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First synthesis and antiprotozoal activities of divinyl sulfone-modified carbohydrates

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ABSTRACT

Divinyl sulfone-modified carbohydrates have been synthesized for the first time by reacting easily available carbohydrate epoxides with thioethanol in a regiospecific fashion. One of the modified divinyl sulfones initiated significant cell death in *Entamoeba* species and the influence of the anomeric configurations on the biological activities of these sugar-derived divinyl sulfones has been highlighted. The most active compound in this series was found to be devoid of any toxicity.

Along with other socio-economic problems in developing countries, diarrhoea is one of the major contributors to childhood mortality and morbidity, causing an estimated 2.5 million deaths per year and long term effect on growth and cognitive functions.[1] Protozoan parasites[2] including *Entamoeba histolytica* are responsible for 34 million to 50 million symptomatic cases of amoebiasis worldwide each year, causing 40–100 thousand deaths annually.[1c–e] It is well established that cysteine proteases are important virulence factors of various infectious agents and the main proteolytic enzymes in many protozoan parasites[1] including *E. histolytica*.[3] *Entamoeba invadens*, the encystation model of parasite *E. histolytica* also reported to have multiple cysteine proteases.[4,5]

Vinyl sulfones in general are known to inhibit cysteine proteases through Micheal addition of the thiol residue on to electron deficient double bond.[6] Although different classes of organic molecules such as peptides, proteins, etc. functionalized with vinyl sulfone group have shown wide-ranging interesting biological compounds, divinyl sulfone (DVS) or 1,1′-sulfonylbisethene ($H_2C{=}CHSO_2CH{=}CH_2$) is capable of reacting with wide-ranging nucleophiles to afford disubstituted or cyclic derivatives. It may be argued therefore that a divinyl sulfone analogue would be more efficient than monovinyl sulfone analogues such that it would be able to alkylate two biological nucleophiles at the same time. Although the biological importance of DVS was recognized way back in 1940s,[7] it was studied recently for its inhibitory properties against glyceraldehyde-3-phosphate dehydrogenase,[8a] inducible VCAM-1 expression,[8b] SrtA, a transpeptidase required for cell wall protein anchoring and virulence in *Staphylococcus aureus*,[8c] cysteine protease of *Plasmodium falciparum*,[8d] etc. Functionalized divinyl sulfones were screened as anti-inflammatory[9a] and tumor cells growth inhibitory[9a] agents.[9b–d] However, the utilization of the DVS functional group in biology is restricted to a great extent because of the non-availability of suitable and efficient strategies for the synthesis of functionalized divinyl sulfones, especially DVS skeleton attached to chiral moieties such as carbohydrates.

Our study required an easy access to relatively large amount of divinyl sulfone-modified carbohydrates. A retrosynthetic analysis of the route to target compounds having divinyl sulfone group in the five-membered rings necessitated the introduction of a $-SCH_2CH_2OH$ group at the C3 position of a furanosyl carbohydrate in. After several trial reactions we concluded that the easiest and general way of forming a C—S bond would be the regioselective opening of epoxides derived from carbohydrates with the sulfur nucleophile generated from mercaptoethanol ($HSCH_2CH_2OH$). Since these vinyl sulfones were to be subjected to biological studies, it was necessary to generate only one regioisomer from these ring opening reactions. We therefore took advantage of the regioselective opening of epoxides imparted by the anomeric configuration of the sugar epoxides. On the other hand, since the reactions of vinyl sulfone-modified pent-2-enopyranosides are influenced to a great extent

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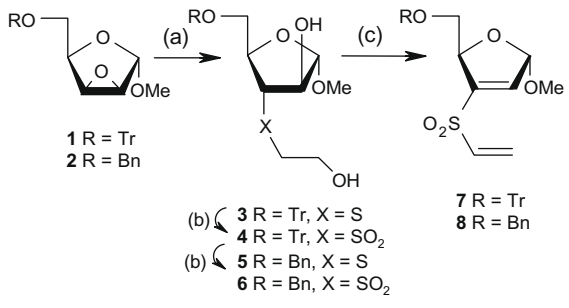
by the anomeric configuration[10] we planned to study the antiparasitic properties of both α- and β-anomeric divinyl sulfones.

Thus, the trityl protected α-anomeric lyxo-epoxide **1**[10] was reacted with mercaptoethanol in the presence of TMG to generate **3**. Compound **3** was oxidized to the sulfone **4** using MMPP. Both the hydroxyl groups of **4** were mesylated and consequent elimination of the mesyl groups in the presence of pyridine produced the desired divinyl sulfone **7** in 64% overall yield. To establish the general applicability of the synthetic strategy and compatibility of this approach with other protecting groups, the benzyl protected α-anomeric epoxide **2**[10] was also reacted with mercaptoethanol in the presence of TMG to generate **5**. Oxidation of **5** to the sulfone **6** and the mesylation of the latter compound afforded the benzyl protected divinyl sulfone **8** in 52% overall yield (Scheme 1).

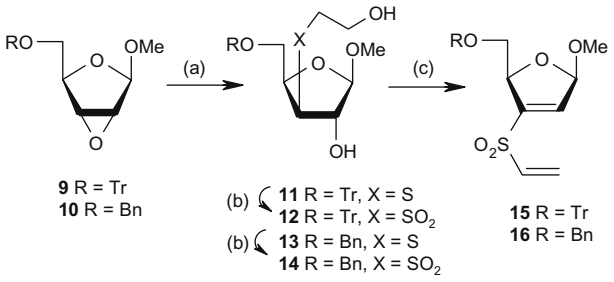
In order to synthesize the β-anomeric analogues of **7** and **8**, the trityl protected β-anomeric ribo-epoxide **9**[10] and the benzyl protected β-anomeric epoxide **10**[10] were converted to thio derivatives **11** and **13**, respectively, following the procedure described in Scheme 1. Compounds **11** and **13** were oxidized to sulfones **12** and **14** in the usual manner. The sulfones were converted to the desired vinyl sulfones **15** (53%) and **16** (45%) via mesylation and elimination (Scheme 2).[11] It should be noted that in both cases (Schemes 1 and 2), only C3-sulfonylated compounds were isolated. Since compounds **3/5** and **11/13** remained contaminated with high-boiling mercaptoethanol, these compounds were directly taken for next synthetic steps and the final vinyl sulfones were obtained as pure materials.

We selected four modified divinyl compounds **7/8** and **15/16** to detect their amoebicidal and growth inhibition effect. *E. histolytica* and *E. invadens* cells were maintained using TYIS-33 medium at 37 and 25 °C, respectively. Stock (10 mM) of **7**, **8**, **15** and **16** was prepared by dissolving them in DMSO. Approximately 5×10^5/mL cells were incubated in presence of modified compounds at concentration ranging from 0 to 100 μM. DMSO and metronidazole were used as negative and positive controls, respectively. After 24 h incubation, cells were washed with 1× PBS and live cells were counted using hemocytometer in presence of trypan blue. All the compounds found to show amoebicidal effect within 12 h. The IC_{50} values of each compound were calculated using Prism Graphpad software (Table 1).

It should be noted that the β-anomeric vinyl sulfones **15**, **16** are more active than their α-anomeric analogues **7**, **8**, respectively (Table 1). This observation highlights the influence of the anomeric configurations on the antiparasitic activities of these compounds which is in line with the reaction patterns of these compounds with nucleophiles such as primary amines. We observed that the α-anomeric vinyl sulfone **7** reacted with one equivalent of benzylamine to produce the cyclic derivative **17** in 48 h whereas the β-anomeric vinyl sulfones **15** under similar reaction conditions produced the cyclic derivative **18** at a much faster rate in 6 h (Scheme 3).[12,13]



Scheme 1. Synthesis of α-anomeric furanosyl-modified divinyl sulfone. Reagents and conditions: (a) mercaptoethanol, TMG, DMF, 5 h, 90 °C; (b) MMPP, MeOH, 6 h, rt; (c) MsCl, py, 0–4 °C, 24 h (for **7**, yield 64%; for **8**, yield 52%) (in three steps).



Scheme 2. Synthesis of β-anomeric furanosyl-modified divinyl sulfone. Reagents and conditions: (a) mercaptoethanol, TMG, DMF, 5 h, 90 °C; (b) MMPP, MeOH, 6 h, rt; (c) MsCl, py, 0–4 °C, 24 h (for **15**, yield 53%; for **16**, yield 45%) (in three steps).

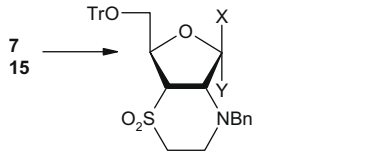
Table 1
IC_{50} values of metronidazole [Mtz], **7, 8, 15**, **16** against *E .histolytica* and *E. invadens*

Compounds	IC_{50} (μM) values	
	E. histolytica	*E. invadens*
Metronidazole	2.5	3.0
7	37.86	25
8	24.35	30.1
15	32.93	17
16	19.5	9.3

IC_{50} values were calculated using Prism Graphpad software. IC_{50} value presented here is the average of at least three experiments done in triplicate.

Cell viability assay for **16**, compound showing the best inhibitory property amongst compounds screened (Table 1) was performed with MCF7 (breast cancer) and AH927 (feline fibroblast) cell lines.[14,15] It has been observed (Fig. 1) that the vinyl sulfone **16** has no cytotoxic effect against both cancer cell (MCF7) and normal cell (AH927).

In summary, the quest for sugar-based antiparasitic new chemical entities has led to the synthesis of densely functionalized divinyl sulfones which are constructed on chiral appendages like pentofuranosyl carbohydrates using easily accessible 2,3-*O*-anhydro sugars as starting materials. One of the divinyl sulfones **16** initiated significant cell death in *E. invadens* and is devoid of any notable toxicity. This preliminary study suggests that the anomeric configuration may have a role in determining the antiparasitic properties of this new class of compounds which is also in line with their chemical properties. This first ever report on the biological properties of a vinyl sulfone-modified carbohydrate in general and divinyl sulfone-modified carbohydrate in particular is expected to trigger research on a new line of antiamoebic agents as well as other antiparasitic drugs.



17 X = H; Y = OMe, Z = Bn
18 X = OMe; Y = H, Z = Bn
19 X = H, Y = OMe, Z = $CH_2CH_2OCOp\text{-}NO_2Ph$
20 X = OMe, Y = H, Z = CH_2CH_2OTr

Scheme 3. Reactions of α- and β-anomeric furanosyl-modified divinyl sulfones. Reagents and conditions: (**17**) benzylamine, MeOH, rt, 48 h, 97%; (**18**) benzylamine, MeOH, rt, 6 h, 95%; (**19**) i—ethanolamine, MeOH, rt, 60 h; ii—*p*-nitrobenzoyl chloride, py, rt, 2 h, 55% (in two steps); (**20**) i—ethanolamine, MeOH, rt, 60 h; ii—trityl chloride, py, rt, 3 days, 58% (in two steps).

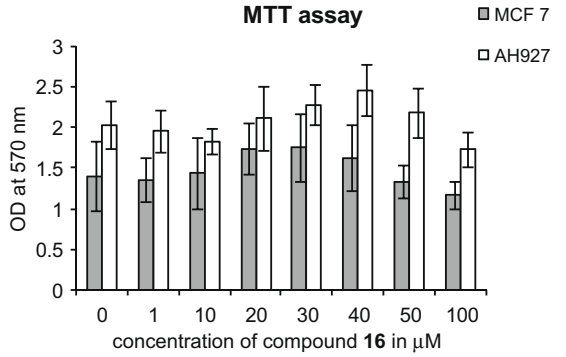


Figure 1. Cell viability assay for **16** using MCF7 (breast cancer) and AH927 (feline fibroblast) cell lines.

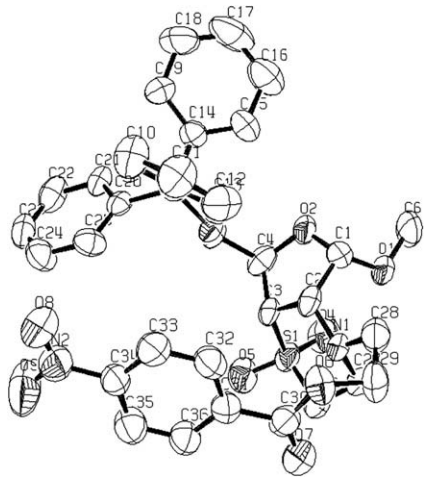


Figure 2. ORTEP diagram of compound **19.**

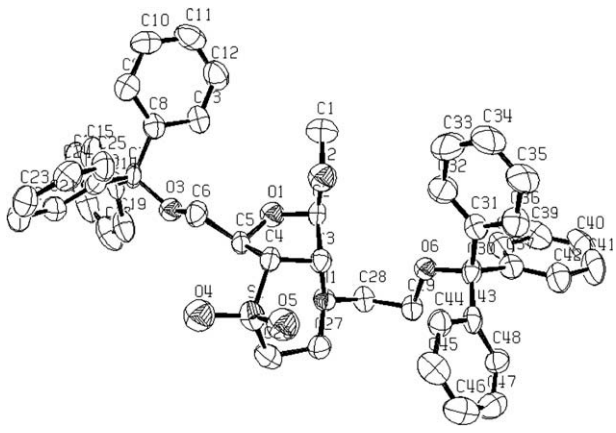


Figure 3. ORTEP diagram of compound **20.**

Acknowledgments

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A. Supplementary data

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication numbers CCDC 682467 and 682468. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk]. Experimental details and spectra of all new compounds associated with this article can be found, in the online version. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.056.

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11. *Compound **16**:* A mixture of mercaptoethanol (2.94 mL, 42.3 mmol) and TMG (3.90 mL, 33.8 mmol) in DMF (10 mL) was heated at 60 °C for 30 min. Compound **10** (1 g, 4.23 mmol) was added to this mixture and the mixture was heated at 90–120 °C for 4–5 h with stirring under N_2. After completion (tlc), the reaction mixture was poured into satd. solution of $NaHCO_3$ (20 mL) and the product was extracted with EtOAc (3× 10 mL). The combined organic layers were dried over anhyd. Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to get a gummy residue. The residue was purified over silica gel column (Eluent/EtOAc/pet ether = 1:1) to get the sulfide **13**. To a well-stirred solution of **13** in dry MeOH (10 mL) was added MMPP (6.22 g, 12.69 mmol) and the mixture was stirred for 6 h under N_2. After completion of reaction (tlc), MeOH was evaporated to dryness under reduced pressure and the residue thus obtained was dissolved in satd. $NaHCO_3$ solution (20 mL). The solution was extracted with EtOAc (3× 10 mL). The combined organic layers were dried over anhyd. Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to get a residue. The residue was purified over silica gel column (Eluent/EtOAc/pet ether = 3:2) to obtain the sulfone **14**. To a well-stirred solution of **14** in pyridine (10 mL) was added a solution of MsCl (1.45 mL, 12.69 mmol) in pyridine (5 mL) dropwise at 0 °C under N_2. After completion of the addition, the reaction mixture was kept at +4 °C. After 24 h (tlc), the reaction mixture was poured into ice-cold water and the solution was extracted with EtOAc (3× 10 mL). The combined organic layers were dried over anhyd. Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to get a residue. The crude material was stirred at room temperature with Et_3N (2 mL) in DCM (10 mL). After 2 h (tlc), the reaction mixture was concentrated under reduced pressure to get a residue. The residue was purified over silica gel column to afford **16** (0.59 g, 45%) in three steps from **10**. Eluent/EtOAc/pet ether (1:3). Gummy liquid. $[\alpha]_D^{25.5}$ (–) 42.24 (c 1.50, $CHCl_3$). ^1H NMR ($CDCl_3$): δ3.45 (s, 3H), 3.62–3.70 (m, 1H), 3.80–3.86 (m, 1H), 4.59 (s, 2H), 5.05–5.08 (m, 1H), 5.77 (br s, 1H), 6.08 (d, J = 9.6 Hz, 1H), 6.42 (d, J = 16.6 Hz, 1H), 6.61–6.74 (m, 2H), 7.31–7.35 (m, 5H). ^{13}C NMR

($CDCl_3$): δ55.4, 71.3 (CH_2), 73.3 (CH_2), 83.1, 107.3, 127.7, 128.3, 130.3 (CH_2), 136.8, 137.7 (C), 138.5, 145.9 (C). HRMS [ES^+, $(M+Na)^+$]: for $C_{15}H_{18}O_5NaS$ Calcd 333.0771, obsd 333.0773.

12. Compounds **8** and **16** also react with benzylamine under similar conditions following the similar pattern. However, in this case, **16** produced a single cyclic product whereas **8** generated an inseparable mixture of cyclic compounds.
13. In order to establish the configurations at C2 and C3 of compounds **17** and **18**, it was necessary to synthesize another set of cyclic compounds **19** and **20** (see Supporting information). The configurations at C2 and C3 of compounds **19** and **20** were unambiguously established with the help of the X-ray analysis of their single crystals (Figs. 2 and 3).
14. Both the cell lines were grown in DMEM supplemented with heat inactivated FBS and antibiotics. Confluent cell from each culture flask was then trypsinized and viable cell count was done by trypan blue solution. Numbers of cells (2×10^3) were seeded in each well in a 96-well flat bottomed cell culture plate and kept at 5% CO_2 and 37 °C. After 36 h compound **16** was added to both the cells with different concentrations ranging from 0 to 100 μM and kept for 48 h. Well without **16** was used as control. After 48 h of treatment with **16**, MTT assay was performed using standard protocol.[15]
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