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PAPER

A straightforward synthetic access to symmetrical glycosyl disulfides and biological evaluation thereof†

Matteo Adinolfi,[a] Domenica Capasso,*[b] Sonia Di Gaetano,[c] Alfonso Iadonisi,*[a] Loredana Leone[a] and Antonello Pastore[a]

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Symmetrical glycosyl disulfides can be prepared within a few hours from per-*O*-acetylated precursors *via* a sequential approach entailing short reactions and no purification of any intermediate. The final thiolate-to-disulfide oxidation step is noticeably accelerated by low amounts of phenyl diselenide under air. Applicability of the strategy to non-saccharidic symmetrical alkyl disulfides has also been examined. A preliminary assay of the cytotoxic activity of symmetrical 1,1′- disulfides was performed on two human tumor cell lines, and a noteworthy activity was recorded for a range of these synthetic compounds.

Introduction

Over recent years glycosyl disulfides have been attracting an increasing interest in view of their biological potential. For example, some members of this class of compounds exhibit good affinity towards lectin,[1] and display promising properties against tumor cells.[2] Indeed, the reversible formation of disulfide linkages provides interesting opportunities for finding bioactive compounds through the generation of dynamic libraries from a set of symmetrical disulfides.[3]

Synthesis of symmetrical 1,1′-glycosyl disulfides is commonly achieved by oxidation of the corresponding glycosyl thiols which can be in turn obtained by elaboration of some sulfurated functionalities (thioesters, xanthates, or thiouronium salts)[1,4] installed at the anomeric positions *via* substitution steps conducted on glycosyl bromides or, less frequently, 1-*O*-acetyl sugars. These procedures require isolation and purification of at least a sulfurated saccharidic intermediate (the glycosyl thiol or/and one of its above mentioned precursors). On the other hand, glycosyl disulfides can also be directly accessed from glycosyl bromides by a prolonged treatment (about 24 h) with tetrathiomolybdate reagents.[5] Synthesis of symmetrical glycosyl disulfides has also been occasionally achieved by procedures extensible to the generation of disulfides bearing different saccharidic moieties at either sulfur: also in these procedures purified glycosyl thiols are needed and a variety of *S*-activators have been described for the purpose.[6]

In this paper we wish to report a more streamlined approach where symmetrical glycosyl disulfides are efficiently accessed from cheap per-*O*-acetylated precursors without isolation of any intermediate, glycosyl thiols included. All the steps of the scheme are fast enough so that the targets can be accessed in a matter of a few hours. Furthermore, we also report the noteworthy *in vitro* cytotoxicity of some of these compounds against two human tumor cell lines.

Results and discussion

Synthesis of symmetrical glycosyl disulfides

The approach proposed here has been inspired by a very efficient protocol for the synthesis of alkyl thioglycosides that we have recently described.[7] This protocol takes advantage of the sequential steps illustrated in Scheme 1 in which a per-*O*-acetylated pyranoside is initially converted into the corresponding glycosyl iodide **1** by short exposure to a slight stoichiometric excess of I_2 and Et_3SiH in refluxing DCM.[8] Crude **1**, obtained by a simple extractive work-up, is then converted into the corresponding isothiouronium derivative **2** by treatment with thiourea in acetonitrile at 60 °C.[9] Addition of a base as mild as TEA and a suitable alkylating agent results in the rapid generation of the corresponding *S*-alkyl thioglycoside (Scheme 1, path A).[9]

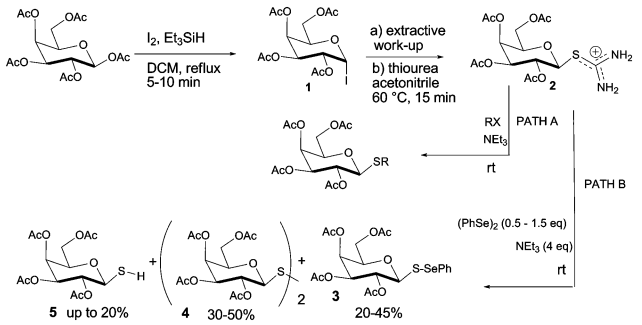
More recently,[10] this scheme was extended to the straightforward synthesis of *S*-(phenylselenyl)thioglycosides[11] as precursors of thioglycosylated indoles which in turn were converted into soluble eumelanin-like polymers. In particular, in the last step of the sequence stoichiometric amounts of phenyl diselenide were added together with TEA to build the S–Se linkage. On performing the synthesis of (phenylselenyl)thio-galactoside **3** (Scheme 1, path

[a]*Dipartimento di Chimica Organica e Biochimica, Università "Federico II" di Napoli, via Cinthia 4, I-80126, Naples, Italy. E-mail: iadonisi@unina.it; Fax: +39 81 674393; Tel: +39 81 674154*

[b]*Facoltà di Scienze Biotecnologiche, Università di Napoli "Federico II", via Mezzocannone 16, 80134, Naples, Italy. E-mail: domenica.capasso@unina.it*

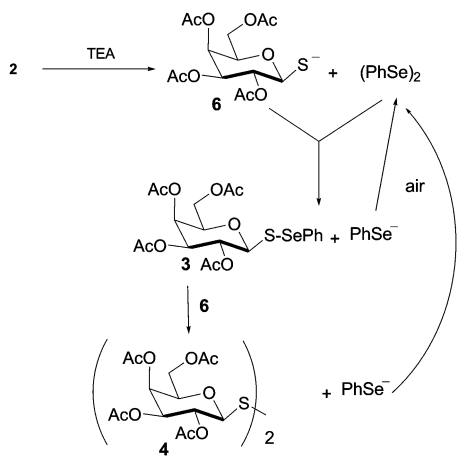
[c]*Istituto di Biostrutture e Bioimmagini, CNR, via Mezzocannone 16, 80134, Naples, Italy*

† Electronic supplementary information (ESI) available: ^1H and ^{13}C NMR spectra of all the synthesized disulfides. See DOI: 10.1039/c1ob05619k



Scheme 1 Synthesis of thioalkyl- and thio(phenylselenyl) galactosides *via* glycosyl iodides.

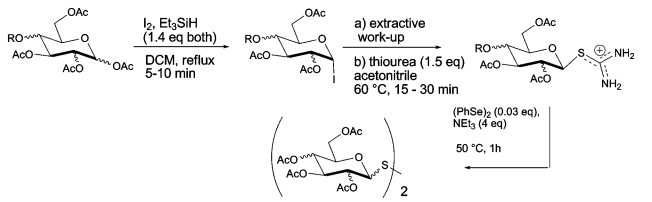
B) under a variety of conditions, we observed that symmetrical galactosyl disulfide **4** was the main by-product of the sequence together with lower amounts of the corresponding glycosyl thiol **5**. This outcome suggested the possibility of generating glycosyl disulfides in high yields by adapting the above sequence and simply adjusting the experimental conditions of the last step. In particular, omitting the addition of phenyl diselenide at this stage of Scheme 1 (path B) was expected, under otherwise identical conditions, to furnish glycosyl disulfides in high yields, logically *via* TEA-induced generation of a glycosyl thiolate and *in situ* oxidation under air. As a matter of fact this idea proved viable, but generation of the desired product took prolonged times at room temperature (overnight stirring). This result appeared rather surprising, as the generation of the same product in acceptable yields (30–50%) took a few minutes when a stoichiometric amount of phenyl diselenide was present. This evidence suggested a possible catalytic role played by phenyl diselenide in generating the symmetrical disulfide **4** through a mechanism in which the *in situ* generated SSe derivative **3** might further react with the glycosyl thiolate **6** as this latter is gradually generated (Scheme 2).[12]

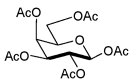
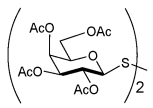
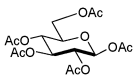
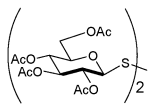
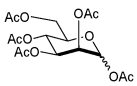
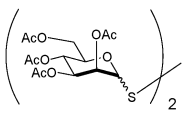
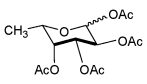
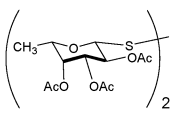
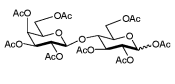
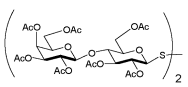


Scheme 2 Hypothesized catalytic role of $(PhSe)_2$ in the synthesis of symmetric glycosyl disulfides.

According to this hypothesis, phenyl selenolate is generated as the leaving group, and this intermediate can be quickly oxidized to the corresponding diselenide performing the reaction under air.[13] As previously suggested by Davis and co-workers,[11] it is interesting to recall that SSe intermediates are also involved in the synthesis of disulfides catalyzed by seleno-enzymes.[14]

Table 1 Synthesis of glycosyl disulfides from per-*O*-acetylated precursors



Entry	Reagent	Product	Yield (dr)[a] (%)
1		 **4**	66 (ββ/βα 9)
2		 **7**	51 (ββ/βα 9)
3		 **8**	44 (αα/αβ 1)[b]
4[c]			46 (αα/αβ 1.5)[b]
5		 **9**	65 (ββ/βα 2.5)
6		 **10**	48 (ββ/βα 16)

[a] Isolated overall yield (diastereoisomeric ratio refers to the anomeric configuration of the two saccharidic residues of the disulfide). [b] Inseparable mixture also containing minor amounts (less than 10%) of the ββ isomer. [c] The last step was performed at rt for 24 h rather than at 50 °C for 1 h.

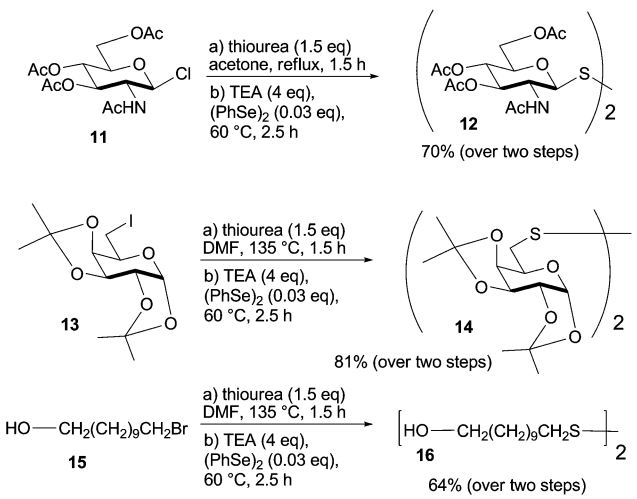
In order to optimize the yields of disulfides, the sequence was repeated starting from per-*O*-acetylated galactose as the model precursor and gradually reducing the amount of phenyl diselenide in the last step, so to minimize the concomitant generation of the (phenylselenyl) thioglycoside **3** while maintaining a faster obtainment of the disulfide product than in the absence of the diselenide catalyst. After several attempts, the best compromise in terms of yield and reduction of experimental time was observed by performing the last step at 50 °C for one hour and in the presence of as low an amount of catalyst as 3%. Under these conditions disulfide **4** was obtained in high overall yield (66% over three steps, Table 1, entry 1).

It is pertinent to note that under otherwise identical conditions, but in the absence of the catalyst, disulfide **4** was obtained in lower yields (less than 50%) within a more complex crude mixture. Also remarkable is that the overall sequence takes shorter experimental times than other described multi-step procedures that often start from more advanced intermediates (glycosyl

bromides rather than per-*O*-acetylated precursors), and that a single purification procedure is required here. On the other hand, minor amounts of the separable αβ-linked disulfide were observed in the resulting mixture.

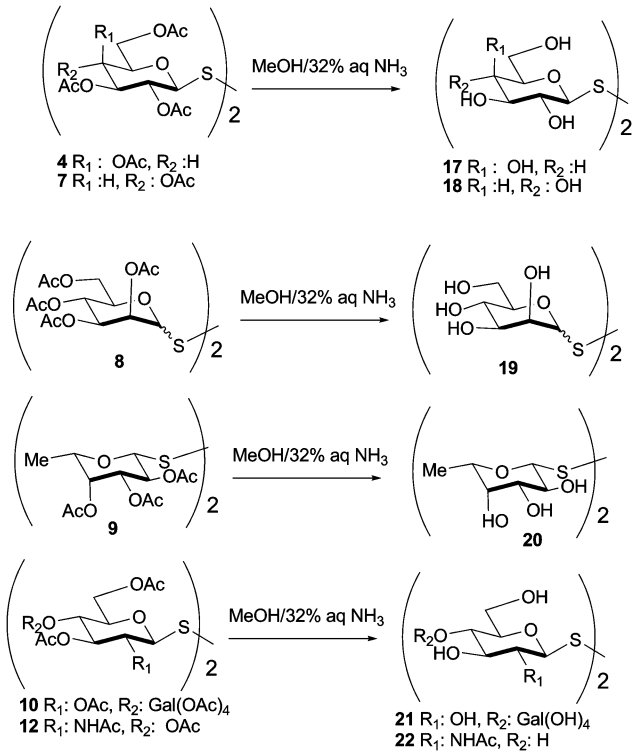
The optimized sequential procedure was applied to a variety of per-*O*-acetylated mono- (Table 1, entries 2–5) and disaccharide (Table 1, entry 6) building blocks and satisfying overall yields were obtained in all cases by performing the last step of the sequence under the same conditions as for the *galacto* precursor. Only in the case of the *manno* precursor (Table 1, entry 3) was the diastereoselectivity of the process poor, with the disulfide products proving to be inseparable. Performing the last step of the sequence at rt for 24 h (Table 1, entry 4) rather than at 50 °C for 1 h (Table 1, entry 3) produced just a slight improvement in the diastereoselectivity and a very similar yield. In all cases, the expected predominance of 1,2-*trans* thioglycoside residues was recorded, consistent with the behaviour observed on applying the procedure to the synthesis of alkyl thioglycosides.[7]

In order to expand the scope of the sequential procedure, structurally different substrates were examined. Due to the easier anomeric functionalization of *N*-acetyl glucosamine with Cl[15] rather than with I,[16] chloride **11**[15] was reacted with thiourea in acetone, according to previously reported conditions[17] and the resulting thiouronium intermediate was submitted to excess TEA and catalytic $(PhSe)_2$ as previously shown in Table 1. Disulfide **12** was obtained in good yield and excellent stereocontrol (Scheme 3). Another interesting application of the protocol was examined on the non-anomeric primary iodide **13** which proved much less reactive than anomeric iodides towards substitution with thiourea, so that a treatment at 135 °C in DMF was required for the first step; subsequent exposure of the thiouronium intermediate to TEA and phenyl diselenide (cat.) at 60 °C yielded disulfide **14** in high overall yield (Scheme 3). The feasible generalization of the strategy to non-saccharidic substrates was tested on 11-bromoundecan-1-ol (**15**) and a satisfying yield of disulfide **16** was again obtained on conducting the initial substitution step at high temperature in DMF (Scheme 3).



Scheme 3 Applications of the strategy to different substrates.

Symmetrical 1,1′-disulfides **4**, **7–10** and **12** were smoothly de-*O*-acetylated in high yields upon exposure to methanol/aq.ammonia (32%) mixtures (Scheme 4), to yield derivatives **17–22** in high yield (over 90% in all cases after chromatographic purification).



Scheme 4 De-*O*-acetylation of symmetrical glycosyl 1,1′-disulfides.

Biological evaluation

The effect of the deprotected symmetric disulfides **17–22** was evaluated for *in vitro* cytotoxicity against two human cancer cell lines (A375 and HepG2) (Fig. 1).

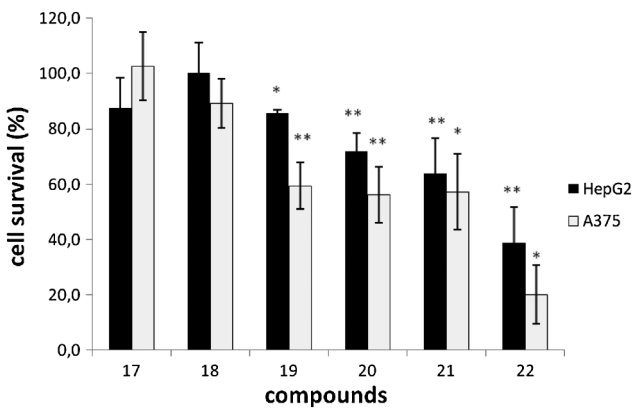


Fig. 1 Cytotoxic effect of **17–22** on A375 and HepG2 cell lines. The cells were incubated in the presence of the compounds at 5 mM concentration at 37 °C for 72 h. Statistical significance was analyzed using Student's *t*-test, paired, two-sided. All experiments were performed in triplicate and repeated at least 3 times (* $p < 0.05$; ** $p \leq 0.005$).

The results obtained showed that some of them highly inhibited the proliferation of the selected cell lines. Experiments performed at 5 mM concentration for 72 h, shown in Fig.1, indicated four molecules (**19–22**) featuring an interesting cytotoxic activity, as

they induced a decrease of cell proliferation ranging from 20% of **19** on HepG2 cells to 80% of **22** on A375 cells. All the active compounds displayed a higher *in vitro* inhibitory activity on the A375 cell line whereas **17** and **18** did not exhibit appreciable activity on the cells tested. The observed cytotoxic effect was not due to osmotic changes, as the parent mono- and disaccharides, devoid of thioglycoside and disulfide linkages, had no effect on cell proliferation at the same concentration (data not shown).

Conclusions

In this paper we have developed a streamlined sequential approach to symmetrical glycosyl disulfides which entails the generation of an isothiouronium key intermediate and its direct elaboration in the presence of a base as mild as TEA and minimal amounts of $(PhSe)_2$. Symmetrical 1,1′-disulfides can be generally obtained from cheap per-*O*-acetylated precursors in a few hours as all the steps are fast enough (especially when intermediate glycosyl iodides can be adopted) and no isolation of any intermediate is required. Additionally, the overall procedure is not especially demanding from an experimental point view, no inert atmosphere being necessary at any stage. Some synthesized disulfides revealed a notable cytotoxic activity against two human tumor cell lines. A more thorough investigation aimed at establishing the mechanism of this inhibitory effect is ongoing and the results will be reported in due course.

Experimental

General methods

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ (internal standard, for ^1H: $CHCl_3$ at δ 7.26; for ^{13}C: $CDCl_3$ at δ 77.0) or D_2O. ^1H NMR assignments were based on homo-decoupling experiments. MALDI-MS spectra were recorded in the positive mode: compounds were dissolved in acetonitrile at a concentration of 0.1 mg mL^{-1} and one microlitre of these solutions was mixed with one microlitre of a 20 mg mL^{-1} solution of 2,5-dihydroxybenzoic acid in 7 : 3 CH_3CN/H_2O. Analytical thin layer chromatography (TLC) was performed on aluminium plates pre-coated with Silica Gel 60 F_{254} as the adsorbent. The plates were developed with 5% H_2SO_4 ethanolic solution and then heating to 130 °C. Column chromatography was performed on silica gel (63–200 mesh). $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}.

General procedure for preparation of symmetrical disulfides 4, 7–10

To a solution of penta-*O*-acetyl galactose (390 mg, 1 mmol) in anhydrous DCM (2 mL) was added I_2 (356 mg, 1.4 mmol), and Et_3SiH (225 μL, 1.4 mmol) (caution: exothermic reaction). The system was refluxed until complete consumption of the starting material (5–10 min as monitored by TLC). The mixture was then diluted with DCM and the organic phase washed with aq. sodium carbonate containing sodium thiosulfate (this latter is added portionwise until consumption of residual iodine in the organic phase). The organic phase was then washed with water, dried, and concentrated. Thiourea (114 mg, 1.5 mmol) was added to the crude residue and the mixture suspended in acetonitrile (2 mL), and then heated to 60 °C. After 15–30 min TLC analysis evidenced complete consumption of the glycosyl iodide and the generation of a polar product. The vessel was cooled to rt and then phenyl diselenide (0.03 mmol, 9.4 mg) and TEA (0.55 mL, 4 mmol) were sequentially added. After 10 min the reaction vessel was placed on an oil bath at 50 °C and kept for an hour at this temperature. The mixture was then concentrated *in vacuo*, the residue dissolved in DCM, and the organic phase washed with water. The aqueous phase was then re-extracted with DCM and combined organic phases were dried and concentrated. Silica gel flash-chromatography of the residue (eluent: hexane/ethyl acetate 6 : 4) yielded disulfide **4** as a foam (240 mg, 66% overall yield).

Synthesis of symmetrical disulfides 12, 14, 16

The compounds were prepared following the same procedures as above described, with differences being essentially in the solvents used, in the temperatures at which the steps are performed and in the corresponding reaction times. These parameters are indicated in each example of Scheme 3.

Bis(2,3,4,6-tetra-*O*-acetyl-1-deoxy-1-thio-β-D-galactopyranosyl) 1,1′-disulfide 4ββ[6c]. R_f 0.4 (3 : 2, ethyl acetate/*n*-hexane); $[\alpha]_D$ −7.6 (*c* 0.90, $CHCl_3$); Found: C, 46.45; H, 5.20. Calcd. for $C_{28}H_{38}O_{18}S_2$: C, 46.28; H, 5.27%); ^1H NMR (500 MHz, $CDCl_3$): δ 5.45 (H-4, bd, J = 3.5 Hz, 1 H), 5.36 (H-2, t, J = 10.0 Hz, 1 H), 5.09 (H-3, dd, J = 3.5 and 10.0 Hz, 1 H), 4.58 (H-1, d, J = 10.0 Hz, 1 H), 4.23 (H-6a, dd, J = 6.5 and 11.5 Hz, 1 H), 4.11 (H-6b, dd, J = 6.5 and 11.5 Hz, 1 H), 4.05 (H-5, bt, J = 6.5 Hz, 1 H), 2.18, 2.09, 2.05, and 1.99 (4 × –$COCH_3$, 4× s, 12 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.9, 169.8, 169.6, and 168.9 (-*C*OCH_3), 87.7 (C-1), 74.3 (C-5), 71.4 (C-3), 67.2, 66.8 (C-2 and C-4), 60.6 (C-6); 20.2 (-CO*C*H_3). MALDI-MS $[M + Na]^+$, found 749.10, calc. 749.15.

Bis(2,3,4,6-tetra-*O*-acetyl-1-deoxy-1-thio-β-D-glucopyranosyl) 1,1′-disulfide 7ββ[4f,5]. R_f 0.4 (3 : 2, ethyl acetate/*n*-hexane); $[\alpha]_D$ –96.8 (*c* 1.49, $CHCl_3$); Found: C, 46.40; H, 5.20. Calcd. for $C_{28}H_{38}O_{18}S_2$: C, 46.28; H, 5.27%); ^1H NMR (500 MHz, $CDCl_3$): δ 5.26 (H-3, t, J = 9.5 Hz, 1H), 5.19 (H-4, t, J = 9.8 Hz, 1H), 5.09 (H-2, t, J = 9.5 Hz, 1H), 4.65 (H-1, d, J = 9.5 Hz, 1H), 4.33 (H-6a, d, J = 4.5 and 12.5 Hz, 1H), 4.22 (H-6b, d, J = 2.0 and 12.5 Hz, 1H), 3.78 (H-5, ddd, 1H), 2.13, 2.10, 2.03, and 2.00 (4× –$COCH_3$, 4× s, 12H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.5, 169.8, 169.1 and 169.0 (-*C*OCH_3), 86.7 (C-1), 75.7 (C-5), 73.5 (C-3), 69.3 (C-2), 67.6 (C-4), 61.3 (C-6); 20.4-20.2 (-CO*C*H_3). MALDI-MS $[M + Na]^+$, found 749.25, calc. 749.15.

Bis(2,3,4,6-tetra-*O*-acetyl-1-deoxy-1-thio-D-mannopyranosyl) 1,1′-disulfide 8 (αα/αβ ca 1 : 1). R_f 0.45 (3 : 2, ethyl acetate/*n*-hexane); ^1H NMR (500 MHz, $CDCl_3$): significant signals of **8αα** anomer at δ 5.40 (H-2, dd, J = 2.0 and 3.0 Hz, 1 H), 5.26 (H-1, d, J = 2.0 Hz, 1 H), 5.25 (H-4, t, J = 10.0 Hz, 1 H), 5.18 (H-3, dd, J = 3.0 and 10.0 Hz, 1 H), 2.09, 2.04, 1.99, 1.94 (4× –$COCH_3$, 4× s, 12 H). Significant signals of **8αβ** anomer at δ 5.51 (H-2 β, bd, J = 3.5 Hz, 1H), 5.42 (H-2 α, bd, J = 1.5 and 3.0 Hz, 1H), 5.33 (H-1 α, d, J = 1.5 Hz, 1 H), 5.11 (H-3 α, dd, J = 3.5 and 10.0 Hz, 1 H), 5.01 (H-3 β, dd, J = 3.5 and 10.0 Hz, 1 H), 4.93 (H-1 β, bs, 1 H), 2.12, 2.08, 2.05, 2.02, 1.99, 1.97, 1.92 and 1.90 (8× –$COCH_3$, 8× s, 24 H). ^{13}C NMR (125 MHz, $CDCl_3$): significant signals of **8αα** at δ 87.2 (C-1), 70.7, 69.4, 68.6 (C-2, C-3, C-5), 65.6 (C-4), 61.7 (C-6); 20.6-20.3 (-CO*C*H_3). Significant signals of **8αβ** at δ 89.4, 89.1 (C-1 α and β), 76.6 (C-5 β), 71.4, 70.3, 69.5 (×2), 68.9 (C-5 α, C-2 and C-3 α and β), 65.9, 64.9 (C-4 α and β), 62.2, and 61.8 (C-6 α and β). MALDI-MS $[M + Na]^+$, found 749.30, calc. 749.15.

Bis(2,3,4,6-tetra-*O*-acetyl-1-deoxy-1-thio-β-L-fucopyranosyl) 1,1′-disulfide 9ββ. R_f 0.55 (3 : 2, ethyl acetate/*n*-hexane); $[\alpha]_D$ −15.9 (*c* 1.93, $CHCl_3$); Found: C, 47.45; H, 5.70. Calcd. for $C_{24}H_{34}O_{14}S_2$: C, 47.21; H, 5.61%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.29 (H-2, t, J = 10.0 Hz, 1 H), 5.24 (H-4, bd, J = 3.0 Hz, 1 H), 5.04 (H-3 dd, J = 3.5 and 10.0 Hz, 1 H), 4.60 (H-1, d, J = 10.0 Hz, 1 H), 3.87 (H-5, bq, J = 6.0 Hz, 1 H), 2.16, 2.06, 1.96 (3× –$COCH_3$, 3× s, 9 H), 1.21 (H_3-6, J = 6.0 Hz, 3 H).^{13}C NMR (50 MHz, $CDCl_3$): δ 170.5, 170.0, 169.4 (-*C*OCH_3), 89.5 (C-1), 73.6 (C-5), 72.2 (C-3), 70.2, 67.6 (C-2 and C-4); 20.7, 20.6, and 20.5 (-CO*C*H_3), 16.3 (C-6). MALDI-MS [M + Na]$^+$, found 633.30, calc. 633.14. Significant data of **9αβ**: ^{1}H NMR (400 MHz, $CDCl_3$) at δ 5.78 (H-1 α, d, J = 5.6 Hz, 1 H), 5.30–5.25 (overlapped signals, 2H), 5.23 (H-4β, bd, J = 3.2 Hz, 1 H), 5.20-5.14 (overlapped signals, 2 H), 5.01 (H-3 β dd, J = 2.8 and 9.8 Hz, 1 H), 4.53 (H-1 β, d, J = 10.0 Hz, 1 H), 4.32 (H-5 α, bq, J = 6.4 Hz, 1 H), 4.32 (H-5 β, bq, J = 6.4 Hz, 1 H), 2.16 (×2), 2.07, 2.05, 1.98, 1.96 (6× –$COCH_3$, 5× s, 18 H), 1.22 and 1.18 (H_3-6 α and β, 2× d, J = 6.4 Hz, 6 H). NMR (100 MHz, $CDCl_3$): δ 170.5, 170.4, 170.0, 169.83, 169.78, 169.6; 90.8 and 88.9 (C-1 α and β), 73.3 (C-5 β), 72.0 (C-3 β), 70.6, 70.1, 68.2, 68.1, 67.3, 66.2 (C-2 and C-4 α and β, C-3 and C-5 α); 20.7, 20.6, 20.5 (-CO*C*H_3).

Bis[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-1-deoxy-1-thio-β-D-glucopyranosyl)] 1,1′-disulfide 10ββ[4d]. R_f 0.45 (4 : 1, ethyl acetate/*n*-hexane); $[\alpha]_D$ −41.4 (*c* 1.52, $CHCl_3$); Found: C, 47.75; H, 5.50. Calcd. for $C_{52}H_{70}O_{34}S_2$: C, 47.92; H, 5.41%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.33 (H-4′, bd, J = 2.8 Hz, 1H), 5.23 (H-3, t, J = 9.2 Hz, 1H), 5.11 (H-2′, dd, J = 8.4 and 10.4 Hz, 1H), 5.05 (H-2, t, J = 10.0 Hz, 1H), 4.96 (H-3′, dd, J = 2.8 and 10.4 Hz, 1H), 4.61 (H-6a, bd, J = 12.0 Hz, 1H), 4.57 (H-1′, d, J = 8.0 Hz, 1H), 4.54 (H-1, d, J = 10.0 Hz, 1H), 4.20–4.00 (H-6b and H_2-6, overlapped signals, 3H), 3.89 (H-5′, bt, J = 6.8 Hz, 1H), 3.69 (H-5, m, 1H), 2.16, 2.14, 2.05, 2.04 (×2), 2.03, 1.96 (7× –$COCH_3$, 6× s, 21H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 170. 1 (×2), 170.0, 169.6, 169.3, 169.0 (-*C*OCH_3), 100.7 (C-1), 86.6 (C-1′), 76.9 (C-5), 75.6 (C-4), 73.7 (C-3), 70.9, 70.5, 70.0, 69.0 (C-2′, C-3′, C-5 and C-2), 66.5 (C-4′), 61.5, 60.5 (C-6 and C-6′); 20.8, 20.7, 20.6, 20.5 (×3), 20.4 (-CO*C*H_3). MALDI-MS [M + Na]$^+$, found 1325.45, calc. 1325.32.

Bis(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-deoxy-1-thio-β-glucopyranosyl) 1,1′-disulfide 12ββ[4d]. R_f 0.45 (92 : 8, dichloromethane/methanol); $[\alpha]_D$ −72.7 (*c* 1.0, $CHCl_3$); Found: C, 46.55; H, 5.45. Calcd. for $C_{28}H_{40}N_2O_{16}S_2$: C, 46.40; H, 5.56%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.24 (NH-2, d, J = 9.0 Hz, 1 H), 5.39 (H-3, t, J = 10.0 Hz, 1 H), 5.00 (H-4, t, J = 10.0 Hz, 1 H), 4.87 (H-1, d, J = 10.5 Hz, 1 H), 4.43 (H-6a, dd, J = 5.5 and 12.5 Hz, 1 H), 4.07 (H-6b, dd, J = 1.5 and 12.5 Hz, 1 H), 4.00 (H-2, m, 1 H), 3.77 (H-5, m, 1 H); 2.12, 2.02 (×2), 2.00 (4× –$COCH_3$, 4× s, 12H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.9, 170.6 (×2) and 169.5 (-*C*OCH_3), 88.7 (C-1), 76.0 (C-5), 72.9 (C-3), 68.4 (C-4), 62.0 (C-6), 53.3 (C-2); 23.3, 20.9, and 20.6 (×2) (-CO*C*H_3). MALDI-MS [M + Na]$^+$, found 747.30, calc. 747.18.

Bis(6-deoxy-6-thio-1,2;3,4-di-*O*-isopropylidene-D-galactopyranose) 6,6′-disulfide 14[18]. R_f 0.40 (3 : 2, *n*-hexane/ethyl acetate); $[\alpha]_D$ −75.3 (*c* 1, $CHCl_3$); Found: C, 52.45; H, 6.85. Calcd. for $C_{24}H_{38}O_{10}S_2$: C, 52.35; H, 6.96%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.51 (H-1, d, J = 4.5 Hz, 1 H), 4.61 (H-3, dd, J = 2.5 and 7.5 Hz, 1 H), 4.34 (H-4, dd, J = 1.5 and 7.5 Hz, 1 H), 4.30 (H-2, dd, J = 2.5 and 4.5 Hz, 1 H), 4.06 (H-5, dt, J = 1.5 and 7.0 Hz, 1 H), 2.92 (H_2-6, d, J = 7.0 Hz, 2 H), 1.55, 1.43, 1.33, and 1.32 (acetonide -CH_3, 4× s, 12H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 109.3 and 108.8 (2× -*C*$(CH_3)_2$), 96.6 (C-1), 71.5 (C-4), 70.9 (C-3), 70.5 (C-2), 66.7 (C-5), 38.2 (C-6); 26.0, 25.9, 25.0 and 24.4 (-C(*C*$H_3)_2$). MALDI-MS [M + Na]$^+$, found 573.25, calc. 573.35.

Bis(11-hydroxyundecanyl) disulfide 16. R_f 0.35 (3 : 2, *n*-hexane/ethyl acetate); Found: C, 65.10; H, 11.570. Calcd. for $C_{22}H_{46}O_2S_2$: C, 64.97; H, 11.40%; ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.62 (t, J = 6.4 Hz, 2H), 2.67 (t, J = 6.4 Hz, 2H), 1.80–1.10 (overlapped signals, 18H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 63.0 (C-1), 39.2 (C-11), 32.7, 29.5, 29.4 (×2), 29.3, 29.2 (×2), 28.5, 25.7 (from C-2 to C-10). MALDI-MS [M + Na]$^+$, found 429.20, calc. 429.28.

General procedure for de-*O*-acetylation of symmetrical 1,1′-disulfides

O-Acetylated 1,1′-disulfides were dissolved at rt in 3 : 1 MeOH/32 aq. ammonia (1.0–1.5 mL/10 mg of substrate). After 3–4 h TLC analysis (eluent AcOEt/MeOH mixtures) typically displayed completion of the reaction and the solvent was removed *in vacuo*. For convenience, some deprotections were occasionally left overnight. The residue was purified by silica gel flash-chromatography (eluent AcOEt/MeOH mixtures, HPLC grade solvents) to yield pure disulfides **17–22**.

Bis(1-deoxy-1-thio-β-D-galactopyranosyl) 1,1′-disulfide 17[3]. R_f 0.15 (3 : 2, ethyl acetate/methanol); ^{1}H NMR (500 MHz, D_2O): δ 4.57 (H-1, d, J = 9.0 Hz, 1 H), 4.00 (H-4, d, J = 3.0 Hz, 1 H), 3.85 (H-4, t, J = 9.0 Hz, 1 H), 3.85–3.75 (overlapped signals, 3H), 3.70 (H-3, dd, J = 3.0 and 9.0 Hz, 1 H). ^{13}C NMR (100 MHz, D_2O): δ 89.9 (C-1), 79.6 (C-5), 73.9 (C-3), 68.8, 68.6 (C-2 and C-4), 61.1 (C-6). MALDI-MS [M + Na]$^+$, found 412.98, calc. 413.06.

Bis(1-deoxy-1-thio-β-D-glucopyranosyl) 1,1′-disulfide 18[3]. R_f 0.15 (3 : 2, ethyl acetate/methanol); ^{1}H NMR (400 MHz, D_2O): δ 4.61 (H-1, d, J = 10.0 Hz, 1 H), 3.93 (H-6a, dd, J = 2.0 and 12.0 Hz, 1 H), 3.75 (H-6b, dd, J = 2.0 and 6.0 Hz, 1 H), 3.62 (t, J = 9.6 Hz, 1 H), 3.55 (t, J = 10.0 Hz, 1 H), 3.52 (H-5, m, 1 H), 3.44 (t, J = 9.6 Hz, 1 H). ^{13}C NMR (100 MHz, D_2O) δ 89.4 (C-1), 80.2 (C-5), 77.0, 71.1, 69.1 (C-3, C-2 and C-4), 60.8 (C-6). MALDI-MS [M + Na]$^+$, found 412.95, calc. 413.06.

Bis(1-deoxy-1-thio-D-mannopyranosyl) 1,1′-disulfide 19 (αα/αβ ca 1 : 1)[3]. R_f 0.15 (3 : 2, ethyl acetate/methanol); Isomer **19 αα**,[3] ^{1}H NMR (400 MHz, D_2O): δ 4.20 (H-2, bs, 1 H), 3.95–3.85 (m, 2 H), 3.85–3.70 (m, 3 H). Significant signals of the αβ diastereoisomer at δ 5.45 (H-1 α, bs, 1H), 5.07 (H-1 β, bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 90.0 (C-1), 74.6, 70.8 (×2) (C-2, C-3 and C-5), 66.8 (C-4), 60.6 (C-6). Significant signals of the αβ diastereoisomer at δ 92.4, 90.6 (C-1 β and α). MALDI-MS [M + Na]$^+$, found 412.96, calc. 413.06.

Bis(1-deoxy-1-thio-β-L-fucopyranosyl) 1,1′-disulfide 20. R_f 0.45 (65 : 35, ethyl acetate/methanol); Found: C, 40.35; H, 6.10. Calcd. for $C_{12}H_{22}O_8S_2$: C, 40.21; H, 6.19%; ^{1}H NMR (400 MHz, D_2O): δ 4.50 (H-1, d, J = 9.6 Hz, 1 H), 3.81 (H-5, bq, J = 6.4 Hz, 1 H), 3.75 (H-4, bs, 1 H), 3.71 (H-2, t, J = 9.6 Hz, 1 H), 3.64 (H-3, bd, J = 9.6 Hz, 1 H), 1.23 (H_3-6, J = 6.0 Hz, 3 H). ^{13}C NMR

(100 MHz, D_2O): δ 90.0 (C-1), 75.5 (C-5), 73.8 (C-3), 71.4, 68.3 (C-2 and C-4), 15.6 (C-6). MALDI-MS [M + Na]$^+$, found 380.98, calc. 381.07.

Bis[β-D-galactopyranosyl-(1→4)-1-deoxy-1-thio-β-D-glucopyranosyl)] 1,1′-disulfide 21[19]. R_f 0.20 (3 : 2, methanol/ethyl acetate); ^{1}H NMR (400 MHz, D_2O): δ 4.63 (H-1′, d, J = 8.8 Hz, 1 H), 4.49 (H-1, d, J = 7.8 Hz, 1 H), 3.99 (H-6a, bd, J = 11.2 Hz, 1 H), 3.94 (H-4′, d, J = 3.2 Hz, 1 H), 3.85–3.70 (overlapped signals, 9H), 3.56 (H-2′, dd, J = 9.6 and 7.8 Hz, 1 H). ^{13}C NMR (100 MHz, D_2O): δ 102.8 (C-1′), 89.2 (C-1), 79.0, 77.6, 75.5, 75.3, 72.5, 70.9 (×2) (C-2′, C-3′, C-5′, C-2, C-3, C-4 and C-5), 68.5 (C-4′), 61.0, 60.0 (C-6 and C-6′). MALDI-MS [M + Na]$^+$, found 737.55, calc. 737.70.

Bis(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-deoxy-1-thio-β-glucopyranosyl) 1,1′-disulfide 22[4d]. R_f 0.5 (1 : 1, ethyl acetate/methanol); ^{1}H NMR (500 MHz, D_2O): δ 4.70 (H-1, d, J = 10.0 Hz, 1H), 3.91 (H-6a, bd, J = 12.0 Hz, 1 H), 3.88 (H-2, t. J = 10.0 Hz, 1 H), 3.91 (H-6a, dd, J = 3.0 and 12.0 Hz, 1 H), 3.52–3.45 (H_2-6, m, 2 H), 2.04 (–$COCH_3$, s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.1 (-*C*OCH_3), 88.5 (C-1), 79.9 (C-5), 74.6 (C-3), 69.2 (C-4), 60.4 (C-6), 53.8 (C-2), 22.0 (CO*C*H_3). MALDI-MS [M + Na]$^+$, found 495.3, calc. 495.11.

Cell culture and proliferation assay

HepG2 and A375 cells were from ATCC. They were maintained in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin, in a humidified 5% CO_2 atmosphere at 37 °C. For the evaluation of the cytotoxic effect of compounds, the exponentially growing A375 and HepG2 cells were seeded at a density of 1.0×10^3 or 3.0×10^3/well respectively, in 96-well flat bottom tissue culture microplates, and incubated with different concentrations of the compounds at 37 °C for 72 h. Each molecule was dissolved in H_2O (except **22** in 5% DMSO). The same volume of vehicle (H_2O or DMSO) was added to cells as control. Cell number was evaluated with crystal violet, which correlates optical density with cell number, according to the procedure described by Gilles *et al.*[20] Cells were fixed by adding a 10% formalin solution and stained with 100 μl of 0.1% crystal violet solution in water for 30 min. Excess dye was removed by washing with deionized water prior to carry out dye solubilization in 10% acetic acid. The optical density of dye extracts was measured directly in plates at 595 nm by using a BioRad microplate Reader Model 680. Statistical significance was analyzed using Student's *t*-test, unpaired, two-sided. All experiments were performed in triplicate and repeated at least three times.

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