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Carbohydrate Research 338 (2003) 11–18

CARBOHYDRATE RESEARCH

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Thio sugars VII. Effect of 3-deoxy-4-*S*-(β-D-gluco- and β-D-galactopyranosyl)-4-thiodisaccharides and their sulfoxides and sulfones on the viability and growth of selected murine and human tumor cell lines

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Received 29 May 2002; accepted 7 October 2002

Abstract

The first conversion of (1→4)-thiodisaccharides into corresponding sulfoxides and sulfones by conventional oxidation with *m*-chloroperoxybenzoic acid (MCPBA) is reported. The effects of α-(1→4)-3′-deoxythiodisaccharides (**8**–**9**) and their sulfoxide (**14**–**15**) and sulfone (**16**–**17**) derivatives on murine leukemia and human colon and pancreatic carcinoma cell viability were studied. Concentrations of thio-sugars that decreased tumor cell line viability by 50% (IC_{50}), measured via the MTT assay, ranged from 6.4 to 38.3 μg/mL. The effect of α-(1→4)-3′-deoxythiodisaccharide derivatives were most profound on human pancreatic epithelial carcinoma (PANC-1) cells with compounds **8** and **9** having IC_{50} values of 6.4 μg/mL and 8.2 μg/mL, respectively. Sulfone derivatives **16** and **17** also had pronounced effects on PANC-1 cell viability ($IC_{50} = 10.2$ μg/mL and 9.6 μg/mL, respectively). These results indicate that deoxythio-disaccharide analogs generated by functionalization of the universal chiral precursor levoglucosenone may have cytotoxic properties and therapeutic potential as anticancer agents. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Levoglucosenone; Cytotoxic activity of S-(1→4)-thiodisaccharides; Sulfones; MTT assay

1. Introduction

Thio-sugars have attracted wide attention as convenient probes for enzyme inhibition studies.[1–3] Recent evidence suggests that these sulfur derivatives may have therapeutic potential in the treatment of variety of pathological conditions, including cancer and infectious diseases.[4] As part of our continuing interest in the biological activity of thio-sugars, especially as enzyme inhibitors[5–7] and components of sugar-containing antibiotics, we turned our attention to a new method of synthesis of (1→4)-thiodisaccharides[5,8] containing biologically important sugar moieties such as galactose, glucose, fucose and mannose. These compounds are efficiently produced by functionalization of the universal precursor levoglucosenone.

While the specific enzyme inhibitory activity of certain thio-disaccharides has been characterized,[1–3,5–7] the effects of functionalized sulfur-containing carbohydrates, including thio-bridged disaccharides, on cancer cell-line viability or cell growth have not yet been explored in detail. Anticancer and antiinfective activity has been reported with the thioglycolipid[9–11] class of sulfur-containing compounds. These sulfur derivatives possess cytostatic capacity in certain tumor cell lines, which is dependent on anomeric stereochemistry. Representative of this selective anomeric effect is the compound 1-*O*-hexadecyl-3-*S*-(D-glucopyranosyl)-2-*O*-methyl-*sn*-3-thioglycerol, of which the α-anomer inhibited the proliferation of leukemic cell lines, while the β-anomer was found to be inactive.[10] The anomeric mixture of the compound inhibited the proliferation of MCF-7, A427, A549 and T84 cancer cells lines in a dose-dependent manner.[11] Another report on the biological activity of thio-disaccharide-type, thiokojibiose

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PII: S0008-6215(02)00394-4

derivatives (octaacetate and thioacetate)[12] showed anti-HIV activity in CEM and MT-2 cells with $IC_{50} = 51$ and 48 μg/mL, respectively.

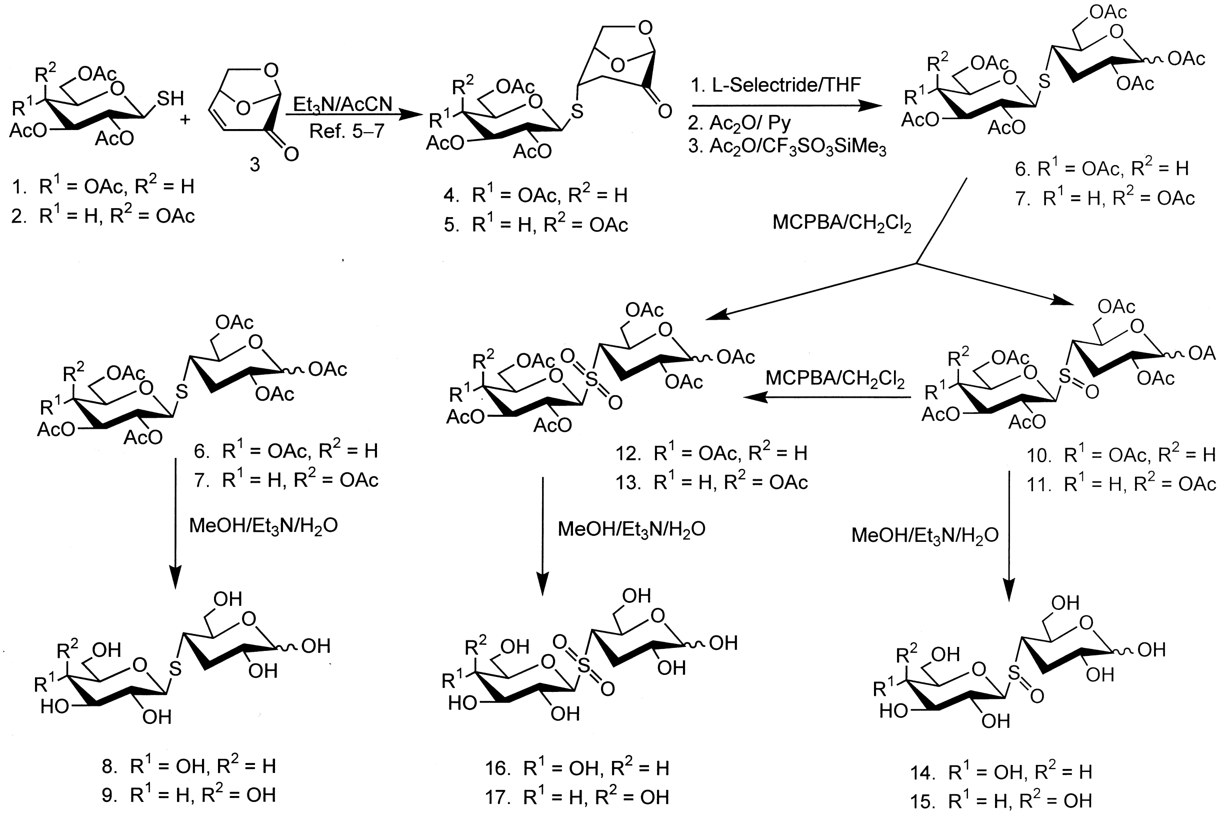
These reports prompted us to test synthesized thio-disaccharides **8**–**9** and their sulfoxide **14**–**15**, and sulfone derivatives **16**–**17** for their cytotoxic activity on cancer cell lines, which is reported in this paper. To date sulfoxides have also been produced by the oxidation of thioglycosides of thio-sugars;[13,14] however, no information on the biological activity of the generated compounds is available. This is a first report on the cytotoxicity of these classes of thio-sugars and (1 → 4)-thiodisaccharide sulfoxides and sulfones (Scheme 1).

2. Materials and methods

2.1. Cell culture and growth assay

Five human and murine tumor cell lines representing leukemia, colon, and pancreatic carcinoma were used in this study. These cell lines are listed in Table 1.

All of the cell lines except for HCT116 were grown in a RPMI-C medium consisting of RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 50 units/mL of penicillin, 50 mg/mL of streptomycin, 25 μg/mL of gentamycin, 0.75% sodium bicarbonate, and 10 mM HEPES buffer



Scheme 1.

Table 1
Tumor cell lines used in this study

Cell Line	Species	Tumor type	Culture	Medium	Trypsin [a]
L1210	Mouse	Lymphocytic leukemia	S [b]	RPMI-C	–
HT-29	Human	Colon adenocarcinoma	ML	RPMI-C	1x
HCT116	Human	Colon carcinoma	ML	RPMI-IC	5x
MiaPaCa-2	Human	Pancreatic carcinoma	ML	RPMI-C	1x
PANC-1	Human	Pancreas epithelioid carcinoma	ML	RPMI-C	5x

[a] Commercially available Trypsin-EDTA (10x) was diluted with phosphate buffered saline to give 1x, 2x, and 5x Trypsin-EDTA solutions.

[b] Suspension cells.

Table 2
Activity of (1 → 4)-thiodisaccharides and their sulfoxides and sulfones on tumor cell viability (IC_{50} in μg/mL)

Compound	L1210	HT-29	HCT116	MiaPaCa-2	PANC-1
8	10.3	30.2	28.4	8.2	6.4
9	14.8	36.4	32.2	10.6	8.2
14	10.1	Not tested	30.3	7.1	7.4
15	13.8	Not tested	36.2	8.1	8.8
16	9.6	Not tested	34.2	9.8	10.2
17	12.8	Not tested	38.3	9.2	9.6

(pH 7.4). The HCT116 cell line was grown in RPMI-IC medium consisting of RPMI-1640. Epithelial tumor cell lines were maintained as confluent monolayers and dissociated by exposure to Trypsin-EDTA prior to seeding on microtiter plates for initiation of the cell viability assay.

2.2. In vitro cell viability assay

Exponentially growing cells ($1.5–2 \times 10^3$ cells) in 0.1-mL of medium were seeded on day 0 in a 96-well microtiter plate. On day 1, 0.1 mL aliquots of medium containing graded concentrations of test agents were added in duplicate to the cell plates. The MTT assay[15,16] was used to measure the effect of thio-sugar analogs **6**–**7** and **8**–**9** activity on cell viability. MTT (3-[4.5-dimethylthiazol-2yl]-2-5-diphenyl-*2H*-tetrazolium bromide, Thiazolyl blue, Sigma Chemical Co, St. Louis, Mo), 5 mg/mL, was added to each culture to equal one tenth the original culture volume, and the plates were incubated for 3 to 4 h at 36 °C. At the end of the incubation period, the medium was removed, and the converted dye was solubilized with acidic 2-propanol (0.04–0.1N HCl in abs 2-propanol). Solubilized dye was measured spectrometrically at 570 nm with background subtraction at 630–690 nm using a Shimadzu UV 1601 spectrophotometer. For the L1210 murine leukemia cell line, the MTT solution was added directly, and samples were triturated. The tetrazolium dye is converted by mitochondrial dehydrogenases[17] of viable cells into purple formazan, and the absorbance of the solubilized formazan solution is proportional to the number of viable cells. In this study, the cells in the well were also inspected under an inverted microscope prior to adding the MTT solution. There was total agreement between manual visual inspection of the plate and the calculated IC_{50} values estimated by the MTT assay. Therefore, the MTT assay was determined to be valid to assess the activity of these new thio-disaccharides on tumor cell viability.

3. Results

The activity of thio-disaccharides **8**–**9**, **14**–**17** on murine leukemia (L1210), human colon tumor cell line (HT-29 and HCT116), and human pancreatic tumor cell lines (PANC-1 and Mia PaCa) was determined by the MTT assay (Table 2).

3.1. Growth inhibitory activity of thio-disaccharide 8

Compound **8** demonstrated modest to good activity on the viability of these cultured tumor cells. A typical example of the concentration-dependant inhibition of the growth of PANC-1 cells is shown in Fig. 1A. Exposure to compound **8** results in a dose-dependent inhibition of PANC-1 cells with an IC_{50} value of 6.4 μg/mL.

3.2. Growth inhibitory activity of thio-disaccharide 9

Compound **9** also demonstrated modest to good growth inhibitory activity against cultured tumor cells. A typical example of the concentration-dependant inhibition of the growth of PANC-1 cells is shown in Fig. 1B. Incubation with compound **9** causes a dose-dependent inhibition of PANC-1 cells with an IC_{50} value of 36.4 μg/mL.

4. Discussion

Both thio-disaccharides (**8** and **9**) are modestly active against the cell lines tested, with concentration that decreased tumor cell viability by 50% (IC_{50} values) ranging from a mean of 10–40 μg/mL, as measured using the MTT assay. According to NCI recommendations,[18] compounds with $IC_{50} < 10$ μg/mL are potentially promising targets for expanded preclinical carcinogenicity testing. These thio-disaccharide compounds are currently being subjected to further in vitro assays in our laboratory.

Since the growth-inhibitory mechanism of action of thio-disaccharides **8**–**9** and their sulfoxide **14**–**15** and sulfone **16**–**17** derivatives remains unknown, it would be important to determine whether compounds **8**–**9** have any antitumor activity *in vivo*. Moreover, the MTT assay may underestimate the intrinsic activity of both thio-disaccharides if these compounds require *in vivo* metabolism to be converted to active molecules. *In vivo* evaluation of these new derivatives would allow this activation to occur in tumor-bearing mice. These studies are currently in progress. These preliminary studies suggest that both thio-disaccharides **8** and **9**, as well as their sulfoxide **14**–**15** and sulfone derivatives **16**–**17**, may have potential as anticancer agents and deserve further biological investigation.

5. Experimental

5.1. General

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. Absorbance was measured at 570 nm on a Schimadzu UV 1601 spectrometer. Thin-layer chromatography (TLC) was performed on precoated Silica Gel 60 F_{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, E. Merck No. 34).

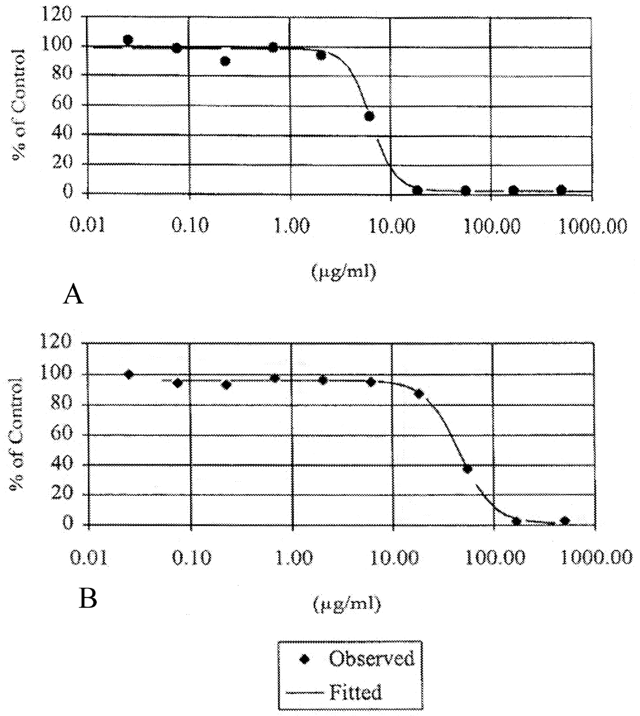


Fig. 1. Concentration-dependent inhibition of Panc-1 cells by thio-disaccharide **8** (A) and HT-29 cells by thio-disaccharide **9** (B).

5.2. ^1H NMR spectra

NMR samples were prepared in $CDCl_3$ (99.8 atom-% D), filtered, freeze-thawed and sealed in 5-mm NMR tubes. 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 the EI mode at 70 eV with a VG 70E mass spectrometer or using CI (NH_3) on a Finnigan 4000 quadrupole high-resolution mass spectrometer.

5.3. General method for the preparation of S-linked 3-deoxy-(1 → 4)-4-thiodisaccharides (4–5)

The general methodology previously reported by us[6] was improved and modified accordingly: To a solution of levoglucosenone[19,20] **3** (126 mg, 1.0 mmol) in acetonitrile (10 mL), a solution of 1-thio-sugar **1**[21] **2**[22] (124 mg, 0.34 mmol) in acetonitrile (5 mL) was added dropwise, followed with addition of Et_3N (0.5 mL). 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 1:4 EtOAc–hexane to give pure syrupy products, which crystallized from ether–hexane.

5.3.1. 1,6-Anhydro-3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-4-thio-β-D-*erythro*-hexopyranos-2-ulose (4)

Yield (149 mg, 89.5%), mp 156–157.5 °C; Lit.[6] mp 157–158.5 °C; R_f 0.41 (1:4, hexane–EtOAc); $[\alpha]_D^{30}$ $-123.2°$ (*c* 0.8, $CHCl_3$); Lit.[6] $[\alpha]_D^{30}$ $-124.21°$ (*c* 0.84, $CHCl_3$).

5.3.2. 1,6-Anhydro-3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-β-D-*erythro*-hexopyranos-2-ulose (5)

Yield (131 mg, 78.5%), mp 151–152.5 °C; R_f 0.45 (1:4, hexane–EtOAc); $[\alpha]_D^{30}$ $-122.2°$ (*c* 0.8, $CHCl_3$); HRMS $(M)^+$ *m*/*z*: Calcd for $C_{20}H_{26}O_{12}S$: 490.1144. Found: 490.1139. The ^1H NMR and ^{13}C NMR spectra of **5** are listed in Tables 3 and 4, respectively.

5.4. General method for the reduction and deprotection of S-linked 3-deoxy-(1 → 4)-4-thiodisaccharides (4–5)

To a cooled and stirred solution of thio-disaccharides **4**–**5** (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 Ac_2O (5 mL) were added and stirred at room temperature overnight. To a cooled solution (0 °C) an additional portion of Ac_2O (10 mL) was added with stirring under Ar, then two drops (3 μL) of trimethylsilyl trifluoromethanesulfonate were added. TLC (1:1 EtOAc–hexane) indicated the completion of the reaction after 12 h. A solution of satd aq $NaHCO_3$ was added, the mixture was stirred for 30 min, and the aq mixture was extracted with EtOAc (3 × 20 mL). The combined extracts were washed with satd aq $NaHCO_3$ (20 mL) and brine (20 mL), and then dried over Na_2SO_4. Evaporation of the solvent under reduced pressure yielded an inseparable anomeric mixture of **6** and **7** (α/β ratio ~ 1:6).

5.4.1. 1,2,6-Tri-*O*-acetyl-3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-α,β-D-*ribo*-hexopyranose (7). Yield (0.62 g, 82%) as a colorless syrup: R_f 0.39, and R_f 0.40 (1:4 hexane–EtOAc); $[\alpha]_D^{30}$ − 12.4° (*c* 0.82, $CHCl_3$); HRMS $(M)^+$ *m*/*z*: Calcd for $C_{26}H_{36}O_{16}S$: 636.1724. Found: 636.1766. The ^1H NMR and ^{13}C NMR spectra of **7** (mainly the β-anomer) are listed in Tables 3 and 4, respectively.

5.5. 3-Deoxy-4-*S*-(β-D-galactopyranosyl)-4-thio-D-*ribo*-hexo-pyranose (9)

Thio-disaccharide **7** (0.250 mg, 0.15 mmol) was dissolved in 4:1:5 MeOH–Et_3N–H_2O (15 mL) and stirred at room temperature. TLC indicated the completion of the reaction after 6 h. Evaporation of the solvent produced an inseparable anomeric mixture (α/β ratio 1:6). Yield (0.117 g, 89%) as a colorless syrup: R_f 0.39 and R_f 0.40 (1:4 hexane–EtOAc); $[\alpha]_D^{30}$ − 12.4° (*c* 0.82, $CHCl_3$); HRMS $(M)^+$ *m*/*z*: Calcd for $C_{12}H_{22}O_9S$: 342.0984. Found: 342.0924. The ^1H NMR and ^{13}C NMR spectra of **9** (mainly the β anomer) are listed in Tables 3 and 4, respectively.

5.6. General method for the oxidation of 3-deoxy-(1 → 4)-*S*-thiodisaccharides (6–7)

Method A. To a cooled and stirred solution of thio-disaccharides **6–7** (421 mg, 0.856 mmol) in ethyl ether (25 mL), MCPBA (0.95 g 2.6 mmol in 25 mL of CH_2Cl_2) was added at 0 °C under an Ar atmosphere. The reaction mixture was stirred for 2 h at room temperature. A solution of satd aq $NaHCO_3$ was added to the reaction mixture and stirred for additional 30 min, and the aq mixture was extracted with EtOAc (3 × 30 mL). The combined extracts were washed with satd aq $NaHCO_3$ (20 mL) and brine (20 mL) and then dried over Na_2SO_4. Removal of the solvent *in vacuo* after co-evaporation with 1:1 toluene–EtOH (5 × 30 mL) produced an oily residue that was subjected to column chromatography on silica gel. The fraction that eluted with 1:4 EtOAc–hexane gave a syrupy epimeric mixture of sulfoxide products **10–11** and a trace amount of the sulfones **12–14**. Yield, 88%.

5.6.1. 3-Deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,2,6-tri-*O*-acetyl-4-thio-α,β-D-*ribo*-hexopyranose *S*-oxide (10). Yield (0.379 g, 88%) as a colorless syrup R_f 0.34, and R_f 0.33 (1:4 hexane–EtOAc); $[\alpha]_D^{30}$ + 10.1° (*c* 0.82, $CHCl_3$); HRMS $(M)^+$ *m*/*z*: Calcd for $C_{26}H_{36}O_{17}S$: 652.1673. Found: 652.1720. The ^1H NMR and ^{13}C NMR spectra of **10** (mainly the β anomer) are listed in Tables 3 and 4, respectively.

5.6.2. 1,2,6-Tri-*O*-acetyl-3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-α,β-D-*ribo*-hexopyranose *S*-oxide (11). Yield (0.379 g, 88%) as a colorless syrup: R_f 0.35, and R_f 0.36 (1:4 hexane–EtOAc); $[\alpha]_D^{30}$ − 13.3° (*c* 0.82, $CHCl_3$); HRMS $(M)^+$ *m*/*z*: Calcd for $C_{26}H_{36}O_{17}S$: 652.1673. Found: 652.1703. The ^1H NMR and ^{13}C NMR spectra of **11** (mainly the β anomer) are listed in Tables 3 and 4, respectively.

Method B. To a cooled and stirred solution of peracetylated sulfoxides of thio-disaccharides **10** and **11** (421 mg, 0.856 mmol) in CH_2Cl_2 (25 mL), MCPBA (0.95 g, 2.6 mL) was added at 0 °C. The reaction mixture was stirred for 3 h and then allowed to continue to reflux for an additional 3 h. The reaction mixture was poured into ice-water and treated with a solution of satd aq $NaHCO_3$. The mixture was stirred for 30 min, and the aq mixture was extracted with EtOAc (3 × 30 mL). The combined extracts were washed with satd aq $NaHCO_3$ (20 mL) and brine (20 mL) and dried over Na_2SO_4. The extract was washed and dried. Removal of the solvent *in vacuo* after co-evaporation with 1:1 toluene–EtOH (5 × 30 mL) afforded an oily residue that was subjected to column chromatography on silica gel. The fraction eluted with 1:4 EtOAc–hexane gave pure syrupy sulfone products **12–13**.

5.6.3. 1,2,6-Tri-*O*-acetyl-3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-4-thio-α,β-D-*ribo*-hexopyranose *S*,*S*-dioxide (12). Yield (0.389 g, 89%) as a colorless syrup: R_f 0.35, and R_f 0.34 (1:4 hexane–EtOAc); $[\alpha]_D^{30}$ + 11.6° (*c* 0.82, $CHCl_3$); HRMS $(M)^+$ *m*/*z*: Calcd for $C_{26}H_{36}O_{18}S$: 668.1622. Found: 668.1723. The ^1H NMR and ^{13}C NMR spectra of **12** (mainly the β anomer) are listed in Tables 3 and 4, respectively.

5.6.4. 1,2,6-Tri-*O*-acetyl-3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-α,β-D-*ribo*-hexopyranose *S*,*S*-dioxide (13). Yield (0.389 g, 88%) as a colorless syrup: R_f 0.35, and R_f 0.34 (1:4 hexane–EtOAc); $[\alpha]_D^{30}$ − 16.1° (*c* 0.82, $CHCl_3$); HRMS $(M)^+$ *m*/*z*: Calcd for $C_{26}H_{36}O_{18}S$: 668.1622. Found: 668.1670. The ^1H NMR and ^{13}C NMR spectra of **13** (mainly the β anomer) are listed in Tables 3 and 4, respectively.

Table 3

^{1}H NMR chemical shifts (δ) and coupling constants (J in Hz) for compounds **5**, **7**, and **9–17** [a]

Compound	H-1′	H-2′	H-3′	H-4′	H-5′	H-6′	H-6′	H-1	H-2	H-3a	H-3e	H-4	H-5	H-6endo	H-6exo	–$COCH_3$
	$J_{1,2}$	$J_{2,3}$	$J_{3',4}$	$J_{4,5}$	$J_{5,6'}$	$J_{5,6''}$	$J_{5,6''}$	$J_{1,2}$	$J_{2,3}$	$J_{3a,4}$	J_{3ae4}	$J_{3,4}$	$J_{5,6ex}$	$J_{5,6endo}$	$J_{6ex, 6en}$	
5	4.66d	5.19dd	5.23t	5.04d	4.82d	4.26dd	4.16d	5.3d		3.12ddd	2.54d	3.56d	4.90dd	3.93dd	4.04dd	2.02–2.1, 4s, 4x OAc
	8.6	9.6	9.9	9.5	5.0	ND [c]	ND [c]	ND [c]	–	7.6	16.6	8.0	4.9	ND [c]	8.2	
7	4.6d	5.19dd	5.23t	5.04d	4.82d	4.26dd	4.06d	4.8d	3.66d	3.12ddd	2.54d	3.56d	4.90dd			2.01–2.1, 7s, 7x OAc
	8.6	9.6	9.9	ND [c]	5.0	ND [c]	ND [c]	8.2	5.0	ND [c]	16.6	8.0	4.9	–	–	
9 [b]	4.66d	5.19dd	5.23t	5.04d	4.82d	3.76dd	4.16d	5.3d	3.66d	3.12ddd	2.64d	3.56d	4.90dd			
	8.6	9.6	9.9	ND [c]	5.0	ND [c]	ND [c]	ND [c]	5.5	ND [c]	16.4	8.0	4.9	–	–	
10	4.66d	5.19dd	5.23t	5.04d	4.82d	4.26dd	4.26d	5.3d	3.66d	3.12ddd	2.54d	3.56d	4.90dd			2.02–2.1, 7s, 7x OAc
	8.6	9.6	9.9	9.5	5.0	ND [c]	ND [c]	ND [c]	5.2	ND [c]	16.6	8.0	4.9	–	–	
11	4.66d	5.19dd	5.23t	5.04d	4.82d	4.26dd	4.26d	5.3d	3.66d	3.12ddd	2.54d	3.56d	4.90dd			2.02–2.1, 7s, 7x OAc
	8.6	9.6	9.9	ND [c]	5.0	ND [c]	ND [c]	8.2	5.0	ND [c]	16.6	8.0	4.9	–	–	
12	4.66d	5.19dd	5.23t	4.65d	4.82d	4.26dd	4.26d	5.4d	3.66d	3.12ddd	2.54d	4.35d	4.48dd			2.02–2.1, 7s, 7x OAc
	8.6	9.6	9.9	9.5	5.0	ND [c]	ND [c]	8.2	5.0	ND [c]	16.6	8.0	4.9	–	–	
13	4.66d	5.19dd	5.23t	5.04d	4.82d	4.26dd	4.26d	4.8d	3.66d	3.12ddd	2.54d	3.56d	4.90dd			2.01–2.1, 7s, 7x OAc
	8.6	9.6	9.9	ND [c]	5.0	ND [c]	ND [c]	8.2	5.0	ND [c]	16.6	8.0	4.9	–	–	
14 [b]	4.6d	5.19dd	5.23t	5.04d	4.82d	4.26dd	4.26d	4.8d	3.66d	3.12ddd	2.54d	3.56d	4.90dd			
	8.6	9.6	9.9	9.5	5.0	ND [c]	ND [c]	8.2	5.0	ND [c]	16.4	8.0	4.9	–	–	
15 [b]	4.86d	5.21dd	5.26t	5.08d	4.84d	3.78dd	4.28d	4.8d	3.58d	3.19ddd	2.54d	3.59d	4.84dd			
	8.2	9.8	10	ND [c]	5.2	ND [c]	ND [c]	8.2	5.2	ND [c]	16.4	8.1	4.8	–	–	
16 [b]	4.86d	5.21dd	5.26t	5.08d	4.84d	3.61dd	4.18d	4.8d	3.58d	3.19ddd	2.54d	3.59d	4.84dd			
	8.2	9.8	10	9.7	5.2	ND [c]	ND [c]	8.2	5.2	ND [c]	16.4	8.1	4.8	–	–	
17 [b]	4.86d	5.21dd	5.26t	5.08d	4.84d	3.78dd	4.18d	4.8d	3.58d	3.19ddd	2.54d	3.59d	4.84dd			
	8.2	9.8	10	ND [c]	5.2	ND [c]	ND [c]	8.2	5.2	ND [c]	16.4	8.1	4.8	–	–	

[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 4
^{13}C NMR chemical shifts (ppm) data for compounds **5**, **7**, and **9**–**17** [a]

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	–OCO	$-CH_3$
5	104.6	207.7	31.2	70.8	78.1	68.6	102.6	67.1	67.4	70.6	76.2	63.8	4x 171.1	17.6, 17.5, 17.5, 17.4
7	96.8	73.7	31.2	65.9	75.0	68.1	101.9	70.7	67.7	69.9	75.0	63.0	7x 176.0	7x 17.6
9 [b]	97.8	74.7	31.2	66.9	78.1	68.1	100.9	67.1	67.7	70.6	76.2	62.8		
10	91.6	73.7	33.1	68.1	78.1	68.6	99.9	67.1	66.8	70.6	76.2	62.8	7x 171.1	2x 17.6, 17.5, 17.5, 2x 17.4
11	91.6	73.7	31.1	68.9	78.0	68.1	100.9	65.7	67.7	72.1	75.0	62.8	7x 176.0	7x 17.6
12	91.6	73.7	33.1	68.1	78.0	68.6	99.9	65.7	66.9	72.1	72.0	62.8	7x 176.0	7x 17.6
13	91.8	73.7	31.2	66.9	78.0	63.1	100.9	70.7	67.7	69.9	72.0	62.8	7x 176.0	7x 17.6
14 [b]	96.8	73.7	33.1	70.6	75.0	68.1	101.8	70.7	66.8	69.9	75.0	63.0		
15 [b]	97.5	74.4	31.7	69.6	75.7	64.8	102.2	75.6	67.2	68.5	75.4	62.8		
16 [b]	96.6	74.4	33.2	70.6	75.7	64.8	101.9	77.0	66.6	68.8	72.4	62.8		
17 [b]	97.6	74.4	31.7	69.6	75.7	64.8	102.1	77.0	67.1	68.8	72.4	62.8		

[a] Determined at 125 MHz in $CDCl_3$ with Me_4Si as the internal reference.

[b] Determined at 125 MHz in D_2O with TMSPA-Na as the internal reference.

5.7. General methods for the deacetylation of sulfoxides 10–11 and sulfones 12–13 of peracetylated 3-deoxy-(1 → 4)-4-thiodisaccharides

Peracetylated sulfoxides (**10**–**11**) and sulfones (**12**–**13**) of the thio-disaccharides (0.250 mg, 0.15 mmol) were dissolved in 4:1:5 MeOH–Et_3N–H_2O (15 mL) and stirred at room temperature. TLC indicated the completion of the reaction after 6 h. Evaporation of the solvent produced an inseparable mixture (α/β, C-1 anomers, in a ratio of 1:3) of sulfoxides **14** and **15** and (α/β, C-1 anomers, in a ratio 1:3) of sulfones **16** and **17**.

5.7.1. 3-Deoxy-4-*S*-(β-D-glucopyranosyl)-4-thio-D-*ribo*-hexopyranose *S*-oxide (14). Yield (122 mg, 89%) as a colorless syrup: $[\alpha]_D^{30}$ $-14.6° \rightarrow -16.2°$ (*c* 0.82, H_2O); HRMS $(M)^+$ *m*/*z*: Calcd for $C_{12}H_{22}O_{10}S$: 358.0933. Found: 358.0956. The ^1H NMR and ^{13}C NMR spectra of **14** are listed in Tables 1 and 2, respectively.

5.7.2. 3-Deoxy-4-*S*-(β-D-galactopyranosyl)-4-thio-D-*ribo*-hexopyranose *S*-oxide (15). Yield (115 mg, 84%) as a colorless syrup: $[\alpha]_D^{30}$ $-12.6° \rightarrow -14°$ (*c* 0.8, H_2O); HRMS $(M)^+$ *m*/*z*: Calcd for $C_{12}H_{22}O_{10}S$: 358.0933. Found: 358.0964. The ^1H NMR and ^{13}C NMR spectra of **15** are listed in Tables 1 and 2, respectively.

5.7.3. 3-Deoxy-4-*S*-(β-D-glucopyranosyl)-4-thio-D-*ribo*-hexopyranose *S*,*S*-dioxide (16). Yield (115 mg, 89%) as a colorless syrup $[\alpha]_D^{30}$ $-10.26° \rightarrow -16.2°$ (*c* 0.82, H_2O); HRMS $(M)^+$ *m*/*z*: Calcd for $C_{12}H_{22}O_{11}S$: 374.0882. Found: 374.0924. The ^1H NMR and ^{13}C NMR spectra of **16** are listed in Tables 1 and 2, respectively.

5.7.4. 3-Deoxy-4-*S*-(β-D-galactopyranosyl)-4-thio-D-*ribo*-hexopyranose *S*,*S*-dioxide (17). Yield (110 mg, 86%) as a colorless syrup: $[\alpha]_D^{30}$ $-12.26° \rightarrow -13°$ (*c* 0.8, H_2O); HRMS $(M)^+$ *m*/*z*: Calcd for $C_{12}H_{22}O_{11}S$: 374.0882. Found: 374.0902. The ^1H NMR and ^{13}C NMR spectra of **17** are listed in Tables 1 and 2, respectively.

Acknowledgements

Financial Support from the American Cancer Society Institutional Grant to University of Connecticut Health Center, ACSIN 152L-132-grant program is gratefully acknowledged.

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