Synthesis and conformational analysis of disaccharide analogues containing disulfide and selenosulfide functionalities in the interglycosidic linkages[1]

Nagasree Chakka, Blair D. Johnston, and B. Mario Pinto

Abstract: The synthesis of novel disaccharides containing disulfide (methyl-4-*S*-(β-D-galactopyranosyl-1′-thio)-4-thio-α-D-glucopyranoside (**1**)) and selenosulfide (methyl-4-*Se*-(β-D-galactopyranosyl-1′-thio)-4-seleno-α-D-glucopyranoside (**2**)) functionalities in the interglycosidic linkages is described. The synthetic strategy relied on the reaction of a β-glycosylthiosulfonate with a carbohydrate thiol or selenol nucleophile. The resulting protected β-dihetero-linked disaccharides were deprotected to give the target compounds. The conformational preferences of these dihetero analogues were inferred from NOESY experiments and line-broadening effects in variable-temperature NMR spectra, and are rationalized in terms of molecular orbital theory. Low-energy conformations of these compounds can populate regions of conformational space not usually occupied by β-linked disaccharides, and offer the possibility for presentation of novel ligand topographies.

Key words: disaccharides, disulfides, selenosulfides, interglycosidic linkages, conformations, MO explanation.

Résumé : On décrit la synthèse de nouveaux disaccharides comportant des fonctionnalités disulfure (4-*S*-(β-D-galactopyranosyl-1′-thio)-4-thio-α-D-glucopyranoside de méthyle (**1**)) et sélénosulfure (4-*Se*-(β-D-galactopyranosyl-1′-thio)-4-séléno-α-D-glucopyranoside de méthyle (**2**)) dans les liaisons interglycosidiques. La stratégie de synthèse se base sur la réaction d'un β-glycosylthiosulfonate avec un carbohydrate comportant un nucléophile thiol ou sélénol. Les disaccharides protégés à liaison β-dihétéro ont été déprotégés pour fournit les composés cibles. Les préférences conformationnelles de ces analogues dihétéro ont été déduites d'expériences « NEOSY » et d'effets d'élargissement de bande dans les spectres de RMN à température variable et elles sont rationalisées en fonction de la théorie des orbitales moléculaires. Les conformations de basse énergie de ces composés occuper des régions de l'espace conformationnel qui ne sont généralement pas occupés par des disaccharides à liaisons β et elles offrent la possibilité de présenter de nouvelles topographies pour les ligands.

Mots clés : disaccharides, disulfures, sélénosulfures, liaisons interglycosidiques, conformations, explication d'orbitales moléculaires.

[Traduit par la Rédaction]

Introduction

Carbohydrates and carbohydrate mimetics are essential tools for the investigation of a variety of biological functions that are mediated by carbohydrate recognition events (1, 2). Furthermore, some of these compounds are known to be therapeutic agents (3–7). It is therefore of interest to examine novel types of carbohydrate derivatives or mimetics to expand the collection of potential drug candidates. The next level of carbohydrate mimetic design should include the potential for presentation of different conformational families on the potential energy surface. Such an approach should provide access to an alternative selection of ligand topographies that might have advantages in drug design.

In general, the conformational preferences about glycosidic linkages in oligosaccharides can be described by the ϕ and ψ torsional angles, as shown in Fig. 1. Excursions about the ψ torsional angles in α- and β-glycosides are pronounced, and are dictated by the exact steric requirements about a particular glycosidic linkage (8). However, the preferences about the ϕ torsional angle are much more restricted by the endo- (9) and exo- (10) anomeric effect. Thus, the preferences in α-glycosides are largely restricted to a ϕ_H gauche+ conformation, whereas those in β-glycosides are re-

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Dedicated to Howard Alper for his contributions to chemistry.

N. Chakka, B.D. Johnston, and B.M. Pinto.[2] Department of Chemistry, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.

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[2]Corresponding author (e-mail: bpinto@sfu.ca).

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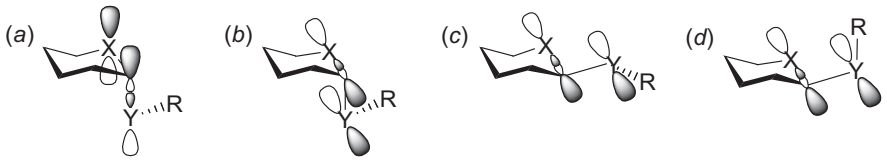
Fig. 1. Stabilizing orbital interactions associated with (*a*) the endo-anomeric effect and (*b*), (*c*), (*d*) the exo-anomeric effect.



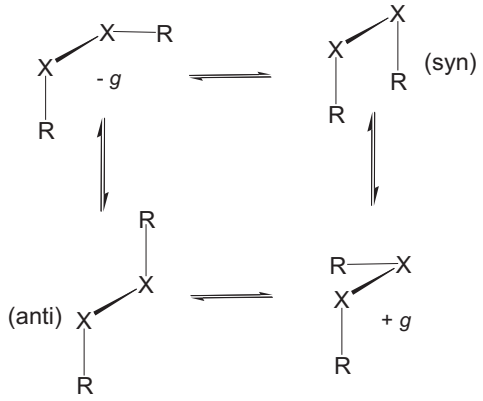
stricted to the ϕ_H gauche– conformation, dictated by the exo-anomeric effect (see structures *b* and *c* in Fig. 1), other conformations about the exocyclic C–O linkage being minimally populated (11).

Thus far, carbohydrate heteroanalogues have been used to advantage to gain access to alternative conformational preferences. For example, disaccharides containing 5-thio sugars, i.e., a sulfur atom in the ring, with different heteroatoms in the interglycosidic linkage, show population of conformations with different ϕ torsional angles (12), and thiooligosaccharides, i.e., those containing a sulfur atom in the interglycosidic linkage, e.g., thiocellobiose, show variations in both ϕ and ψ angles (13). Similarly, the pseudodisaccharide moiety in the tetrasaccharide dihydroglucoacarbose, containing a nitrogen atom in an α-glycosidic linkage, also displays variations in the ψ angle when free in solution (14). However, examples of β-glycosides containing ϕ angles that differ from the gauche conformation shown, e.g., a ϕ_H anti conformation (structure *d* in Fig. 1), have been elusive (15). It is worthy of note that the ϕ_H anti conformation will still permit operation of the exo-anomeric effect, i.e., the $n_Y \rightarrow \sigma^*_{C-X}$ stabilizing orbital interaction.

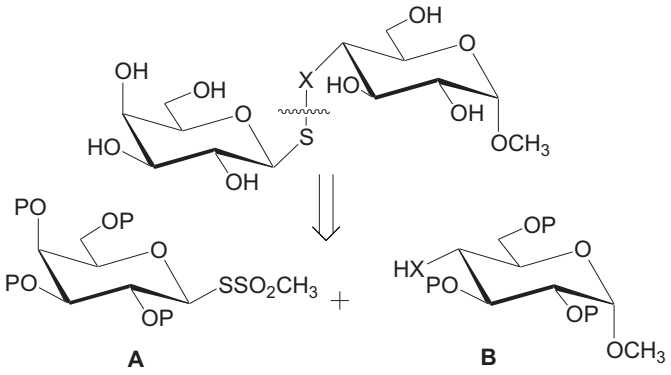
We were intrigued by the reported conformational preferences of oligosaccharides containing dihetero functionalities in the interglycosidic linkage, for example, those about the N–O glycosidic linkage in the naturally occurring calicheamycins (16), a family of enediyne antitumor antibiotics. These newly discovered compounds with unusual molecular structure have striking biological activity. This unusual N–O linkage has attracted the attention of several groups, and disaccharide mimics wherein two monosaccharide units are linked by a N—O bond have recently been reported (17–19). We propose here to investigate the corresponding compounds containing the S–S and S–Se functionalities in the glycosidic linkages.

Fraser et al. (20) have reported a variable-temperature NMR study of the effects of substitution on the barriers to rotation about the sulfur–sulfur bond in acyclic disulfides, and proposed that interconversion between the preferred ground-state enantiomeric +*g* and –*g* conformations proceeds by way of either syn or anti transition states (Fig. 2). A study of the influence of structure on the height of the interconversion barrier for a series of different R groups showed that the syn transition state, previously assumed to be less stable, was actually of lower energy than the anti transition state. The barrier to rotation about the S—S bond was found to increase from E_a = 7.0 to E_a = 9.4 kcal/mol with an increase in substituent size. The apparent steric rate retardation was explained in terms of a strong preference for the syn over the anti transition state. However, Jorgensen and Snyder (21) concluded on the basis of molecular mechanics calculations on dialkyl disulfides that rotation proceeds by way of an anti transition state. Although syn/anti

Fig. 2. Interconversion between preferred ground-state conformations.



Scheme 1.



X = S for disulfide **1**

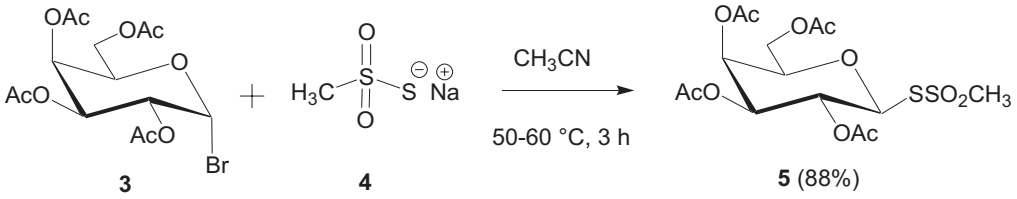
X = Se for selenosulfide **2**

P = protecting group

barriers for simple, uncongested disulfides (e.g., HSSH and CH_3SSCH_3) have been computed at various levels of sophistication (22), the anti energy maximum was always lower than the syn energy maximum. Our own previous study of restricted rotation in hindered dichalcogenides showed that diselenides and ditellurides exhibit similar conformational preferences to disulfides (23).

We chose to examine in detail the conformational preferences in β-linked disaccharides containing disulfide and selenosulfide moieties in the interglycosidic linkage and report herein that these compounds take up the nomal ϕ_H gauche+ conformation about the C-1′—S bond but exhibit restricted rotation about the S—X bond and preferentially populate either the (+*g*) or the (–*g*) conformation about this linkage. Szilágyi et al. (24) and Davis et al. (25) have separately reported the syntheses of symmetric, nonreducing di-

Scheme 2.



saccharides containing interglycosidic (1→1′) disulfide linkages; these compounds also display unusual conformational preferences. In addition, the syntheses of monosaccharides containing sulfenamide aglycons have been described recently (26).

Results and discussion

Synthesis

Retrosynthetic analysis of the target compounds showed that they could be synthesized by the coupling reaction of monosaccharide **A** (a glycosylthio donor) with a monosaccharide **B** containing an appropriate nucleophile at C-4 (Scheme 1).

The synthesis of the glycosyl donor **5** was first attempted by the reaction of acetobromogalactopyranose **3** with sodium thiomesylate **4** (27, 28) in ethanol, as described for the corresponding glucose derivative (29). No desired product was observed even after refluxing. A similar result was obtained when DMF was used as the solvent. However, the displacement reaction proceeded smoothly in acetonitrile at room temperature, to give thiomesylate **5** (Scheme 2).

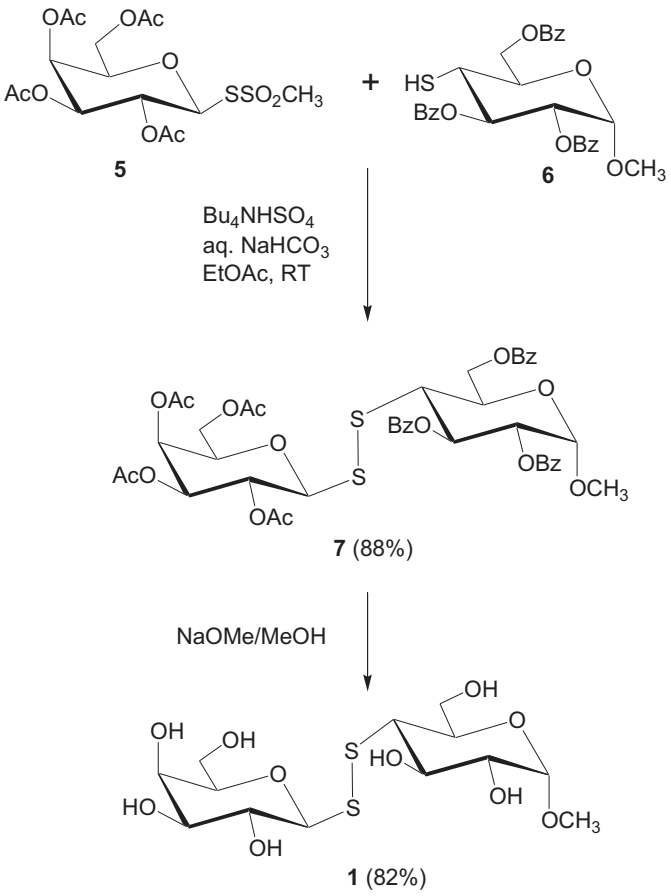
The coupling reaction of thiomesylate **5** with thiol **6** (30) in the presence of a phase-transfer catalyst (tetrabutylammonium hydrogensulfate) and saturated aqueous sodium bicarbonate in EtOAc at room temperature gave the (1′→4) disulfide **7** in excellent yield (Scheme 3). Methanolysis then afforded the target disulfide **1**.

Analogously, the coupling reaction of thiomesylate **5** with selenol **8** (31) gave selenosulfide **9**, (4→4′) diselenide **10** being formed as an oxidative by-product. The target selenosulfide **2** was obtained by deprotection of compound **9** by methanolysis. Similarly, diselenide **11** was obtained by deprotection of (4→4′) diselenide **10** under Zemplén conditions (Scheme 4).

Conformational analysis

The conformational preferences of compounds **7** and **9** may be defined in terms of the φ, ψ, and ω angles shown in Fig. 3. These preferences were probed by means of ^{1}H NMR spectroscopy. The NOESY spectrum for each compound showed cross peaks for H-3/H-1′, H-3/H-5′, and OCH_3/H-5′, in addition to the cross peaks generally expected for the two monosaccharide units. COSY-type cross peaks for strongly coupled resonances could be distinguished from true NOESY correlations by their anti-phase character. The specific NOESY cross peaks suggest that the disaccharides have a preferred –gauche (–*g*) conformation about the ψ angle, although the ω angle could not be defined explicitly with the limited number of observed NOE cross peaks (Fig. 3). A more rigorous analysis by computational methods in the absence of experimental data did not seem warranted for the purposes of this study. Inspection of molecular models suggested that the observed NOEs would be consistent with a conformation in which the $C_1'SXC4$ (X = S, Se) torsion angle is about –60° (Fig. 3). Our assignment of conformational preferences is consistent with those reported by Szilágyi et al. (24) for 1→1′-linked disaccharides containing a disulfide moiety in the interglycosidic linkage.

Scheme 3.



The ^{1}H NMR spectra for compounds **7** and **9** in deuterated toluene showed broad resonances for H-1′, H-5′, H-6A′, and H-6B′ in ring **A**, suggesting that there is exchange on the NMR timescale between conformations in the intermediate exchange regime at room temperature. Heating the samples to about 350 K led to sharp NMR signals, as indicated in Fig. 4 for the case of **7**. As the temperature was lowered below room temperature, the spectra showed coalescence behaviour and eventually, below 200 K, resolution into resonances for two separate conformers at the slow-exchange limit. The ratio of the major conformer to the minor conformer was 3:1 based on integrations of the separate H-4

Scheme 4.

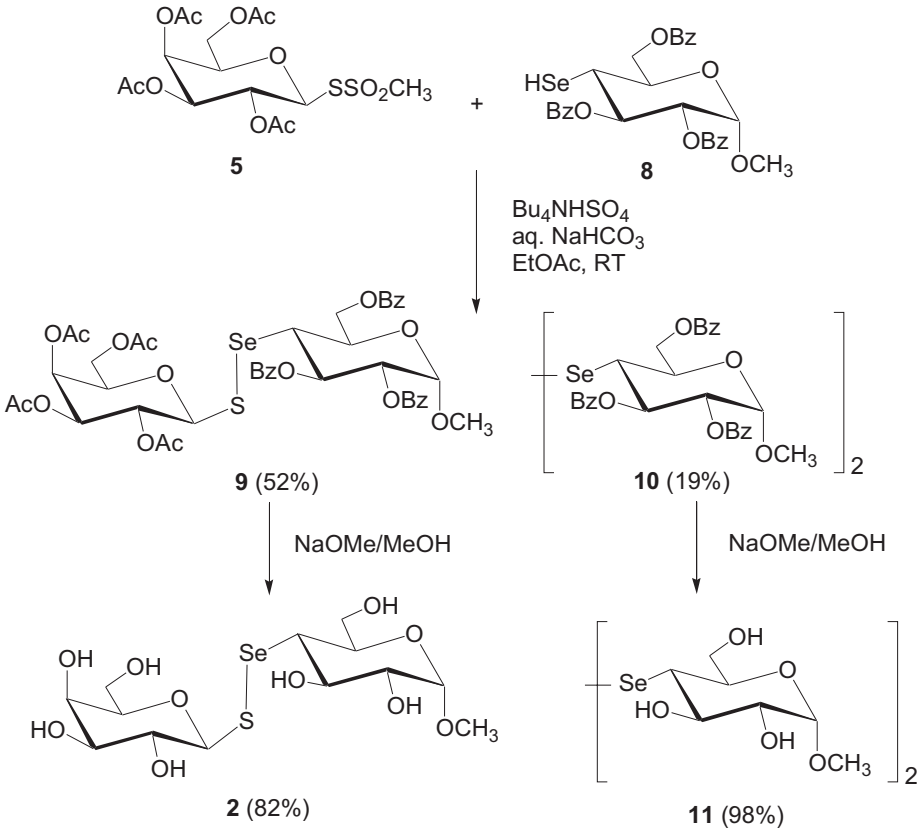
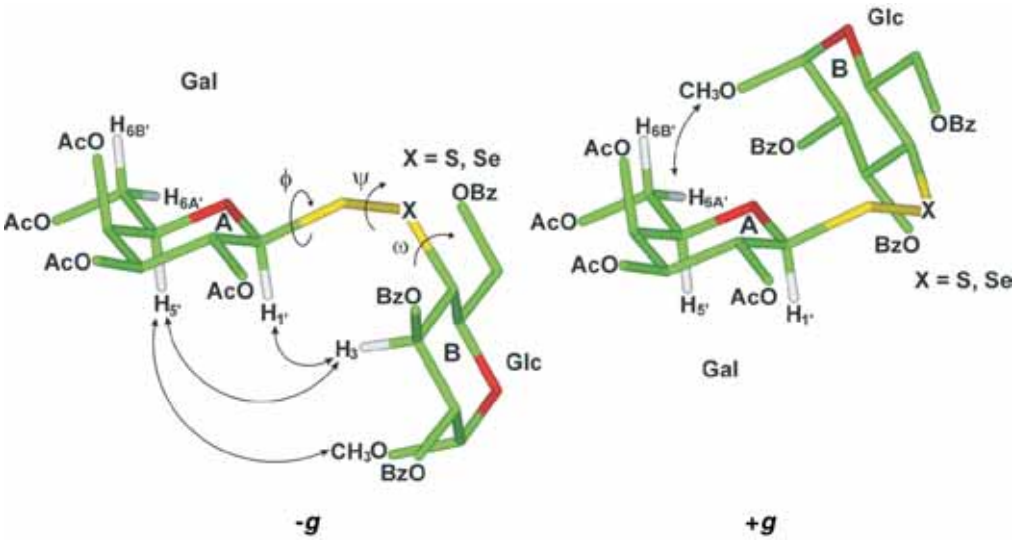


Fig. 3. Preferred conformations of compounds **7** and **9**.



resonances at δ 2.95 and δ 2.45 in the spectrum recorded at 193 K (Fig. 4). Based on these variable-temperature NMR results, we conclude that a second conformation, namely the +gauche (+*g*) conformation (Fig. 3) is also populated. Broadening of the H-1′ and H-5′ signals can then be accounted for by the proximity of ring **B** to ring **A**, in the (–*g*) conformation, where ring **B** is under ring **A**, and broadening of the H-6′ signal by the proximity of the two rings in the (+*g*) conformation, where ring **B** is above ring **A** (Fig. 3). The observations of weak OCH_3/H-6′ correlations in the NOESY spectra lend additional support to the importance of the (+*g*) conformation about the S—X bond in these compounds.

The NOESY spectra for target compounds **1** and **2** showed cross peaks for OCH_3 with the H-5′/H-6′ multiplet. However, the high degree of spectral overlap did not permit unambiguous assignment of the other signals. It seems reasonable that compounds **1** and **2** will have similar

Fig. 4. Variable-temperature ^{1}H NMR spectra of compound **7** in toluene-d_8.

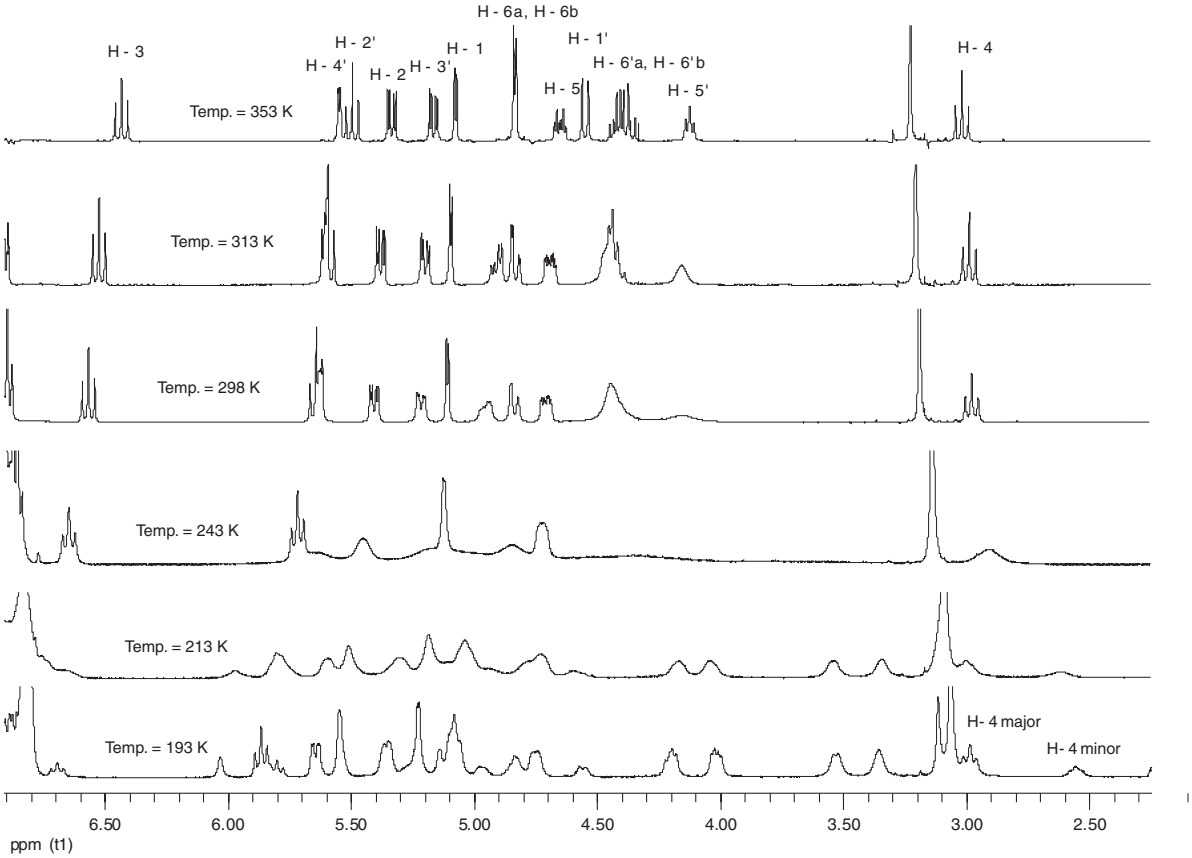
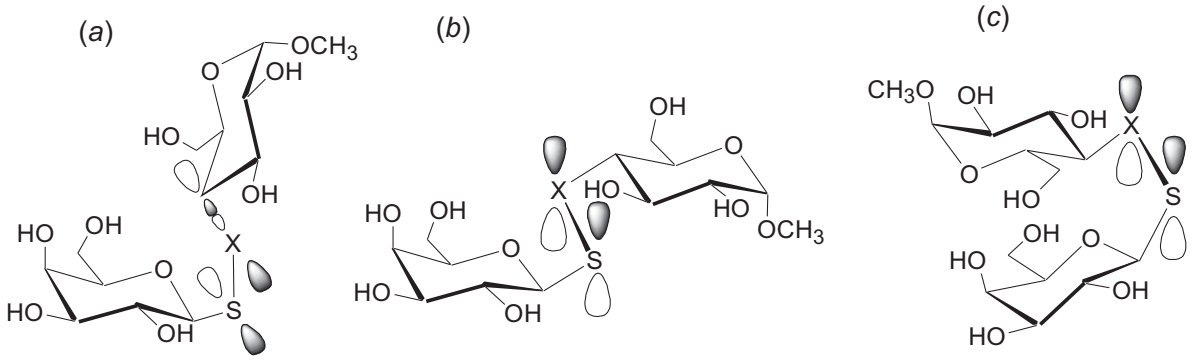


Fig. 5. Fragment molecular orbital interactions in compounds **1** and **2** (X = S, Se).



conformational preferences as their protected precursors. However, in these cases, no significant line-broadening effects were observed at room temperature, indicating that the barrier to rotation about the S—X (X = S, Se) bond is less than in the corresponding protected compounds.

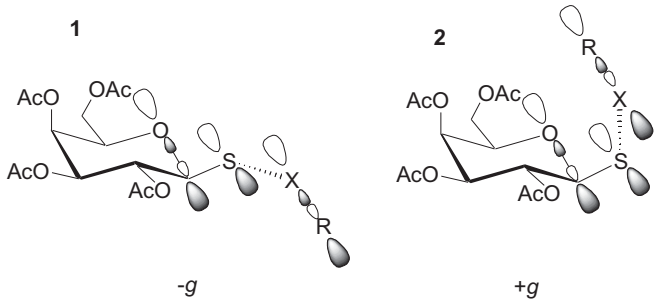
The gauche conformational preferences about the S—S and S—Se bonds are consistent with arguments based on perturbational molecular orbital theory. Thus, an anti or syn preference is disfavored based on a 4e repulsive interaction between the 3*p* orbitals on the sulfur atom with either the S (3*p*) or Se (4*p*) orbital on the adjacent atom (Figs. 5*b*, 5*c*) (32). In contrast, the gauche conformations (e.g., Fig. 5*a*) permit a 2e stabilizing $n_S \rightarrow \sigma^*_{X-C}$ hyperconjugative interaction.

Interestingly, both (+*g*) and (–*g*) conformations also permit expression of the $n_S \rightarrow \sigma^*_{C1-O5\,Gal}$ exo-anomeric interaction (Fig. 6). These two conformations are therefore stabilized by both the exo-anomeric effect and the hyperconjugative interactions associated with the RS–SR′ and RS—SeR′ linkages (Fig. 6). Importantly, the (+*g*) conformation (Fig. 6*b*) places the glucose ring above the galactose ring in a manner similar to the elusive ϕ_H anti conformation in β-linked disaccharides while the (–*g*) conformation places the two sugar rings in a similar orientation to that observed for ordinary disaccharides.

Conclusions

Disaccharide analogues in which the interglycosidic linkage is either a disulfide or selenosulfide moiety have been

Fig. 6. Combined exo-anomeric $n_S \rightarrow \sigma^*_{C\text{-}1\text{-}O\text{-}5}$ and $n_S \rightarrow \sigma^*_{X\text{-}R}$ interactions (X = S, Se).



synthesized. The compounds display interesting conformational preferences, as determined by NOE spectroscopy and line-broadening effects. These preferences are consistent with the operation of the destabilizing and stabilizing orbital interactions associated with the RS–SR′ and RS–SeR′ linkages as well as with the stabilizing $n_S \rightarrow \sigma^*_{C\text{-}1'\text{–}O\text{-}5'}$ exo-anomeric orbital interaction. The conformational preferences suggest a means of expanding the repertoire of carbohydrate topographies for use as biological probes.

Experimental section

General

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured with a Rudolph Research Autopol II automatic polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX-400 NMR spectrometer at 400.13 MHz and 100.6 MHz for proton and carbon, respectively. Chemical shifts are given in ppm downfield from TMS for those spectra measured in $CDCl_3$, CD_3OD, $(CH_3)_2CO$, or toluene-d_8 and from 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) for those spectra measured in D_2O. Chemical shifts and coupling constants were obtained from a first-order analysis of the spectra. All assignments were confirmed with the aid of two-dimensional ^{1}H/^{1}H (COSYDFPT), ^{1}H/^{13}C (INVBTP), ^{1}H (NOESYTP), and ^{1}H (MLEVTP) experiments using standard Bruker pulse programs. Processing of the spectra was performed with standard UXNMR (Bruker) and WINNMR software. Zero filling of the acquired data (512 t_1 values and 2 K data points in t_2) led to a final data matrix of 1 K × 1 K ($F_1 \times F_2$) data points. MALDI-TOF mass spectra were obtained for samples dispersed in a 2,5-dihydroxybenzoic acid matrix using a PerSeptive Biosystems Voyager-DE instrument. Analytical TLC was performed on aluminum plates precoated with Merck silica gel 60F-254 as the adsorbent. The developed plates were air-dried, exposed to UV light and (or) sprayed with a solution containing 1% $Ce(SO_4)_2$ and 1.5% molybdic acid in 10% aq. H_2SO_4 and heated. Compounds were purified by flash column chromatography on Kieselgel 60 (230–400 mesh). Solvents were distilled before use and were dried, as necessary. Solvents were evaporated under reduced pressure and below 50 °C.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl methanethiosulfonate (5)

Acetobromogalactose **3** (1.00 g, 2.43 mmol) was added to a solution of sodium methanethiosulfonate **4** (0.38 g, 2.84 mmol) in acetonitrile (25 mL) at room temperature under nitrogen. The temperature was then slowly raised to 50–60 °C and the reaction mixture was stirred for 3 h until TLC showed completion of the reaction. The suspension was cooled and the solvent was removed. The residue was purified by chromatography on silica gel, (EtOAc–hexane, 7:12) to give the title compound as a white solid (0.95 g, 88%); mp 124–126 °C. $[\alpha]_D^{23}$ +3 (*c* 1.9, $CHCl_3$). ^{1}H NMR (400 MHz, acetone-d_6) δ: 5.50 (1H, dd, $J_{3,4}$ = 3.5 Hz, $J_{4,5}$ = 1.0 Hz, H-4), 5.47 (1H, d, $J_{1,2}$ = 10.4 Hz, H-1), 5.39 (1H, dd, $J_{2,3}$ = 9.9 Hz, H-3), 5.19 (1H, dd, H-2), 4.44 (1H, ddd, H-5), 4.22 (1H, dd, $J_{5,6a}$ = 4.4 Hz, $J_{6a,6b}$ = 11.6 Hz, H-6a), 4.13 (1H, dd, $J_{5,6b}$ = 8.0 Hz, H-6b), 3.56 (3H, s, SSO_2CH_3), 2.15–1.92 (12H, 4s, 4 × OAc). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 170.17–169.69 (CO, 4 × OAc), 86.98 (C-1), 75.37 (C-5), 71.33 (C-3), 67.07 (C-4), 65.79 (C-2), 61.86 (C-6), 52.76 (SSO_2CH_3), 20.62–20.47 (Me, 4 × OAc). Anal. calcd. for $C_{15}H_{22}O_{11}S_2$: C 40.72, H 5.01; found: C 41.01, H 5.05.

Methyl 2,3,6-tri-*O*-benzoyl-4-*S*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl-1′-thio)-4-thio-α-D-glucopyranoside (7)

To a mixture of thiosulfonate **5** (2.14 g, 4.8 mmol) and thiol **6** (2.75 g, 5.1 mmol) in EtOAc (58 mL) was added phase-transfer catalyst, tetrabutylammonium hydrogensulfate (2.16 g, 6.4 mmol), and saturated aqueous sodium hydrogencarbonate (13 mL). The reaction mixture was stirred at room temperature under nitrogen for 18 h and then diluted with EtOAc (100 mL). The organic layer was then washed with saturated sodium chloride solution (2 × 30 mL) followed by water (2 × 30 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated. The crude product was purified by column chromatography (EtOAc–hexane, 1:2). The title compound **7** was obtained as an amorphous white solid (3.97 g, 88%). $[\alpha]_D^{23}$ –30.3 (*c* 3.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.09–7.94 (6H, 3d, 3 × OBz ortho Hs), 7.64–7.34 (9H, m, 3 × OBz meta + para H), 6.23 (1H, dd, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 10.4 Hz, H-3), 5.50 (1H, d, $J_{3',4'}$ = 3.4 Hz, $J_{4',5'} \approx 0.0$ Hz, H-4′), 5.31 (1H, dd, $J_{1',2'}$ = 10.0 Hz, H-2′), 5.20 (1H, dd, $J_{1,2}$ = 3.6 Hz, H-2), 5.17 (1H, d, H-1), 5.13 (1H, dd, $J_{2',3'}$ = 10.1 Hz, H-3′), 4.82 (1H, broad dd, $J_{5,6a}$ = 4.0 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6a), 4.73 (1H, dd, $J_{5,6b}$ = 2.1 Hz, H-6b), 4.53–4.51 (2H, m, H-1′, H-5), 4.44–4.27 (3H, m, H-5′, H-6a′, H-6b′), 3.48 (3H, s, OMe), 3.11 (1H, dd, $J_{4,5}$ = 10.4 Hz, H-4), 2.20–1.99 (12H, 4s, 4 × OAc). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 170.38–169.42 (CO, 4 × OAc), 166.17–165.72 (CO, 3 × OBz), 133.29–128.36 (15C, Ph), 97.18 (C-1), 89.50 (br, C-1′), 74.51 (C-5′), 73.35 (C-2), 71.81 (C-3′), 69.62 (br, C-3), 67.58 (C-5), 66.87 (2C, C-2′, C-4′), 63.56 (C-6), 60.35 (C-6′), 55.67 (OMe), 47.91 (C-4), 20.72–20.56 (Me, 4 × OAc). MALDI-TOF-MS calcd. for $C_{42}H_{44}O_{17}S_2$: 884.20; found: 907.07 $(M + Na)^+$. Anal. calcd. for $C_{42}H_{44}O_{17}S_2$: C 57.01, H 5.01; found: C 57.28, H 5.08.

Methyl-4-*S*-(β-D-galactopyranosyl-1′-thio)-4-thio-α-D-glucopyranoside (1)

The blocked disulfide **7** (1.06 g, 1.19 mmol) was treated with 1 mol/L methanolic sodium methoxide (4 mL) at room temperature for 10 min in dry methanol (40 mL). The reaction mixture was then neutralized to pH 6.4 with Rexyn 101

H^+. The neutralized solution was filtered and evaporated to dryness in vacuo to give a white solid. The crude product was purified by column chromatography on silica gel (EtOAc–methanol–water, 6:3:1) to give the title compound as a white crystalline solid (0.39 g, 82%); mp 151–153 °C (EtOH). $[\alpha]_D^{23}$ –100 (*c* 1.15, D_2O). ^1H NMR (400 MHz, D_2O) δ: 4.81 (1H, d, $J_{1,2}$ = 3.7 Hz, H-1), 4.36 (1H, d, $J_{1',2'}$ = 9.5 Hz, H-1′), 4.07 (1H, dd, $J_{2,3} \approx J_{3,4} \approx$ 9.9 Hz, H-3), 4.00–3.94 (4H, m, H-4′, H-5′, H-6a, H-6b), 3.77 (1H, dd, $J_{2',3'}$ = 9.6 Hz, H-2′), 3.76 (1H, dd, $J_{5,6a}$ = 6.9 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6a′), 3.73–3.66 (3H, m, H-5, H-3′, H-6b′), 3.62 (1H, dd, H-2), 3.40 (3H, s, OMe), 2.65 (1H, dd, $J_{4,5}$ = 10.2 Hz, H-4). ^{13}C NMR (400 MHz, D_2O) δ: 102.06 (C-1), 91.84 (C-1′), 82.23 (C-5′), 76.53 (C-3′), 75.18 (C-2), 73.77 (C-4′), 71.22 (C-5), 71.09 (C-2′), 70.52 (C-3), 64.22 (C-6′), 63.96 (C-6), 57.84 (OMe), 52.61 (C-4). MALDI-TOF-MS calcd. for $C_{13}H_{24}O_{10}S_2$: 404.1; found: 427.3 $(M + Na)^+$. Anal. calcd. for $C_{13}H_{24}O_{10}S_2$: C 38.61, H 5.98; found: C 38.55, H 6.00.

Methyl 2,3,6-tri-*O*-benzoyl-4-*Se*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl-1′-thio)-4-seleno-α-D-glucopyranoside (9) and methyl 2,3,6-tri-*O*-benzoyl-4-seleno-α-D-glucopyranoside diselenide (10)

To a mixture of thiosulfonate **5** (2.20 g, 4.98 mmol) and selenol **8** (3.68 g, 6.29 mmol) in EtOAc (55 mL) was added phase-transfer catalyst, tetrabutylammonium hydrogensulfate (2.56 g, 7.5 mmol), and saturated aqueous sodium hydrogencarbonate (40 mL). The reaction mixture was stirred at room temperature under nitrogen overnight and diluted with EtOAc (150 mL). The organic layer was then washed with saturated sodium chloride solution (2 × 50 mL) followed by water (2 × 100 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated. The crude product was then purified by column chromatography on silica gel (EtOAc–hexane, 1:2.5). The title compound **9** was obtained as a colorless amorphous solid (2.4 g, 52%) and the protected (4→4′) diselenide **10**, a by-product, was obtained as a yellow solid (1.43 g, 38% with respect to selenol **8**). Compound **9**: $[\alpha]_D^{23}$ +66 (*c* 0.5, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ: 8.10–7.94 (6H, 3d, 3 × OBz ortho H), 7.62–7.32 (9H, m, 3 × OBz meta + para H), 6.21 (1H, dd, $J_{2,3}$ = 9.2 Hz, $J_{3,4}$ = 10.7 Hz, H-3), 5.51 (1H, d, $J_{3',4'}$ = 3.4 Hz, $J_{4',5'}$ = 0.0 Hz, H-4′), 5.30 (1H, dd, $J_{1',2'}$ = 10.0 Hz, H-2′), 5.22 (1H, dd, $J_{1,2}$ = 3.6 Hz, H-2), 5.20 (1H, d, H-1), 5.13 (1H, dd, $J_{2',3'}$ = 10.0 Hz, H-3′), 4.89 (1H, broad dd, $J_{6a,6b}$ = 12.1 Hz, H-6a), 4.73 (1H, dd, $J_{5,6b}$ = 2.0 Hz, H-6b), 4.65 (1H, broad d, H-1′), 4.57 (1H, ddd, H-5), 4.40–4.28 (3H, br m, H-5′, H-6a′, H-6b′), 3.48 (3H, s, OMe), 3.30 (1H, dd, H-4), 2.21, 2.02, 2.00, 1.99 (12H, 4s, 4 × OAc). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 170.45–169.54 (CO, 4 × OAc), 166.20, 165.81, 165.57 (CO, 3 × OBz), 133.29–128.37 (18C, 3 × Ph), 97.35 (C-1), 87.0 (C-1′), 74.60 (C-5′), 73.35 (C-2), 71.77 (C-3′), 69.86 (C-3), 68.26 (C-5), 66.85 (C-2′,4′), 64.39 (C-6), 60.36 (C-6′), 55.69 (OMe), 42.60 (C-4), 20.80–20.60 (Me, 4 × OAc). MALDI-TOF-MS calcd. for $C_{42}H_{44}O_{17}SSe$: 932.1; found: 955.5 $(M + Na)^+$. Anal. calcd. for $C_{42}H_{44}O_{17}SSe$: C 54.14, H 4.76; found: C 54.03, H 4.90. Compound **10**: This product was found to have identical physical constants and NMR data to those previously reported (31).

Methyl-4-*Se*-(β-D-galactopyranosyl-1′-thio)-4-seleno-α-D-glucopyranoside (2)

The blocked selenosulfide **9** (2.0 g, 2.15 mmol) was treated with 1 mol/L methanolic sodium methoxide (6 mL) at room temperature for 1 h in dry methanol (80 mL). The reaction mixture was then adjusted to pH 6.4 with Rexyn 101 H^+. The solution was filtered and evaporated to dryness in vacuo to give a white solid. The crude product was purified by column chromatography on silica gel (EtOAc–methanol–water, 6:3:1); the title compound was obtained as a white crystalline solid (0.75 g, 77%); mp 122–124 °C (EtOH). $[\alpha]_D^{23}$ –114 (*c* 2.75, D_2O). ^1H NMR (400 MHz, D_2O) δ: 4.63 (1H, d, $J_{1,2}$ = 3.7 Hz, H-1), 4.37 (1H, d, $J_{1',2'}$ = 8.3 Hz, H-1′), 4.03 (1H, dd, $J_{2,3} \approx J_{3,4}$ = 9.6 Hz, H-3), 4.01–3.93 (4H, m, H-5, H-4′, H-6a, H-6b), 3.79–3.65 (5H, m, H-5′, H-6a′, H-6b′, H-2′, H-3′), 3.62 (1H, dd, H-2), 3.39 (3H, s, OMe), 2.94–2.83 (1H, second order multiplet, H-4). ^{13}C NMR (400 MHz, D_2O) δ: 102.15 (C-1), 90.56 (C-1′), 82.20 (C-5′), 76.39 (C-3′), 75.19 (C-2), 74.31 (C-4′), 72.68 (C-5), 71.39 (C-2′), 71.11 (C-3), 64.94 (C-6′), 63.94 (C-6), 57.87 (OMe), 48.62 (C-4). MALDI-TOF-MS calcd. for $C_{13}H_{24}O_{10}SSe$: 452.0; found: 475.1 $(M + Na)^+$. Anal. calcd. for $C_{13}H_{24}O_{10}SSe$: C 34.59, H 5.36; found: C 34.27, H 5.50.

Methyl 4-seleno-β-D-glucopyranoside diselenide (11)

The protected (4→4′) diselenide **10** (0.725 g, 0.62 mmol) was treated with 1 mol/L methanolic sodium methoxide (4 mL) at room temperature for 2.5 h in dry methanol (40 mL). The reaction mixture was then neutralized to pH 6.2 with Rexyn 101 H^+. The neutralized solution was filtered and evaporated to dryness in vacuo to give a yellow solid. The crude product was purified by column chromatography on silica gel (EtOAc–methanol–water, 6:3:1) to give the title compound as a yellow crystalline solid (0.31 g, 98%); mp 95–97 °C. $[\alpha]_D^{21}$ –77 (*c* 0.4, D_2O). ^1H NMR (400 MHz, D_2O) δ: 4.83 (1H, d, $J_{1,2}$ = 3.4 Hz, H-1), 3.99 (2H, d, H-6a, H-6b), 3.85 (1H, dd, $J_{2,3}$ = 9.7 Hz, H-3), 3.72 (1H, ddd, $J_{5,6a}$ = 2.9 Hz, H-5), 3.64 (1H, dd, H-2), 3.78 (3H, s, OCH_3), 2.85 (1H, dd, $J_{3,4}$ = 10.7 Hz, H-4). MALDI-TOF-MS calcd. for $C_{14}H_{26}O_{10}Se_2$: 514.0; found: 537.2 $(M + Na)^+$. Anal. calcd. for $C_{14}H_{26}O_{10}Se_2$: C 32.82, H 5.12; found: C 33.09, H 5.37.

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