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Carbohydrate Research 342 (2007) 1929–1933
Carbohydrate RESEARCH

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A click chemistry approach to glycomimetics: Michael addition of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose to 4-deoxy-1,2-*O*-isopropylidene-L-*glycero*-pent-4-enopyranos-3-ulose – a convenient route to novel 4-deoxy-(1→5)-5-*C*-thiodisaccharides

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Received 26 April 2007; received in revised form 30 May 2007; accepted 2 June 2007
Available online 9 June 2007

Abstract—The base catalyzed conjugate Michael addition of the 1-thiosugar, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose, **1**, to a new highly reactive enone 4-deoxy-1,2-*O*-isopropylidene-L-*glycero*-pent-4-enopyranos-3-ulose, **2**, proceeds steroselectively with formation of adduct **3** in 94% yield. Convenient stereoselective reduction of the C-3 keto function of **3** with L-Selectride® followed by in situ acetylation produces thiodisaccharide **4** in good 82% yield. Cleavage of the 1,2-*O*-isopropylidene protecting group with *p*-toluenesulfonic acid in methanol, followed by de-O-acetylation, produced an inseparable anomeric mixture of methyl 4-deoxy-5-*C*-(β-D-glucopyranosyl)-thio-α/β-L-*ribo*-pyranoside **5** in 72% overall yield. This approach constitutes a new general two-step click chemistry route to the previously unknown class of 4-deoxy-(1→5)-5-*C*-thiodisaccharides as stable and biologically important glycomimetics.

Keywords: Sugar enones; Michael addition; 1,5-Linked-4-deoxy-5-*C*-thiodisaccharides

Click chemistry is a new molecular approach[1,2] that uses reactive building blocks through simple and reliable chemical transformations and is allowing rapid exploration in the drug discovery process, especially via combinatorial synthesis of new lead prototypes. In our efforts toward preparing glycomimetics[3] and thiodisaccharides,[4,5] we previously employed reactive building blocks such as the enones levoglucosenone[6–11] (1,6-anhydro-3,4-dideoxy-β-D-*glycero*-hex-3-enopyranos-2-ulose) and isomeric isolevoglucosenone[12–14] (1,6-anhydro-2,3-dideoxy-β-D-*glycero*-hex-2-enopyranos-4-ulose) as universally reactive Michael addition acceptors. This general Michael addition approach was originally developed in our laboratory[4,5] and was followed by others.[15–18]

As our need for larger quantities and variety of conventionally functionalized enones increased, we have been constantly exploring methods that would make them more readily available for exploratory studies and multistep syntheses for click chemistry approaches. One such reactive enone, originally synthesized by Klemer and Jung[19] and currently explored by us, is 4-deoxy-1,2-*O*-isopropylidene-L-*glycero*-pent-4-enopyranos-3-ulose (**2**). The compound serves as a convenient new building block for stereoselective functionalization reactions, especially as a Michael addition acceptor (Fig. 1).

As noted previously, our recent results[4,5] on Michael addition of sugar thiols to reactive enones such as levoglucosenone show complete stereoselective addition reactions due to its rigid bicyclic framework and steric shielding of the upper face of the pyranose ring by the 1,6-anhydro ring. The high stereoselectivity observed

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doi:10.1016/j.carres.2007.06.005

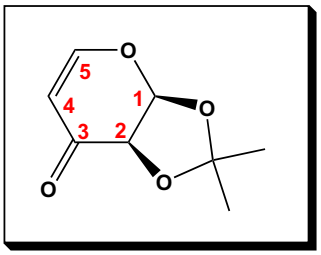


Figure 1.

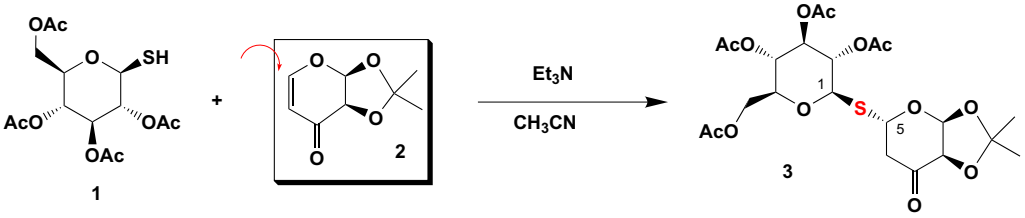
with this enone and the rationale for the selectivity makes reactions with the enone highly predictable in the Michael addition of organometalics[20] and other carbon nucleophiles.[21,11] These important observations prompted us to expand this study to the exploration of the synthetic utility of 4-deoxy-1,2-*O*-isopropylidene-L-*glycero*-pent-4-enopyranos-3-ulose (**2**) by expecting a similar type of steroselectively in introducing a sulfur bridge between two sugar units at C-(1→5) by stereoselective Michael addition of 1-thio-sugars at the C-5 position.

Thiodisaccharides[22] are specific nonhydrolyzable glycomimetics containing sulfur in the glycosidic linkage and have been synthesized previously by a variety of methods[23–30] including S_N2-type reactions involving the action of a thiolate anion and a glycosyl halide, the displacement of a leaving group by 1-thio-glucopyranose. The Michael addition of thiol **1**[31] to the enone **2** proceeded smoothly with the formation of β-(1→5)-4-deoxy-5-*C*-thiodisaccharide **3** in 94% yield. The proton–proton coupling constants in the ^{1}H NMR spectrum of **3** confirmed that only the 5-axial adducts were obtained as a single addition product. This stereoselectivity, as was observed previously in levoglucosenone conjugate additions,[4–12] proceeds by the attack of incoming nucleophile at the top of alkene face of enone **2** ring as depicted in Scheme 1.

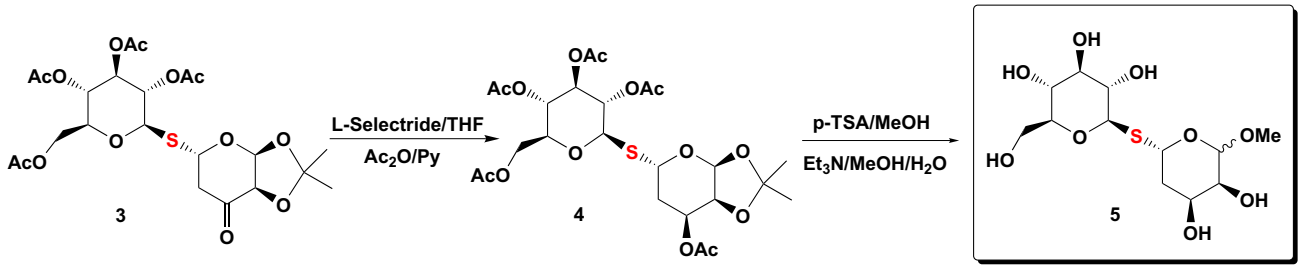
The ^{1}H NMR spectrum of adduct **3** did not show signals corresponding to potential D-*threo* isomer, clearly demonstrating that the stereochemistry of the addition of thiol **1** to enone **2** is completely stereoselective. The ^{13}C NMR spectrum of adduct **3** showed no alkene signals, and the C-4 signal appeared upfield at ~42.5–42.8 ppm, respectively.

Ketone **3** offers potential in the synthesis of precursors of certain amino sugars as reported by us earlier[4,5] through conventional oximation and highly stereoselective reduction of the acetamido function. The reduction of the C-3 keto function of ketone **3**, with L-Selectride®, followed by in situ conventional acetylation, proceeded stereoselectively with the formation of the L-*ribo*-isomer **4** in 89% yield. Only a trace amount of the corresponding L-*xylo*-isomer was detected by ^{1}H NMR spectroscopy. Coupling constants of reduction product **4**, $J_{3,4a} = 7.6$ Hz and $J_{3,4e} = 4.8$ Hz, indicated the axial disposition of the new substituent at C-3. This is in full agreement with earlier observation by Horton and coworkers[14,15] of high stereoselectivity and yet another classical example of the preferential attack of the reducing agent from the top face on C-3 keto group (Scheme 2). Moreover, the ^{1}H and ^{13}C NMR spectrum of **4** firmly support the assignment as the L-*ribo*-configuration. In particular, the coupling constants between equatorially disposed H-5 and the axially disposed H-4, $J_{4a,5} =$ 4.7 Hz, are of great diagnostic value.

The cleavage of the 1,2-*O*-isopropylidene ring in **4** was examined under various reaction conditions. The meth-



Scheme 1. Stereolelective Michael addition of **1**–**2** with formation of **3**.



Scheme 2. Reduction of keto function at C-3 in **3** and deprotection of **4** with formation of 1,5-*C*-thiodisaccharide **5**.

Table 1. ^{1}H NMR chemical shifts (δ) and coupling constants (J in Hz) for **3**–**5**[a]

Compound	H-1′ $J_{1,2}$	H-2′ $J_{2,3}$	H-3′ $J_{2,3'}$	H-4′ $J_{3,4}$	H-5′ $J_{5,6'}$	H-6′ $J_{5,6''}$	H-6′ $J_{5,6''}$	H-1 $J_{1,2}$	H-2 $J_{2,3}$	H-3 $J_{3a,4}$	H-4a $J_{4a,3a}$	H-4e $J_{4e,3a}$; $J_{4e,4a}$	H-5 J	–COCH$_3$
3	4.6 d	5.1 dd	5.2 t	5.1 d	4.8 d	4.2 d	4.1 d	4.2 d	4.25 d	—	3.5 d	2.6 d	4.50 dd	4 × 1.89
	8.6	9.6	9.8	9.5	ND[c]	ND[c]	ND[c]	3.0	5.0		8.0	4.8; 16.0	4.9	
4	4.8 d	5.2 dd	5.4 t	5.3 d	4.9 d	4.2	4.2 d	4.3 d	4.3 d	3.12 dd	3.5 d	2.6 d	4.50 dd	2.02
	8.2	9.6	9.6	9.3	5.2	ND[c]	ND[c]	3.0	ND[c]	7.6	8.0	4.8; 16.0	4.9	5 × OAc
5[b]	4.8 d	5.1 dd	5.2 t	5.1 d	4.8 d	4.26 dd	4.26 dd	5.3 d	4.6 d	3.12 dd	3.5 d	2.6	4.9 dd	OMe
	8.6	9.6	9.9	9.5	5.0	ND[c]	ND[c]	ND[c]	ND[c]	7.6	8.0	4.8; 16.0	4.9	1.25

[a] Determined at 500 MHz in $CDCl_3$ with Me_4Si as internal reference.
[b] Determined at 500 MHz in D_2O with TMSPA-Na as internal reference.
[c] ND the coupling constant was not determined.

Table 2. ^{13}C NMR Chemical shifts (ppm) data for **3**–**5**[a]

Compound	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	C-1	C-2	C-3	C-4	C-5	–OCO	–CH$_3$	–COCH$_3$
3	102.6	67.1	67.4	70.1	76.3[c]	65.3	92.8	69.4	200.1	42.5	46.2	106.8	2 × 26.3	4 × 20.5
4	96.8	67.1	66.9	70.2	73.1[c]	65.5	92.8	69.2	67.8	42.8	46.5	105.9	2 × 26.5	20.6 5 × OAc
5[b]	101.4	76.2	67.1	68.8	72.2	64.4	92.2	66.5	68.6	38.6	46.1	—	–OMe 55.7	—

[a] Determined at 125 MHz in $CDCl_3$ with Me_4Si as internal reference.
[b] Determined at 125 MHz in D_2O with TMSPA-Na as internal reference.
[c] Assignments can be mutually interchanged.

od of choice was conventional hydrolysis using catalytic amount of *p*-toluenesulfonic acid in anhydrous methanol solution, performed according to the convenient protocol of Zhu and Vogel,[32] followed by in situ conventional deacetylation with an aqueous methanolic solution of triethylamine (4:1:5 MeOH–Et_3N–H_2O) at room temperature for 6 h. This resulted in the formation of an anomeric mixture of methyl (1→5)-5-*C*-thiodisaccharide **5** (α/β 1:5) in 91% yield. Separation of the anomers proved impossible because of their almost identical R_f values. However, the ^{1}H NMR and ^{13}C NMR spectra and mass spectral data for the mixture firmly established their identity (Tables 1 and 2).

Molecular modeling of methyl 4-deoxy-5-*S*-(β-D-glucopyranosyl)-5-thio-α/β-L-ribopyranose **5** (Fig. 2) clearly confirms the stereochemistry as determined by NMR. The length of C-1–S bond is 1.8121 Å, whereas the C-5–S bond is 1.8104 Å and the dihedral angle for C–S–C bridge is 108.833°. Complete data will be published under separate communication. All the measurements are quite similar to the calculations for the sulfur bridge of other thiodisaccharides and are substantially different than that of the oxygen counterpart as previously postulated in the literature.[33,34] Moreover, the critically important value of lipophilicity of the target molecule (**5**) for the potential affinity effect to cell membrane and the level of penetration/diffusion through membrane was also calculated through molecular modeling as $\log P = -1.76$. This particular data is essential for preliminary biological screening of thio-glycomimetics for selective in vitro cell viability assays as reported by us earlier.[22d]

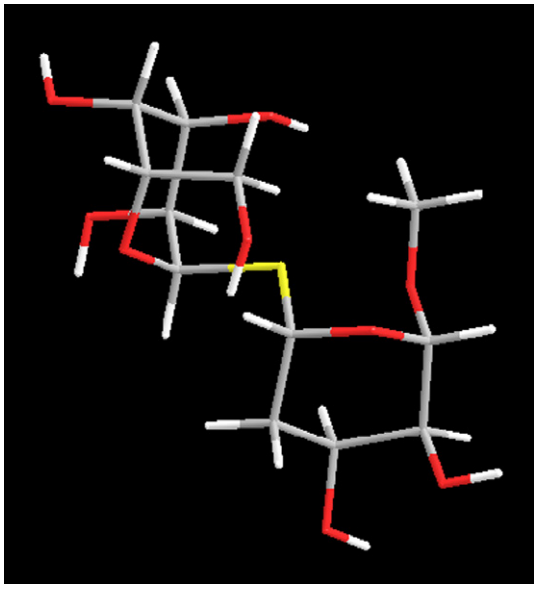


Figure 2. Molecular model of **5** generated by Chem Draw 3D Ultra 8.0 and minimized.

1. Experimental

1.1. General methods

Unless otherwise noted, starting materials were obtained from commercial suppliers and used without purification. All melting points were uncorrected and were measured in open capillary tubes. Optical rotations were determined on a Jasco Model DIP-370 Polarimeter in $CHCl_3$ solutions. Thin-layer chromatography (TLC)

was performed on precoated Silica gel $60F_{254}$ plates from E. Merck and visualized by spraying with 10% ethanolic sulfuric acid and subsequent heating. Column chromatography was performed on Silica Gel 60 (70–230 mesh, Merck No. 34). ^{1}H NMR samples were prepared in $CDCl_3$ (99.8 at % D), filtered, freeze–thawed, and sealed in a 5 mm NMR tube. Tetramethylsilane (TMS) was used as an internal chemical shift reference. High-resolution NMR spectra were obtained on a Bruker DMX-500 spectrometer. Mass spectra were obtained either in EI mode at 70 eV or using CI (NH_3). Molecular modeling calculations were measured with ChemDraw Office, Ultra 8.0 by CambridgeSoft, Cambridge, MA.

1.2. 4-Deoxy-1,2-*O*-isopropylidene-L-*glycero*-pent-4-enopyranos-3-ulose (2)

Compound **2** was produced by the methodology of Klemer and Jung[19] $R_f = 0.59$ (EtOAc), mp 60–61.5 °C, lit. 58–59 °C,[16] $[\alpha]_D$ +318 (*c* 1.0, $CHCl_3$), $[\alpha]_D$ +318 (*c* 1.0, $CHCl_3$): for ^{1}H NMR and ^{13}C NMR data see Ref. 19.

1.3. 4-Deoxy-1,2-*O*-isopropylidene-5-*C*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-thio-α-L-*erythro*-pentopyranos-3-ulose (3)

To a solution of enone **2**[19] (170 mg, 0.1 mmol) in acetonitrile (10 mL) a solution of 1-thio-sugar **1**[31] (125 mg, 0.34 mmol) in 5 mL of acetonitrile was added dropwise. The reaction mixture was stirred at room temperature for 24 h. After evaporation of the solvent, the syrupy residue was purified by column chromatography on silica gel. The fraction eluted with 20% EtOAc in hexane (v/v) to give pure syrupy products, which crystallized from Et_2O–hexane. Yield (475 mg, 89%), colorless syrup; $R_f = 0.69$ (1:4, hexane–EtOAc); $[\alpha]^{30}$ +134.2 (*c* 0.84, $CHCl_3$); HRMS $(M)^+$ *m*/*z*: Calcd for $C_{22}H_{30}O_{13}S$: 534.14. Found: 534.11. ^{1}H NMR: δ; 4.6 (d, 1H, H-1′, $J_{1,2} = 8.6$ Hz) $J_{1'5} = 10.5$ Hz; data for other protons and carbon resonances are listed in Tables 1 and 2, respectively.

1.4. 3-*O*-Acetyl-4-deoxy-1,2-*O*-isopropylidene-5-*C*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-thio-α-L-*ribo*-pentopyranoside (4)

To a cooled and stirred solution of thiodisaccharide **3** (210 mg, 0.428 mmol) in THF, L-Selectride® (1 M in THF, 1.0 mL) was added at −78 °C under an Ar atmosphere. The reaction mixture was stirred for 3 h and then pyridine (4 mL) and acetic anhydride (5 mL) were added and stirred at room temperature overnight. The reaction mixture was poured into ice water and extracted with ethyl acetate. The extract was washed and dried. Removal of the solvent in vacuo after coevaporation with 1:1 toluene–EtOAc (5 × 30 mL) afforded an oily residue that was subjected to column chromatography on silica gel. The fraction eluted with 20% EtOAc in hexane (v/v) gave syrupy product **4**. Yield (190 mg, 82%), $R_f = 0.42$, (1:4, hexane–EtOAc); $[\alpha]^{30}$ +65.2 (*c* 0.84, $CHCl_3$); HRMS $(M)^+$ *m*/*z*: Calcd for $C_{24}H_{34}O_{14}S$: 578.2. Found: 578.1. The ^{1}H NMR and ^{13}C NMR data for **4** are listed in Tables 1 and 2, respectively.

1.5. Methyl 4-deoxy-5-*C*-(β-D-glucopyranosyl)-thio-α/β-L-*ribo*-pentopyranoside (5)

To a cooled solution (0 °C) of **4** (0.5 g, 0.93 mmol) in anhydrous methanol (20 mL) stirred under argon, *p*-toluenesulfonic acid (25 mg) was added. TLC (1:1 EtOAc–hexane) indicated the completion of the reaction after 12 h. A solution of saturated sodium bicarbonate was added, the mixture was stirred for 30 min, and the aqueous mixture was extracted three times (3 × 20 mL) with EtOAc. The combined extracts were washed with saturated sodium hydrogen carbonate (20 mL) and brine (20 mL) and then dried over sodium sulfate. Evaporation of the solvent under reduced pressure yielded crystalline residue, which was dissolved in a 15 mL solution of 4:1:5 MeOH–Et_3N–H_2O and stirred at room temperature. TLC indicated the completion of the deprotection reaction after 6 h. Evaporation of the solvent produced an inseparable anomeric mixture (α/β in a ratio 1:6). Yield (119 mg, 89%) as a colorless syrup $[\alpha]^{30}$ +130.2 (*c* 0.82, H_2O); HRMS $(M)^+$ *m*/*z*: Calcd for $C_{12}H_{22}O_9S$: 342.1. Found: 342.0. The ^{1}H NMR and ^{13}C NMR data for **5** are listed in Tables 1 and 2, respectively.

Acknowledgment

Financial Support from the Wilkes University Internal Grant Program is gratefully acknowledged.

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