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Synthesis of Sulfur-Containing Analogues of αGalNAc (Tn-Antigen) and βGal1,3αGalNAc (T-Antigen)

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Summary. A method for the preparation of thio-analogues of the T- and Tn-antigen was developed. Thus, starting from a known N-acetamido-glucoside derivative, the epidithio analogue of the Tn-antigen was accessible in a four-step reaction sequence. The corresponding epidithio analogue and the thioanhydro derivative of the T-antigen were synthesized starting from a disaccharide derivative. For the preparation of the epidithio analogue the sulfur atoms were introduced *via* thiocyanates in a stepwise fashion, using mesylate as the leaving group at C-6 and triflate as the leaving group at C-4 in the reducing carbohydrate moiety. The synthesis of the thioanhydro analogue was achieved by introducing a thiocyanate group at C-6 into the glucose moiety, followed by subsequent displacement of a mesylate group at C-4 under inversion of configuration utilizing sodium methoxide.

Keywords. Carbohydrates; T-Antigen; Tn-Antigen; Thio analogues; Glycosides.

Introduction

The development of synthetic strategies towards sulfur-containing carbohydrates remains a challenging task in organic synthesis [1]. Thiosugar derivatives are produced by the introduction of sulfur in the place of oxygen atoms in defined positions of carbohydrate molecules. Due to specific interactions with enzymes and other proteins they are of interest as enzyme inhibitors or pharmaceutical lead structures [1–3]. Here we report on the synthesis of thio analogues of the Tn-(α*Gal*NAc) and T-antigen (β*Gal*1,3α*Gal*NAc) prepared as the allyl glycosides. The T-antigen, first described by *Thomsen* [4] and *Friedenreich* [5], has been identified to be responsible for erythrocyte agglutination with serum antibodies [6]. Subsequently, this antigen has been discovered on the surface of various carcinoma cells [7, 8]. Therefore, the synthesis of thio analogues of the T- and Tn-antigen is of interest for medicinal chemistry as well as immunological studies. These

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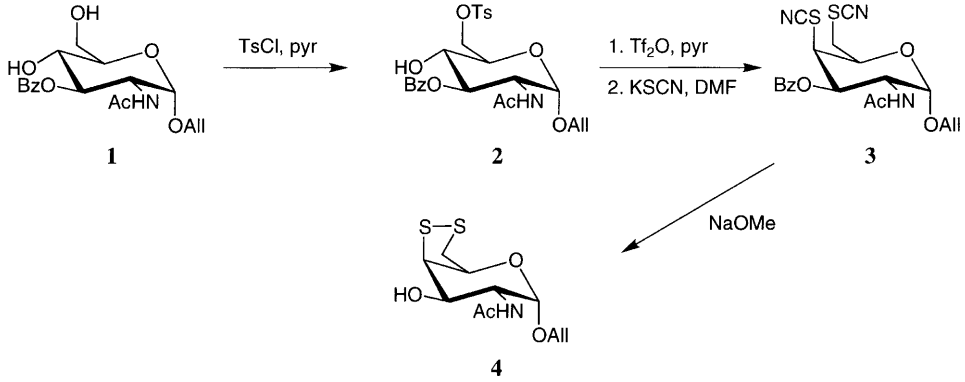
compounds may potentially act as photooxidants [9] and immunomodulators [10]. Furthermore, they should be suitable for studies of various lectin binding sites [11, 12]. Indeed, thiosugar derivatives of the T-antigen have been shown to specifically bind to legume lectins [13].

Results and Discussions

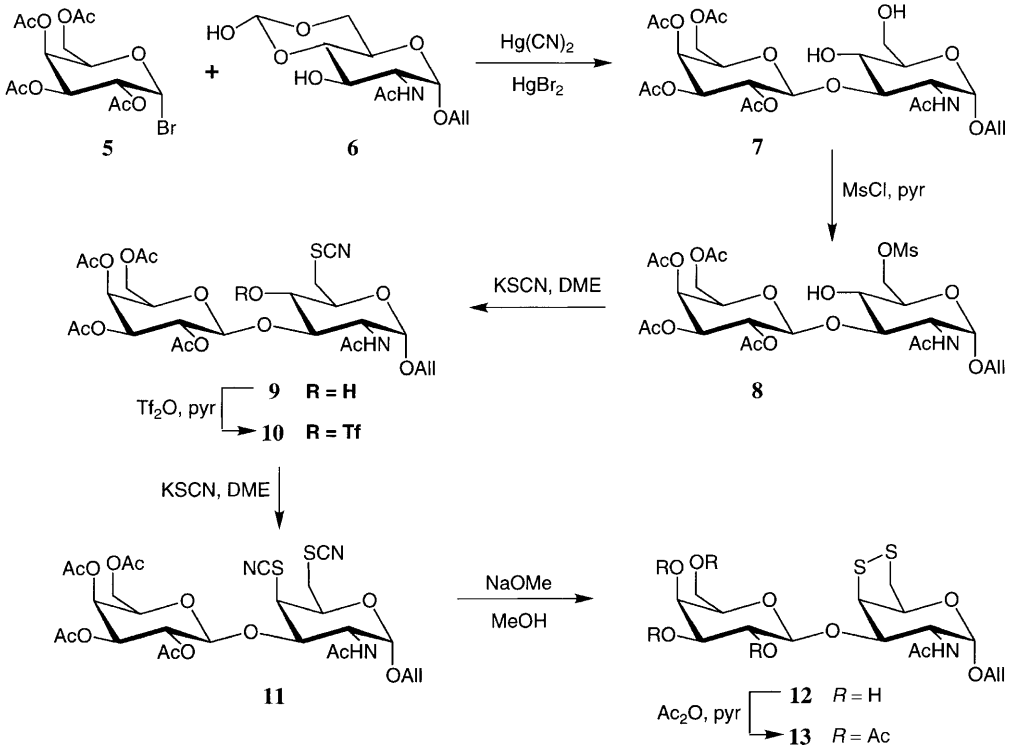
Recently we have reported on the synthesis of the allyl N-acetyl-4,6-epidithio-galactosamine analogue **4** [14], which was prepared following essentially a procedure developed by *Hill et al.* [15]. Here we first present an improved synthesis in terms of overall yield of the target compound **4**. We started the reaction sequence with the well known 2-acetamido-3-O-benzoyl-2-deoxy-α-D-glucopyranoside **1** [16], which upon treatment with 1.1 equivalents of toluenesulfonyl chloride gave the monotosylate **2** (Scheme 1). Esterification of the hydroxyl group on C-4 afforded its corresponding triflate. This was followed by double displacement of the leaving groups by thiocyanate to yield the galacto derivative **3**. Conversion of the dithiocyanate derivative **3** to the 4,6-epidithio analogue **4** was achieved by applying the *Zemplén* saponification procedure, providing **4** in 27% overall yield from **1**.

The synthesis of the epidithio analogue **12** of the T-antigen was performed starting from the known disaccharide **7** [17, 18], which was easily prepared by *Helferich* glycosylation [19] of the saccharide **6** with acetobromogalactose (**5**) [20] (Scheme 2). The sulfur atoms of the target **10** were introduced in a stepwise fashion as thiocyanate groups, a strategy significantly superior over the one-step substitution procedure described above for the Tn-analogue. Thus, mesylating the primary hydroxyl group of the disaccharide **7** yielded the monomesylate **8**. The leaving group at the primary position was subjected to a thiocyanate displacement in dimethoxy ethane (*DME*) at elevated temperature to yield the derivative **9**. Alcohol **9** was transformed to the corresponding triflate **10**, which in a second displacement step using potassium thiocyanate was converted into the dithiocyanato disaccharide derivative **11**. For confirmation of the change in configuration at



1 **2** **3** **4**

Scheme 1

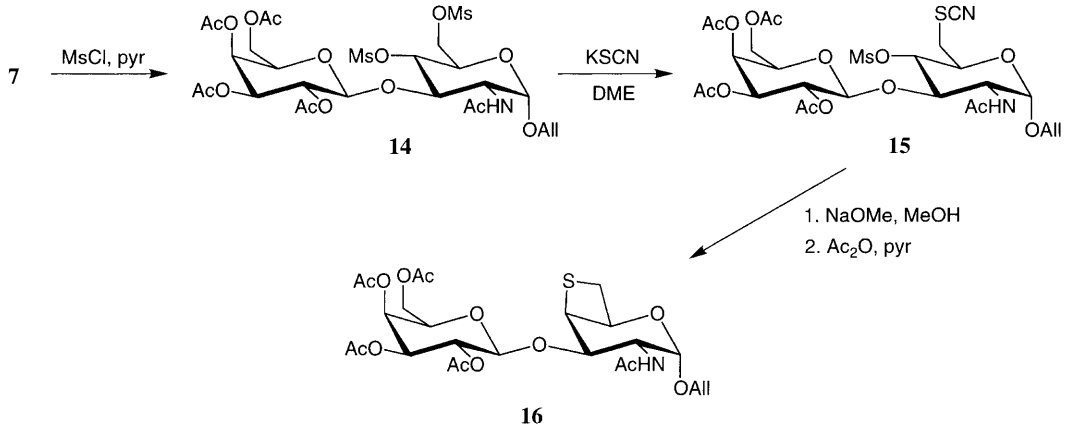


Scheme 2

C-4 of derivative **9**, various two dimensional NMR techniques were employed [21, 22]. Thus, all ^{1}H and ^{13}C signals were assigned, and small coupling constants $J_{3,4}$ and $J_{4,5}$ (4.0 and 1.1 Hz, respectively) indicate the galacto-configuration of the dithiocyanato moiety of **11**. Conversion into the epidithio analogue **12** was achieved by treatment of **11** with sodium methoxide in dry methanol. The presence of the intramolecular disulfide bridge in the T-antigen thio analogue **12** was assigned unambiguously on the basis of NMR and electrospray mass spectroscopy. To facilitate structural assignment, compound **12** was also transformed into its peracetylated derivative **13**.

The synthesis of the 4,6-thioanhydro derivative **16** was achieved starting from disaccharide **7** and applying a slightly modified strategy (Scheme 3). Thus, mesylation of both hydroxyl groups of derivative **7** followed by monosubstitution of the primary mesylate leaving group using potassium thiocyanate gave compound **15**. Treatment of **15** with sodium methoxide in methanol at elevated temperature followed by reacetylation of the crude thioanhydro analogue for simplification of structural assignments afforded compound **16** in moderate yields.

In conclusion, various sulfur-containing analogues of the T- and Tn-antigen were synthesized in a straightforward way. These compounds show selective binding to plant lectins [13] and are interesting candidates for further bioorganic studies. Investigations aiming at the exploration of carbohydrate binding sites of various biomolecules are under way.



Scheme 3

Experimental

IR spectra were recorded on a Perkin Elmer 1600 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ unless otherwise given using $CHCl_3$ as internal standard on a Bruker DRX 400 NMR spectrometer at 400.13 and 100.61 MHz, or on a Bruker DPX 250 NMR spectrometer at 250.13 and 62.90 MHz. Primed assignments for disaccharide derivatives refer to the galactose moiety, double primed ones to the allyl aglycon, and all others to the *Glc*NAc residues, respectively. Mass spectra were recorded on a Finnigan MAT 900S spectrometer. Electrospray interface mass spectra were measured on a LC-MS/MS PE Sciex API 365 using MeOH (10% NH_4OAc) as solvent. Melting points were determined on a Kofler apparatus and are uncorrected. Optical rotations were measured on a Perkin Elmer 361 polarimeter in a 1 dm cell. TLC was performed on precoated Merck plates (silica F_{254}). Detection was performed using UV light, I_2, and by dipping the plates into a solution of 2% $Ce(SO_4)_2 \cdot 4H_2O$ in 5% H_2SO_4 with subsequent heating. Flash chromatography was performed with Merck silica gel 60 (230–400 mesh). Elemental analyses agreed favourably with the calculated data. All solvents were of reagent grade. Reagents were obtained from commercial suppliers. Compound **1** was prepared according to Ref. [16], compound **5** according to Ref. [20], and compound **6** according to Ref. [23].

Allyl 2-acetamido-3-O-benzoyl-2-deoxy-6-p-toluenesulfonyl-α-D-glucopyranoside (**2**; $C_{25}H_{29}NO_9S$)

To a stirred solution of 624 mg of **1** (1.71 mmol) in 7 cm^3 dry pyridine and 10 mg of dimethylamino pyridine (*DMAP*), a solution of 358 mg *p*-toluenesulfonic acid (1.88 mmol) in 2 cm^3 dry pyridine was added at 0°C. The reaction mixture was kept at 0°C for 5 days. The solvent was removed by coevaporation with toluene, and the crude product was purified by flash chromatography (gradient: hexanes:ethyl acetate = 1:1 to 1:2) to yield 80% of **2** (12% of starting material were recovered).

TLC (ethyl acetate): $R_f = 0.74$; ^{1}H NMR (400 MHz, δ, $CDCl_3$): 7.98–7.32 (m, $C_6H_4CH_3$, C_6H_5), 5.84 (m, $H_{2'}$), 5.78 (d, $J_{NH,2} = 8.86$, NHAc), 5.24 (m, H_3), 4.80 (d, $J_{1,2} = 3.45$, H_1), 4.34 (m, $J_{5,6a} = 1.97$, $J_{6a,6b} = 11.32$, H2, H_{6a}, H_{6b}), 4.13 (m, $H_{1'}$), 3.93 (m, $H_{1'}$), 3.88 (ddd, $J_{4,5} = 9.85$, $J_{5,6b} = 4.92$, H_5), 3.78 (dt, $J_{4,OH} = 4.43$, H_4), 3.11 (br s, OH), 2.42 (s, $C_6H_4CH_3$), 1.81 (s, $NHCOCH_3$) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 169.95, 167.92, 144.97, 133.59, 133.09, 132.82, 129.95, 129.82, 129.03, 128.51, 127.97, 118.32, 96.43, 74.81, 70.69, 70.08, 68.66, 68.57, 68.54, 51.36, 23.10, 21.62 ppm.

Allyl 2-acetamido-3-O-benzoyl-4,6-di-thiocyanato-2,4,6-trideoxy-α-D-galactopyranoside (**3**; $C_{20}H_{21}N_3O_5S_2$)

To a solution of 710 mg (1.37 mmol) of derivative **2** in 18 cm^3 dry pyridine, 0.35 cm^3 (2.08 mmol) of triflic anhydride were added dropwise at 30°C. After 10 min of stirring the reaction flask was transferred to an ice bath, and stirring was continued for 4 h. The reaction was quenched by the addition of 14 cm^3 saturated aq. NaCl solution and 25 cm^3 ethyl acetate. The phases were separated, and the aqueous phase was extracted twice with 25 cm^3 of ethyl acetate. The combined organic phases were dried over $MgSO_4$, filtered, and evaporated to dryness. The crude material obtained was dissolved in 10 cm^3 *DMF*, and 1.33 g of KSCN (13.7 mmol) were added. The reaction mixture was stirred for 16 h at room temperature (r.t.), followed by heating to 110°C for 72 h. The mixture was cooled to r.t., diluted with 10 cm^3 H_2O, and extracted with three portions of 20 cm^3 ethyl acetate. The combined organic phases were dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. After flash chromatography (hexanes:ethyl acetate = 1:1), 360 mg of dithiocyanate **3** (59%) were obtained. Additionally, 76 mg of **2** (12%) were recovered.

TLC (ethyl acetate:hexanes = 4:1): $R_f = 0.77$; $[\alpha]_D^{20} = +33.78°$ ($c = 1.19$, $CHCl_3$); IR: $\nu = 3306$, 2941, 2158, 1724, 1666, 1537 cm^{-1}; ^1H NMR (400 MHz, δ, $CDCl_3$): 8.08–7.45 (m, C_6H_5), 5.89 (m, $H_{2'}$), 5.77 (d, $J_{NH,2} = 9.35$, NHAc); 5.56 (dd, $J_{2,3} = 11.32$, $J_{3,4} = 3.94$, H_3), 5.33 (m, $H_{3'}$), 4.97 (d, $J_{1,2} = 3.94$, H_1), 4.71 (ddd, H_2), 4.60 (ddd, $J_{4,5} = 1.48$, $J_{5,6b} = 3.94$, $J_{5,6a} = 8.86$, H_5), 4.33 (m, $H_{1'}$), 4.25 (dd, H_4), 4.09 (m, $H_{1'}$), 3.28 (ddd, $J_{6a,6b} = 13.78$, H_{6a}, H_{6b}), 1.89 (s, NHCOC*H*$_3$) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 170.01, 166.31, 134.01, 132.32, 130.20, 128.66, 128.21, 119.56, 110.92, 110.57, 96.65, 69.69, 69.30, 68.05, 55.58, 48.06, 35.72, 23.16 ppm; MS (FI, 7 kV, 3 mA, 140°C): $m/z = 448.3$ (M^+H), 395.2.

Allyl 2-acetamido-4,6-epidithio-2,4,6-trideoxy-α-D-galactopyranoside (**4**; $C_{11}H_{17}NO_4S_2$)

To a stirred solution of 557 mg dithiocyanate **3** (1.24 mmol) in 16 cm^3 dry MeOH, 9 cm^3 of a 0.1 *M* solution of MeONa in dry MeOH were added. The reaction mixture was heated for 10 min to reflux, followed by stirring at r.t. for 1 h. The mixture was neutralized by the addition of Dowex 50 W (H^+). The resin was filtered off, and the filtrate was evaporated to dryness. The crude product obtained was purified by flash chromatography (ethyl acetate:hexanes = 4:1; $R_f = 0.32$) to yield 200 mg (55%) **4** as yellowish crystals.

M.p.: 195°C (decomposition); $[\alpha]_D^{20} = +202.58°$ ($c = 1.24$, CH_3OH); IR: $\nu = 3294.3$, 1648.2, 1549.4 cm^{-1}; ^1H NMR (250 MHz, δ, D_2O/CD_3OD): 5.94 (m, $H_{2'}$), 5.26 (m, $H_{3'}$), 4.88 (d, $J_{1,2} = 3.34$, H_1), 4.85 (m, H_5), 4.45 (dd, $J_{2,3} = 10.96$, $J_{3,4} = 4.79$, H_3), 4.35 (dd, H_2), 4.22 (m, $H_{1'}$), 4.10 (dd, $J_{4,5} = 2.74$, H_4), 4.02 (m, $H_{1'}$), 3.43 (dd, $J_{6a,5} = 4.57$, $J_{6a,6e} = 11.88$, H_{6a}), 3.24 (dd, $J_{6b,5} = 1.37$, H_{6b}), 1.99 (s, NHCOC*H*$_3$) ppm; ^{13}C NMR (62.9 MHz, δ, D_2O/CD_3OD): 135.37, 117.73, 99.08, 75.82, 69.70, 66.80, 66.76, 51.57, 44.45, 22.61 ppm; MS (FI, 7 kV, 3 mV, 120°C): m/z (rel. intensity) = 291.1 (100, M^+-H), 267.2 (18).

Allyl tetra-O-acetyl-β-D-galactopyranosyl-(1-3)-2-acetamido-2-deoxy-α-D-glucopyranoside (**7**; $C_{25}H_{37}NO_{15}$)

To 80 cm^3 of a mixture of toluene and nitromethane (1:1), 800 mg glucopyranoside **6** (2.29 mmol) were added in one portion and dissolved by heating to reflux. The solution was transferred under Ar into a flask containing 580 mg $Hg(CN)_2$ (2.30 mmol), 83 mg $HgBr_2$ (0.23 mmol), and 2.7 g molecular sieves (3 Å). After stirring for 1 h at 60°C, 940 mg galactosylbromide **5** (2.29 mmol) dissolved in 4 cm^3 nitromethane were added dropwise over a period of 2 h. After stirring for 16 h at 60°C, further additions were made of 580 mg of $Hg(CN)_2$ (2.30 mmol), 83 mg $HgBr_2$ (0.23 mmol), and 940 mg bromide **5** dissolved in 4 cm^3 nitromethane, and heating was continued until TLC (toluene:acetone = 3:1) showed that all starting material **6** had been consumed (2 h). The suspension

obtained was filtered over Celite®, and the solvent was removed by evaporation. The residue was redissolved in CH_2Cl_2 (50 cm^3) and washed with a 5% solution of $NaHCO_3$. The organic phase was dried over $MgSO_4$, filtered, and the solvent was removed under vacuum. After flash chromatography (toluene:acetone = 3:1), 1.37 g of benzylidene protected disaccharide were obtained. The product obtained was dissolved in 120 cm^3 of 80% acetic acid and heated to 90°C for 45 min. The solvent was removed by coevaporation with toluene (3 times); the crude product obtained (1.16 g; 86%) was pure enough for further conversions. The physical and spectroscopic data were in full agreement with those reported in Refs. [17] and [18].

Allyl tetra-O-acetyl-β-D-galactopyranosyl-(1-3)-2-acetamido-2-deoxy-6-methanesulfonyl-α-D-glucopyranoside (**8**; $C_{26}H_{39}NO_{17}S$)

To a solution of 460 mg (0.78 mmol) of compound **7** in 6 cm^3 dry pyridine, 0.06 cm^3 (0.78 mmol) methanesulfonyl chloride were added dropwise at 0°C. After 2 h of stirring the solvent was removed by coevaporation with toluene, and the crude product obtained was purified by flash chromatography (ethyl acetate:hexanes = 9:1) to yield 461 mg of **8** (88%).

M.p.: 92°C; $[\alpha]_D^{20} = +56.2°$ ($c = 0.87, CH_2Cl_2$); IR: $\nu = 3468.1$, 2938.2, 1751.9, 1654.7 cm^{-1}; ^{1}H NMR (400 MHz, δ, $CDCl_3$): 5.87 (m, $H_{2''}$), 5.53 (d, $J_{NH,2} = 9.85$, NHAc), 5.35 (d, $J_{3',4'} = 2.95$, $H_{4'}$), 5.27 (m, $H_{3''}$), 5.20 (dd, $J_{1',2'} = 7.88$, $J_{2',3'} = 10.34$, $H_{2'}$), 4.97 (dd, $J_{3',4'} = 3.44$, $H_{3'}$), 4.80 (d, $J = 3.94$, H_1), 4.52 (d, $H_{1'}$), 4.50 (dd, $J = 1.48$), 4.38 (dd, $J = 5.42$, $J = 10.83$), 4.28 (ddd, $J_{2,3} = 9.85$, H_2), 4.13 (m, $H_{1''}$), 3.97 (m, $H_{1''}$), 3.80 (m, 1H), 3.71 (s, 1H, OH), 3.56 (m, $H_{6'}$), 3.03 (s, SO_2CH_3), 2.14, 2.06, 2.04, 1.99, 1.96 (5s, $COCH_3$) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 170.40, 170.10, 169.40, 169.34, 133.02, 118.78, 101.58, 96.62, 82.72, 71.08, 70.66, 69.81, 68.62, 68.58, 68.51, 68.32, 66.79, 61.23, 51.17, 37.56, 23.40, 20.63, 20.59, 20.56, 20.48 ppm; MS (FI, 7 kV, 3 mA, 230°C): m/z (rel. intensity) = 670.2 (82), 612.0 (18), 347.0 (20), 331.0 (36), 166.9 (36), 95.8 (63), 59.9 (100).

Allyl tetra-O-acetyl-β-D-galactopyranosyl-(1-3)-2-acetamido-2,6-dideoxy-6-thiocyanato-α-D-glucopyranoside (**9**; $C_{26}H_{36}N_2O_{14}S$)

A suspension of 445 mg (0.66 mmol) of monomesylate **8** and 276 mg (2.84 mmol) of KSCN in 10 cm^3 1,2-dimethoxyethane (*DME*) was heated at 70°C for 12 h. Additional 280 mg (2.88 mmol) of KSCN and 10 cm^3 *DME* were added, and heating at reflux was continued for 20 h. The solvent was removed under vacuum, and the residue was dissolved in CH_2Cl_2 (35 cm^3) and washed 3 times with 20 cm^3 portions of H_2O. The organic layer was dried over $MgSO_4$, filtered, and the solvent was evaporated. The crude product obtained was purified by flash chromatography (toluene:acetone = 3:1) to yield 381 mg (91%) of **9** as colorless crystals.

M.p.: 177°C; $[\alpha]_D^{20} = +76.6°$ ($c = 1.23, CH_2Cl_2$); IR: $\nu = 3468.0$, 2933.9, 2156.4, 1752.6, 1658.6 cm^{-1}; ^{1}H NMR (400 MHz, δ, $CDCl_3$): 5.89 (m, $H_{2''}$), 5.53 (d, $J_{NH,2} = 9.53$, NHAc), 5.35 (dd, $J_{4',5'} = 1.00$, $J_{3',4'} = 3.51$, $H_{4'}$), 5.28 (m, $H_{3''}$), 5.20 (dd, $J_{1',2'} = 8.03$, $J_{2',3'} = 10.54$, $H_{2'}$), 4.97 (dd, $H_{3'}$), 4.79 (d, $J_{1,2} = 4.02$, H_1), 4.51 (d, $H_{1'}$), 4.30 (ddd, $J_{2,3} = 10.04$, H_2), 4.25 (m, $H_{1''}$), 4.12 (m, $H_{6'}^*$), 4.00 (m, $H_{5'}^*$, $H_{1''}$), 3.84 (ddd, $J_{5,6a} = 2.51$, H_5), 3.73 (s, OH), 3.56 (dd, $J_{3,4} = 8.53$, H_3^*), 3.51 (dd, $J_{6a,6b} = 14.06$, H_{6a}), 3.43 (t, $J = 9.03$, H_4), 3.01 (dd, $J_{5,6b} = 8.53$, H_{6b}), 2.13, 2.06, 2.05, 2.00, 1.95 (5s, $COCH_3$) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 170.41, 170.09, 169.42, 169.30, 132.87, 119.10, 112.17, 101.61, 96.41, 82.60, 71.21, 71.16, 70.65, 70.45, 68.61, 68.45, 66.82, 61.23, 51.18, 35.95, 23.42, 20.62, 20.59, 20.47 ppm; assignments marked with an asterisk may be interchanged.

Allyl tetra-O-acetyl-β-D-galactopyranosyl-(1-3)-2-acetamido-2,6-dideoxy-6-thiocyanato-4-trifluoromethanesulfonyl-α-D-glucopyranoside (**10**; $C_{27}H_{35}F_3N_2O_{16}S_2$)

To a solution of 237 mg (0.37 mmol) of **9** in 6 cm^3 dry pyridine, 0.11 cm^3 (0.65 mmol) trifluoromethanesulfonic anhydride were added dropwise at −15°C. After 15 min the temperature

was raised to 0°C, and stirring was continued for 3 h. The reaction mixture was diluted with 30 cm^3 CH_2Cl_2 and washed twice with saturated aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and evaporated to dryness. The crude product obtained could be purified by flash chromatography (hexanes:ethyl acetate = 1:2) to yield 178 mg (63%) of **10** as colorless crystals.

M.p.: 139–140°C; $[\alpha]_D^{20} = +45.67°$ $(c = 0.9, CH_2Cl_2)$; IR: $\nu = 3369.2$, 2938.0, 2158.8, 1750.1, 1683.2 cm^{-1}; ^{1}H NMR (400 MHz, δ, $CDCl_3$): 5.91 (m, $H_{2''}$), 5.59 (d, $J_{NH,2} = 10.04$, NHAc), 5.35 (m, $H_{4'}$, $H_{3''}$), 5.10 (dd, $J_{1',2'} = 8.03$, $J_{2',3'} = 10.54$, $H_{2'}$), 4.92 (dd, $J_{3',4'} = 3.51$, $H_{3'}$), 4.80 (d, $J_{1,2} = 4.02$, H_1), 4.64 (d, $H_{1'}$), 4.57 (t, $J = 9.53$, H_5), 4.47 (ddd, $J_{2,3} = 10.54$, H_2), 4.30 (m, $H_{1''}$), 4.20 (dd, $J = 6.03$, $J = 11.55$), 4.15 (dt, $J_{5a,6} = 2.51$, H_5), 4.05 (m, 3 H), 3.85 (t, $J = 7.03$), 3.40 (dd, $J_{6a,6b} =$ 14.06, H_{6a}), 3.07 (dd, $J_{5,6b} = 9.04$, H_{6b}), 2.10, 2.05, 2.03, 1.95 (4s, $COCH_3$) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 170.12, 169.61, 169.27, 132.24, 120.00, 111.31, 100.41, 96.03, 83.16, 72.82, 70.82, 70.45, 69.29, 68.35, 68.27, 66.52, 60.69, 52.40, 35.12, 23.36, 20.65, 20.52, 20.34 ppm.

Allyl tetra-O-acetyl-β-D-galactopyranosyl-(1-3)-2-acetamido-4,6-di-thiocyanato-2,4,6-trideoxy-α-D-galactopyranoside (**11**; $C_{27}H_{35}N_3O_{13}S_2$)

A suspension of 73 mg (0.095 mmol) of **10** and 46 mg (0.473 mmol) of KSCN in 10 cm^3 *DME* was heated to reflux for 12 h. Then the solvent was removed under vacuum. The crude material obtained was redissolved in 30 cm^3 of CH_2Cl_2 and washed 3 times with 20 cm^3 portions of H_2O. The organic layer was dried over $MgSO_4$, filtered, and evaporated. After purification by flash chromatography (toluene:acetone = 3:1), 45 mg (83%) of **11** were obtained as colorless crystals.

M.p.: 142°C; $[\alpha]_D^{20} = +86.30°$ $(c = 0.52, CH_2Cl_2)$; IR: $\nu = 3306.4$, 2936.2, 2157.6, 1750.3, 1659.9 cm^{-1}; ^{1}H NMR (600 MHz, δ, $CDCl_3$): 5.91 (m, $H_{2''}$), 5.58 (d, $J_{NH,2} = 9.5$, NHAc), 5.37 (dd, $J_{4',5'} = 0.8$, $J_{3',4'} = 3.3$, $H_{4'}$), 5.34 (m, $H_{3''}$), 5.32 (dd, $J_{1',2'} = 8.4$, $J_{2',3'} = 10.4$, $H_{2'}$), 5.01 (dd, $H_{3'}$), 4.90 (d, $J_{1,2} = 3.8$, H_1), 4.74 (d, $H_{1'}$), 4.41 (m, $J_{4,5} = 1.1$, $J_{5,6a} = 3.9$, $J_{5,6b} = 8.8$, H_5), 4.38 (ddd, $J_{2,3} = 9.7$, H_2), 4.30 (m, $J_{6a',6b'} = 11.5$, $H_{6a'}$, $H_{1''}$), 4.18 (dd, $J_{4,5} = 1.1$, $J_{3,4} = 4.0$, H_4), 4.14 (dd, $J_{5',6b'} = 5.6$, $H_{6b'}$), 4.06 (m, H_3, $H_{1''}$), 3.89 (m, $H_{5'}$), 3.29 (m, $J_{6a,6b} = 14.4$, H_{6a}, H_{6b}), 2.15 (s, $COCH_3$), 2.02 (s, $2 \times COCH_3$), 2.00, 1.98 (2s, $COCH_3$) ppm; ^{13}C NMR (150 MHz, δ, $CDCl_3$): 170.63, 170.58, 170.31, 169.37, 169.33, 132.55 (C2″), 119.72 (C3″), 111.29 (–SCN), 110.94 (–SCN), 101.91 (C1′), 96.70 (C1), 74.96 (C3), 71.80 (C5′), 70.65 (C3′), 69.29 (C1″), 68.90 (C2′), 68.03 (C5), 67.17 (C4′), 61.86 (C6′), 57.75 (C4), 48.55 (C2), 35.87 (C6), 23.37, 20.93, 20.74, 20.67, 20.64 ppm; MS (FI, 7 kV, 3 mA, 190°C): m/z (rel. intensity) = 674.5 (78), 621.6 (100), 589.6 (19).

Allyl β-D-galactopyranosyl-(1-3)-2-acetamido-4,6-epidithio-2,4,6-trideoxy-α-D-galactopyranoside (**12**; $C_{17}H_{27}NO_9S_2$)

To a solution of 101 mg (0.15 mmol) of dithiocyanate derivative **11** in 50 cm^3 dry MeOH, 0.45 cm^3 of a 1 *M* methanolic MeONa solution were added. After 4 h of stirring the mixture was neutralized by addition of solid CO_2. The solvent was concentrated under vacuum to a volume of 2 cm^3, filtered over silica gel (2 g), and the silica gel layer was eluted with 10 cm^3 of MeOH. The product was obtained by removing the solvent under vacuum as a colorless glass.

Yield: 54 mg (79%); IR: $\nu = 3319.5$, 2948.3, 2162.7, 1629.0, 1538.1 cm^{-1}; ^{1}H NMR (400 MHz, δ, D_2O): 6.03 (m, $H_{2''}$), 5.37 (m, $H_{3''}$), 5.09 (s, H_5), 5.01 (d, $J_{1,2} = 3.01$, H_1), 4.59 (m, $J_{2,3} = 10.54$, H_2, H_3, H_4), 4.48 (d, $J_{1',2'} = 8.0$, $H_{1'}$), 4.31 (m, $H_{1''}$), 4.13 (m, $H_{1''}$), 3.95 (d, $J_{3',4'} = 3.51$, $H_{4'}$), 3.81 (m, $H_{6'}$), 3.71 (dd, $J_{5,6a} = 4.51$, $J_{5,6b} = 7.53$, $H_{5'}$), 3.65 (dd, $J_{2',3'} = 10.03$, $H_{3'}$), 3.52 (m, $H_{2'}$, H_{6a}), 3.42 (dd, $J_{5,6b} = 1.01$, $J_{6a,6b} = 12.55$, H_{6b}), 2.06 (s, NHCOCH_3) ppm; ^{13}C NMR (100 MHz, δ, D_2O): 175.13, 133.91 (C2″), 118.58 (C3″), 105.43 (C1′), 97.80 (C1), 75.54 (C5′), 75.29 (C3′), 75.11 (C5), 72.95, 70.93 (C2′), 69.24 (C1″), 69.01 (C4′), 63.06 (C4), 61.37 (C6′), 48.81 (C2), 43.70 (C6), 22.38 ppm; ES-MS (negative mode): $m/z = 452.0$ (calcd.: 452.5; M-H^-).

Allyl tetra-O-acetyl-β-D-galactopyranosyl-(1-3)-2-acetamido-4,6-epidithio-2,4,6-trideoxy-α-D-galactopyranoside (**13**; $C_{25}H_{35}NO_{13}S_2$)

To a solution of 50 mg (0.11 mmol) of **12** in 4 cm^3 dry pyridine, 0.4 cm^3 acetic anhydride were added, and the reaction mixture was stirred at r.t. for 16 h. The solvents were removed under vacuum, and the crude product was purified by flash chromatography (ethyl acetate) to yield 65 mg (96%) of **13**.

M.p.: 223–224°C; $[\alpha]_D^{20} = +87.65°$ ($c = 0.85, CHCl_3$); IR: $\nu = 3306.8$, 2931.0, 1751.1, 1654.2 cm^{-1}; ^{1}H NMR (400 MHz, δ, $CDCl_3$): 5.84 (m, $H_{1''}$), 5.51 (d, $J_{NH,2} = 9.03$, NHAc), 5.31 (d, $H_{4'}$), 5.22 (m, $H_{3''}$), 5.08 (dd, $J_{1',2'} = 8.03$, $J_{2',3'} = 10.54$, $H_{2'}$), 4.91 (dd, $J_{3',4'} = 3.51$, $H_{3'}$), 4.87 (d, $J_{1,2} = 3.52$, H_1), 4.72 (m, H_5^*), 4.58 (m, H_2, $H_{1'}$), 4.27 (dd, $J = 5.02$, $J = 10.5$, H_3^*), 4.14 (m, $H_{6a'}^*$, $H_{1''}$), 4.05 (m, H_4^*, $H_{6b'}^*$), 3.96 (m, $H_{1''}$), 3.83 (dt, $J = 6.53$, $H_{5'}^*$), 3.24 (m, H_{6a}, H_{6b}), 2.11, 2.01, 1.98, 1.93, 1.90 (5s, $COCH_3$) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 170.35, 170.24, 170.15, 169.51, 169.36, 133.33, 118.34, 101.45, 98.04, 74.68, 73.34, 70.88, 70.73, 68.74, 68.50, 66.86, 62.88, 61.18, 48.23, 43.51, 23.40, 20.66, 20.64, 20.61, 20.50 ppm; MS (FI, 7 kV, 3 mA, 130°C): m/z (rel. intensity) = 621.36 (100; M^+), 530.63 (18); assignments marked with an asterisk may be interchanged.

Allyl tetra-O-acetyl-β-D-galactopyranosyl-(1-3)-2-acetamido-2-deoxy-4,6-dimethanesulfonyl-α-D-glucopyranoside (**14**; $C_{27}H_{41}NO_{19}S_2$)

To a solution of 15 mg (0.144 mmol) of disaccharide **7** in 3 cm^3 dry pyridine, 0.06 cm^3 (0.775 mmol) methanesulfonyl chloride and 10 mg *DMAP* were added at 0°C. After stirring for 20 min the reaction mixture was allowed to warm to r.t. Stirring was continued for 6 h; then the reaction was quenched by addition of 3 cm^3 H_2O. The solvents were removed by coevaporation with toluene. The residue was redissolved in 10 cm^3 of H_2O and extracted 3 times with 10 cm^3 portions of ethyl acetate. The combined organic phases were dried over $MgSO_4$, filtered, and evaporated to dryness. The dimesylated derivative **14** was obtained after flash chromatography (ethyl acetate:hexanes = 5:1).

Yield: 90 mg (84%); colorless glass; $[\alpha]_D^{20} = +45.45°$ ($c = 0.77$, $CHCl_3$); IR: $\nu = 3386.9$, 2940.0, 1752.6, 1680.0 cm^{-1}; ^{1}H NMR (400 MHz, δ, $CDCl_3$): 5.88 (m, $H_{2''}$), 5.60 (d, $J_{NH,2} = 10.04$, NHAc), 5.36 (d, $H_{4'}$), 5.30 (m, $H_{3''}$), 5.10 (dd, $J_{1',2'} = 8.03$, $J_{2',3'} = 10.54$, $H_{2'}$), 4.93 (dd, $J_{3',4'} = 3.51$, $H_{3'}$), 4.75 (d, $J_{1,2} = 3.51$, H_1), 4.64 (d, $H_{1'}$), 4.46 (dd, $J = 2.51$, $J = 12.04$), 4.37 (dt, $J_{2,3} = 10.54$, H_2), 4.32 (m, 2 H), 4.20 (dd, $J = 6.03$, $J = 11.55$), 4.14 (m, H1″), 3.98 (m, 4 H), 3.85 (t, $J = 7.02$), 3.22, 3.04 (2s, SO_2CH_3), 2.10, 2.09, 2.04, 2.02, 1.95 (5s, $COCH_3$) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 170.37, 170.05, 170.01, 169.56, 169.29, 132.65, 119.46, 100.86, 96.36, 76.54, 74.63, 70.53, 70.40, 68.96, 68.13, 67.92, 67.63, 66.88, 60.61, 52.15, 38.69, 37.58, 23.38, 20.62, 20.55, 20.47 ppm; MS (FI, 7 kV, 3 mA, 175°C): m/z (rel. intensity) = 748.4 (79; M^+), 712.7 (64), 687.4 (100), 651.4 (30).

Allyl tetra-O-acetyl-β-D-galactopyranosyl-(1-3)-2-acetamido-2,6-dideoxy-4-methanesulfonyl-6-thiocyanato-α-D-glucopyranoside (**15**; $C_{27}H_{38}N_2O_{16}S_2$)

A suspension of 64 mg (0.086 mmol) of **14** and 82 mg (0.84 mmol) of KSCN in 10 cm^3 *DME* was heated to 100°C for 15 h. Subsequently, the solvent was removed under vacuum. The residue was redissolved in 10 cm^3 H_2O and extracted 3 times with 15 cm^3 portions of ethyl acetate. The combined organic phases were dried over $MgSO_4$, filtered, and evaporated to dryness. The crude product obtained was purified by flash chromatography (ethyl acetate:hexanes = 5:1) to yield 50 mg (82%) of **15** as a colorless glass.

$[\alpha]_D^{20} = +68.67°$ ($c = 1.20$, $CHCl_3$); IR: $\nu = 3350.7$, 3019.8, 2938.1, 2157.6, 1753.8, 1681.8 cm^{-1}; ^{1}H NMR (400 MHz, δ, $CDCl_3$): 5.90 (m, $H_{2''}$), 5.60 (d, $J_{NH,2} = 10.04$, NHAc), 5.33 (m, $H_{4'}$, $H_{3''}$), 5.01 (dd, $J_{1',2'} = 8.04$, $J_{2',3'} = 10.54$, $H_{2'}$), 4.93 (dd, $J_{3',4'} = 3.51$, $H_{3'}$), 4.79 (d, $J_{1,2} = 4.02$, H_1), 4.62 (d, $H_{1'}$), 4.44 (dt, $J_{2,3} = 10.54$, H_2), 4.27 (m, 3 H), 4.05 (m, 3 H), 4.00 (dd, $J_{3,4} = 9.53$, H_3), 3.89 (t, 1H), 3.47 (dd, $J_{5,6a} = 2.51$, $J_{6a,6b} = 14.06$, H_{6a}), 3.22 (s, SO_2CH_3), 3.10 (dd,

$J_{5,6b} = 8.03$, H_{6b}), 2.10, 2.09, 2.05, 2.02, 1.95 (5s, $COCH_3$) ppm; ^{13}C NMR (62.9 MHz, δ, $CDCl_3$): 170.04, 169.53, 169.29, 132.43 ($C_{2''}$), 119.75 ($C_{3''}$), 112.03 (SCN), 100.91 ($C_{1'}$), 96.20 (C_1), 78.62, 74.54, 70.47, 70.39, 69.02 ($C_{1''}$), 68.90, 67.87, 66.86, 60.59 ($C_{6'}$), 52.17 (C_2), 38.56 (SO_2CH_3), 35.23 (C_6), 23.38, 20.64, 20.55, 20.47 ppm; MS (FI, 7 kV, 3 mA): m/z (rel. intensity) = 711.3 (48, M^+), 650.3 (100).

Allyl tetra-O-acetyl-β-D-galactopyranosyl-(1-3)-2-acetamido-4,6-thioanhydro-2,4,6-trideoxy-α-D-galactopyranoside (**16**; $C_{25}H_{35}NO_{13}S$)

To a solution of 27 mg (0.038 mmol) of **15** in 3 cm^3 dry MeOH, 0.08 cm^3 of a 1 *M* methanolic MeONa solution were added at r.t. The reaction mixture was heated at reflux for 2 h and subsequently neutralized by addition of Dowex 50 W (H^+). The resin was filtered off, and the solvent was removed under vacuum. The crude product obtained was redissolved in 2 cm^3 dry pyridine, and 0.5 cm^3 acetic anhydride as well as 5 mg *DMAP* were added. The reaction mixture was stirred for 20 h at r.t. The solvents were removed by coevaporation with toluene, and the product obtained was purified by flash chromatography (ethyl acetate:hexanes = 6:1).

Yield: 8 mg (36%); IR: $\nu = 3308.0$, 2938.0, 1750.1, 1654.2, 1544.2 cm^{-1}; ^1H NMR (400 MHz, δ, $CDCl_3$): 5.84 (m, $H_{2''}$), 5.52 (d, $J_{NH,2} = 8.95$, NHAc), 5.34 (dd, $J_{3',4'} = 3.53$, $J_{4',5'} = 0.95$, $H_{4'}$), 5.23 (m, $H_{3''}$), 5.06 (dd, $J_{1',2'} = 8.01$, $J_{2',3'} = 10.36$, $H_{2'}$), 4.99 (d, $J_{1,2} = 2.83$, H_1), 4.94 (dd, $H_{3'}$), 4.72 (ddd, $J_{2,3} = 10.13$, H_2), 4.59 (d, $H_{1'}$), 4.56 (t, $J_{5,6a} = 4.71$, H_5), 4.40 (t, H_4^*), 4.14 (m, $H_{6'}^*$, $H_{1''}$), 3.97 (m, $H_{1''}$), 3.91 (dd, $J = 6.12$, H_3^*), 3.85 (dt, $H_{5'}$), 3.42 (dd, H_{6a}), 2.59 (d, $J_{6a,6b} = 10.12$, H_{6b}), 2.19, 2.10, 2.08, 2.03, 1.99 (5s, $COCH_3$) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 170.37, 170.27, 170.18, 169.54, 133.54 ($C_{2''}$), 117.94 ($C_{3''}$), 100.95 ($C_{1'}$), 97.21 (C_1), 72.96, 72.04, 70.86, 70.84, 68.63 ($C_{1''}$), 68.55, 66.92, 61.27 ($C_{6'}$), 49.54, 43.45, 28.48 (C_6), 23.45, 20.68, 20.60, 20.55 ppm; MS (FI, 7 kV, 3 mA, 120–170°C): m/z (rel. intensity) = 590.2 (100, M^+), 488.3 (10), 142.1 (20); assignments marked with an asterisk may be interchanged.

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References

[1] Witzak ZJ (1999) Current Medicinal Chemistry **6**: 163
[2] Defaye J, Gelas J (1991) In: Atta-ur-Rahman (ed) Studies in Natural Product Chemistry, vol 8E. Elsevier, Amsterdam, p 315
[3] Driguez H (1997) Top Curr Chem **187**: 85
[4] Thomsen O (1927) Z Immun Forsch **52**: 85
[5] Friedenreich V (1930) The Thomsen Hemagglutination Phenomenon. Levin & Munksgaard, Copenhagen
[6] Vaith P, Uhlenbruck G (1978) Z Immun Forsch **154**: 1
[7] Springer GF (1984) Science **224**: 1198
[8] Springer GF, Taylor CR, Howard DR, Tegtmeyer H, Desai PR, Murthy SM, Felder B, Scanlon EF (1985) Cancer **55**: 561
[9] Prompers JJ, Hilbers CW, Pepermans HAM (1999) FEBS Lett **456**: 409
[10] Unger FM, Liehl E (1985) German Patent Appl DE 3438585, A1, May 2
[11] Sharma V, Vigayon M, Surolia A (1996) J Biol Chem **271**: 21209
[12] Surolia A, Sharon N, Schwarz FP (1996) J Biol Chem **271**: 17697
[13] Streicher H, Schmid W, Wenzl I, Fiedler C, Kählig H, Unger FM (2000) Bioorg Med Chem Lett **10**: 1369

[14] Wenzl I, Kählig H, Unger FM, Schmid W (1999) Monatsh Chem **130**: 1137
[15] Hill J, Hough L, Richardson AC (1968) Carbohydr Res **8**: 19
[16] Jeanloz RW (1954) J Am Chem Soc **76**: 555
[17] Lee RT, Lee YC (1994) Carbohydr Res **251**: 69
[18] Gambert U, Lio RG, Farkas E, Thiem J, Bencomo VV, Liptak A (1997) Bioorg Med Chem **5**: 1285
[19] Helferich B, Zirner J (1962) Chem Ber **34**: 957
[20] Jaenloz RW, Stoffyn PJ (1962) Methods Carbohydr Chem **1**: 224
[21] Kessler H, Gehrke M, Griesinger C (1988) Angew Chem Int Ed Engl **27**: 490
[22] Rance M, Sörensen OW, Bodenhausen G, Wagner G, Ernst RR, Wüthrich K (1983) Biochem Biophys Res Commun 479
[23] Warren CD, Jeanloz RW (1977) Carbohydr Res **53**: 67

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