





Fig. 2 Structure of putative disaccharide inhibitors.

We propose that the IdoA(2S)-GlcNS(6Sulfamate) disaccharide will have superior potency as a minimal fragment Sulf inhibitor compared to the beforementioned reported disaccharide because the substrate specificity studies of the Sulfs point towards this disaccharide unit as being the most frequently desulfated among HS.<sup>8</sup> In this study, we report the re-evaluation of monosaccharide glucosamine-6-*O*-sulfamates as inhibitors of Sulf-2, and the synthesis of putative disaccharide inhibitors using the IdoA(2S)-GlcNS(6S) scaffold as a guide in order to determine whether this fragment size is the shortest effective moiety for HS inhibition.

Putative inhibitors **1** and **2** (Fig. 2) were synthesised according to literature protocols.<sup>5,6</sup> Additionally, a non-hydrolysable analogue **3**, bearing a methylene sulfonamide in the 6-position, was designed and prepared. The key step was the installation of the methylsulfonyl functional group which was achieved *via* nucleophilic substitution of an orthogonally-protected triflate **9**, which was synthesized from glucosamine hydrochloride according to Scheme 1. The 3- and 4-hydroxyls of intermediate **7**<sup>6</sup> were protected by reaction with 2,3-butanedione, trimethyl orthoformate and catalytic sulfonic acid in refluxing methanol, to give **8** as a single diastereomer in 83% yield.

Triflation of **8** was achieved with triflic anhydride in the presence of Et<sub>3</sub>N at 0 °C to give **9** in 58% yield. The nucleophile LiCH<sub>2</sub>SO<sub>2</sub>(DMB)<sub>2</sub> was generated *in situ* by deprotonation of methylsulfonyl CH<sub>3</sub>SO<sub>2</sub>(DMB)<sub>2</sub> with *n*-BuLi at -78 °C.<sup>9</sup> The methylene sulfonamide unit was then introduced by nucleophilic substitution of triflate **9** with LiCH<sub>2</sub>SO<sub>2</sub>N(DMB)<sub>2</sub>

to give fully protected intermediate **10** in 65% yield. Finally, a three-reaction sequence was performed: (1) the carboxybenzyl group was deprotected in 46% yield by palladium-catalyzed hydrogenation; (2) addition of sulfur trioxide pyridine complex to the amine intermediate in water at pH 9–10 resulted in the sulfation of the amino group in 37% yield; (3) deprotection of the two *N*-2,4-dimethoxybenzyl and 3,4-bisacetal units was achieved using TFA in water in 52% yield. Following purification the putative non-hydrolysable inhibitor **3** was isolated as a sodium salt in 7% yield over three steps.

Two disaccharide inhibitors, **4** and **5**, were designed based on the trisulfated disaccharide fragment of HS identified by Sulf substrate specificity studies, incorporating the 6-*O*-sulfamate group, Fig. 2. Retrosynthetic analysis of inhibitor **4** identified key intermediates *p*-tolyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-1-thio-*D*-glucopyranoside **12** prepared from diacetone *D*-glucose, 6 steps, 22% yield<sup>10</sup> and glucosamine glycosyl acceptor **16**, which was synthesized according to Scheme 2. First, the 4-OH and 6-OH of intermediate **7** were protected using a benzylidene acetal, which formed **13** as a single diastereoisomer in 87% yield. Next, the 3-OH was protected using sodium hydride and benzyl bromide in DMF to obtain **14** in 58% yield. Benzylidene acetal **14** was hydrolysed to **15** using 70% acetic acid at 65 °C (63% yield) and finally, silyl ether formation using *tert*-butyldiphenylchlorosilane gave acceptor **16** in 90% yield.

The glycosylation reaction between **12** and **16** was achieved using the NIS/TfOH reagent system to activate the thioglycoside donor to give the desired disaccharide intermediate **17** isolated in 84% yield as a single (alpha) anomer (Scheme 3). Anomeric stereochemistry was assigned by <sup>1</sup>H NMR spectroscopy. Next, the acetate esters were removed under Zemplén conditions to give triol **18** in 98% yield. The primary alcohol was oxidised using catalytic TEMPO and stoichiometric PIDA which produced lactone **19** in 61% yield. Desilylation of compound **19** initially proved challenging due to the instability of the lactone with nucleophiles causing low yields under TBAF deprotection conditions. Even when buffered with acetic acid, only a low yield (45%) of **20** was isolated. The optimised conditions used tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF) that gave an isolated yield of 75%. Next, regioselective sulfamoylation of **20** was achieved under



Scheme 1 Synthesis of inhibitors **1α**, **1β**, **2** and **3**.





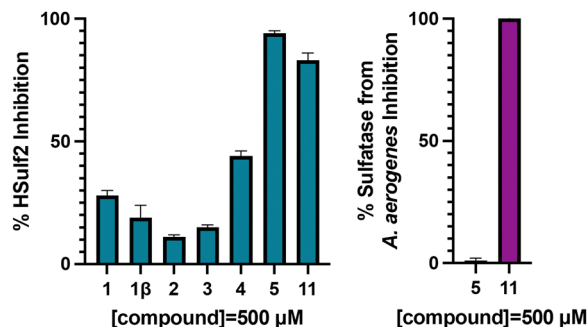


Fig. 3 (left) Sulf-2 and (right) sulfatase from *A. aerogenes* inhibition data for glucosamine-based inhibitors and biphenyl trichloroethylsulfamate **11**. Data represented as the mean  $\pm$  SD, ( $n = 2$ ). Inhibition values are reported as percentages of the uninhibited control values.

complex in basic aqueous medium to afford final compound **5** in 39% over two steps as the tri-sodium salt.

The inhibition of HSulf-2 with inhibitors **1**, **2**, **3**, **4** and **5** was determined using a competition assay with purified, recombinant HSulf-2 and a fluorogenic substrate 4-methylumbelliferyl sulfate (4-MUS) (Fig. 3). HSulf-2 shows pro-tumoral behaviour and therefore is a prime target for the design of inhibitors. Compounds were tested at a single concentration (500  $\mu$ M) to compare inhibitory activity and the  $IC_{50}$  value was determined for the most potent inhibitor. Biphenyl trichloroethylsulfamate **11**<sup>13</sup> (Scheme 4), was included as a benchmark Sulf-2 inhibitor. Inhibition of sulfatase from *Aerobacter aerogenes*, a bacterial sulfatase was used to assess selectivity of the compounds.

In the monosaccharide series, parent glucosamine-6-*O*-sulfamate **1** was found to display weak inhibition of 28% at 500  $\mu$ M, and **1β**, **2** and **3** inhibited Sulf 2 by <15% at 500  $\mu$ M. As predicted, the extension of fragment size to the disaccharide scaffold led to an increased inhibition at 500  $\mu$ M. Disulfated disaccharide **4** inhibited Sulf-2 by 44% and trisulfated disaccharide **5** inhibited Sulf-2 almost completely (95%) at 500  $\mu$ M.

The inhibition of Sulf-2 by compound **11** was evaluated over a concentration range and the  $IC_{50}$  was found to be 39.8  $\mu$ M  $\pm$  17.6 (Fig. S1, ESI<sup>†</sup>). In comparison, the best biphenyl inhibitor reported by Reuillon *et al.*, compound **11**, was reported of having an  $IC_{50}$  value of 167  $\pm$  5  $\mu$ M against Sulf-2. In the present study, compound **11** was used as a benchmark compound and it was found to be less potent than compound **11** (80% vs. 95% inhibition of Sulf-2 at 500  $\mu$ M, Fig. 3). Furthermore, at this single concentration compound **11** exhibited potent inhibition of sulfatase from *A. aerogenes* (100%) compared to compound **5** (1%  $\pm$  1). This shows that compound **5** is more potent and more selective than the previous best inhibitor of Sulf-2 reported in the literature.

A small library of saccharide-based endosulfatase inhibitors was prepared incorporating a 6-sulfamate group in place of the glucosamine 6-*O*-sulfate. The presented study supports previous findings that the replacement of the glucosamine-6-*O*-sulfate with the 6-sulfamate group leads to effective inhibition of HSulf-2 activity. The putative inhibitors were evaluated in a competition assay with recombinant HSulf-2 and a fluorogenic substrate 4-methylumbelliferyl sulfate (4-MUS). Trisulfated **5** was found to be superior to the other inhibitors investigated and is more potent against Sulf-2 and more selective for Sulf-2 vs. other sulfatases than a biphenyl trichloroethylsulfamate inhibitor reported in the literature.<sup>13</sup> We propose that compound **5**, and consequently the disaccharide IdoA(2S)-GlcNS(6S), may represent the minimal-size fragment of HS required for effective inhibition of the endosulfatases. The disaccharide IdoA2S-GlcNS(6S) is not a substrate of the Sulfs,<sup>14</sup> however this fragment-size does efficiently bind to the active site (evidenced by inhibition in the 4-MUS assay), making it a good scaffold for inhibitor design. While inhibitor **5** displays effective inhibition, the fate of **5** in the presence of Sulf-2 remains unknown: whether the C(6)O-S bond is hydrolyzed or **5** simply binds to the active site and functions as a competitive inhibitor requires further investigation.

A. K. is grateful to the EPSRC and OxStem for financial support. S. E. thanks EPSRC SBM CDT (EP/L015838/1) for a studentship. This work was also supported by the "Investissements d'avenir" program Glyco@Alps (ANR-15-IDEX-02) and a grant from the Agence Nationale de la Recherche (ANR-17-CE11-0040). I. B. S. acknowledges integration into the Interdisciplinary Research Institute of Grenoble (IRIG, CEA).

## Conflicts of interest

There are no conflicts to declare.

## References

- (a) E. H. Pempe, T. C. Burch, C. J. Law and J. Liu, *Glycobiology*, 2012, 22, 1353–1362; (b) A. Seffouh, *et al.*, *FASEB J.*, 2013, 27, 2431–2439.
- A. Seffouh, *et al.*, *Cell. Mol. Life Sci.*, 2019, 76, 1807–1819.
- P. C. Billings and M. Pacifici, *Connect. Tissue Res.*, 2015, 56, 272–280.
- (a) E. Hammond, A. Khurana, V. Shridhar and K. Dredge, *Front. Oncol.*, 2014, 4, 195; (b) S. D. Rosen and H. Lemjabbar-Alaoui, *Expert Opin. Ther. Targets*, 2010, 14, 935–949.
- M. Schelwies, *et al.*, *ChemBioChem*, 2010, 11, 2393–2397.
- D. C. Miller, *et al.*, *Org. Biomol. Chem.*, 2015, 13, 5279–5284.
- L. T. Chiu, *et al.*, *J. Am. Chem. Soc.*, 2020, 142, 5282–5292.
- X. B. Ai, *et al.*, *J. Cell Biol.*, 2003, 162, 341–351.
- L. Navidpour, W. Lu and S. D. Taylor, *Org. Lett.*, 2006, 8, 5617–5620.
- T. H. Li, *et al.*, *ChemMedChem*, 2014, 9, 1071–1080.
- K. M. Sureshan, *et al.*, *J. Med. Chem.*, 2012, 55, 1706–1720.
- Y. P. Hu, *et al.*, *Nat. Chem.*, 2011, 3, 557–563.
- T. Reuillon, *et al.*, *Chem. Sci.*, 2016, 7, 2821–2826.
- O. M. Saad, *et al.*, *Glycobiology*, 2005, 15, 818–826.

