (m, 2H, Ar–H), 5.71 (s, 1H, C*H*Ph), 5.56 (d, 1H, $J_{1,2}$ 8.6 Hz, H-1), 4.40–4.26 (m, 4H, H-2, H-3, H-5, H-6a), 3.86 (dd, 1H, $J_{5,6b}$ 3.0 Hz, $J_{6a,6b}$ 9.3 Hz, H-6b), 3.81 (dd, 1H, $J_{3,4} = J_{4,5}$ 9.9 Hz, H-4), 1.94 (s, 3H, CH_3CO). ^{13}C NMR (75 MHz, Me_2SO-d_6): δ 169.5 (C=O); 162.6, 142.5, 138.2, 129.4, 128.6, 126.9, 126.3, 117.3 (Ar–C); 101.2 (*C*HPh), 97.3, 78.5, 68.6, 67.7, 63.7, 53.3; 23.2 (*C*H_3CO). HRESIMS: calcd for $[C_{21}H_{22}N_2O_8+Na]^+$: 453.1274. Found *m*/*z*: 453.1279.

3.4.3. 4-Nitrophenyl 4,6-*O*-benzylidene-2-deoxy-2,3-sulfamido-β-D-allopyranoside (16). To a suspension of **15** (1.35 g, 3.14 mmol) in THF (50 mL) at rt was added NaH (0.8 g, 60% in mineral oil, 20 mmol, 6.4 equiv). After 15 min, a soln of 1,1′-sulfonyldiimidazole (1.5 g, 7.58 mmol, 2.4 equiv) in THF (30 mL) was added dropwise and the mixture was stirred for 20 h at rt. The reaction was quenched with MeOH and the suspension filtered through a short pad of Celite column that was washed with methylene chloride. The solvents were evaporated, and the resulting residue was purified by flash column chromatography (100:1 CH_2Cl_2–CH_3OH) to give **16** in 82% yield (1.14 g). ^1H NMR (400 MHz, $CDCl_3$): δ 8.23 (m, 2H, Ar–H), 7.48 (m, 2H, Ar–H), 7.39 (m, 3H, Ar–H), 7.11 (m, 2H, Ar–H), 5.60 (d, 1H, $J_{1,2}$ 7.3 Hz, H-1), 5.59 (s, 1H, C*H*Ph), 5.31 (dd, 1H, $J_{2,3}$ 4.1 Hz, H-3), 5.25 (br s, 1H, N*H*Ac), 4.47 (dd, 1H, $J_{5,6a}$ 5.2 Hz, $J_{6a,6b}$ 10.6 Hz, H-6a), 4.15 (ddd, 1H, H-5), 4.10 (br dd, 1H, H-2), 3.99 (dd, 1H, $J_{3,4}$ 2.7 Hz, $J_{4,5}$ 9.6 Hz, H-4), 3.78 (t, 1H, $J_{5,6b}$ 10.4 Hz, H-6b). ESIMS: calcd for $[C_{19}H_{18}N_2O_9S+Na]^+$: 473.1. Found *m*/*z*: 473.0.

3.4.4. 4-Nitrophenyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-2,3-sulfamido-β-D-allopyranoside (17). To a suspension of **16** (0.88 g, 1.96 mmol) in CH_2Cl_2 (40 mL) and pyridine (2 mL) at rt was added dropwise AcCl (0.33 mL, 4.64 mmol, 2.4 equiv), and the mixture was stirred for 1 h at rt. The reaction mixture was diluted with CH_2Cl_2 (150 mL), washed with water and brine, dried over $MgSO_4$, filtered and concentrated to give a residue. To the residue were added EtOAc and petroleum ether and the precipitate was filtered and washed with petroleum ether to give 710 mg of product **17**. The mother liquor was concentrated and purified by flash column chromatography (250:1 and 100:1 CH_2Cl_2-EtOAc) to give a second portion of product (60 mg). Totally 770 mg (80%) of **17** was obtained as a white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 8.22 (m, 2H, Ar–H), 7.48 (m, 2H, Ar–H), 7.39 (m, 3H, Ar–H), 7.09 (m, 2H, Ar–H), 5.61 (s, 1H, C*H*Ph), 5.57 (d, 1H, $J_{1,2}$ 6.1 Hz, H-1), 5.34 (dd, 1H, $J_{2,3}$ 4.8 Hz, $J_{3,4}$ 2.7 Hz, H-3), 5.04 (br t, 1H, H-2), 4.47 (dd, 1H, $J_{5,6a}$ 4.9 Hz, $J_{6a,6b}$ 10.6 Hz, H-6a), 4.24 (ddd, 1H, $J_{4,5}$ 9.9 Hz, H-5), 4.15 (dd, 1H, H-4), 3.77 (dd, 1H, $J_{5,6b}$ 10.2 Hz, H-6b), 2.49 (s, 3H, CH_3CO). ESIMS: calcd for $[C_{21}H_{20}N_2O_{10}S+Na]^+$: 515.1. Found *m*/*z*: 515.2.

3.4.5. 4-Nitrophenyl 2-acetamido-3-*S*-acetyl-4,6-*O*-benzylidene-2-deoxy-3-thio-β-D-glucopyranoside (18). To a soln of sulfamidate **17** (0.77 g, 1.57 mmol) in dry DMF (50 mL) was added KSAc (0.89 g, 7.8 mmol, 5 equiv), and the mixture was stirred for 1 h at rt, after which the solvent was evaporated under diminished pressure to give a yellowish solid. The solid was suspended in THF (20 mL), and treated with 6 mL of THF–H_2SO_4–H_2O (5 mL:220 μL:70 μL) for 0.5 h. The mixture was extracted with CH_2Cl_2 (400 mL) and the organic phase washed with satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated to give a residue. The product was precipitated by the addition of acetone and petroleum ether and filtered to give 600 mg of **18**. The mother liquor was purified by flash column chromatography (20:1 CH_2Cl_2–acetone) to give a second portion of product (43 mg). In total 643 mg (84%) of **18** was obtained as a yellowish solid. $[\alpha]_D^{22}$ −15.4 (*c* 0.5, acetone). ^1H NMR (400 MHz, $CDCl_3$): δ 8.19 (m, 2H, Ar–H), 7.44 (m, 2H, Ar–H), 7.36 (m, 3H, Ar–H), 7.05 (m, 2H, Ar–H), 5.75 (d, 1H, N*H*Ac), 5.53 (s, 1H, C*H*Ph), 5.23 (d, 1H, $J_{1,2}$ 8.1 Hz, H-1), 4.38 (m, 2H, H-2,

H-6a), 3.93 (dd, 1H, $J_{2,3} = J_{3,4}$ 11.4 Hz, H-3), 3.82–3.68 (m, 2H, H-5 and H-6b), 3.67 (dd, 1H, $J_{4,5}$ 8.3 Hz, H-4), 2.37 (s, 3H, CH_3CO), 1.93 (s, 3H, CH_3CO). ^{13}C NMR (75 MHz, Me_2SO-d_6): δ 194.1 (*S*-acetyl), 169.3 (C=O); 161.6, 142.2, 137.3, 128.9, 128.1, 126.0, 125.8, 116.7 (Ar–C); 100.6 (*C*HPh); 99.0, 77.3, 69.3, 67.6, 52.1, 47.2; 30.6, 22.6 (*C*H_3CO). HRESIMS: calcd for $[C_{23}H_{24}N_2O_8S+Na]^+$: 511.1151. Found *m*/*z*: 511.1152.

3.4.6. 4-Nitrophenyl 2-acetamido-3-*S*-acetyl-4,6-di-*O*-acetyl-2-deoxy-3-thio-β-D-glucopyranoside (19). A suspension of **18** (0.3 g, 0.6 mmol) in acetic acid (8 mL) and water (2 mL) was stirred for 3.5 h at 60 °C. The solvents were then evaporated to give a yellowish solid, which was dried under diminished pressure. To the solid were added pyridine (5 mL) and Ac_2O (5 mL), and the mixture was stirred overnight at rt. The mixture was poured into icy water, stirred for 0.5 h, extracted with CH_2Cl_2 (200 mL) and the organic phase washed with 1 M HCl, satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The resulting residue was purified by flash column chromatography (1:1 and 1:2 petroleum ether–EtOAc) to give **19** in 81% overall yield (241 mg). $[\alpha]_D^{22}$ −10.4 (*c* 1.0, acetone). ^1H NMR (400 MHz, $CDCl_3$): δ 8.17 (m, 2H, Ar–H), 6.92 (m, 2H, Ar–H), 5.71 (d, 1H, *J* 8.9 Hz, N*H*Ac), 5.30 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1), 5.09 (dd, 1H, $J_{3,4}$ 10.5 Hz, $J_{4,5}$ 9.8 Hz, H-4), 4.26 (m, 2H, H-2, H-6a), 4.13 (dd, 1H, $J_{5,6b}$ 2.1 Hz, $J_{6a,6b}$ 12.2 Hz, H-6b), 3.96 (m, 2H, H-3 and H-5), 2.37 (s, 3H, CH_3CO), 2.05 (s, 6H, $2 \times CH_3CO$), 1.93 (s, 3H, CH_3CO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.0 (*S*-acetyl), 170.7, 170.5, 169.5 (C=O); 161.7, 143.1, 125.9, 116.7 (Ar–C); 99.7, 75.2, 66.8, 62.5, 54.2, 47.6; 30.9, 23.4, 20.9, 20.8 (*C*H_3CO). HRESIMS: calcd for $[C_{20}H_{24}N_2O_{10}S+Na]^+$: 507.1049. Found *m*/*z*: 507.1048.

3.5. 4-Nitrophenyl 2-acetamido-2-deoxy-3-thio-β-D-glucopyranoside (20)

Into a soln of compound **19** (160 mg, 0.33 mmol) in dry MeOH (15 mL) was bubbled argon for 0.5 h, and then sodium methylate soln (5.4 M, 0.2 mL, 1.08 mmol, 3.3 equiv) was added. The soln was stirred for 0.5 h at rt, neutralised with Amberlite IR-120H ion exchange resin, filtered and washed with MeOH and concentrated. The product was precipitated by addition of acetone and petroleum ether to give a white solid in quantitative yield. $[\alpha]_D^{22}$ −24.1 (*c* 0.5, MeOH). ^1H NMR (400 MHz, CD_3OD): δ 8.14 (m, 2H, Ar–H), 7.10 (m, 2H, Ar–H), 5.14 (d, 1H, $J_{1,2}$ 8.1 Hz, H-1), 3.89 (dd, 1H, H-2), 3.84 (dd, 1H, $J_{5,6a}$ 1.7 Hz, H-6a), 3.67 (dd, 1H, $J_{5,6b}$ 5.6 Hz, $J_{6a,6b}$ 12.2 Hz, H-6b), 3.47 (m, 1H, H-5), 3.34 (dd, 1H, $J_{3,4} = J_{4,5}$ 9.7 Hz, H-4), 2.97 (dd, 1H, $J_{2,3}$ 9.9 Hz, H-3), 1.91 (s, 3H, CH_3CO). ^{13}C NMR (75 MHz, CD_3OD): δ 173.8 (C=O); 163.7, 144.2, 126.8, 117.8 (Ar–C); 100.9, 81.1, 72.7, 62.7, 57.1, 48.9, 22.9 (*C*H_3CO). HRESIMS: calcd for $[C_{14}H_{18}N_2O_7S+Na]^+$: 381.0732. Found *m*/*z*: 381.0722. Anal. Calcd for $C_{14}H_{18}N_2O_7S$: C, 46.92; H, 5.06; N, 7.82. Found: C, 46.56; H, 5.20; N, 8.04.

3.6. Preparation of 4-nitrophenyl 2-acetamido-2-deoxy-3-thio-β-D-galactopyranoside (33)

3.6.1. 4-Nitrophenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranoside (24). A mixture of **21** (4.1 g, 18.55 mmol) and acetyl chloride (20 mL) was stirred overnight at rt, diluted with CH_2Cl_2 (200 mL) and poured into icy water. The organic phase was washed with icy cold $NaHCO_3$ and brine, dried over $MgSO_4$, concentrated, and the solid dried under diminished pressure. ^1H NMR analysis of the white solid showed that it was a mixture of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl chloride (**23**, ~60%) and 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-galactopyranose (**22**, ~40%). To this solid were added acetone (50 mL, HPLC-grade), 4-nitrophenol (6.0 g, 43.17 mmol) and K_2CO_3 (8.0 g, 57.97 mmol), and the suspension was stirred for 1 h at 50 °C. After evaporation, the residue was dissolved in CH_2Cl_2 (300 mL), washed with satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The resulting residue was crystallised from MeOH to give **24** in 29% yield (2.5 g). ^1H NMR (400 MHz, $CDCl_3$): δ 8.17 (m, 2H, Ar–H), 7.08 (m, 2H, Ar–H), 5.66 (d, 1H, *J* 8.4 Hz, N*H*Ac), 5.48 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1), 5.47–5.43 (m, 2H, H-3 and H-4), 4.25–4.10 (m, 4H, H-2, H-5, H-6a and H-6b), 2.16 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 1.94 (s, 3H, CH_3CO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.0, 170.7, 170.6, 170.4 (C=O); 161.8, 143.1, 125.9, 116.8 (Ar–C); 98.2, 71.6, 69.5, 66.8, 61.9, 51.6, 23.6, 20.9 (3C) (*C*H_3CO). ESIMS: calcd for $[C_{20}H_{24}N_2O_{11}+Na]^+$: 491.1. Found *m*/*z*: 491.1.

3.7. 4-Nitrophenyl 2-acetamido-2-deoxy-β-D-galactopyranoside (25)

Compound **24** (2.0 g, 4.27 mmol) was deacetylated as described for **20** to give **25** in quantitative yield. ^1H NMR (400 MHz, Me_2SO-d_6): δ 8.20 (m, 2H, Ar–H), 7.77 (d, 1H, *J* 9.0 Hz, N*H*Ac), 7.16 (m, 2H, Ar–H), 5.14 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1), 4.83 (d, 1H, *J* 6.2 Hz, OH), 4.71 (d, 1H, *J* 8.8 Hz, OH), 4.70 (d, 1H, *J* 10.2 Hz, OH), 4.03 (m, 1H, H-2), 3.75–3.52 (m, 5H, H-3, H-4, H-5, H-6a and H-6b), 1.80 (s, 3H, CH_3CO). ^{13}C NMR (75 MHz, Me_2SO-d_6): δ 169.7 (C=O); 162.4, 141.7, 125.8, 116.6 (Ar–C); 98.8, 75.9, 71.0, 67.4, 60.3, 51.7, 23.1 (*C*H_3CO). HRESIMS: calcd for $[C_{14}H_{18}N_2O_8+Na]^+$: 365.0961. Found *m*/*z*: 365.0961.

3.7.1. 4-Nitrophenyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (26). Compound **26** was prepared as described for **14** by the treatment of compound **24** (1.74 g, 5.09 mmol) with benzaldehyde dimethyl acetal and 4-toluenesulfonic acid monohydrate in DMF. The product was obtained as a white solid in 92% yield (2.01 g). ^{1}H NMR (400 MHz, Me_2SO-d_6): δ 8.22 (m, 2H, Ar–H), 7.83 (d, 1H, J 8.7 Hz, N*H*Ac), 7.51 (m, 2H, Ar–H), 7.41 (m, 3H, Ar–H), 7.24 (m, 2H, Ar–H), 5.65 (s, 1H, C*H*Ph), 5.32 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1), 5.16 (br s, 1H, OH), 4.24–3.81 (m, 6H, H-2, H-3, H-4, H-5, H-6a and H-6b), 1.82 (s, 3H, CH_3CO). ^{13}C NMR (75 MHz, Me_2SO-d_6): δ 169.7 (C=O); 162.2, 141.8, 138.5, 128.7, 128.0, 126.3, 125.8, 116.6 (Ar–C); 99.8 (*C*HPh), 99.4, 75.0, 69.1, 68.3, 66.5, 51.5, 23.1 (*C*H_3CO). HRESIMS: calcd for $[C_{21}H_{22}N_2O_8+Na]^+$: 453.1274. Found m/z: 453.1263.

3.7.2. 4-Nitrophenyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-gulopyranoside (28). A mixture of **26** (2.1 g, 4.88 mmol), anhydrous CH_2Cl_2 (60 mL) and pyridine (12 mL) was cooled to 0 °C under argon, and to this soln was added, dropwise, Tf_2O (2.5 mL, 14.87 mmol, 3 equiv) with stirring. The reaction mixture was stirred for another period of 1.5 h at 0 °C, diluted with CH_2Cl_2 (250 mL), washed with icy 1 M HCl, satd aq $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was evaporated to give a residue, which was dried under diminished pressure to afford a yellowish foam (**27**). To this foam were added dry DMF (65 mL) and $NaNO_2$ (3.6 g, 52.17 mmol), and the suspension was stirred for 4 days at rt under argon. The reaction mixture was diluted with CH_2Cl_2 (500 mL), washed with icy 1 M HCl, satd aq $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated. The resulting residue was purified by flash column chromatography (20:1 CH_2Cl_2–CH_3OH = 20:1) to give a yellowish foam from an ether solution of which the product was precipitated by the addition of acetone and petroleum. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (m, 2H, Ar–H), 7.52 (m, 2H, Ar–H), 7.38 (m, 3H, Ar–H), 7.11 (m, 2H, Ar–H), 6.08 (d, 1H, J 7.3 Hz, N*H*Ac), 5.80 (d, 1H, $J_{1,2}$ 8.7 Hz, H-1), 5.61 (s, 1H, C*H*Ph), 4.39–4.12 (m, 5H, H-2, H-3, H-5, H-6a and H-6b), 2.05 (s, 3H, CH_3CO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.2 (C=O); 162.2, 142.7, 137.7, 129.4, 128.5, 126.4, 125.7, 116.7 (Ar–C); 101.0 (*C*HPh), 97.2, 75.7, 69.8, 69.4, 66.3, 50.8; 23.4 (*C*H_3CO). HRESIMS: calcd for $[C_{21}H_{22}N_2O_8+Na]^+$: 453.1274. Found m/z: 453.1280.

3.7.3. 4-Nitrophenyl 4,6-*O*-benzylidene-2-deoxy-2,3-sulfamido-β-D-gulopyranoside (29). Compound **28** (340 mg, 0.79 mmol) was converted to its sulfamidate and purified as described for **16** to yield compound **29** as a yellowish solid (280 mg, 79%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.23 (m, 2H, Ar–H), 7.50 (m, 2H, Ar–H), 7.39 (m, 3H, Ar–H), 7.13 (m, 2H, Ar–H), 5.67 (s, 1H, C*H*Ph), 5.57 (d, 1H, $J_{1,2}$ 8.1 Hz, H-1), 5.11 (dd, 1H, $J_{2,3}$ 3.1 Hz, $J_{3,4}$ 4.4 Hz, H-3), 5.04 (d, 1H, J 5.2 Hz, N*H*Ac), 4.47 (m, 1H, H-5), 4.46 (dd, 1H, $J_{5,6a}$ 1.3, $J_{6a,6b}$ 12.7 Hz, H-6a), 4.22 (dd, 1H, $J_{5,6b}$ 1.8 Hz, H-6b), 4.14 (dt, 1H, H-2), 4.00 (br d, 1H, H-4). ^{13}C NMR (75 MHz, $CDCl_3$): 163.2, 143.5, 139.6, 129.7, 129.0, 127.3, 126.5, 117.5 (Ar–C); 101.5 (*C*HPh); 96.9, 76.9, 70.2, 69.8, 66.9, 57.0. HRESIMS: calcd for $[C_{19}H_{18}N_2O_9S+Na]^+$: 473.0631. Found m/z: 473.0634.

3.7.4. 4-Nitrophenyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-2,3-sulfamido-β-D-gulopyranoside (30). Compound **29** (276 mg, 0.61 mmol) was acetylated and purified as described for **17** to yield compound **30** as a white solid (297 mg, 98%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.23 (m, 2H, Ar–H), 7.50 (m, 2H, Ar–H), 7.41 (m, 3H, Ar–H), 7.11 (m, 2H, Ar–H), 5.68 (s, 1H, C*H*Ph), 5.53 (d, 1H, $J_{1,2}$ 7.7 Hz, H-1), 5.15 (dd, 1H, $J_{2,3}$ 2.4, $J_{3,4}$ 4.5 Hz, H-3), 5.04 (m, 1H, H-2), 4.48 (m, 1H, H-5), 4.47 (dd, 1H, $J_{5,6a}$ 1.4 Hz, H-6a), 4.23 (dd, 1H, $J_{5,6b}$ 1.8 Hz, $J_{6a,6b}$ 13.0 Hz, H-6b), 4.02 (br d, 1H, H-4), 2.49 (s, 3H, CH_3CO). ^{13}C NMR (75 MHz, Me_2CO-d_6): 162.1, 144.3, 138.9, 130.0, 129.0, 127.3, 126.7, 117.8 (Ar–C); 101.8 (*C*HPh); 99.2, 79.6, 70.5, 68.9, 67.7, 58.1, 23.1 (*C*H_3CO). HRESIMS: calcd for $[C_{21}H_{20}N_2O_{10}S+Na]^+$: 515.0736. Found m/z: 515.0742.

3.7.5. 4-Nitrophenyl 2-acetamido-3-*S*-acetyl-4,6-*O*-benzylidene-2-deoxy-3-thio-β-D-galactopyranoside (31). A mixture of compound **30** (142 mg, 0.29 mmol) and KSAc (165 mg, 1.45 mmol, 5 equiv) in dry DMF (10 mL) was heated to 65 °C under argon and stirred overnight. The solvent was evaporated under diminished pressure to give a yellow residue, which was suspended in THF (4 mL), and acid (1.5 mL, 5 mL:220 μL:70 μL THF–H_2SO_4–H_2O) was added. The suspension was stirred for 0.5 h at rt, diluted with CH_2Cl_2 (75 mL), washed with satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated to give crude product. The product was purified by flash column chromatography (30:1 and 20:1 CH_2Cl_2–acetone) (**31**, 120 mg, 85% with a trace of impurity). $[\alpha]_D^{22}$ −12.0 (*c* 0.5, acetone). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (m, 2H, Ar–H), 7.51 (m, 2H, Ar–H), 7.38 (m, 3H, Ar–H), 7.07 (m, 2H, Ar–H), 5.84 (d, 1H, J 9.0 Hz, N*H*Ac), 5.56 (s, 1H, C*H*Ph), 5.42 (d, 1H, $J_{1,2}$ 8.1 Hz, H-1), 4.43 (dt, 1H, H-2), 4.33 (br d, 1H, $J_{6a,6b}$ 12.4 Hz, H-6a), 4.22 (dd, 1H, $J_{2,3}$ 12.0 Hz, $J_{3,4}$ 3.0 Hz, H-3), 4.16 (d, 1H, H-4), 4.09 (br d, 1H, H-6b), 3.84 (m, 1H, H-5), 2.40 (s, 3H, CH_3CO), 1.92 (s, 3H, CH_3CO). ^{13}C NMR (75 MHz, Me_2SO-d_6): δ 196.8 (*S*-acetyl), 170.6 (C=O); 162.1, 142.9, 137.3, 129.4, 128.5, 126.4, 125.9, 116.9 (Ar–C); 101.3 (*C*HPh); 100.2, 74.9, 69.1, 68.9, 50.1, 46.4; 30.8, 23.6 (*C*H_3CO). HRESIMS: calcd for $[C_{23}H_{24}N_2O_8S+Na]^+$: 511.1151. Found m/z: 511.1162.

3.7.6. 4-Nitrophenyl 2-acetamido-3-*S*-acetyl-4,6-di-*O*-acetyl-2-deoxy-3-thio-β-D-galactopyranoside (32). Compound **31** (320 mg) was hydrolysed, acetylated and purified as described for **19** to yield compound **32** (215 mg, 68%) as a white solid. $[\alpha]_D^{22}$ +9.4 (*c* 0.5, acetone). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (m, 2H, Ar–H), 7.09 (m, 2H, Ar–H), 5.59 (d, 1H, *J* 9.0 Hz, N*H*Ac), 5.40 (d, 1H, $J_{3,4}$ 2.6 Hz, H-4), 5.33 (d, 1H, $J_{1,2}$ 8.1 Hz, H-1), 4.35 (dt, 1H, $J_{2,3}$ 12.2 Hz, H-2), 4.24–4.06 (m, 4H, H-3, H-5, H-6a and H-6b), 2.38 (s, 3H, CH_3CO), 2.18 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 1.96 (s, 3H, CH_3CO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 195.3 (*S*-acetyl), 170.7, 170.5, 170.1 (C=O); 161.8, 143.0, 125.8, 116.7 (Ar–C); 99.9, 74.0, 68.3, 62.3, 50.5, 46.6; 30.7, 23.4, 20.8, 20.7 (*C*H_3CO). HRESIMS: calcd for $[C_{20}H_{24}N_2O_{10}S+Na]^+$: 507.1049. Found *m*/*z*: 507.1058.

3.7.7. 4-Nitrophenyl 2-acetamido-2-deoxy-3-thio-β-D-galactopyranoside (33). Compound **32** (115 mg, 0.24 mmol) was deacetylated as described for **20** to yield compound **33** as a white solid (83 mg, 98%). $[\alpha]_D^{22}$ +30.9 (*c* 0.5, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 8.12 (m, 2H, Ar–H), 7.10 (m, 2H, Ar–H), 5.10 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1), 4.03 (dd, 1H, $J_{2,3}$ 11.8 Hz, H-2), 3.79 (d, 1H, H-4), 3.73–3.65 (m, 3H, H-5, H-6a and H-6b), 3.07 (dd, 1H, $J_{3,4}$ 2.5 Hz, H-3), 1.87 (s, 3H, CH_3CO). ^{13}C NMR (75 MHz, Me_2SO-d_6): δ 169.3 (C=O), 162.2, 141.8, 125.8, 116.6 (Ar–C); 99.7, 78.2, 67.9, 60.5, 52.0, 44.8; 22.9 (*C*H_3CO). HRESIMS: calcd for $[C_{14}H_{18}N_2O_7S+Na]^+$: 381.0732. Found *m*/*z*: 381.0743. Anal. Calcd for $C_{14}H_{18}N_2O_7S$: C, 46.92; H, 5.06; N, 7.82. Found: C, 46.73; H, 5.08; N, 8.18.

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