

---

Miller DC, Carbain B, Beale GS, Alhasan SF, Reeves HL, Baisch U, Newell DR,  
Golding BT, Griffin RJ.

[Regioselective sulfamoylation at low temperature enables concise syntheses  
of putative small molecule inhibitors of sulfatases.](#)

*Organic & Biomolecular Chemistry* 2015, 13(18), 5279-5284.

This article was published in its final definitive form by the Royal Society of Chemistry, 2015.

**DOI link to published article:**

<http://dx.doi.org/10.1039/c5ob00211g>

**Date deposited:**

22/12/2015

**Embargo release date:**

31 March 2016



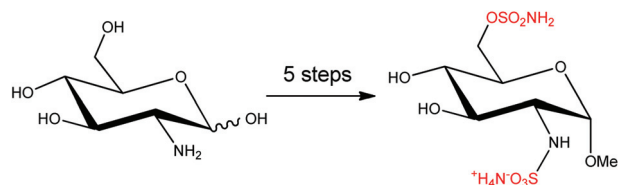
This work is licensed under a [Creative Commons Attribution-NonCommercial 3.0 Unported License](#)

1

**Regioselective sulfamoylation at low temperature enables concise syntheses of putative small molecule inhibitors of sulfatases**

Duncan C. Miller,\* Benoit Carbain, Gary S. Beale, Sari F. Alhasan, Helen L. Reeves, Ulrich Baisch, David R. Newell, Bernard T. Golding\* and Roger J. Griffin

An improved synthesis from D-glucosamine of a purported Sulf-2 inhibitor is described, with assay data for this compound and analogues showing only weak inhibition of Sulf-2, and sulfatases ARSA or ARSB.





## Regioselective sulfamoylation at low temperature enables concise syntheses of putative small molecule inhibitors of sulfatases†

Cite this: DOI: 10.1039/c5ob00211g

Duncan C. Miller,<sup>\*a</sup> Benoit Carbain,<sup>a</sup> Gary S. Beale,<sup>b</sup> Sari F. Alhasan,<sup>b</sup> Helen L. Reeves,<sup>b</sup> Ulrich Baisch,<sup>c,d</sup> David R. Newell,<sup>b</sup> Bernard T. Golding<sup>\*a</sup> and Roger J. Griffin<sup>†a</sup>

Regioselective sulfamoylation of primary hydroxyl groups enabled a 5-step synthesis (overall yield 17%) of the first reported small molecule inhibitor of sulfatase-1 and 2, (2*S*,3*R*,4*R*,5*S*,6*R*)-4,5-dihydroxy-2-methoxy-6-((sulfamoyl-oxy)methyl)tetrahydro-2*H*-pyran-3-yl)sulfamate (methyl 2-deoxy-2-sulfamino-6-*O*-sulfamoyl- $\alpha$ -D-glucopyranoside, **1**), which obviated the use of hydroxyl protecting groups and is a marked improvement on the reported 9-step synthesis (overall yield 9%) employing hazardous trifluoromethylsulfonyl azide. The sulfamoylation methodology was used to prepare a range of derivatives of **1**, and inhibition data was generated for Sulf-2, ARSA and ARSB.

Received 2nd February 2015,  
Accepted 31st March 2015

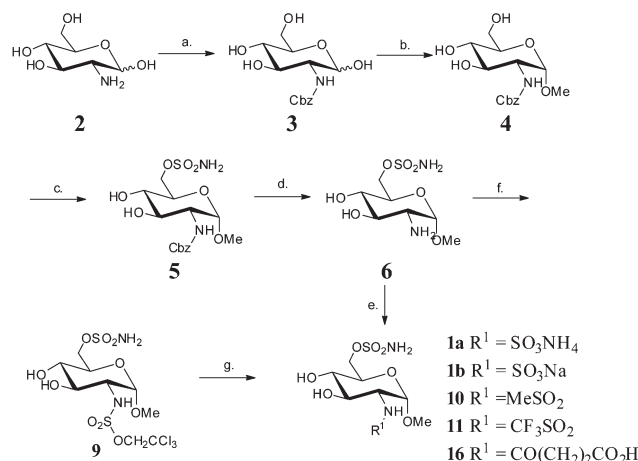
DOI: 10.1039/c5ob00211g

www.rsc.org/obc

### Introduction

The human sulfatase enzymes, sulfatase-1 (Sulf-1) and sulfatase-2 (Sulf-2), were first cloned in 2002<sup>1</sup> and have been implicated in both the FGF and wnt signalling pathways.<sup>2</sup> The FGF signalling pathway is known to affect cell proliferation, invasion, migration and angiogenesis,<sup>3</sup> while the wnt signalling pathway has been implicated in cell growth and proliferation.<sup>4</sup> Sulf-1 and Sulf-2 have selectivity for removing sulfates from the 6-position of glucosamine residues in heparan sulfate proteoglycans (HSPGs), which consist of a protein core conjugated to multiple heparan sulfate polysaccharide chains. The HSPG chains are composed to a large extent of repeating disaccharide units of hexuronic acid (glucuronic acid or iduronic acid) and glucosamine. Weakly active small molecule dual inhibitors of Sulf-1 and Sulf-2 have been reported.<sup>5</sup> The most active inhibitor was (2*S*,3*R*,4*R*,5*S*,6*R*)-4,5-dihydroxy-2-methoxy-6-((sulfamoyl-oxy)methyl)tetrahydro-2*H*-pyran-3-yl)sulfamate (methyl 2-deoxy-2-sulfamino-6-*O*-sulfamoyl- $\alpha$ -D-glucopyranoside, **1**, see

Scheme 1), a derivative of glucosamine sulfate, which bears an *O*-sulfamate at the 6-position and a *N*-sulfate on the amino function. The reported IC<sub>50</sub> values for **1** are 95  $\mu$ M and 130  $\mu$ M against Sulf-1 and Sulf-2, respectively.



**Scheme 1** Synthesis of **1a**, **1b**, **10**, **11** and **16** from D-glucosamine. Reagents and conditions: (a) CbzCl, NaHCO<sub>3</sub>, H<sub>2</sub>O, 92%; (b) HCl–MeOH, 80 °C, 18 h, 73%; (c) Method 1 H<sub>2</sub>NSO<sub>2</sub>Cl, DMA–toluene, –15 °C, 43%; Method 2 H<sub>2</sub>NSO<sub>2</sub>Cl, DMF–CH<sub>3</sub>CN, –40 °C, 55%; Method 3 H<sub>2</sub>NSO<sub>2</sub>Cl, 10% DMA–MeCN, –40 °C, 19%. (d) H<sub>2</sub>/Pd/C, MeOH, 40 °C, 2 h, 99%; (e) R<sup>1</sup> = SO<sub>3</sub>NH<sub>4</sub>: (i) Py·SO<sub>3</sub>, H<sub>2</sub>O, pH 9–10, (ii) EtOAc–MeOH–NH<sub>4</sub>OH, 43%; R<sup>1</sup> = MeSO<sub>2</sub>: MeSO<sub>2</sub>Cl, Et<sub>3</sub>iPrNH, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C–r.t., 1 h, 28%; R<sup>1</sup> = CF<sub>3</sub>SO<sub>2</sub>: (CF<sub>3</sub>SO<sub>2</sub>)<sub>2</sub>O, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>–dioxane, 0 °C, 1 h, 33%. R<sup>1</sup> = CO–(CH<sub>2</sub>)<sub>2</sub>CO<sub>2</sub>H: succinic anhydride, H<sub>2</sub>O–dioxane, r.t., 18 h, 18%; (f) 1,1,1-trichloroethylsulfonil imidazolium tetrafluoroborate (1 eq.), THF, 60 °C, 18 h, 61%; (g) R<sup>1</sup> = SO<sub>3</sub>Na (i) Zn (15 eq.), MeOH, H<sub>2</sub>O, 60 °C, 1 h; (ii) ion exchange chromatography on Dowex 50W8 × 200 Na form, H<sub>2</sub>O, 93%.

<sup>a</sup>Newcastle Cancer Centre, Northern Institute for Cancer Research, School of Chemistry, Bedson Building, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK. E-mail: bernard.golding@newcastle.ac.uk; Fax: +44 (0)191 2226929; Tel: +44 (0)191 2226647

<sup>b</sup>Northern Institute for Cancer Research, Paul O’Gorman Building, Medical School, Newcastle University, Newcastle upon Tyne, NE2 4HH, UK

<sup>c</sup>School of Chemistry, Bedson Building, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK

<sup>d</sup>Department of Chemistry, University of Malta, Msida, MSD 2080, Malta

† Electronic supplementary information (ESI) available. CCDC 1043886. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c5ob00211g

‡ Deceased 24 September 2014.

The published synthesis<sup>5</sup> from (D)-(+)-glucosamine **2** provided compound **1** by a 9-step route in 9% overall yield. The amino group of **2** was converted into azido through a diazo transfer reaction with triflic azide, a reagent with a well-documented explosion hazard.<sup>6</sup> The use of this reagent in the presence of dichloromethane further exacerbates the risk, as this combination can form diazidomethane, another high energy compound with an inherent explosion hazard.<sup>7</sup> The anomeric methoxy group was installed, followed by a sequence of 3 steps requiring benzyl protection of the 3- and 4-hydroxy groups. After sulfamate formation on the unprotected 6-hydroxyl group, the azido group was reduced to an amino function, which was converted to an *N*-sulfate. Finally, deprotection of the 3- and 4-benzyl ethers provided **1**.

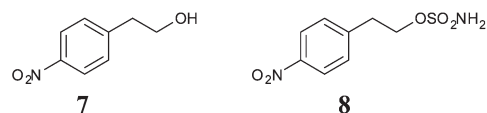
## Improved synthesis of **1**

In order to prepare inhibitor **1** as a tool for further study of the biological implications of inhibition of Sulf-1 and Sulf-2, we investigated an alternative route that avoids triflic azide and minimises the use of protecting groups, thus reducing the number of steps and improving the efficiency of the synthesis. We sought a strategy to compound **1** that dispensed with protection/deprotection at the 3- and 4-positions of glucosamine.<sup>5</sup>

Selective sulfamoylation of the primary hydroxyl groups in di- and tri-hydroxylated compounds using (*N*-(*tert*-butoxycarbonyl)-*N*-[(triethylenedi-ammonium) sulfonyl]azanide) gave only moderate yields and regioselectivity on simple alkanediols, required the synthesis of the reagent and a subsequent acidic deprotection step to liberate the primary sulfamate.<sup>8</sup> We therefore investigated an alternative approach using sulfamoyl chloride as the sulfamoylating reagent, exploiting the differential reactivity of primary *versus* secondary alcohols to achieve direct regioselective sulfamoylation of the *O*<sup>6</sup>-position (Scheme 1).

Protection of glucosamine **2** with a benzylcarbamate (Cbz) group under standard conditions,<sup>9</sup> gave intermediate **3** in 92% yield. Heating **3** in methanolic hydrogen chloride gave a 5 to 1 ratio of  $\alpha$  to  $\beta$  anomers under thermodynamic control. The desired  $\alpha$  anomer **4** was easily separated in 73% yield.

Regioselective sulfamoylation was initially attempted using sulfamoyl chloride (H<sub>2</sub>NSO<sub>2</sub>Cl) prepared by reaction of sulfamoyl isocyanate with formic acid.<sup>10</sup> Suitable solvent systems for sulfamate formation were identified using 4-nitrophenethyl alcohol **7** as a model substrate. For optimisation studies it was preferable to generate H<sub>2</sub>NSO<sub>2</sub>Cl in solution in acetonitrile, prior to addition to substrate in DMA, to allow control of gas evolution on scale-up. Reaction of the acetonitrile solution of H<sub>2</sub>NSO<sub>2</sub>Cl with **7** in DMA (Method 1) resulted in efficient conversion to **8** within 5 minutes at room temperature. Further studies showed that DMF (Method 2) and 10% DMA in acetonitrile (Method 3) were also suitable for the sulfamoylation of **7** to **8**, and allowed the reaction to be performed at -40 °C, giving high conversions after 24 hours and similar reaction profiles.



The developed conditions were applied for sulfamoylation of **4**. After 18 hours at -40 °C, a further 0.15 equivalent of H<sub>2</sub>NSO<sub>2</sub>Cl was added to each reaction and stirring was continued for a further 3 hours. Chromatographic purification led to isolation of 55% product **5** using Method 2, but only 19% with Method 3. Full details of sulfamoylation methods 1–3 are given in the general procedures in the ESI.†

The Cbz protecting group was removed from **5** in quantitative yield under palladium-catalysed flow hydrogenation conditions in methanol to give **6**. Addition of sulfur trioxide-pyridine complex to **6** in an aqueous medium (pH 9–10), resulted in chemoselective sulfation of the amino group to give **1** in 43% yield.<sup>11</sup> Thus, the target monosaccharide **1** was obtained in a 17% overall yield in only five steps (Scheme 1).

An alternative *N*-sulfation process *via* a trichloroethyl protected sulfate provided **1** in a superior yield of 56% over two steps from **6** *via* **9** (Scheme 1). Incorporation of this sulfation protocol into the synthesis of **1** improved the overall yield for the preparation of this key Sulf-2 inhibitor to 21% over six steps.

## Synthesis of analogues of **1**

From the homology model of *F. heparinum* sulfatase, it has been proposed that the sulfate on *N*<sup>2</sup> of the glucosamine residue in heparan sulfate may affect binding solely by enhancing the hydrogen bond donor ability of the *N*<sup>2</sup>-hydrogen atom.<sup>12</sup> To explore this hypothesis for Sulf-2, sulfonamides **10** and **11** that would affect NH acidity were synthesised as shown in Scheme 1.

Slow evaporation of a dilute methanolic solution of **10** provided a crystal suitable for small molecule X-ray crystallography. The structure (Fig. 1) confirmed the assignment of the *O*<sup>6</sup>-sulfamate regiochemistry and that as expected, the pyranose core adopts a chair conformation. The amino hydrogen atoms of *N*<sup>1</sup> and *N*<sup>2</sup> and the oxygen atoms of the sulfonyl

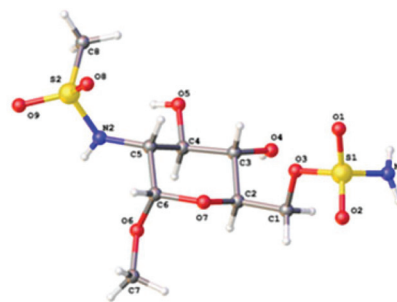


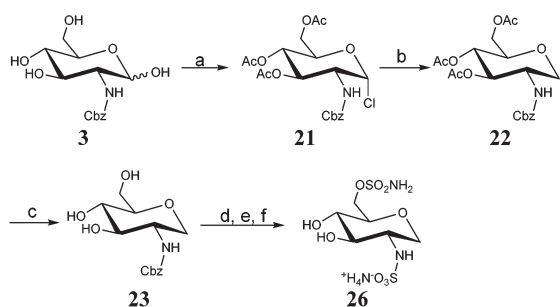
Fig. 1 Small molecule X-ray crystal structure of **10**.

groups are in ideal positions to form moderate to strong hydrogen bonds. Sulfonamides are amongst the strongest H-bond donors, with N(H)⋯O distances ranging between 2.7 Å (270 pm) and 3.2 Å.<sup>13</sup> Molecules of **10** form a 2-dimensional network of double layered sheets comprising hydrogen bonds (O–H⋯O and NH⋯O) with donor–acceptor distances of 2.85–3.10 Å. The H-bond distances are in the range reported for amido-functionalised monosaccharides.<sup>14</sup>

Succinamide derivative **16** was prepared by reaction of **6** with succinic anhydride (Scheme 1). The importance of the anomeric stereochemistry of the monosaccharide template was investigated by preparing β anomer **20** (see ESI: Scheme S1†). Reaction of **3** with a 1.25 M methanolic HCl solution for 1 hour gave a 3 : 2 ratio of α to β anomers, allowing isolation of a 37% yield of **4** and 21% of **17**. Sulfamoylation of **17** using Method 1 gave a 19% yield of **18**, which was converted into target **20** with no epimerisation at the anomeric centre.

To assess whether the *N*-sulfate could be replaced by a hydroxyl group, analogues were prepared in a single step from commercially available methyl α-D-glucopyranoside **12** and methyl α-D-mannopyranoside **14** using sulfamoylation Method 2. The reaction did not proceed cleanly and isolated yields of only 25% of **13** and 9% of **15** were obtained. Sulfamoylation of the 2-position is a likely confounding factor in this reaction, consistent with the empirically observed order of reactivity of the hydroxyl groups of glucopyranosides (6 > 2 > 3 > 4) in reactions with benzoyl chloride.<sup>15</sup>

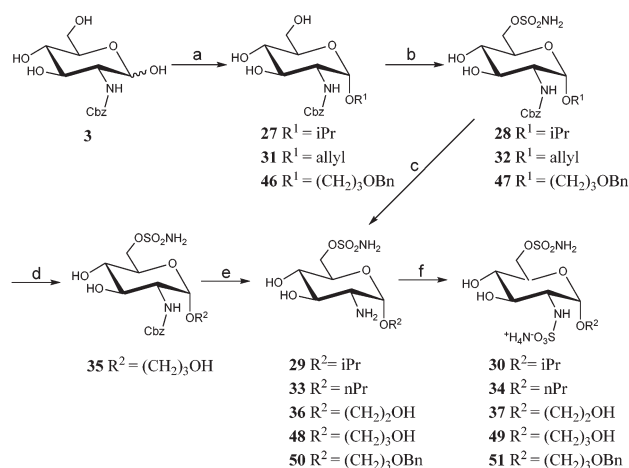
For removal of the anomeric substituent of **1** (Scheme 2), treatment of **3** with acetyl chloride<sup>16</sup> gave **21**. Radical hydrodechlorination<sup>16</sup> with tributyltin hydride and AIBN gave a 47% isolated yield of **22**. Tributyltin hydride could be replaced by the less toxic tris(trimethylsilyl)silane,<sup>17</sup> resulting in a cleaner reaction profile and a straightforward purification on silica, leading to an improved isolated yield of 90% for **22**. Deprotection of the acetoxy groups under Zemplén conditions<sup>18</sup> proceeded in high yield to triol **23**. The use of sulfamoylation Method 1, gave a 34% yield of **24**, which was progressed using the conditions described for the previous analogues, to provide **26**.



**Scheme 2** Reagents and conditions: (a) AcCl, r.t., 48 h, 55%; (b) (i) Bu<sub>3</sub>SnH–AIBN, 110 °C, 1.5 h, 47%, or (ii) (TMS)<sub>3</sub>SiH–AIBN, 110 °C, 1.5 h, 92%; (c) NaOMe (cat.), MeOH, r.t., 2 h, 85%; (d) ClSO<sub>2</sub>NH<sub>2</sub>, Tol–DMA, –15 °C, 2 h, 34%; (e) H<sub>2</sub>/10% Pd/C, MeOH–CH<sub>2</sub>Cl<sub>2</sub>, 40 °C, 2 h, 98%; (f) SO<sub>3</sub>·Py, H<sub>2</sub>O, pH 9–10, r.t., 2 h, 24%.

From consideration of the structure of heparan sulfate, it was concluded that the binding site of Sulf-2 accommodates further saccharide units at the reducing end of the monosaccharide template. In an effort to develop SARs for this region, alternative anomeric substituents were investigated. Thus, reaction of **3** with isopropyl alcohol and 4 M HCl–dioxane gave predominantly α-anomer **27** in 73% isolated yield. Sulfamoylation using Method 1 gave a 37% yield of **28**, which was progressed through deprotection/sulfation steps (Scheme 3) to provide target **30**.

The anomeric position of glucosamine in HSPGs is linked to an iduronic acid residue. Hence, polar groups at this position may be able to mimic interactions of the polar functionality of the iduronate residue with the Sulf-2 protein. The allyloxy group was introduced into the anomeric position using allyl alcohol and 4 M HCl–dioxane at 70 °C for 18 h, to give a 52% yield of α anomer **31**, together with 20% of the β anomer, which were readily separable. Sulfamate formation using Method 1 on **31** gave a 40% yield of **32**. Reduction of the alkene was achieved concurrently with hydrogenolysis of the Cbz-protected amine to give **33**, which was sulfated to provide **34** (Scheme 3). Ozonolysis of **32**, with reductive work-up, gave **35**, which was carried through the standard deprotection/sulfation methodology to provide **37**.



**Scheme 3** Reagents and conditions: R<sup>1</sup> = R<sup>2</sup> = *i*Pr: (a) HCl–dioxane, IPA, 60 °C, 4 h, 73%; (b) ClSO<sub>2</sub>NH<sub>2</sub>, Tol–DMA, –15 °C, 2 h, 37%; (c) H<sub>2</sub>/10% Pd/C, MeOH–CH<sub>2</sub>Cl<sub>2</sub>, 40 °C, 1 h, 100%; (f) SO<sub>3</sub>·Py, H<sub>2</sub>O, pH 9–10, r.t., 90 min, 27%; R<sup>1</sup> = OCH<sub>2</sub>CH=CH<sub>2</sub>, R<sup>2</sup> = *n*Pr: (a) allyl alcohol, HCl–dioxane, 60 °C, 4 h, 52%; (b) ClSO<sub>2</sub>NH<sub>2</sub>, Tol–DMA, –15 °C, 2.5 h, 40%; (c) H<sub>2</sub>/10% Pd/C, MeOH–CH<sub>2</sub>Cl<sub>2</sub>, 40 °C, 2 h, 100%; (f) SO<sub>3</sub>·Py, H<sub>2</sub>O, pH 9–10, r.t., 90 min, 39%; R<sup>1</sup> = OCH<sub>2</sub>CH=CH<sub>2</sub>, R<sup>2</sup> = CH<sub>2</sub>CH<sub>2</sub>OH: (a) allyl alcohol, HCl–dioxane, 60 °C, 4 h, 52%; (b) ClSO<sub>2</sub>NH<sub>2</sub>, Tol–DMA, –15 °C, 2.5 h, 40%; (d) (i) O<sub>3</sub>/MeOH, –78 °C, 30 min; (ii) NaBH<sub>4</sub>, 1 h, 69%; (e) H<sub>2</sub>/10% Pd/C, MeOH–CH<sub>2</sub>Cl<sub>2</sub>, 40 °C, 3 h, 78%; (f) SO<sub>3</sub>·Py, H<sub>2</sub>O, pH 9–10, r.t., 1 h, 30%. R<sup>1</sup> = R<sup>2</sup> = O(CH<sub>2</sub>)<sub>3</sub>OBn: (a) 3-(benzyloxy)propan-1-ol, HCl–dioxane, 75 °C, 5 h, 31%; (b) ClSO<sub>2</sub>NH<sub>2</sub>, DMF, –40 °C, 18 h, 57%; (c) H<sub>2</sub>/5% Pd/C, EtOH, 20 °C, 1 h, 75%; (f) SO<sub>3</sub>·Py, H<sub>2</sub>O, pH 9–10, r.t., 18 h, 62%. R<sup>1</sup> = O(CH<sub>2</sub>)<sub>3</sub>OBn, R<sup>2</sup> = O(CH<sub>2</sub>)<sub>3</sub>OH: (a) 3-(benzyloxy)propan-1-ol, HCl–dioxane, 75 °C, 5 h, 31%; (b) ClSO<sub>2</sub>NH<sub>2</sub>, DMF, –40 °C, 18 h, 57%; (c) H<sub>2</sub>/5% Pd/C, AcOH, 20 °C, 1 h, 83%; (f) SO<sub>3</sub>·Py, H<sub>2</sub>O, pH 9–10, r.t., 1 h, 41%.

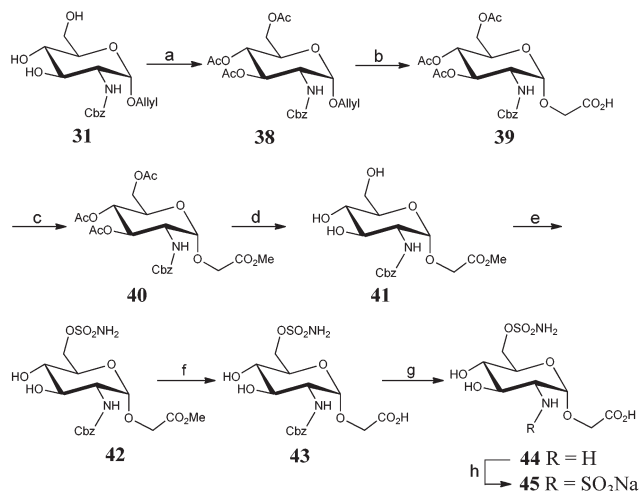


Incorporation of 3-benzyloxypropanol at the  $C^1$ -position of **3** gave a 31% yield of  $\alpha$  anomer **46** (Scheme 3). Sulfamoylation using Method 2 gave **47** in 57% yield. Palladium catalysed hydrogenation allowed deprotection of both *O*-benzyl and *N*-Cbz groups to give **48**, which was sulfated on nitrogen to yield target **49**. Chemoselective removal of the carbamate from **47** was achieved by flow hydrogenation over 5% palladium on carbon at room temperature, providing **50**, which was sulfated using the pH-controlled sulfation conditions to give benzyloxypropyl derivative **51** in 62% yield (Scheme 3).

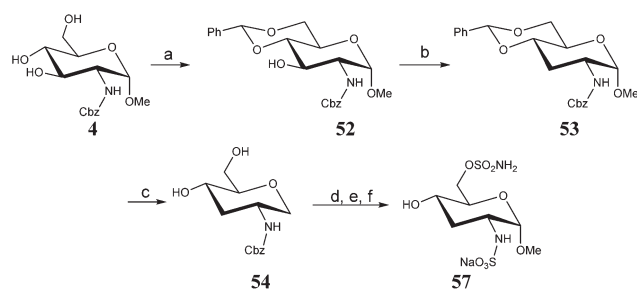
Oxidative cleavage of the allyl group of **38** using  $\text{NaIO}_4$  with catalytic  $\text{RuCl}_3$ <sup>19</sup> afforded **39** (Scheme 4). Alkylation of the carboxylic acid **39** gave ester **40**. Deprotection of the acetate groups using Zemplén conditions proceeded in high yield to **41**, which was sulfamoylated to **42** in 67% yield using Method 2. Hydrolysis of ester **42** to the corresponding carboxylic acid **43** prior to deprotection of the amino functionality afforded **44**. The latter was sulfamoylated to acid **45**.

An approach employing Barton–McCombie radical deoxygenation to allow preparation of  $C^3$ - and  $C^4$ -methylene targets **57** and **63** was inspired by a similar strategy described for the synthesis of a series of activators of the glms-riboswitch of *Staphylococcus aureus*.<sup>20</sup>

The 4- and 6-positions of **4** were selectively protected as benzylidene acetal **52** (Scheme 5)<sup>21</sup> which gave an isolated yield of 79%. Radical deoxygenation of **52** under modified Barton–McCombie conditions<sup>22,23</sup> using tris(trimethylsilyl)silane<sup>17</sup> gave **53** in 83% isolated yield.<sup>24</sup> Complete removal of the benzylidene acetal from **53** afforded diol **54**. Regioselective sulfamate formation using Method 2 gave a 36% yield of **55**. Hydrogenation followed by *N*-sulfation, gave **57**. The synthesis of  $C^4$ -methylene derivative **63** required selective protection of

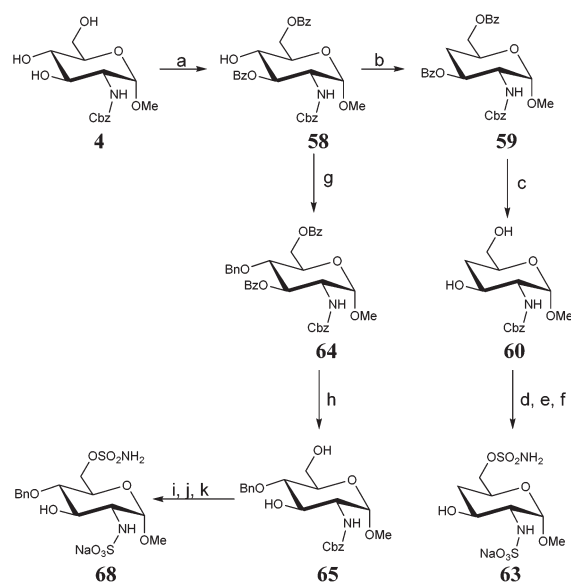


**Scheme 4** Reagents and conditions: (a) pyridine,  $\text{Ac}_2\text{O}$ , r.t. 8 h, 96%; (b)  $\text{RuCl}_3$ ,  $\text{NaIO}_4$ , MeCN,  $\text{CH}_2\text{Cl}_2$ ,  $\text{H}_2\text{O}$ , r.t. 30 min, 56–62%. (c)  $\text{MeI}-\text{Cs}_2\text{CO}_3-\text{CH}_3\text{CN}$ , r.t., 18 h, 77–95%; (d)  $\text{NaOMe}$  (cat.), MeOH, r.t., 1 h, 100%; (e)  $\text{ClSO}_2\text{NH}_2$ , DMF,  $-40^\circ\text{C}$ , 18 h, 67%; (f) 2 M  $\text{NaOH}_{(\text{aq})}$ , THF, r.t., 2 h, 82%; (g)  $\text{H}_2/10\%$  Pd/C, MeOH,  $40^\circ\text{C}$ , 3 h, 100%; (h)  $\text{SO}_3\cdot\text{Py}$ ,  $\text{H}_2\text{O}$ , pH 9–10, r.t., 24 h, 38%.



**Scheme 5** Reagents and conditions: (a)  $\text{PhCH}(\text{OMe})_2$ , *p*-TsOH, DMF,  $75^\circ\text{C}$ , 3 h, 79%; (b) (i)  $\text{CS}(\text{Im})_2$ , toluene,  $110^\circ\text{C}$ , 3 h; (ii)  $\text{TMS}_3\text{SiH}$ , AIBN,  $110^\circ\text{C}$ , 1 h, 83%; (c) (i) *p*-TSA (cat),  $\text{MeOH}-\text{CH}_2\text{Cl}_2$ ,  $\mu\text{W}$ ,  $80^\circ\text{C}$ , 20 min; (ii) 10%  $\text{K}_2\text{CO}_3$  (aq), 72%; (d)  $\text{ClSO}_2\text{NH}_2$ , DMF,  $-40^\circ\text{C}$ , 24 h, 36%; (e)  $\text{H}_2/10\%$  Pd/C, AcOH,  $40^\circ\text{C}$ , 2 h, 97%; (f)  $\text{SO}_3\cdot\text{Py}$ ,  $\text{H}_2\text{O}$ , pH 9–10, r.t., 2 h, 21%.

the 3- and 6-hydroxyl groups. The order of reactivity of the hydroxyl groups of methyl- $\text{D}$ -glucopyranoside<sup>15</sup> is conserved in  $\text{D}$ -glucosamine systems, enabling selective protection at the 3- and 6-positions.<sup>20</sup> Using the literature conditions at room temperature resulted in no selectivity, with only 7% of **58** being obtained along with 69% of tris(benzoyl) product. Reducing the reaction temperature to  $-40^\circ\text{C}$  allowed **58** to be isolated in a 72% yield (Scheme 6). Radical deoxygenation of **58** provided **59**, which was deprotected to give a 68% yield of diol



**Scheme 6** Reagents and conditions: (a)  $\text{BzCl}$  (2.2 eq.),  $\text{CH}_2\text{Cl}_2$ -pyridine,  $-40^\circ\text{C}$ , 3 h, 72%; (b) (i)  $\text{CS}(\text{Im})_2$ , toluene,  $110^\circ\text{C}$ , 3 h; (ii)  $\text{TMS}_3\text{SiH}$ , AIBN,  $110^\circ\text{C}$ , 1 h, 79%; (c)  $\text{NaOMe}$  (cat), MeOH, r.t., 18 h, 68%; (d)  $\text{ClSO}_2\text{NH}_2$ , DMF,  $-40^\circ\text{C}$ , 36 h, 51%; (e)  $\text{H}_2/10\%$  Pd/C, AcOH,  $40^\circ\text{C}$ , 2 h, 70%; (f)  $\text{SO}_3\cdot\text{Py}$ ,  $\text{H}_2\text{O}$ , pH 9–10, r.t., 2 h, 8%; (g) benzyl-2,2,2-trichloroacetimidate (8 eq.), TfOH, dioxane,  $60^\circ\text{C}$ , 4 h, 78%; (h) 3 eq.  $\text{LiAlH}_4$ ,  $0^\circ\text{C}$ , 2 h, 69%; (i)  $\text{ClSO}_2\text{NH}_2$ , Tol-DMA,  $-40^\circ\text{C}$ , 22 h, 52% (j) 10 bar  $\text{H}_2/5\%$  Pd/C, MeOH, r.t. 35 min, 100% (k)  $\text{SO}_3\cdot\text{Py}$ ,  $\text{H}_2\text{O}$ , pH 9–10, r.t., 2 h, 25%.

Table 1 Sulfatase inhibition data

Cmpd	Sulf-2 <sup>a</sup>	ARSA <sup>a</sup>	ARSB <sup>a</sup>	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>
1	0	0	0	α-OMe	NHSO <sub>3</sub> Na	OH	OH
26	0	0	32	H	NHSO <sub>3</sub> NH <sub>4</sub>	OH	OH
20	0	70	67	β-OMe	NHSO <sub>3</sub> Na	OH	OH
37	0	0	0	α-O(CH <sub>2</sub> ) <sub>2</sub> OH	NHSO <sub>3</sub> Na	OH	OH
49	0	0	0	α-O(CH <sub>2</sub> ) <sub>3</sub> OH	NHSO <sub>3</sub> NH <sub>4</sub>	OH	OH
45	0	0	0	α-CH <sub>2</sub> CO <sub>2</sub> H	NHSO <sub>3</sub> Na	OH	OH
51	n.t.	40	77	α-O(CH <sub>2</sub> ) <sub>2</sub> OBn	NHSO <sub>3</sub> NH <sub>4</sub>	OH	OH
30	0	0	19	α-O <sup>i</sup> Pr	NHSO <sub>3</sub> NH <sub>4</sub>	OH	OH
34	0	0	0	α-O <sup>n</sup> Pr	NHSO <sub>3</sub> NH <sub>4</sub>	OH	OH
10	0	0	12	α-OMe	NHSO <sub>2</sub> Me	OH	OH
11	n.t.	29	55	α-OMe	NHSO <sub>2</sub> CF <sub>3</sub>	OH	OH
16	0	0	33	α-OMe	NHCO(CH <sub>2</sub> ) <sub>2</sub> CO <sub>2</sub> H	OH	OH
6	0	0	44	α-OMe	NHCbz	OH	OH
5	0	86	3	α-OMe	NH <sub>2</sub>	OH	OH
13	7	0	11	α-OMe	OH <sup>eq</sup>	OH	OH
15	0	0	14	α-OMe	OH <sup>ax</sup>	OH	OH
57	0	0	12	α-OMe	NHSO <sub>3</sub> Na	H	OH
63	0	0	21	α-OMe	NHSO <sub>3</sub> Na	OH	H
68	0	0	0	α-OMe	NHSO <sub>3</sub> Na	OH	OBn

<sup>a</sup> % inhibition at 1 mM; n.t. = not tested.

60. Sulfamoylation gave **61** in 51% yield, with subsequent N-deprotection and sulfate formation providing target **63**.

Introduction of alkoxy substituents at the 4-position of **1** was investigated starting from 3,6-dibenzoyl protected intermediate **58**. Benzoylation using Bundle's reagent (benzyl-2,2,2-trichloroacetimidate) with catalytic triflic acid<sup>26,27</sup> provided *O*<sup>4</sup>-benzyl ether **64** in 78% yield (Scheme 6). Reaction of **64** with lithium aluminium hydride allowed isolation of **65** in 69% yield. Sulfamoylation using Method 2 gave **66** in 52% yield, which was deprotected selectively in the presence of the benzyl ether under mild palladium-catalysed flow hydrogenation conditions, to give **67** which was sulfated to **68**.

## Biological data

Inhibition of Sulf-2, and counter-screening against aryl sulfatases A (ARSA) and B (ARSB) was assessed. Compounds were assayed for their ability to inhibit the desulfation of 4-methylumbelliferyl sulfate (4-MUS) to the fluorescent phenol, 4-methylumbelliferone (4-MU), at a single concentration of 1 mM. In this assay format, lead monosaccharide sulfamate **1** exhibited less than 10% inhibition of Sulf-2 activity, and also demonstrated no inhibition of ARSA or ARSB. The measured Sulf-2 inhibition of **1** was in contrast to the reported literature value (IC<sub>50</sub> = 130 μM), which may reflect differences in the assay procedures. Specifically, the assay employed by Schelwies and colleagues<sup>5</sup> involved pre-incubation of the inhibitor and Sulf-2 followed by a 10-fold dilution into the assay mixture prior to determination of the residual enzyme activity; however, the IC<sub>50</sub> cited relates to the inhibitor concentration in the undiluted sample and not in the final enzymatic reaction. In the data presented here, all values relate to inhibitor concentrations in the final enzymatic assay and as indicated at the

concentration of **1** tested, 1 mM, there was no significant Sulf-2 inhibition. All analogues prepared also displayed poor inhibition of Sulf-2.

Only two compounds exhibited significant inhibition of ARSA. The β anomer of **1** inhibited 70% of ARSA activity at 1 mM, and also inhibited ARSB to a similar extent (67% inh@1 mM). The unsubstituted 2-amino derivative of **1** was selective for ARSA (86% inh@1 mM) over ARSB (3% inh@1 mM). The *N*-Cbz derivative of **1** exhibited some degree of selectivity for inhibition of ARSB (44% inh@1 mM) with no inhibition of ARSA at this concentration (Table 1).

## Conclusions

A short synthesis of the purported inhibitor **1** of Sulf-1 and Sulf-2 has been developed. Optimised low temperature conditions were developed with a model substrate and applied to a monosaccharide template, resulting in the first regioselective sulfamoylation of a carbohydrate. The developed route allows access to **1** in five steps and 17% overall yield compared to 9 steps and 9% yield for the previously published procedure. A range of analogues has been prepared using the regioselective sulfamate formation methodology, exploring diversification at the 1, 2, 3, and 4-positions of the glucosamine template. Compound **1**, and all derivatives prepared were found to have minimal inhibition of Sulf-2, in contrast to claims in ref. 5.

## Acknowledgements

The authors thank: Cancer Research UK and Pfizer Global Research and Development for financial support, the EPSRC National Mass Spectrometry Service at the University of Wales



(Swansea) for mass spectrometric determinations, Drs C. Cano and I. R. Hardcastle for helpful discussions and Dr R. Harrington for assistance with the crystallography.

## Notes and references

- 1 M. Morimoto-Tomita, K. Uchimura, Z. Werb, S. Hemmerich and S. D. Rosen, *J. Biol. Chem.*, 2002, **277**, 49175.
- 2 R. Nawroth, A. van Zante, S. Cervantes, M. McManus, M. Hebrok and S. D. Rosen, *PLoS One*, 2007, **2**, e392.
- 3 N. Turner and R. Grose, *Nat. Rev. Cancer*, 2010, **10**, 116.
- 4 A. Klaus and W. Birchmeier, *Nat. Rev. Cancer*, 2008, **8**, 387.
- 5 M. Schelwies, D. Brinson, S. Otsuki, Y.-H. Hong, M. K. Lotz, C.-H. Wong and S. R. Hanson, *ChemBioChem*, 2010, **11**, 2393.
- 6 A. D. Yoffe, *Proc. R. Soc. London, Ser. A, Math. Phys. Sci.*, 1951, **208**, 188.
- 7 A. Hassner, M. Stern, H. E. Gottlieb and F. Frolow, *J. Org. Chem.*, 1990, **55**, 2304.
- 8 I. Armitage, A. M. Berne, E. E. Elliott, M. Fu, F. Hicks, Q. McCubbin and L. Zhu, *Org. Lett.*, 2012, **14**, 2626.
- 9 E. Kamst, K. Zegelaar-Jaarsveld, G. A. van der Marel, J. H. van Boom, B. J. J. Lugtenberg and H. P. Spaink, *Carbohydr. Res.*, 1999, **321**, 176.
- 10 R. Appel and G. Berger, *Chem. Ber.*, 1958, **91**, 1339.
- 11 D. T. Warner and L. L. Coleman, *J. Org. Chem.*, 1958, **23**, 1133.
- 12 J. R. Myette, V. Soundararajan, Z. Shriver, R. Raman and R. Sasisekharan, *J. Biol. Chem.*, 2009, **284**, 35177.
- 13 T. Steiner, *Angew. Chem., Int. Ed.*, 2002, **41**, 48.
- 14 I. J. Bruno, J. C. Cole, P. R. Edgington, M. Kessler, C. F. Macrae, P. McCabe, J. Pearson and R. Taylor, *Acta Crystallogr., Sect. B: Struct. Sci.*, 2002, **58**, 389.
- 15 J. M. Williams and A. C. Richardson, *Tetrahedron*, 1967, **23**, 1369.
- 16 M. J. Bamford, J. C. Pichel, W. Husman, B. Patel, R. Storer and N. G. Weir, *J. Chem. Soc., Perkin Trans. 1*, 1995, 1181.
- 17 C. Chatgililoglu, *Acc. Chem. Res.*, 1992, **25**, 188.
- 18 G. Zemplen, *Matematikai es Termeszettudományi Ertesito*, 1937, **55**, 432.
- 19 W. P. Griffith, A. G. Shoair and M. Suriaatmaja, *Synth. Commun.*, 2000, **30**, 3091.
- 20 C. E. Lünse, M. S. Schmidt, V. Wittmann and G. n. Mayer, *ACS Chem. Biol.*, 2011, **6**, 675.
- 21 D. H. R. Barton, S. Augy-Dorey, J. Camara, P. Dalko, J. M. Delaunéy, S. D. Géro, B. Quiclet-Sire and P. Stütz, *Tetrahedron*, 1990, **46**, 215.
- 22 D. H. R. Barton and S. W. McCombie, *J. Chem. Soc., Perkin Trans. 1*, 1975, 1574.
- 23 D. H. R. Barton, D. Crich, A. Löbberding and S. Z. Zard, *Tetrahedron*, 1986, **42**, 2329.
- 24 K. Daragics and P. Fügedi, *Tetrahedron Lett.*, 2009, **50**, 2914.
- 25 N. Karst and J.-C. Jacquinet, *J. Chem. Soc., Perkin Trans. 1*, 2000, 2709.
- 26 T. Iversen and D. R. Bundle, *J. Chem. Soc., Chem. Commun.*, 1981, 1240.
- 27 W. J. Sanders, D. D. Manning, K. M. Koeller and L. L. Kiessling, *Tetrahedron*, 1997, **53**, 16391.