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Note

Synthesis of S-linked glucosaminyl-4-thiohex-2-enopyranosides via allylic S_N2' substitution of a tosylhexenose

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Abstract

Treatment of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranose with 1,6-anhydro-3,4-dideoxy-2-*O*-*p*-toluenesulfonyl-β-D-*erythro*-hex-3-enopyranose gave 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-1,6-anhydro-2,3-dideoxy-4-thio-β-D-*erythro*-hex-2-enopyranose in 86% yield. Its 1,6-anhydride bond was cleaved with methanol to give a mixture of methyl glycosides (α/β ~ 5:1), from which the α anomer was separated by crystallization and converted into its 6-acetate, 6-methanesulfonate, or deacetylated to obtain the corresponding free methyl thiodisaccharide. The structure of the new compounds was confirmed by ^{1}H and ^{13}C NMR spectra.

Keywords: Thiosugars; 1,6-Anhydro-2,3-dideoxy-hex-2-enopyranoses, 4-substituted; (1→4)-Thiodisaccharides, unsaturated; 1-Thio-*N*-acetylglucosamine; Allylic substitution

1. Introduction

Thiooligosaccharides[1] constitute an important class of sulfur analogs of naturally occurring oligosaccharides and thus their potential is exploited in glycotherapy, drug-preparation, and in studies concerning the mechanisms of action of various enzymes (especially hydrolases–glycosidases) on oligosaccharides and their glycoconjugates. In the latter case, it is often necessary to prepare a simple, enzymatically non-cleavable analog of the target molecule which acts as a competitive inhibitor.[1] The glycoconjugates containing 2-acetamido-2-deoxy-D-glucose (GlcNAc) play an important role in living organisms. The synthesis of their thioanalogs attracts much attention and many thiooligosaccharides of this type have been synthesized recently.[1] The principal reaction involved in this synthesis was the nucleophilic displacement of the triflate group at C-4 of a suitably protected hexopyranose with 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucosamine.[2,3] 4-Thiodisaccharides thus obtained were used for the syntheses of more complex oligosaccharides[4–7] and their inhibitor activity towards various cellulases and α-amylases has been extensively studied.[4,5,7–9] Until now, not any unsaturated thiooligosaccharides containing GlcNAc unit have been reported in the literature.

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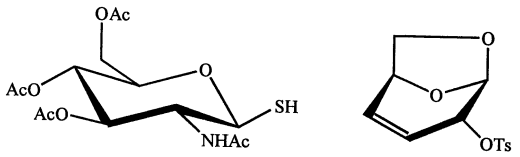
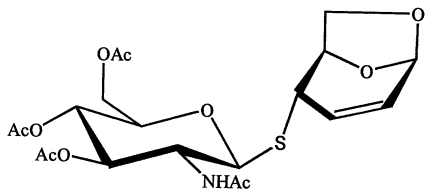
2. Results and discussion

In the present work, we describe the synthesis of the title compound using the readily accessible and reactive 1,6-anhydro-3,4-dideoxy-2-*O*-*p*-toluenesulfonyl-β-D-*erythro*-hex-3-enopyranose (**2**)[10,11] for the S-alkylation of the thioglucosamine derivative **1** according to the procedure described earlier.[11]



1

2



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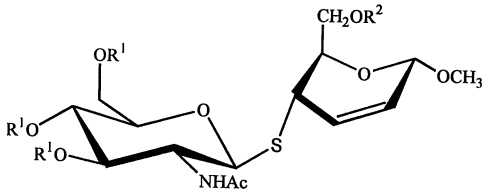

	R^1	R^2
4	Ac	H
5	Ac	Ac
6	Ac	Ms
7	H	H

The coupling of **1** with the unsaturated tosylhexenose **2** proceeded with high regio- and stereoselectivity as a S_N2' type reaction with retention of the configuration. Potassium carbonate in acetone proved to be the best reagent, whereas lithium carbonate in acetone or 1,8-diazabicyclo[5.4.0]undec-7-ene in oxolane were much less effective. It is worth mentioning that addition of a small amount of water into the reaction mixture accelerated the reaction dramatically. On the other hand, dry K_2CO_3 in absolute acetone gave almost no conversion. The coupling reaction proceeded readily at room temperature to give the unsaturated 4-thiodisaccharide **3** in 86% yield and a small amount of the unreacted tosylhexenose **2** and a disulfide originating from **1**, which were separated by chromatography. Methanolysis of the 1,6-anhydride bond under catalysis of pyridinium *p*-toluenesulfonate (PPTS) gave a mixture of anomeric methyl glycosides in a ratio $\alpha/\beta \sim 5{:}1$ (estimated from ^{1}H NMR spectra). If the methanolysis was performed longer (>2 h), further conversion of α to β anomer was observed in a small extent. From this mixture, the pure α anomer **4** could be crystallized out. The isomerization was catalyzed by PPTS, and hence its traces had to be carefully removed from the crystalline α anomer. On the other hand, the corresponding 6-*O*-acetate **5** and also the 6-*O*-methanesulfonyl derivative **6** prepared by conventional procedures, were stable compounds. We also tried tosylation of **4** with tosyl chloride in pyridine, but the reaction was very slow, the 6-*O*-tosyl derivative was formed in only 10% yield, and the major part of the starting glycoside **4** decomposed. The free glycoside **7** was obtained by catalytic de-O-acetylation according to Zemplén.

Preferred conformations of compounds **4**–**7** (calculated by MM2+ method) can be characterized by the twisted half chair form $^{O}H_5$ of the unsaturated hexopyranose ring and the chair form $^4C_1$ of the 2-acetamidoglucose ring. Theoretical calculations are supported by observed interproton coupling constants. The *J*-values are in a good semiquantitative agreement with the interproton torsion angles in calculated energy minimized conformation, e.g., for compound **7**: $J_{1,2}$ 3.1 Hz (−43°), $J_{3,4}$ 2.2 Hz (+74°), $J_{4,5}$ 10.6 Hz (−170°), $J_{1',2'}$ 10.4 Hz (+177°), $J_{2',3'}$ 10.2 Hz (−170°), $J_{3',4'}$ 9.4 Hz (+167°), $J_{4',5'}$ 10.0 Hz (−174°).

3. Experimental

General methods.—Melting points were determined with a Boëtius micromelting-point apparatus and are uncorrected. Optical rotations were measured at 25 °C with an AUTOPOL III polarimeter (Rudolph Research, Flanders, NJ). NMR spectra were recorded on Varian UNITY-500 instrument (^{1}H at 500 MHz; ^{13}C at 125.7 MHz) in $CDCl_3$ (except for compound **7**, insoluble in $CDCl_3$, which was measured in $(CD_3)_2SO$) (Tables 1 and 2). For structural assignment of protons and carbons

Table 1
Proton NMR data for compounds **3**–**7**

Proton	Chemical shifts (ppm)					H,H	Coupling constants (Hz)				
	3 ($CDCl_3$)	**4** ($CDCl_3$)	**5** ($CDCl_3$)	**6** ($CDCl_3$)	**7** (Me_2SO)		**3** ($CDCl_3$)	**4** ($CDCl_3$)	**5** ($CDCl_3$)	**6** ($CDCl_3$)	**7** (Me_2SO)
H-1	5.49 dd	4.90 m	4.90 m	4.90 m	4.81 m	1,2	3.4	3.0	3.0	3.1	3.1
H-2	6.03 ddd	5.88 ddd	5.90 ddd	5.85 ddd	5.76 ddd	1,3	0.8	~0.6	?	?	1.0
H-3	5.68 dddd	5.93 ddd	5.93 bdd	6.00 ddd	5.95 ddd	1,4	~0	~1.5	~1.5	1.6	~1.5
H-4	3.30 ddd	3.58 dq	3.45 dq	3.53 dm	3.43 dm	2,3	9.4	10.0	10.0	10.1	10.0
H-5	4.89 ddt	4.01 ddd	4.20 ddd	4.17 ddd	3.72 ddd	2,4	1.7	2.4	2.2	2.4	2.4
H-6a	3.98 dd	3.94 dd	4.51 dd	4.74 dd	3.75 dd	3,4	4.3	2.0	1.8	2.1	2.2
H-6b	3.63 dd	3.91 dd	4.40 dd	4.49 dd	3.64 dd	3,5	1.8	~0	~0	~0	~0
						4,5	1.0	10.4	10.5	10.7	10.6
						5,6a	6.2	2.6	5.0	4.0	1.8
						5,6b	2.0	4.1	2.2	1.9	5.4
						6a,6b	7.9	12.0	12.0	11.1	11.7
H-1′	4.68 d	4.72 d	4.68 d	4.73 d	4.44 d	1′,2′	10.5	10.4	10.5	10.4	10.4
H-2′	4.15 dt	4.06 ddd	4.05 dt	4.10 m	3.49 q	2′,3′	10.2	10.0	10.0	10.2	9.8
H-3′	5.16 dd	5.15 t	5.14 t	5.12 m	3.25 dd	2′,NH	9.4	9.4	9.3	9.4	9.4
H-4′	5.09 dd	5.09 t	5.08 t	5.08 m	3.11 dd	3′,4′	9.4	9.2	9.3	~10	8.3
H-5′	3.71 ddd	3.68 ddd	3.65 ddd	3.68 m	3.06 ddd	4′,5′	10.0	10.0	9.9	~10	9.8
H-6′a	4.17 dd	4.22 dd	~4.17 m	~4.18 m	3.64 dd	5′,6′a	2.7	2.6	2.8	[a]	1.9
H-6′b	4.20 dd	4.17 dd	~4.17 m	~4.18 m	3.44 dd	5′,6′b	4.8	4.4	4.5	[a]	5.3
						6′a,6′b	12.3	12.4	[a]	[a]	11.8
NH	5.62 bd	5.57 bd	5.64 bd	5.75 d	7.78 d						
Ac	2.06 s	2.10 s	2.11 s	2.10 s	1.80 s						
	2.04 s	2.04 s	2.10 s	2.03 s							
	2.035 s	2.02 s	2.03 s	2.02 s							
	1.95 s	1.96 s	2.02 s	1.96 s							
			1.96 s								
OMe		3.42 s	3.42 s	3.42 s							
OMs				3.10 s							

[a] The *J*-value could not be determined.

both 1D NMR experiments (^{1}H and ^{13}C-APT) and 2D-methods (^{1}H,^{1}H-COSY and 2D-^{1}H,^{13}C-HMQC) were combined. Thin-layer chromatography (TLC) was performed on DC Alufolien plates (E. Merck, type 5554) coated with Kieselgel 60 F_{254}, detection with 50% H_2SO_4 or 3% EtOH solution of anisaldehyde acidified with concd H_2SO_4, and heating. For preparative column chromatography, Silica Gel Kieselgel 60 (E. Merck, 60–230 mesh) was used. Reactions were performed in an Ar atmosphere. Solutions were evaporated under reduced pressure at temperature below 40 °C. Analytical samples were dried over phosphorus pentoxide at rt under reduced pressure.

2-Acetamido-3,4,6-tri-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl - (1→4) - 1,6 - anhydro - 2,3-dideoxy-4-thio-β*-D-erythro-*hex-2-enopyranose* (**3**).—Tosylhexenose **2**[11] (430 mg, 1.52 mmol) was added to the solution of the thioglucosamine **1**[12] (600 mg, 1.65 mmol) in acetone (45 mL) followed by the addition of K_2CO_3 (700 mg) and water (0.1 mL). The suspension was stirred under an Ar atmosphere at rt for 24 h. The reaction was monitored by TLC in EtOAc. After consumption of **1**, the reaction mixture was poured onto crushed ice (100 mL) and extracted three times with CH_2Cl_2 (150 mL). The combined organic layers were washed with water, dried over anhyd Na_2SO_4 and evaporated to dryness. The solid residue was chromatographed on silica gel (20 g). Elution with EtOAc gave regenerated tosylhexenose **2** (74 mg, 17%) and 619 mg (86%), the product **3**, and traces of a disulfide originating from the thioglucosamine **1** (mp 240–241 °C, lit.[13] gives 240–241 °C). Recrystallization of **3** from acetone–ether–light petroleum mixture gave 486 mg (67%) needles of mp 177–179 °C, $[\alpha]_D + 86°$ (*c* 0.38, $CHCl_3$). Anal. Calcd for $C_{20}H_{27}NO_{10}S$: C, 50.73; H, 5.75; N, 2.96; S, 6.77. Found: C, 50.65; H, 5.82; N, 2.86; S, 6.69.

Methyl 2-acetamido-3,4,6-tri-O-*acetyl-2-deoxy - β - D - glucopyranosyl - (1→4) - 2,3-dideoxy-4-thio-α*-D-erythro-*hex-2-enopyranoside* (**4**).—The 1,6-anhydro derivative **3** (95 mg, 0.2 mmol) and pyridinium *p*-toluenesulfonate (PPTS, 5 mg freshly recrystallized from abs acetone) were refluxed in abs MeOH (5

Table 2
^{13}C chemical shifts for compounds **3**–**7**

Carbon	**3** ($CDCl_3$)	**4** ($CDCl_3$)	**5** ($CDCl_3$)	**6** ($CDCl_3$)	**7** (Me_2SO)
1	95.41	95.08	95.09	95.36	94.31
2	129.89	127.75	127.89	127.11	126.84
3	125.76	131.75	131.22	131.49	132.77
4	44.10	38.85	39.77	39.82	38.22
5	76.81	70.88	68.72	68.65	71.99
6	67.16	62.32	63.81	69.28	61.20
1′	83.06	83.60	83.81	84.75	83.79
2′	53.01	53.37	53.33	53.08	54.68
3′	73.74	73.71	73.66	73.76	75.51
4′	68.34	68.02	68.12	68.09	70.28
5′	75.96	75.73	75.62	75.72	81.12
6′	62.17	61.70	62.02	61.98	61.02
OMe		55.64	55.62	55.86	54.79
Ac: C=O	171.13	171.60	171.10	171.00	169.15
	170.67	170.73	170.86	170.62	
	170.08	169.94	170.60	170.08	
	169.33	169.21	169.97	169.22	
			169.20		
Ac: CH_3	23.25	23.27	23.23	23.17	23.19
	20.68	20.74	20.89	20.73	
	20.65	20.66	20.70	20.64	
	20.58	20.58	20.65	20.57	
			20.57		
SO_2CH_3				37.67	

mL) under an Ar atmosphere. After 1 h, the starting compound was nearly consumed. The course of methanolysis was monitored by TLC in 10:1 EtOAc–dioxane to visualize the α and β anomers. Both anomers were formed in the approximate ratio $\alpha/\beta = 5:1$ (as estimated from integrated signals of *O*-methyl group protons in ^{1}H NMR spectrum of the reaction mixture). Prolonged refluxing (> 2 h) led to the formation of a larger amount of the β anomer. After evaporation of the reaction mixture to dryness, the residue was dissolved in EtOAc (30 mL), the solution was filtered through a short column (5 cm) of silica gel in order to separate PPTS. The resulting filtrate was evaporated and the residue (93 mg, 92%) crystallized from MeOH–ether–light petroleum to yield the pure α anomer **4** (76 mg, 75%); mp 180–181 °C, $[\alpha]_D + 78°$ (*c* 0.64, $CHCl_3$). Anal. Calcd for $C_{21}H_{31}NO_{11}S$: C, 49.89; H, 6.18; N, 2.77; S 6.34. Found: C, 49.71; H, 6.25; N, 2.65; S 6.40.

Methyl 2-acetamido-3,4,6-tri-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl-(1→4)-6*-O-*acetyl-2,3-dideoxy-4-thio-α*-D-erythro-*hex-2-enopyranoside* (**5**).—To a mixture of the α-methyl glycoside **4** (150 mg, 0.3 mmol), dry pyridine (50 μL) and dry THF (3 mL), Ac_2O (60 μL) was added with stirring under Ar atmosphere at rt. After 19 h, TLC (10:1 EtOAc–dioxane) showed no starting material. The reaction mixture was evaporated and coevaporated three times with 5 mL of dry toluene. The solid residue was recrystallized from acetone–ether–light petroleum to give **5** (152 mg, 94%), mp 158–159 °C, $[\alpha]_D + 74.8°$ (*c* 0.21, $CHCl_3$). Anal. Calcd for $C_{23}H_{33}NO_{12}S$: C, 50.45; H, 6.07; N, 2.56; S, 5.86. Found: C, 50.63; H, 6.09; N, 2.51; S, 5.82.

Methyl 2-acetamido-3,4,6-tri-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl-(1→4)-6*-O-*methanesulfonyl-2,3-dideoxy-4-thio-α*-D-erythro-*hex-2-enopyranoside* (**6**).—To a solution of the α-methyl glycoside **4** (150 mg, 0.3 mmol) in 20 mL of dry pyridine mesyl chloride (40 μL) was added with stirring under an Ar atmosphere and cooling at −15 °C. The stirred reaction mixture was brought up to rt in 2 h and monitored with TLC (10:1 EtOAc–dioxane). Because of the presence of the unreacted starting material, the mixture was recooled at −15 °C and another portion of mesyl chloride (40 μL) was added. After warming up to rt, the reaction mixture was stirred for 2 days at this temperature. The reaction mixture was poured onto crushed ice (100 mL) and extracted three times with 60 mL of CH_2Cl_2. The combined organic layers were washed consecutively with 2 × 5% HCl, 1 × 5% $NaHCO_3$ and water, dried over anhyd Na_2SO_4 and evaporated to dryness. The solid residue was chromatographed on silica gel (12 g). Elution with EtOAc and crystallization (acetone–ether–light petroleum) gave **6** (120 mg, 70%), mp 140–141 °C, $[\alpha]_D + 64°$ (*c* 0.23, $CHCl_3$). Anal. Calcd for $C_{22}H_{33}NO_{13}S_2$: C, 45.27; H, 5.70; N, 2.40; S, 10.99. Found: C, 45.40; H, 5.84; N, 2.29; S, 10.89.

Methyl 2-acetamido-2-deoxy-β-D-*glucopyranosyl-(1→4)-2,3-dideoxy-4-thio-α*-D-erythro-*hex-2-enopyranoside* (**7**).—The 6-*O*-acetate **5** (62 mg, 0.11 mmol) was dissolved in abs MeOH (5 mL) and 100 μL of 1 M MeONa was added with stirring at rt. After 24 h standing, the deacetylation was completed (monitored by TLC in 1:2 MeOH–EtOAc). Then the solution was evaporated and the residue several times coevaporated with toluene (5 mL). The solid residue was crystallized from MeOH–ether to give **7** (36 mg, 89%), mp 119–121 °C, $[\alpha]_D + 130°$ (*c* 0.5, water). Anal. Calcd for $C_{15}H_{25}NO_8S$: C, 47.48; H, 6.64; N, 3.69; S, 8.45. Found: C, 47.53; H, 6.61; N, 3.78; S, 8.37.

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