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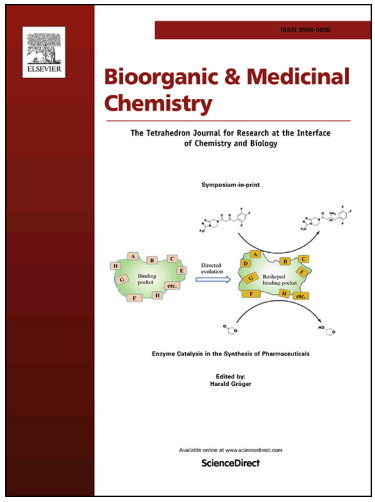

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PII: S0968-0896(18)31521-9
DOI: https://doi.org/10.1016/j.bmc.2018.10.008
Reference: BMC 14567

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 28 August 2018
Revised Date: 3 October 2018
Accepted Date: 11 October 2018

Please cite this article as: DiFrancesco, B.R., Morrison, Z.A., Nitz, M., Monosaccharide inhibitors targeting Carbohydrate Esterase family 4 de-*N*-acetylases, *Bioorganic & Medicinal Chemistry* (2018), doi: https://doi.org/10.1016/j.bmc.2018.10.008

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Monosaccharide inhibitors targeting carbohydrate esterase family 4 de-N-acetylases

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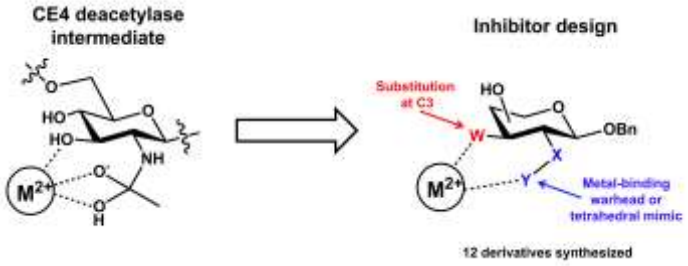
Benjamin R. DiFrancesco[a,‡], Zachary A. Morrison[a,‡], Mark Nitz[a*]
Department of Chemistry, University of Toronto, Toronto, ON, M5S 3H6, Canada



Bioorganic & Medicinal Chemistry
journal homepage: www.elsevier.com

Monosaccharide inhibitors targeting Carbohydrate Esterase family 4 de-*N*-acetylases

Benjamin R. DiFrancesco[a,‡], Zachary A. Morrison[a,‡], Mark Nitz[a*]

[a]*Department of Chemistry, University of Toronto, Toronto, ON, M5S 3H6, Canada*
‡ These authors contributed equally to the work

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Deacetylase
Phosphonate
Inhibitor
Metallohydrolase

ABSTRACT

The Carbohydrate Esterase family 4 contains virulence factors which modify peptidoglycan and biofilm-related exopolysaccharides. Despite the importance of this family of enzymes, a potent mechanism-based inhibition strategy has yet to emerge. Based on the postulated tridentate binding mode of the tetrahedral de-*N*-acetylation intermediate, GlcNAc derivatives bearing metal chelating groups at the 2 and 3 positions were synthesized. These scaffolds include 2-C phosphonate, 2-C sulfonamide, 2-thionoacetamide warheads as well as derivatives bearing thiol, amine and azide substitutions at the 3-position. The inhibitors were assayed against a representative peptidoglycan deacetylase, Pgda from *Streptococcus pneumonia,* and a representative biofilm-related exopolysaccharide deacetylase, PgaB from *Escherichia. coli*. Of the inhibitors evaluated, the 3-thio derivatives showed weak to moderate inhibition of Pgda. The strongest inhibitor was (2,3-dideoxy-2-thionoacetamide-3-thio)-benzyl-β-D-glucoside, whose inhibitory potency showed an unexpected dependence on metal concentration and was found to have a partial mixed inhibition mode ($K_i = 2.9 \pm 0.6$ μM).

1. Introduction

Many members of the carbohydrate esterase family 4 (CE4) act as virulence factors.[1] In bacteria, CE4 enzymes catalyze the de-*N*-acetylation of *N*-acetylglucosamine and muramic acid residues in peptidoglycan conferring lysozyme resistance to the cell wall and reducing host recognition of peptidoglycan fragments.[2] Deletion or mutagenesis of peptidoglycan deacetylase results in reduced pathogenicity in many bacterial species including *Streptococcus pneumoniae*[3], *Bacillus anthracis* [4], *Enterococcus faecalis*[5], *Listeria monocytogenes*[6] and *Helicobacter pylori*[7]. CE-4 deacetylases are also found in fungi and have been postulated to play a variety of roles in pathogenicity including host invasion in *Colletotrichum lindemuthianum*[8] and spore formation in *Schizosaccharomyces pombe*[9]. The modification of biofilm-related exopolysaccharides is another mechanism by which CE-4 enzymes act as virulence factors. In *Escherichia coli*[10], *S. epidermis*[11], and *Bordetella brontiseptica*[12] a CE4 enzyme partially de-*N*-acetylates β(1-6)-N-acetylglucosamine (PNAG) homopolymers, a common biofilm matrix exopolysaccharide. Similarly, CE4 enzymes are responsible for the deacetylation of biofilm matrix exopolysaccharides in *Pseudomonas aeruginosa*[13] and *Aspergillus fumigatus*[14].

The CE4 family members contain a divalent metal at the core of their active site, which is coordinated by a conserved His-His-Asp triad. In solved structures of CE4 enzymes, an octahedral coordination geometry is usually observed.[8,16,23] Structural comparisons show strong conservation of key catalytic residues with differences observed in active site architecture, largely depending on substrate recognition.[15] Mechanistically, linear free energy relationships support CE4-catalyzed de-N-acetylation proceeding through a high energy tetrahedral oxyanion intermediate (Figure 1).[8, 15, 16]

Despite the relevance of this class of metalloenzymes, potent inhibition strategies have yet to emerge. The development of inhibitors against this important enzyme family will help further elucidate their structural features and mechanism. Additionally, successful inhibitors would be useful probes of biological function, and perhaps even as novel antimicrobial agents.

Reported inhibitor development efforts targeting the CE4 enzymes have had modest success. High-micromolar affinity inhibition of PgaB, the *E. coli* PNAG de-*N*-acetylase, has been achieved using a glucosamine scaffold displaying metal chelating groups such as a *N*-thioglycolylamide.[17] More recently, monosaccharide transition state analogues bearing a methylphosphonamidate have afforded micromolar affinity inhibition of PgdA, the *S. pneumoniae* peptidoglycan de-*N*-acetylase, but were ineffective against PgaB.[18] This was surprising given these enzymes' presumed mechanistic similarities. Reports of high throughput in silico screening and screening hydroxamic acid derivatives have yielded inhibitors in the mid-micromolar range.[19, 20] In this work we further elucidate the binding determinants and mechanisms of these CE4 enzymes by exploring two new inhibitor scaffolds.

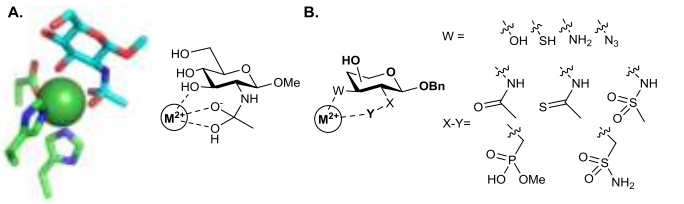


Figure 1. A. Docked structure of de-*N*-acetylation tetrahedral intermediate in PgaB showing active site metal and ligating residues. B. Proposed metal ligating inhibitor scaffolds investigated.

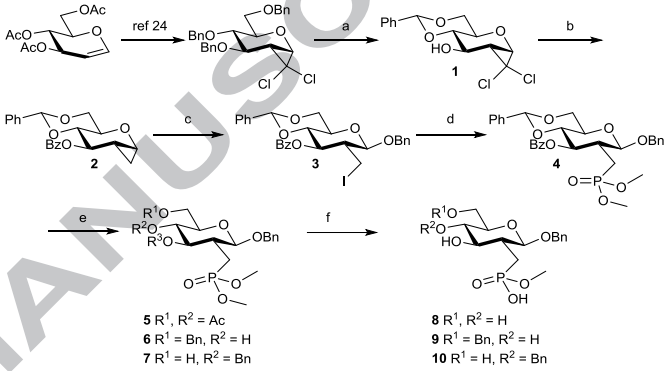
The methylphosphonamidate inhibitors are promising scaffolds against peptidoglycan deacetylase.[18] Unfortunately, these compounds are prone to degradation below pH 7.0, limiting their use *in vivo*.[21] Seeking more chemically stable alternatives, we hypothesized that methylphosphinates, methylphosphonates or sulfonamides, installed on a 2-C-gluco scaffold, could function as analogues of the phosphonamidates (Fig. 1). These moieties have been used successfully as tetrahedral transition state mimics of enzymes and have superior stability when compared to the corresponding phosphonamidates.[22]

The C3 hydroxyl of GlcNAc is thought to coordinate the active site metal ion during substrate binding and catalysis by CE4 enzymes (Fig. 1A). It may also hydrogen bond to the metal-ligating Asp residue. Metal binding by 3-OH is supported by docking studies of substrates and tetrahedral intermediates of CE4 enzymes.[16, 23] Furthermore, a metal-O3 interaction was observed with bound chitosan oligosaccharides upon structural analysis of an active site mutant of the CE4 enzyme *Vibrio cholera* chitin de-N-acetylase (*Vc*CDA).[15] Lastly, substitution of the C3 hydroxyl for a methoxy group led to 4-fold weaker binding among the previously reported methylphosphonamidate inhibitors of *Sp*PgdA.[18] Based on this evidence, it was hypothesized that this binding mode could be exploited in the design of more potent CE4 inhibitors. The substitution of the C3 hydroxyl group of GlcNAc with a metal chelating functionality was hypothesized to increase binding affinity (Fig. 1). In addition, derivatives with modification at C2 and C3 were explored, modifying an acetamido to thioacetamido or a methyl sulfonamide moiety. It was envisioned that these would afford tighter metal coordination or mimicry of the tetrahedral intermediate, respectively.

2. Results

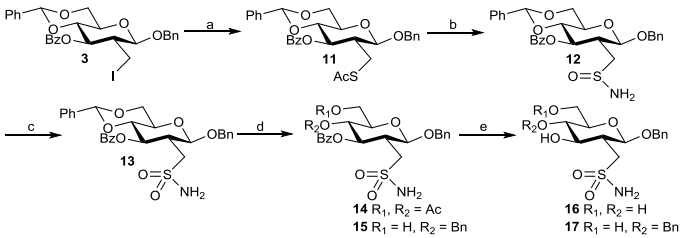
2.1. Synthesis of 2-C-gluco derivatives

To access the 2-C-gluco scaffold, we chose to follow a modified literature protocol by Ramana et al (Scheme 1).[24] The most potent phosphonamidate inhibitor of Pgda was a functionalized benzyl 4-*O*-benzyl-β-D-glucoside likely due to contact with hydrophobic residues flanking the active site.(18) To enable access to both 4-*O*-benzyl and 6-*O*-benzyl 2-C-glucosides a 4,6-*O*-benzylidene protecting group was installed, which could subsequently be selectively reduced to the desired benzyl derivatives. Efforts to install the 4,6-*O*-benzylidene on the glucal scaffold were not successful under a range of acidic and basic conditions. Thus, the 4,6-*O*-benzylidene was installed subsequent to 2-C functionalization. Using conditions reported by Ramana *et al.* the perbenzylated glucal was dichlorocyclopropanated in good yield and selectivity.[25] The benzyl protecting groups were then reductively cleaved and the 4,6-*O*-benzylidene was introduced on the triol to give the desired product **1**. The reduction of the dichloromethylene with $LiAlH_4$ required more forcing conditions than reported on the per-benzyl protected substrate but proceeded to completion after 72 hrs at reflux to give the reduced cyclopropane. The remaining hydroxyl was then protected with a benzoyl group to provide **2**. The NIS-promoted glycosidation of **2** gave the desired benzyl glycoside in a 1:3 (α:β) ratio. The desired 2-C-iodo β-D-benzyl glycoside (**3**) could be separated by column chromatography in 68% yield. Unfortunately submitting the primary iodide (**3**) to Arbuzov conditions with diethyl methylphosphinite at a range of temperatures up to 120 ºC gave only a complex mixture. It is known that phosphinites are more challenging substrates for Arbuzov reactions than the corresponding phosphonates.[26] Thus, we hypothesized that the more easily synthesized methylphosphonate would still be a good mimic of the tetrahedral intermediate despite the larger size of the methyl ester. Gratifyingly, the 2-C-iodo (**3**) was smoothly converted into the methyl phosphonate (**4**) via an Arbuzov reaction with trimethyl phosphite.



Scheme 1. Synthesis of methylphosphonate inhibitors a) i) H_2, Pd/C, THF/MeOH. ii) benzaldehyde dimethyl acetal, pTSA, MeCN, 62% over two steps. b) i) $LiAlH_4$, THF, reflux, 72h ii) BzCl, py, 78% over two steps. c) NIS, BnOH, DCM, 68%. d) $P(OMe)_3$, reflux, 69%. e) for **5**, 8:1:1 AcOH:THF:H_2O, reflux, quant., for **6** Et_3SiH, BF_3•Et_2O, DCM, 0 ºC, 70% for **7** BH_3•THF, TMSOTf, DCM, RT, 26% f) NaOMe/MeOH, **8** 78%, **9** 85%, **10** 50%.

The selective benzylidene reduction of **4** to the 4-*O*-benzyl derivative **7** was achieved with BH_3·THF and TMSOTf at ambient conditions. The complimentary 6-*O*-benzyl derivative **6** accessed in low yield with Et_3SiH and BF_3·Et_2O at 0 °C with a significant amount of complete benzylidene hydrolysis being observed. The 3-O-benzoyl group and the phosphonate diester were hydrolyzed under Zemplén conditions to give the desired methylphosphonates **8**-**10**.



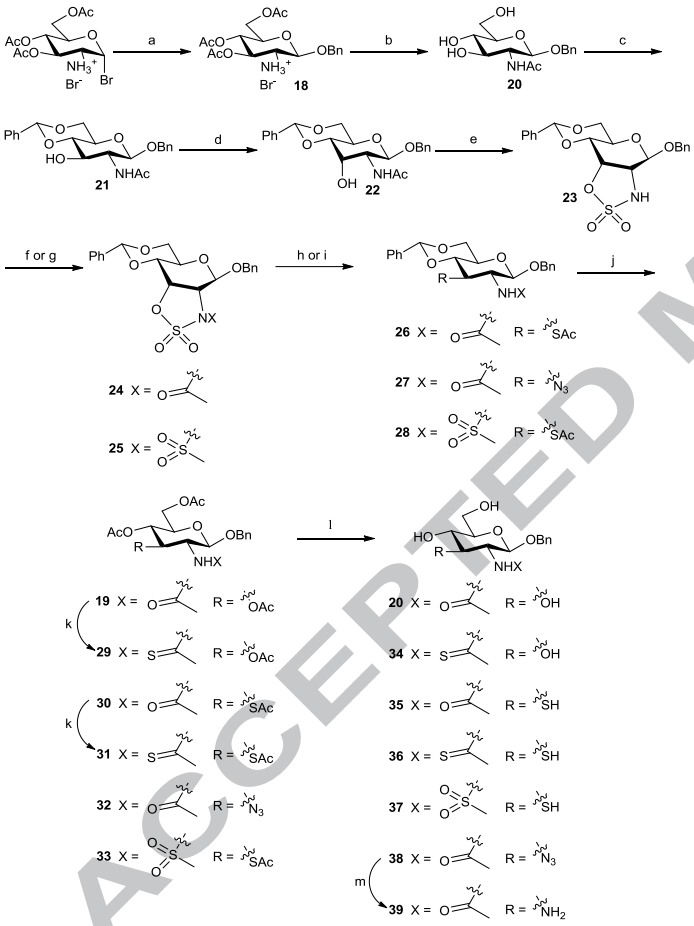
Scheme 2. Synthesis of sulfonamide inhibitors. a) potassium thioacetate, THF, 60 ºC, 6h, 93%. b) i) SO_2Cl_2, Ac_2O, DCE, -10 ºC, 1h. ii) NH_4OH(aq), 86% over two steps c) mCPBA, DCM, 1h, 93%. d) for **14** i) 8:1:1 AcOH:THF:H2O, reflux. ii) Ac_2O, py, 3h, 35%., for **15,** BH_3•THF, TMSOTf, DCM, RT, 26% e) NaOMe/MeOH, **16** 78%, **17** 17%.

To access the desired 2-C-gluco sulfonamide inhibitors, the primary iodide was cleanly displaced from compound **3** with potassium thioacetate to give **11** in good yield (Scheme 2). Treatment of the thioester with sulfuryl chloride and acetic anhydride oxidized **11** to the sulfinyl chloride.[27] Subsequent

reaction of this intermediate with ammonium hydroxide gave the sulfinamide **12** as an inseparable mixture of diasteromers. Attempts were made to deprotect the sulfinamide (condition e), however all attempts resulted in degradation, suggesting that this compound is unstable. The sulfinamide **12** was found to readily oxidize when left exposed to air over the course of several hours. In light of these findings, **12** was quantitatively oxidized to sulfonamide **13** using mCPBA. Selective benzylidene reduction to give the 4-*O*-benzyl derivative **15**, required an extra equivalent of Lewis acid likely due to the Lewis basicity of the sulfonamide nitrogen. Unfortunately, treatment of compound **13** with Et_3SiH, BF_3•Et_2O yielded only a complex mixture even after short reaction times at -10 °C. Compounds **14** and **15** could be deprotected under standard Zemplén conditions to yield the desired sulfonamides **16** and **17.**

2.2. Synthesis of C3 and amido derivatives

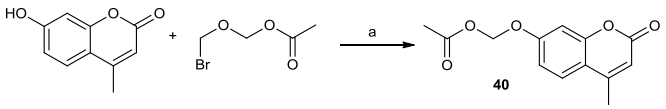
The substitution of the C3-hydroxyl of GlcNAc for a thiol or an amine is synthetically challenging due to neighboring group participation by the resident C2 acetamido group. This complication can be avoided by through installation of a 2,3 cyclic sulfamidate, which can be opened by nucleophilic displacement.[28] The 2,3 cyclic sulfamidate **23** was readily synthesized from the known glycosyl bromide with minor modifications and an improved yield (Scheme 3).[29] Condensation of the glycosyl bromide with benzyl alcohol gave the crystalline β-benzyl glycoside **18** without chromatography.[30] Acetylation of the amine and a Zemplén deprotection afforded the unprotected β-benzyl glycoside **20**. Installation of a benzylidene acetal gave the highly insoluble **21** in good yield. The inversion of stereochemistry at C3 was achieved by mesylation followed by hydrolysis in 1:10 H_2O/1-methoxy-2-propanol with sodium acetate, giving **22**. Finally, the 2,3 cyclic sulfamidate **23** was produced using sodium hydride and 1,1'-sulfonyldiimidazole.



Scheme 3. Synthesis of chelating inhibitors. a) BnOH, pyr, 45 ºC, 24h, 72%. b) I) Ac_2O, Et_3N, CH_2Cl_2, 3h. II) NaOMe/MeOH, 78% over two steps. c) $PhC(OMe)_2$, pTsOH, CH_3N, 4h, 92%. d) I) MsCl, pyr, CH_2Cl_2. II) 10:1 methoxyl-2-propanol/H_2O, NaOAc, 115 ºC, 24h, 77% over two steps. e) 1,1-sulfonyldiimidazole, NaH, THF, 17h, 91%. f) for **24**, AcCl, pyr, CH_2Cl_2, 88%. g) for **25**, MsCl, Et_3N, CH_2Cl_2, 0 ºC, 0.5h, 92%. h) for **26** and **28**, I) KSAc, DMF, 1h. II) THF/H_2SO_4/H_2O, 0.5h. 64-76% over two steps. i) for **27**, I) NaN_3, DMF, 2h. II) THF/H_2SO_4/H_2O, 1h. 85% over two steps. j) I) AcOH/H_2O, 60 ºC, 4-6h. II) Ac_2O/pyr, 64-96% over two steps. k) 0.6 eq Lawesson's reagent, toluene, 80 ºC, 4h, 51-53%. l) NaOMe/MeOH, 54-100%. m) Ph_3P, THF/H_2O, rt, 72h, 92%.

2.3. Inhibition assays

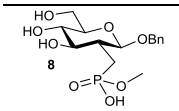
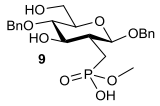
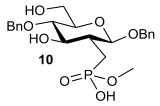
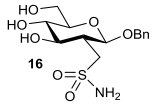
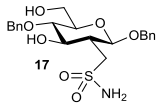
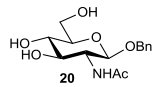
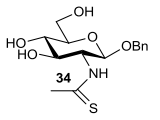
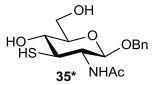
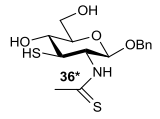
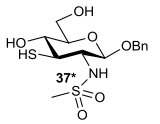
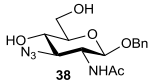
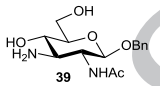
The inhibition of PgaB and PgdA were evaluated with the pseudosubstrate acetoxymethyl-4-methylumbelliferone (AMMU) (**40**). This substrate was available in one step via a condensation of 4-methylumbelliferone and (bromomethoxy)methylacetate (Scheme 4). Although the synthesis yielded a mixture of the desired acetoxymethyl derivative and the acetoxy-4-methylumbelliferone these could be readily separated by selective hydrolysis. AMMU showed significantly reduced levels of background hydrolysis under the assay condition than previously employed 3-carboxyumbelliferyl acetate.[17] Although AMMU was not sufficiently soluble to yield complete Michaelis-Menten parameters it could be effectively used in the preliminary determination of IC_{50} values.



Scheme 4. Synthesis of pseudo substrate (AMMU) for evaluation of de-N-acetylase activity. a) I) DIPEA, $CHCl_3$. II) $BnNH_2$, $CHCl_3$, 18% over two steps.

Analysis of the methylphosphonate inhibitors (**8**-**10**) against PgaB gave similar results to the previously investigated methylphosphonamidate inhibitors with no significant inhibition observed at low millimolar concentrations. Against PgdA some inhibition was observed with compound **8** (IC_{50} = 4.9 mM) however this inhibition was less potent than the corresponding methylphosphonamidate (K_i = 580 μM).[18] The 4-*O* and 6-*O*-benzyl functionalized monosaccharides, compounds **9** and **10**, showed no significant inhibition against PgdA at 5 mM concentrations. This was surprising given the corresponding 4-*O*-benzyl functionalized methylphosphonamidate previously reported is the most potent inhibitor of *Sp*Pgda to date

Table 1. IC_{50} values measured for inhibitors.

Compound	IC_{50} (mM)[a]	
	PgaB	*Sp*Pgda
 **8**	>10	4.9 ± 0.7
 **9**	>5	>5
 **10**	>5	>5
 **16**	>10	>10
 **17**	>5	>5
 **20**	>20	11 ± 1
 **34**	>10	>10
 **35***	>8 (1.1 ± 0.4)[b]	>8 (5 ± 2)[c]
 **36***	>1 (0.14 ± 0.04)[b]	>1 (0.011 ± 0.003)[c]
 **37***	>1 (>1)[b]	>1 (>1)[c]
 **38**	>5	>5
 **39**	>10	>10

[a] 1.5 μM PgdA or 2 μM PgaB, 100 μM AMMU. Except where otherwise stated, the enzymes were supplemented with one equivalent of $CoCl_2$. The reaction was conducted in anoxic conditions.

[b] 4 μM $ZnCl_2$, 2 μM PgaB, 500 μM AMMU

[c] 3 μM $ZnCl_2$, 1.5 μM PdgA, 100 μM AMMU

[c] Compounds with * were incubated with 1 eq of DTT prior to assay. DTT did not inhibit either enzyme at 10 mM.

(K_I = 80 μM).[18] Overall, these results suggest that the methylphosphonate is not recognized at the deacetylase active site as effectively as the methylphosphonamidates. This may be due to either the hydrogen bonding potential of the amine present in the phosphonamidates or the decreased size of the methyl phosphonamidate. We hypothesize that the relatively constrained methyl binding pocket found in the active site of Pgda is unable to accommodate the extra atom in the methylphosphonate derivative resulting in the severely attenuated binding observed versus the previously synthesized phosphonamidate derivatives.

Analysis of the sulfonamide inhibitors (**16**-**17**) did not show promising inhibition of PgaB or PgdA. These results were surprising given that sulfonamides are common metal binding motifs in metalloenzyme inhibitors. One explanation is that the sulfonamide is unable to achieve the correct orientation to chelate the metal in concert with the 3-OH of the scaffold, or that the penalty for the absence of the methyl substituent is substantial.

Prior to evaluation of the C3-substitued derivatives as inhibitors they were analyzed as potential substrates of PgdA or PgaB. None of the sugars showed appreciable turnover when analyzed at low millimolar concentrations with high enzyme concentrations (3 or 4 μM) as determined by a fluorescamine assay (Fig. S1). The inhibition (IC_{50}) of the compounds (**34**-**39**) were then investigated using the AMMU substrate (Table 1). No substantial inhibition was observed with the amine (**39**), azide (**38**) or thiocarbonyl (**34**) derivatives. The inability of the 3-azido derivative **38** to inhibit the enzymes was expected, given the steric bulk and non-coordinating nature of the azide substituent. However, the failure of the amine **39** to bind was unexpected. The pKa of the amine is calculated to be ~7.9 and thus should be available for metal binding. Weak inhibition was observed with the 3-thio (**35**), and the 3-thio thiocarbonyl (**36**), supporting an interaction between the -OH position and the active site metal. The 3-thio sulfonamide (**37**) showed no significant inhibition up to its solubility limit (~ 1 mM). This suggests that the methyl sulfonamide is a poor mimic of the tetrahedral intermediate, perhaps due to its lack of negative charge.

CE4 enzymes are known to be promiscuous with respect to their active site metal. Generally, optimal enzyme activity is observed with Co^{2+}-loaded enzyme but substantial activity is also observed with Zn^{2+}, Fe^{2+} and in some cases Ni^{2+}. The dependence of the observed inhibition on the identity of the active site metal was investigated through the addition of Zn^{2+} (2 eq) or Co^{2+} (1 eq) to the as isolated enzymes. Interestingly in the presence of added zinc, but not cobalt, more potent inhibition was observed for the 3-thio derivatives **35** and **36**. Consistent with this result, thiols are known to be better ligands for zinc than cobalt.[31] In the presence of zinc, **36** provided micromolar IC_{50} values for both PgaB and PgdA, though was most potent against the latter. However, its dose-response curve against PgdA was unusual, plateauing at 30-40% initial activity (Fig. 2).

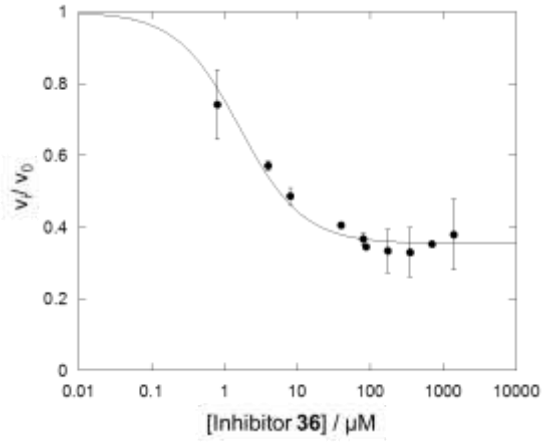


Figure 2. IC_{50} curve for PgdA inhibition by **36**. Conditions: 100 μM AMMU, 1.5 μM PgdA supplemented with 3 μM $ZnCl_2$.

Given the observed differences between inhibition in the presence of Co^{2+} and Zn^{2+} further studies were undertaken to determine the mechanism of action of **36** under controlled metal loading conditions. Apo-Pgda was prepared by exhaustive dialysis against EDTA-containing buffer. From this preparation Pgda could be isolated and its activity restored with the addition of Zn^{2+} (Fig. S2).

Surprisingly, when the apo-PgdA (1.5 μM) was loaded with only two equivalents of zinc (3 μM), no inhibition of AMMU hydrolysis by **36** was observed (Fig. 3). Increasing the zinc concentration to 6 μM restored inhibition, and further increases up to 200 μM Zn^{2+} did not further improve the inhibition observed. These observations are consistent with a complex of zinc and **36** as the active inhibitor species.

A detailed kinetic study of PgdA inhibition with **36** was conducted using a substrate, N,N',N''-triacetyl chitotriose, which closely mimics the natural substrate (Fig 4). Comparison of the kinetics of chitotriose deacetylation, relative to previously reported values for the Co^{2+}-loaded enzyme, showed the Zn^{2+}-loaded enzyme to have a lower k_{cat} and higher K_m values (K_m = 14 ± 3 mM versus 5.9 ± 0.3 mM, and k_{cat} = 0.51 ± 0.06 s^{-1} versus 1.1 ± 0.1 s^{-1}).[16] This is expected, as PgdA is known to have greatest catalytic activity when loaded with Co^{2+} .

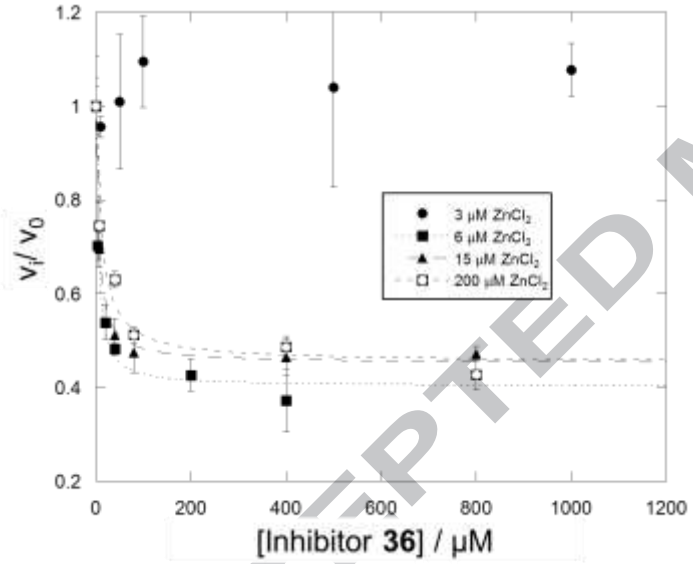


Figure 3. Metal dependence of PgdA inhibition by compound **36**. Conditions: 1.5 μM apo-PgdA, 100 μM AMMU.

Analysis of a range of concentrations of **36** using the chitotriose substrate on the zinc loaded PgdA gave inhibition consistent with partial mixed inhibition mode (Fig. 5). In this model the α and β parameters quantify the extent to which the inhibitor acts by perturbing substrate binding or turnover, respectively. The ratio of these constants (β/α) gives the fraction of residual catalytic efficiency the enzyme-inhibitor complex retains. This inhibition mode is supported by the hyperbolic appearance of a Dixon plot of these data (Fig. S3). In the absence of inhibitor, sufficient substrate saturation could be achieved to determine K_m and V_{max}. However, at high inhibitor concentrations the apparent K_m values exceeded the maximum substrate concentration, preventing accurate measurement of these parameters. Nevertheless, replotting the apparent V_{max}/K_m values at each inhibitor concentration permitted determination of K_i and the β/α ratio. Fitting eq. 1 to the data by nonlinear regression analysis gave inhibition constant K_i = 2.9 ± 0.6 μM and β\/α = 0.32 ± 0.01 (Fig. S4).

$$\frac{\left(\frac{V_{max}}{K_m}\right)^{app}}{\left(\frac{V_{max}}{K_m}\right)^{0}} = \frac{1}{1+\frac{[I]}{K_i}} + \frac{\beta/\alpha}{1+\frac{K_i}{[I]}} \qquad \text{(eq. 1)}$$

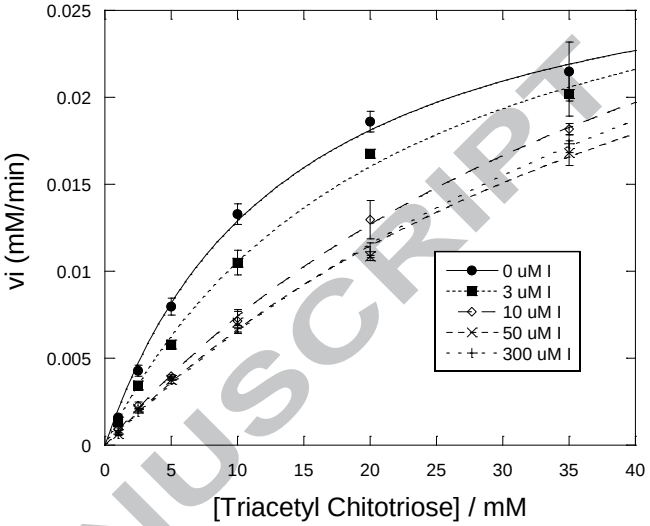


Figure 4. PgdA inhibition kinetics by compound **36**. Conditions: 1 μM apo-PgdA supplemented with 6 μM $ZnCl_2$.

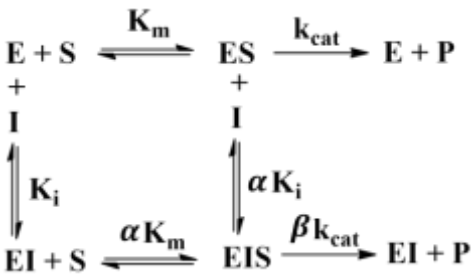


Figure 5. Kinetic scheme of a partial mixed type inhibition.

We hypothesize that a Zn^{2+}-**36** complex binds to an allosteric site of PgdA and reduces its catalytic efficiency to roughly 30% of the uninhibited enzyme. Since individual K_m and V_{max} values could not be determined at higher inhibitor concentrations, it is challenging to evaluate the relative contribution of the β and α parameters. However, it is clear that the inhibition increased the apparent K_m, indicating α>1. Thus although **36** was the most successful inhibitor synthesized, its binding mode and mechanism are entirely different than anticipated.

3. Conclusions

In conclusion, we have synthesized and evaluated a library of GlcNAc analogues with metal chelating substituents as potential CE4 esterase inhibitors. A 2-C gluco scaffold was efficiently accessed using iodinium-mediated cyclopropane opening. The resulting primary iodide was a versatile synthon to produce methylphosphonate and sulfonamide groups. In addition, glucosamine derivatives functionalized with metal coordinating groups at both C2 and C3 have been accessed through nucleophilic ring opening of 2,3-cyclic sulfamidates. Evaluation of this inhibitor library has highlighted the level of specificity of substrate recognition in CE4 enzymes. The lack of inhibition by the methylphosphonate inhibitors suggests the PgdA binding site cannot accommodate functional groups larger than a methyl substituent. Furthermore, our results suggest only minor

contributions to substrate binding can be attributed to the OH-3 hydroxyl metal coordination as no significant binding was observed with the substitution with a thiol at C3. Lastly, a potent but mechanistically unusual CE4 inhibitor was identified. In the presence of excess Zn^{2+}, the 2-thioacetamido 3-thio derivative **36** was a partial mixed type inhibitor of PgdA.

Interestingly, our IC_{50} and K_m data suggest that the triacetylchitotriose substrate and the benzyl glycoside of GlcNAc (**20**) bind PgdA's active site with similar affinity. Nevertheless, de-N-acetylation activity is orders of magnitude higher on chitotriose. This differential catalytic competence between the monosaccharide and trisaccharide substrates suggests orientation or the enzyme conformation is suboptimal with the monosaccharides and larger substrate analogues may be necessary to build potent inhibitors.

4. Experimental

4.1. Enzyme expression and purification

PgaB was expressed and purified as previously reported.(23) Briefly, a culture of E. coli BL21 (DE3) cells transformed with a pET28 plasmid coding for PgaB42-655 was grown in LB media with streptomycin (100 mg L-1). The culture was grown at 37 °C to an OD600 of 0.6, induced with IPTG (1 mM), and grown overnight at 15 °C. Cells were collected by centrifugation (3750 g, 45 min). The cells were suspended in PgaB lysis buffer (50 mM phosphate, 200 mM NaCl, pH 8) and a protease inhibitor cocktail tablet (Roche) then lysed by sonication. The solution was clarified by centrifugation (16,000 g, 1 h). PgaB was purified by immobilized metal ion affinity chromatography using HIS-Select Nickel Affinity resin (Sigma–Aldrich). The column was washed with 10 volumes of PgaB lysis buffer then PgaB was eluted with an increasing imidazole gradient (20 mM to 200 mM). The protein was concentrated by centrifugal filtration (Milipore, 30K cutoff) and buffer exchanged using the same method into PgaB lysis buffer. Protein concentration was quantified by 280 nm absorbance (ε = 152 000 M-1 cm-1).

*Sp*PgdA was expressed and purified as previously reported [4]. A culture of E. coli BL21 (DE3) cells transformed with a pET28b-*Sp*PgdA232–431 plasmid was grown in LB media with kanamycin (50 mg/L). The culture was grown at 37 °C to an OD600 of 0.6, induced with IPTG (1 mM), and grown for 3 h at 37 °C. Cells were collected by centrifugation (3750 g, 1 h). The cells were suspended in Tris buffer (25 mM, pH 7.5) with NaCl (100 mM) and a protease inhibitor cocktail tablet (Roche) then lysed by sonication. The solution was clarified by centrifugation (16 000 g, 1 h). *Sp*PgdA was purified by immobilized metal ion affinity chromatography using HIS-Select Nickel Affinity resin (Sigma–Aldrich). The column was washed with 10 volumes of buffer then *Sp*PgdA was eluted with an increasing imidazole gradient (5 mM to 200 mM). The protein was dialyzed against HEPES buffer (25 mM, pH 7) and concentrated by centrifugal filtration (Milipore, 3K cutoff). Protein concentration was quantified by 280 nm absorbance (ε = 254 440 M-1 cm-1). Metal-free Apo *Sp*PgdA was produced by dialysis against EDTA (5 mM) in HEPES buffer (25 mM, pH 7) with three dialysate changes over 18 h. Next, EDTA was removed by dialysis against HEPES buffer with four dialysate changes over 36 h.

4.2. Fluorogenic AMMU esterase assay

Reactions were performed in a black 96-well plate (50 µL total volume). PgaB assays used 2 µM enzyme in phosphate buffer (50 mM, pH 8) with NaCl (300 mM), while *Sp*PgdA assays employed 1.5 µM enzyme in HEPES buffer (25 mM, pH 7.0). The enzymes were supplemented with either one equivalent of $CoCl_2$ or two equivalents of $ZnCl_2$ as specified. The substrate AMMU was dissolved in DMSO and added to a final concentration of 100 µM AMMU and 2% DMSO, unless otherwise stated. AMMU is subject to slow auto-hydrolysis, which was measured alongside all enzymatic reactions. The enzymatic reaction rates were corrected by subtraction of this auto-hydrolysis rate. All inhibitor stocks were prepared in assay buffer and any thiol-containing compounds were incubated with one equivalent of DTT prior to assaying. The fluorescence was measured at 2 minutes intervals over 10 minutes for Co^{2+}-loaded PgaB (λex = 386 nm, λem = 460 nm). Alternatively, it was monitored for 60 minutes with readings at 5 minute intervals for Zn^{2+}-loaded PgaB, Co^{2+}-loaded *Sp*PgdA, and Zn^{2+}-loaded *Sp*PgdA. IC_{50} values were determined by fitting eq. 2 to the data using nonlinear regression analysis.

$$\frac{v_i}{v_0} = \frac{IC_{50}{}^n}{IC_{50}{}^n + [I]^n} \qquad \text{(eq. 2)}$$

Where v_i and v_0 are the velocities of the inhibited and uninhibited reactions, n is the Hill coefficient, and [I] is the inhibitor concentration. All nonlinear regression analysis was done using the program *KaleidaGraph* v. 4.03 from Synergy Software (Reading, PA).

4.3. Fluorescamine assay deacetylase assay

A fluorescamine-based assay was used to monitor *Sp*PgdA de-*N*-acetylation of chitotriose, modified from a previously reported method.(23) Assays were conducted in HEPES buffer (25 mM, pH 7.0) with 50 µL reaction volumes. *Sp*PgdA (1 µM) was added to solutions with varied concentrations of chitotriose (1 to 35 mM) and inhibitor **21** (0 to 300 µM). The reaction was monitored for 1.5 h by removing an aliquot (10 µL) every 0.5 h increment. The reaction was stopped by adding the aliquot to a 96-well plate containing quenching buffer (20 µL, 0.4 M borate, 0.1 M EDTA, pH 9.0). The mixture was reacted with fluorescamine (10 µL, 20 mg/mL in DMF) for 10 min, and then diluted with DMF/H2O (1:1, 60 µL). A range of glucosamine standards (0.05 to 10 mM) were also prepared according to this protocol to quantitatively relate fluorescence intensity to the amount of de-N-acetylation. The fluorescence of all samples were measured (λex = 360 nm, λem = 460 nm). Chitotriose auto-hydrolysis in the assay buffer with or without **21** was negligible (data not shown).

The ability of PgaB or PgdA to catalyze hydrolysis of C3-substitued derivatives was evaluated using a fluorescamine-based assay. The hydrolysis of these sugars would generate a primary amine which forms a fluorescent product upon reaction with fluorescamine. PgaB (3 µM) or PgdA (4 µM) was incubated with each sugar (1 mM or 5 mM) at 37 °C for 24 h. These reactions were conducted in each enzyme's respective assay buffer described previously. Aliquots (10 µL) of each reaction were added to borate buffer to adjust pH (0.4 M, pH 9), reacted with fluorescamine (10 µL, 20 mg/mL in DMF) for 10 min, and diluted with water (80 µL). The samples were transferred to a 96-well plate and the fluorescence was measured (λex = 360 nm, λem = 460 nm). As positive controls PNAG pentamer and triacetylchitotriose were used as substrates of PgaB and PgdA, respectively.

4.4. *Chemical Synthesis*

4,6- *O*-Benzylidene-1,5-anhydro-2-deoxy-1,2-*C*-(dichloromethylene)-D-*glycero*-D-*gulo*-hexitol (1)

The required benzyl-protected dichlorocyclopropane sugar was prepared according to a published procedure. (24) The dichlorocyclopropane derivative (5.4 g, 10.8 mmol) was dissolved in 50% MeOH/THF (500 mL), the solution was degassed before careful addition of 10% palladium on carbon (50 mg). Flask was purged with N_2 three times prior to three purges with H_2. The flask was maintained with a positive pressure of H_2, sealed and vigorously stirred overnight. The hydrogen gas was then evacuated and the reaction mixture was filtered through celite, the celite was washed exhaustively with MeOH and the resulting filtrate was concentrated to dryness under reduced pressure to give the desired triol. This product was immediately carried forward to the next step.

The triol was dissolved in dry MeCN (50 mL) and benzaldehyde dimethyl acetal (1.5 eq, 2.4 mL, 16 mmol) was added along with catalytic *p*-toluenesulfonic acid (0.1 eq, 0.19 g, 1.1 mmol). The reaction was sealed under N_2 and stirred overnight. When complete by TLC, the reaction was quenched with $NaHCO_{3(aq)}$ and extracted into DCM (75 mL). The organic layers were pooled and dried over $Na_2SO_{4(s)}$ and dried and concentrated. The resulting residue was purified using silica gel chromatography (5-20% EtOAc/pentane) to give the pure compound (2.0 g, 59%) over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.50 – 7.44 (m, 2H), 7.39 (dt, *J* = 4.5, 2.7, 3H), 5.51 (s, 1H), 4.27 (dd, *J* = 10.4, 4.9, 1H), 4.04 (dd, *J* = 9.4, 2.4, 1H), 3.89 (d, *J* = 9.2, 1H), 3.80 (td, *J* = 9.9, 4.9, 1H), 3.64 – 3.50 (m, 2H), 1.98 (dd, *J*=9.1, 2.5, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.9, 129.3, 128.3, 126.2, 101.8, 80.5, 69.9, 68.1, 67.6, 62.7, 34.3. DART-MS calc for $C_{14}H_{15}Cl_2O_4$ $[M+H]^+$: 317.0347; found 317.0346.

1,5-Anhydro-4,6-*O*-benzylidene-3-*O*-benzoyl-2-deoxy-1,2-*C*-methylene-D-*glycero*-D-*gulo*-hexitol (2)

To a stirred solution of lithium aluminum hydride (20 eq, 19.1 g, 503 mmol) in dry THF (200 mL), a 0.1 M solution of **1** (7.98 g, 25.2 mmol) in THF was slowly added. Reaction was fitted with a condenser and allowed to reflux for 72 h. After this time, the reaction was cooled to 0 °C in a large water/ice bath. Maintaining this temperature, EtOAc was slowly added over 1 h until quenching was complete. The mixture was then filtered and the solid was thoroughly washed with EtOAc, retaining all filtrate. The combined filtrates were washed with water (500 mL) and brine (500 mL). The organic layer was dried over $Na_2SO_{4(s)}$ and evaporated to dryness. Crude product was used in the next reaction without purification.

Residue was dissolved in dry pyridine (150mL) and treated with benzoyl chloride (2.5 eq, 6.9 mL, 59.6 mmol) and left until complete by TLC (approximately 3 hours). The reaction was quenched with $NH_4Cl_{(aq)}$ and extracted 3x with EtOAc (50 mL). Organic layers were pooled and washed with $NaHCO_{3(aq)}$, $NH_4Cl_{(aq)}$, water, and brine. Finally, the organic layer was dried, concentrated and purified using silica gel chromatography to give the title compound in (6.0 g, 68%) as a white crystalline solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.18-8.11 (m, 3H), 7.68-7.58 (m, 1H), 7.54-7.45 (m, 5H), 7.38-7.32 (m, 2H) 5.59 (s, 1H), 5.38 (d, *J* = 8.6 Hz, 1H), 4.34 (dd, *J* = 10.6, 5.1 Hz, 1H), 3.94 (dd, *J* = 9.9, 8.7 Hz, 1H), 3.81-3.71 (m, 2H), 3.54 (ddd, *J* = 9.8, 5.1 Hz, 1H), 1.24 (ddd, *J* = 7.0, 7.0, 4.1 Hz, 1H), 1.05 (m, 1H), 0.83 (ddd, *J* = 10.6, 7.2, 7.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.0, 137.1, 133.8, 133.1, 130.2, 129.8, 129.0, 128.5, 128.4, 128.2, 126.1, 101.5, 78.0, 71.7, 69.4, 61.4, 52.8, 13.8, 5.6. DART-MS calc for $C_{21}H_{21}O_5$ $[M+H]^+$: 353.1389; found 353.1398.

Benzyl 4,6- *O*-benzylidene-3-*O*-benzoyl-2-deoxy-2-*C*-(iodomethyl)-*β*-D-glucopyranoside (3)

Compound **2** (0.25 g, 0.71 mmol), was dissolved in dry DCM (4 mL) along with benzyl alcohol (5.0 eq, 0.36 mL, 3.5 mmol). To this solution was added *N*-iodosuccinimide (1.2 eq, 0.192 g, 0.852 mmol) at room temperature. The flask was then sealed and purged with N_2 and allowed to react overnight. Upon completion by TLC, the reaction was quenched by the dropwise addition of $Na_2S_2O_{3(aq)}$ until the solution became colourless. The reaction was diluted with DCM (20 mL) and extracted. The organic layer was washed with water and brine before being dried and evaporated. Purification by silica gel chromatography (100% $CHCl_3$) provided **3** (0.27 g, 66%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.01 – 7.93 (m, 3H), 7.51 – 7.43 (m, 1H), 7.39 – 7.25 (m, 10H), 7.20 (dt, *J*=6.1, 2.7, 4H), 5.43 (s, 1H), 4.88 (d, *J*=11.2, 1H), 4.70 (d, *J*=8.0, 1H), 4.59 (d, *J*=11.2, 1H), 4.32 (dd, *J*=10.4, 5.0, 1H), 3.80 (dt, *J*=16.9, 9.8, 2H), 3.55 (td, *J*=9.7, 5.0, 1H), 3.43 (dd, *J*=10.5, 3.2, 1H), 3.18 – 3.12 (m, 1H), 1.70 (ddt, *J*=10.7, 8.0, 2.9, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.1, 137.0, 136.8, 133.2, 129.9, 129.6, 128.9, 128.6, 128.5, 128.4, 128.2, 128.2, 126.1, 103.5, 101.5, 80.3, 72.1, 72.0, 68.8, 66.4, 45.5, 3.6. DART-MS calc for $C_{28}H_{28}IO_6$ $[M+H]^+$: 587.0931; found 587.0934.

Benzyl 4,6- *O*-benzylidene-3-*O*-benzoyl-2-deoxy-2-*C*-((dimethylphosphono)methyl)-*β*-D-glucopyranoside (4)

Compound **3** (0.25 g, 0.43 mmol) was dissolved in trimethylphosphite (2.5 mL, 21 mmol), fitted with a condenser and sealed under N_2, the reaction was allowed to reflux overnight. The reaction was allowed to cool to RT at which point water (50 mL) was added and left to stir for 30 min. The mixture was then extracted into DCM (25 mL). The organic layers were pooled and washed with brine before being dried and evaporated. The residue left under high vacuum overnight before purification via silica gel chromatography (10-20% EtOAc/$CHCl_3$) to give **4** (0.12 g, 49%) as a clear oil in. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.02-7.98 (m, 2H), 7.50-7.44 (m, 1H), 7.34-7.17 (m, 12H), 5.48 (dd, *J* = 11.0, 9.2 Hz, 1H), 5.43 (s, 1H), 4.77 (d, *J* = 8.7 Hz, 1H), 4.72 (dd, *J* = 121, 11.3 Hz, 2H), 4.32 (dd, *J* = 10.4, 4.9 Hz, 1H), 3.78 (t, *J* = 10.2 Hz, 1H) 3.69 (t, *J* = 9.3 Hz, 1H) 3.61-3.51 (m, 2H), 3.45 (m, 6H), 2.41 (m, 1H), 2.03-1.93 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.6, 137.1, 136.9, 133.0, 129.8, 128.8, 128.5, 128.3, 128.3, 128.1, 128.1, 126.0, 101.9, 101.8, 101.3, 80.6, 72.7, 72.7, 71.6, 68.8, 66.4, 52.4, 52.4, 52.2, 52.1, 42.4, 42.3, 23.1, 21.7. ^{31}P NMR (162 MHz, $CDCl_3$) δ: 31.6. DART-MS calc for $C_{30}H_{24}O_9P$ $[M+H]^+$: 569.1940; found 569.1949.

Benzyl 4,6- *O*-acetyl-3-*O*-benzoyl-2-deoxy-2-*C*-((dimethylphosphono)methyl)-*β*-D-glucopyranoside (5)

Compound **4** (0.10 g, 0.18 mmol) was dissolved in an 8:1:1 acetic acid:THF:water (2 mL). The solution was brought to reflux and left overnight. Upon disappearance of starting material, the solvent was removed under vacuum. The resulting residue was dissolved in pyridine (2 mL) to which was added acetic anhydride (0.35 mL, 3.5 mmol). The reaction stirred for 3 hours before being quenched through the addition of $NaHCO_{3(aq)}$ (10 mL). The mixture was extracted with EtOAc (10 mL) and organic layers pooled then washed with $NH_4Cl_{(aq)}$, water, and brine prior to removal of the solvent via rotary evaporation. Purification by silica gel chromatography (50-100% EtOAc/pentane) provided **5** (0.095 g, 98%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.96 – 7.91 (m, 2H), 7.52 – 7.45 (m, 1H), 7.40 – 7.24 (m, 7H), 5.36 (dd, *J*=11.2, 9.1 Hz, 1H), 5.09

(dd, *J*=10.0, 9.1 Hz, 1H), 4.88 (d, *J*=11.3 Hz, 1H), 4.67 (d, *J*=8.7 Hz, 1H), 4.58 (d, *J*=11.3 Hz, 1H), 4.26 (dd, *J*=12.2, 4.8 Hz, 1H), 4.12 (dd, *J*=12.2 Hz, 2.4, 1H), 3.72 (ddd, *J*=10.0, 4.9, 2.4 Hz, 1H), 3.43 (m, 6H), 2.38 (m, 1H), 2.04 (s, 3H), 1.99 – 1.90 (m, 2H), 1.82 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.8, 169.7, 166.0, 136.8, 133.3, 129.8, 129.5, 128.5, 128.5, 128.4, 128.1, 101.1, 101.0, 74.0, 74.0, 71.8, 71.4, 69.9, 62.4, 52.4, 52.4, 52.1, 52.0, 41.9, 41.8, 29.7, 20.8, 20.6. ^{31}P NMR (162 MHz, $CDCl_3$) δ: 31.4. DART-MS calc for $C_{27}H_{34}O_{11}P$ $[M+H]^+$: 565.1839; found 565.1838.

Benzyl 6-*O*-Benzyl-3-*O*-benzoyl-2-deoxy-2-*C*-((dimethylphosphono)methyl)-*β*-D-glucopyranoside (6)

Phosphonate sugar **4** (0.165 g, 0.290 mmol) was dissolved in dry DCM (3 mL) under Ar and cooled to 0 °C. To this solution was added triethylsilane (10 eq, 0.46 mL, 2.9 mmol) and boron trifluoride diethyl etherate (2.0 eq, 0.075 mL, 0.58 mmol). The reaction was left at 0 °C for 4 h before being allowed to warm to RT overnight. The reaction was quenched with $NaHCO_{3(aq)}$ and extracted three times into DCM (10 mL). The organic layers were pooled, dried and evaporated. Purification via silica gel chromatography (100% EtOAc) provided the title compound (0.043 g, 26%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.14-8.06 (m, 2H), 7.60 (m, 1H), 7.51-7.30 (m, 13H), 5.29 (dd, *J*=11.2, 8.7 Hz, 1H), 4.98 (d, *J*=11.4 Hz, 1H), 4.75 (d, *J*=8.6 Hz, 1H), 4.65 (m, 3H), 3.92-3.81 (m, 2H), 3.77 (ddd, *J*=9.5, 3.4 Hz, 1H), 3.68-3.60 (m, 2H), 3.53 (m, 6H) 2.52-2.35 (m, 1H), 2.08 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.7, 138.0, 137.2, 133.4, 129.9, 129.6, 128.5, 128.4, 128.4, 128.3, 127.9, 127.7, 127.7, 127.7, 101.0, 101.0, 77.7, 77.6, 74.9, 73.7, 71.9, 71.2, 70.1, 52.4, 52.3, 52.1, 52.0, 41.5, 41.5, 22.8, 21.4.^{31}P NMR (162 MHz, $CDCl_3$) δ: 31.8. ESI-MS calc for $C_{30}H_{35}NaO_9P$ $[M+Na]^+$: 593.1911; found 593.1909.

Benzyl 4-*O*-benzyl-3-*O*-benzoyl-2-deoxy-2-*C*-((dimethylphosphono)methyl)-*β*-D-glucopyranoside (7)

Phosphonate **4** (0.165 g, 0.29 mmol) was dissolved in dry DCM (5 mL) under Ar. To this solution was added 1 M borane tetrahydrofuran complex solution (5.0 eq, 1.45 mL) and trimethylsilyl triflate (0.15 eq, 0.008 mL, 0.044 mmol) and reaction was left overnight at room temperature. The reaction was quenched with $NaHCO_{3(aq)}$ and extracted three times into DCM (10 mL). The organic layers were pooled, dried and evaporated. Purification via silica gel chromatography (20% EtOAc/pentane) provided the title compound (0.12, 70%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.11 – 8.05 (m, 2H), 7.60 (m, 1H), 7.47 (m, 2H), 7.41 – 7.27 (m, 5H), 7.21 – 7.07 (m, 5H), 5.62 (dd, *J*=11.2, 8.5 Hz, 1H), 5.31 (d, *J*=3.3 Hz, 1H), 4.74 (d, *J*=11.6 Hz, 1H), 4.58 (d, *J*=2.9 Hz, 2H), 4.54 (d, *J*=11.6 Hz, 1H), 3.90 – 3.76 (m, 5H), 3.62 (m, 6H), 2.47 (m, 1H), 2.16 – 2.02 (m, 1H), 1.83 (ddd, *J*=20.8, 15.6, 2.8 Hz, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.1, 137.4, 137.3, 133.3, 129.8, 129.7, 129.7, 129.7, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 98.0, 98.0, 76.7, 76.6, 74.9, 74.8, 74.6, 71.4, 70.0, 61.5, 52.3, 52.3, 52.3, 52.3, 40.6, 40.6, 22.8, 21.3.^{31}P NMR (162 MHz, $CDCl_3$) δ: 32.5. ESI-MS calc for $C_{30}H_{35}NaO_9P$ $[M+Na]^+$: 593.1911; found 593.1901.

General procedure for Zemplén deacetylation.

The starting peracetylated sugar was dissolved in MeOH. NaOMe solution (25% wt) was added dropwise until the solution was highly basic. The reaction was stirred at RT and monitored by TLC. Once complete, Dowex resin (H^+-form) was added until the solution was neutral on pH paper. The mixture was filtered, concentrated, and lyophilized.

Benzyl 2-deoxy-2-*C*-((methylphosphono)methyl)-*β*-D-glucopyranoside (8)

Compound **5** (0.012 g, 0.021 mmol) was deprotected according to the general Zemplén deacetylation procedure. The product was dissolved in water and washed with EtOAc. The aqueous layer was then lyophilized and subsequently purified by size-exclusion chromatography on a P2 resin in water to give the deprotected product **8** (0.006 g, 78%) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ = 7.55 – 7.36 (m, 5H), 4.83 (dd, *J*=89.9, 11.3 Hz, 2H), 4.62 (d, *J*=8.7 Hz, 1H), 3.95 (dd, *J*=12.3, 2.1 Hz, 1H), 3.79 – 3.70 (m, 1H), 3.66 – 3.57 (m, 1H), 3.52 (d, *J*=10.6 Hz, 3H), 3.46 (ddd, *J*=8.6, 6.1, 2.0 Hz, 1H), 3.39 – 3.30 (m, 1H), 2.05 (ddd, *J*=20.5, 15.4, 2.5 Hz, 1H), 1.88 – 1.74 (m, 1H), 1.68 (td, *J*=16.0, 8.1 Hz, 1H). ^{13}C NMR (100 MHz, D_2O) δ: 136.7, 128.9, 128.7, 128.5, 101.8, 101.7, 75.7, 75.6, 75.5, 71.7, 70.6, 61.1, 51.4, 51.3, 43.8, 43.8, 24.7, 23.4.^{31}P NMR (162 MHz, D_2O) δ: 27.1. ESI-MS calc for $C_{15}H_{23}NaO_8P$ $[M+Na]^+$: 385.1023; found 385.1017.

Benzyl 6-*O*-benzyl-2-deoxy-2-*C*-((methylphosphono)methyl)-*β*-D-glucopyranoside (9)

Compound **6** (0.043 g, 0.075 mmol) was deprotected according to the general Zemplén deacetylation procedure. The product was dissolved in water and washed with EtOAc (5 mL). The aqueous layer was then lyophilized and subsequently purified by size-exclusion chromatography on a P2 resin with water as the eluent to give the deprotected product **9** (0.006, 18%) yield. ^{1}H NMR (500 MHz, D_2O) δ = 7.37 – 7.15 (m, 11H), 4.63 (dd, *J*=93.8, 11.4 Hz, 2H), 4.45 – 4.41 (m, 1H), 3.79 (dd, *J*=11.4, 2.1 Hz, 1H), 3.48 – 3.40 (m, 2H), 3.37 (d, *J*=10.5 Hz, 3H), 3.18 (dd, *J*=10.0, 8.8 Hz, 1H), 1.90 (ddd, *J*=20.1, 15.6, 2.8 Hz, 1H), 1.71 – 1.60 (m, 1H), 1.53 (ddd, *J*=16.9, 15.5, 8.1 Hz, 1H).^{13}C NMR (126 MHz, D_2O) δ: 137.3, 136.6, 128.8, 128.6, 128.4, 128.3, 128.2, 101.8, 101.7, 75.4, 75.4, 74.4, 73.1, 71.7, 70.8, 69.3, 51.2, 51.2, 43.7, 43.7, 24.4, 23.4.^{31}P NMR (162 MHz,D_2O) δ: 27.1. ESI-MS calc for $C_{22}H_{29}NaO_8P$ $[M+Na]^+$: 475.1492; found 475.1496.

Benzyl 4-*O*-benzyl-2-deoxy-2-*C*-((methylphosphono)methyl)-*β*-D-glucopyranoside (10)

Compound **7** (0.11 g, 0.19 mmol) was deprotected according to the general Zemplén deacetylation procedure. The product was dissolved in water and washed with EtOAc (5 mL). The aqueous layer was then lyophilized and subsequently purified by size-exclusion chromatography on a P2 resin with water as the eluent to give the deprotected product (0.001 g, 15%). ^{1}H NMR (D_2O, 500 MHz) δ: 7.35-7.24 (m, 10H), 5.09 (d, *J*=3.9 Hz, 1H), 4.74 (dd, *J*=161, 10.4 Hz, 2H), 4.49 (dd, *J*=10.6, 1.4 Hz 2H), 3.61 (m, 2H), 3.58-3.50 (m, 2H) 3.41-3.35 (m, 4H), 1.93-1.83 (m, 2H) 1.61 (ddd, 1H); ^{13}C NMR (126 MHz, D_2O) δ: 137.2, 137.1, 128.9, 128.8, 128.7, 128.6, 128.4, 128.4, 128.4, 128.2, 98.6, 78.7, 78.6, 74.8, 71.1, 70.0, 60.4, 51.0, 50.9, 42.5, 42.5, 22.9, 21.80.^{31}P NMR (162 MHz,D_2O) δ: 27.4. ESI-MS calc for $C_{22}H_{29}NaO_8P$ $[M+Na]^+$: 475.1492; found 475.1490.

Benzyl 4,6- *O*-benzylidene-3-*O*-benzoyl-2-deoxy-2-*C*-(acetylthiomethyl)-*β*-D-glucopyranoside (11)

Potassium thioacetate (2.0 eq, 0.012 g, 0.10 mmol) was added to a solution of iodo sugar **3** (0.03 g, 0.051 mmol) in THF (2 mL).

The solution was heated to 60 °C under N_2 and stirred overnight. Upon complete consumption of starting material by TLC, reaction was allowed to return to RT before solvent was removed by rotary evaporation. The resulting residue was resuspended in EtOAc (25 mL) and washed with $NaHCO_{3(aq)}$, water, and brine. The organic layer was dried and solvent was removed under vacuum. Purification by silica gel chromatography (100% $CHCl_3$) provided the thioacetate product (0.025 g, 93%) as a tan solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.01 – 7.94 (m, 2H), 7.53 – 7.44 (m, 1H), 7.41 – 7.25 (m, 9H), 7.21 (m, 3H), 5.47 – 5.39 (m, 2H), 4.84 (d, *J*=11.3 Hz, 1H), 4.57 – 4.48 (m, 2H), 4.31 (dd, *J*=10.5, 5.0 Hz, 1H), 3.81 – 3.67 (m, 2H), 3.47 (ddd, *J*=9.7, 9.7, 5.0 Hz, 1H), 3.25 (dd, *J*=14.2, 3.5 Hz, 1H), 3.10 (dd, *J*=14.1, 4.2 Hz, 1H), 2.33 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 194.1, 165.6, 137.1, 136.7, 133.1, 129.9, 129.8, 128.9, 128.5, 128.4, 128.4, 128.1, 128.1, 126.0, 101.4, 101.1, 80.4, 71.5, 70.8, 68.8, 66.3, 46.1, 30.5, 25.4. ESI-MS calc for $C_{30}H_{34}NO_7S$ $[M+NH_4]^+$: 552.2050; found 552.2058.

Benzyl 4,6- *O*-benzylidene-3-*O*-benzoyl-2-deoxy-2-*C*-((aminosulfinyl)methyl)-*β*-D-glucopyranoside (12)

Acetic anhydride (1.1 eq, 0.004 mL, 0.04 mmol) and sulfuryl chloride (2.0 eq, 0.006 mL, 0.07 mmol) were added at -10 °C to a solution of thioacetate **11** (0.020 g, 0.037 mmol) in DCE (0.5 mL) and stirred for 30 min. The reaction mixture was then concentrated to an oil to which was added an ice-cold solution of 28% NH_4OH (0.25 mL). After 5 min of vigorous stirring, reaction was diluted with water (10 mL) and extracted three times with EtOAc (25 mL). Organic layers were dried and removed under rotary evaporation to give the title compound (0.017 g, 86%) as a mixture of diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.12 – 8.02 (m, 2H), 7.60 – 7.52 (m, 1H), 7.43 (m, 2H), 7.37 (m, 8H), 7.31 – 7.27 (m, 2H), 5.54 – 5.45 (m, 2H), 4.96 (d, *J*=11.6 Hz, 1H), 4.65 (dd, *J*=10.1, 1.5 Hz, 1H), 4.41 (dd, *J*=10.5, 5.0 Hz, 1H), 3.92 – 3.77 (m, 3H), 3.58 (ddd, *J*=9.7, 9.7, 5.0 Hz, 1H), 2.88 – 2.78 (m, 2H), 2.69 (ddd, *J*=10.8, 8.6, 5.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.9, 136.3, 133.4, 130.0, 128.9, 128.7, 128.6, 128.5, 128.4, 128.3, 128.1, 126.0, 101.5, 100.9, 80.1, 72.4, 71.1, 68.7, 66.8, 41.9. DART-MS calc for $C_{28}H_{30}NO_7S$ $[M+H]^+$: 524.1743; found 524.1760.

Benzyl 4,6-*O*-benzylidene-3-*O*-benzoyl-2-deoxy-2-*C*-(sulfamoylmethyl)-*β*-D-glucopyranoside (13)

To a solution of sulfinamide **12** (0.34 g, 0.65 mmol) in DCM (7 mL) was added a commercial mixture of 70% mCPBA (estimated 1.5 eq, 0.21 g, 0.97 mmol). Reaction was complete within 15 min and quenched with $NaHCO_{3(aq)}$. The mixture was diluted with DCM (15 mL) and aqueous layer removed. The organic layer was dried and the solvent removed to provide the title compound as a white solid (0.33 g, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.07 – 8.02 (m, 2H), 7.59 – 7.52 (m, 1H), 7.46 – 7.26 (m, 12H), 5.61 (dd, *J*=10.9, 9.1 Hz, 1H), 5.50 (s, 1H), 4.95 (d, *J*=10.9 Hz, 1H), 4.86 (d, *J*=8.6 Hz, 1H), 4.61 (d, *J*=10.9 Hz, 1H), 4.41 (dd, *J*=10.4, 4.9 Hz, 1H), 3.84 (ddd, *J*=12.7, 9.7, 9.7 Hz, 2H), 3.62 (ddd, *J*=9.7, 9.7, 4.9 Hz, 1H), 3.44 (dd, *J*=15.2, 3.8 Hz, 1H), 3.31 (dd, *J*=15.2, 5.2 Hz, 1H), 2.64 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.3, 136.9, 136.2, 133.4, 130.0, 129.3, 128.9, 128.8, 128.6, 128.5, 128.5, 128.4, 128.2, 126.0, 101.4, 101.1, 80.2, 77.3, 77.0, 76.7, 71.9, 71.5, 68.6, 66.4, 52.9, 44.0. ESI-MS calc for $C_{28}H_{33}N_2O_8S$ $[M+NH_4]^+$: 557.1952; found 557.1952.

Benzyl 4,6-di-*O*-Acetyl-3-*O*-benzoyl-2-deoxy-2-*C*-(sulfamoylmethyl)-*β*-D-glucopyranoside (14)

Compound **13** (0.050 g, 0.093 mmol) was dissolved in an 8:1:1 acetic acid:THF:water (1 mL). The solution was brought to reflux and left overnight, then the solvent was removed under vacuum. The resulting residue was dissolved in pyridine (1 mL) to which was added acetic anhydride (0.1 mL, 1.06 mmol). The reaction was allowed to progress for 3 hours before being quenched with the addition of $NaHCO_{3(aq)}$ (10 mL). The mixture was extracted with EtOAc (15 mL) and organic layers pooled then washed with $NH_4Cl_{(aq)}$, water, and brine prior to removal of the solvent via rotary evaporation. Purification by silica gel chromatography (50% EtoAc/pentane) provided the product as a white solid in (0.017 g, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.65 (m, 2H), 8.02 – 7.96 (m, 2H), 7.61 – 7.51 (m, 1H), 7.39 – 7.32 (m, 6H), 5.49 (dd, *J*=11.1, 9.0 Hz, 1H), 5.22 (dd, *J*=9.9, 9.0 Hz, 1H), 4.81 (dd, *J*=133.2, 11.0 Hz, 2H), 4.80 (d, *J*=8.6 Hz, 1H), 4.38 – 4.30 (m, 1H), 4.21 (dd, *J*=12.2, 2.5 Hz, 1H), 3.79 (ddd, *J*=9.9, 4.9, 2.6 Hz, 1H), 3.40 (dd, *J*=15.1, 4.0 Hz, 1H), 3.29 (dd, *J*=15.1, 5.3 Hz, 1H), 2.64 (m, 1H), 2.12 (s, 3H), 1.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.8, 169.6, 166.4, 148.9, 136.8, 136.1, 133.7, 133.0, 130.1, 130.0, 129.7, 128.8, 128.7, 128.6, 128.6, 128.1, 124.1, 100.3, 77.3, 77.2, 77.0, 76.7, 72.7, 71.9, 71.7, 69.5, 62.2, 52.6, 43.6, 20.8, 20.5. ESI-MS calc for $C_{25}H_{29}NNaO_{10}S$ $[M+Na]^+$: 558.1404; found 558.1412.

Benzyl 4-*O*-benzyl-3-*O*-benzoyl-2-deoxy-2-*C*-(sulfamoylmethyl)-*β*-D-glucopyranoside (15)

Sulfonamide **13** (0.050 g, 0.093 mmol) was dissolved in dry DCM (2 mL) under Ar. To this solution was added 1 M borane tetrahydrofuran complex solution (5.0 eq, 0.46 mL) and trimethylsilyl triflate (0.15 eq, 0.025 mL, 0.139 mmol) and reaction was left overnight. The reaction was quenched with $NaHCO_{3(aq)}$ and extracted three times into DCM (15 mL). The organic layers were pooled, dried and evaporated. Purification via silica gel chromatography (50-100% EtOAc/pentane) provided the product (0.013 g, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.06 (m, 2H), 7.62 – 7.53 (m, 1H), 7.44 (m, 2H), 7.39 – 7.28 (m, 7H), 7.25 – 7.19 (m, 2H), 7.12 – 7.06 (m, 2H), 5.39 (dd, *J*=11.3, 8.8 Hz, 1H), 4.92 (d, *J*=11.2 Hz, 1H), 4.86 (d, *J*=8.7 Hz, 1H), 4.63 (d, *J*=11.2 Hz, 1H), 4.04 (dd, *J*=28.6, 6.2 Hz, 1H), 3.93 (d, *J*=3.5 Hz, 1H), 3.86 (d, *J*=4.6 Hz, 1H), 3.76 (t, *J*=9.2 Hz, 1H), 3.49 (ddd, *J*=9.6, 4.7, 3.5 Hz, 1H), 3.28 (dd, *J*=14.9, 4.1 Hz, 1H), 3.19 (dd, *J*=14.8, 4.5 Hz, 1H), 2.48 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.1, 136.7, 136.7, 133.8, 130.1, 129.0, 128.7, 128.7, 128.6, 128.5, 128.4, 128.3, 127.9, 127.7, 127.3, 100.4, 76.3, 75.7, 71.9, 71.3, 62.5, 50.1, 47.0, 43.3. ESI-MS calc for $C_{28}H_{31}NNaO_8S$ $[M+Na]^+$: 564.1663; found 564.1669.

Benzyl 2-deoxy-2-*C*-(sulfamoylmethyl)-*β*-D-glucopyranoside (16)

Compound **14** (0.018 g, 0.033 mmol) was deprotected according to the general Zemplén deacetylation procedure. The residue was subsequently purified by size-exclusion chromatography on a P2 resin using water as the eluent to give the deprotected product as a white solid (0.009, 78%). ^{1}H NMR (500 MHz, D_2O) δ = 7.36 – 7.25 (m, 5H), 4.81 (d, *J*=11.3 Hz, 1H), 4.60 – 4.53 (m, 2H), 3.81 (dd, *J*=12.3, 2.2 Hz, 1H), 3.63 (dd, *J*=12.4, 5.8 Hz, 1H), 3.46 (dd, *J*=11.0, 8.6 Hz, 1H), 3.35 – 3.22 (m, 4H), 1.94 (ddt, *J*=11.0, 8.9, 4.5 Hz, 1H); ^{13}C NMR (126 MHz, D_2O) δ: 136.4, 131.1, 129.7, 128.9, 128.7, 128.5, 127.1, 100.3, 75.8, 73.2, 71.5, 70.7, 60.8, 52.2, 44.5. ESI-MS calc for $C_{14}H_{21}NNaO_7S$ $[M+Na]^+$: 370.0931; found 370.0931.

Benzyl 4-*O*-Benzyl-2-deoxy-2-*C*-(sulfamoylmethyl)-*β*-D-glucopyranoside (17)

Compound **15** (0.013 g, 0.0246 mmol) was deprotected according to the general Zemplén deacetylation procedure. The residue was subsequently purified by silica gel chromatography (100% EtOAc) to give the product as a white solid (0.002 g, 17%). ^{1}H NMR (500 MHz, CD_3OD) δ = 7.41 (m, 2H), 7.36 – 7.20 (m, 8H), 4.79 (dd, *J*=180.7, 11.1 Hz, 2H), 4.72 (d, *J*=8.8 Hz, 1H), 4.19 – 4.08 (m, 2H), 3.92 (dd, *J*=12.2, 1.6 Hz, 1H), 3.72 (dd, *J*=12.0, 5.6 Hz, 1H), 3.66 (dd, *J*=11.3, 7.7 Hz, 1H), 3.37 (dd, *J*=14.6, 3.9Hz, 1H), 3.30 – 3.25 (m, 3H), 2.01 (m, 1H);. ^{13}C NMR (126 MHz, CD_3OD) δ: 137.9, 137.6, 128.1, 128.0, 127.9, 127.5, 127.4, 127.0, 100.5, 76.6, 73.7, 71.5, 70.8, 61.4, 49.2, 46.3, 45.1. ESI-MS calc for $C_{21}H_{27}NNaO_7S$ $[M+Na]^+$: 460.1400; found 460.1399.

Benzyl 2-amino-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (18)

The required glycosyl bromide was prepared according to a published procedure (30). This glycosyl donor (19.5 g, 43.4 mmol) was added to dry benzyl alcohol (250 mL) and pyridine (4.5 mL). The solution was stirred at 45 °C for 24 h, then poured into 1 L of diethyl ether and vigorously stirred to precipitate the product. It was recrystallized from methanol/diethyl ether, yielding the product as white crystals. The mother liquor was subjected to a second round of recrystallization to afford an additional crop (14.9, 72%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.48 – 7.30 (m, 5H, Ph), 5.26 (dd, *J* = 10.6, 9.1, 1H, H-3), 5.07 (dd, *J* = 10.1, 9.1 Hz, 1H, H-4), 4.94 (d, *J* = 11.5 Hz, 1H, CH_2Ph), 4.79 (d, *J* = 8.3 Hz, 1H, H-1), 4.75 (d, *J* = 11.5 Hz, 1H, CH_2Ph), 4.33 (dd, *J* = 12.4, 4.7 Hz, 1H, H-6_a), 4.19 (dd, *J* = 12.4, 2.4 Hz, 1H, H-6_b), 3.90 (ddd, *J* = 10.1, 4.7, 2.4 Hz, 1H, H-5), 3.41 (dd, *J* = 10.6, 8.3 Hz, 1H, H-2), 2.08 (2 s, 6H, $COCH_3$), 2.03 (s, 3H, $COCH_3$).^{13}C NMR (100 MHz, CD_3OD) δ 172.2, 172.0, 171.2, 137.4, 129.9, 129.6, 129.4, 98.7, 73.4, 72.4, 62.9, 55.6, 24.2, 20.8, 20.5.

Benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (19)

Compound **18** (8.73 g, 22.1 mmol) was dissolved in dry DCM (120 mL) with Et_3N (9.3 mL) and acetic anhydride (10.5 mL). The solution was stirred for 3 h at rt. The mixture was diluted with additional DCM, and washed with 1 M HCl, water, then brine. Removal of solvent *in vacuo* gave a white powder (7.85 g, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24-7.37 (m, 5H, Ph), 5.96 (d, *J*=9.9 Hz, 1H, NH), 5.25 (dd, *J*= 10.5, 9.3 Hz, 1H, H-3), 5.08 (t, *J*=9.8 Hz, 1H, H-4), 4.88 (d, *J* = 12.2 Hz, 1H, CH_2Ph), 4.69 (d, *J* = 8.4 Hz 1H, H-1), 4.60 (d, *J* = 12.2 Hz, 1H, CH_2Ph), 4.27 (dd, *J* = 4.9, 12.3 Hz, 1H, H-6_a), 4.15 (dd, *J* = 2.5, 12.3 Hz, 1H H-6_b), 3.98 (dt, *J* = 8.6, 10.5, 1H, H-2), 3.7 (m, 1H, H-5), 2.09 (s, 3H, $COCH_3$), 2.00(s, 6H, 2 $COCH_3$), 1.88 (s, 3H, $COCH_3$). ^{13}C NMR (400 MHz, $CDCl_3$) δ 170.8, 170.7, 170.3, 169.4, 137.0, 128.4, 127.9, 99.6, 72.5, 71.8, 70.7, 68.8, 62.2, 54.4, 23.2, 20.8, 20.7, 20.6.

Benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (21)

To a slurry of **20** (5.50 g, 17.7 mmol) in dry acetonitrile (150 mL) was added benzaldehyde dimethyl acetal (2.1 eq, 5.5 mL, 36.6 mmol) followed by catalytic *p*-toluenesulfonic acid (monohydrate, 150 mg, 0.8 mmol). The mixture was stirred for 4 h at rt. The highly insoluble product was further precipitated by cooling to 0 °C. Filtration of the slurry afforded the product as a white powder (6.50 g, 92%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.87 (d, *J* = 7.8 Hz, 1H, NH), 7.53 – 7.19 (m, 10H, Ph), 5.62 (s, 1H, CHPh), 5.31 (bs, 1H, OH), 4.78 (d, *J* = 12.5 Hz, 1H, CH_2Ph), 4.58 (d, *J*= 8.0 Hz, 1H, H-1), 4.53 (d, *J* = 12.5 Hz, 1H, CH_2Ph), 4.24 (dd, *J* = 10.2, 4.9 Hz, 1H, H-6_a), 3.77 (t, *J* = 10.1 Hz, 1H, H-6_b), 3.62 (m, 2H, H-2 and H-3), 3.47 (t, *J* = 9.0 Hz, 1H, H-4), 3.38 (m, 1H, H-5), 1.83 (s, 3H, $COCH_3$). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.1, 137.9, 137.7, 128.9, 128.2, 128.0, 127.4, 127.2, 126.3, 101.4, 100.7, 81.3, 70.3, 70.0, 67.9, 66.0, 56.2, 23.1.

Benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-allopyranoside (22)

Compound **21** (9.79 mmol, 3.92 g) was stirred with dry DCM (40 mL) and pyridine (40 mL) for 5 min, then MsCl (3 eq, 3.81 mL, 29.4 mmol) was added dropwise. The reaction was stirred at room temperature under Ar(g) for 4 h, then quenched by addition of MeOH. Volatiles components were removed *in vacuo*, facilitated by co-evaporation with toluene. The resulting orange residue was stirred in cold methanol for 30 min. The precipitate was collected by filtration and washed with cold methanol, affording the mesylate as a white solid (3.75 g). It was used without further purification. The mesylate was suspended in 10:1 methoxy-2-propanol/H_2O (120 mL) and reacted with sodium acetate (5 g) at 115 °C for 24 h. The solvent was removed *in vacuo*. The residue was triturated with H_2O (200 mL) and filtered, yielding the product as a white foam (3.02 g, 77%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.97 (d, *J* = 8.8 Hz, 1H, NH), 7.56 – 7.09 (m, 10H, Ph), 5.66 (s, 1H, CHPh), 5.50 (d, *J* = 4.4 Hz, 1H, OH), 4.79 (d, *J* = 7.9 Hz, 1H, H-1), 4.76 (d, *J* = 11.6 Hz, 1H, CH_2Ph), 4.54 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.28 (dd, *J* = 9.9, 5.0 Hz, 1H, H-6_a), 3.99 (bs, 1H, H-3), 3.95 – 3.84 (m, 2H, H-2 and H-5), 3.74 (t, *J* = 10.2 Hz, 1H, H-6_b), 3.68 (dd, *J* = 9.4, 2.4 Hz, 1H, H-4), 1.86 (s, 3H, $COCH_3$). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.7, 137.9, 137.7, 128.8, 128.1, 127.9, 127.4, 126.4, 100.6, 99.4, 78.5, 70.3, 68.3, 67.4, 62.9, 52.8, 22.7.

Benzyl 4,6-*O*-benzylidene-2-deoxy-β-D-allopyranoside-2,3-sulfamidate (23)

To a suspension of **22** (1.00 g, 2.51 mmol) in dry THF (40 mL) was added sodium hydride (6.6 eq, 660 mg, 60% dispersion,16.5 mmol). The solution was stirred for 15 min under Ar(g). Then, a solution of 1,1-sulfonyldiimidazole (2.5 eq, 1.24 g, 6.26 mmol) in dry THF (24 mL) was added dropwise over 1 h. The reaction mixture was stirred at rt for 17 h, then quenched by addition of MeOH. Solvent was removed *in vacuo*. The residue was suspended in DCM and washed with water then brine. The product was purified by column chromatography (gradient: 100% DCM to 10:1 DCM/EtOAc). Impure column fractions were re-crystalized (10:10:1 DCM/EtOAc/MeOH). The product was obtained as a white solid (950.5 mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 – 7.31 (m, 10H, Ph), 5.56 (s, 1H, CHPh), 5.12 (dd, *J* = 4.5, 3.0 Hz, 1H, H-3), 5.02 – 4.96 (m, 2H, NH and H-1), 4.92 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.65 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.45 (dd, *J* = 10.5, 5.1 Hz, 1H, H-6_a), 3.98 (td, *J* = 9.8, 5.1 Hz, 1H, H-5), 3.84 (dd, *J* = 9.6, 3.1 Hz, 1H, H-4), 3.78 (t, *J* = 10.3 Hz, 1H, H-6_b), 3.71 (dd, *J* = 7.4, 4.5 Hz, 1H, H-2).^{13}C NMR (101 MHz, $CDCl_3$) δ 136.5, 136.4, 129.7, 128.8, 128.6, 128.3, 126.4, 102.8, 100.2, 80.3, 75.6, 72.3, 68.9, 63.1, 59.5, 53.6.

Benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-allopyranoside-2,3-sulfamidate (24)

Compound **23** (566 mg, 1.4 mmol) was dissolved in dry DCM (30 mL) with dry pyridine (1.43 mL). AcCl (2.4 eq, 0.24 mL, 3.38 mmol) was added dropwise and the reaction mixture was stirred for 1 h at rt. It was diluted with DCM (100 mL), and washed with water then brine. Evaporation *in vacuo* gave an orange residue which was purified by silica column chromatography (10:1 DCM/EtOAc), yielding a white solid (547 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.27 (m, 10H, Ph), 5.60 (s, 1H, CHPh), 5.21 (dd, *J* = 4.4, 2.6 Hz, 1H, H-3), 4.96 (d, *J* = 7.0 Hz, 1H, H-1), 4.92 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.71 (bs, 1H, H-2), 4.63 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.48 (dd, *J* = 10.5, 4.8 Hz, 1H, H-6_a), 4.03 (td, *J* = 9.6, 4.8 Hz, 1H, H-5), 3.97 (dd, *J* = 9.6, 2.7 Hz, 1H, H-4), 3.82 (dd, *J* = 10.5, 9.7 Hz, 1H, H-6_b), 2.37 (s, 3H, $COCH_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.3, 136.2, 129.8, 128.8, 128.6, 128.5, 128.1, 126.4, 103.0, 99.9, 78.1, 77.4, 75.1, 71.9, 69.0, 63.4, 22.7.

Benzyl 2-methylsulfonamido-4,6-*O*-benzylidene-2-deoxy-β-D-allopyranoside-2,3-sulfamidate (25)

Compound **23** (150 mg, 0.358 mmol) was dissolved in dry DCM with Et_3N (6.5 eq, 325 µL, 2.33 mmol) and cooled to 0°C. MsCl (9 eq, 250 µL, 3.23 mmol) was added dropwise and the reaction stirred for 0.5 h at 0 °C. The solution was diluted with DCM and washed with water then brine. The product was purified by silica chromatography (solvent: DCM), giving the product as a white solid (160.8 mg, 92%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.68 – 7.15 (m, 10H), 5.79 (s, 1H, CHPh), 5.65 (dd, *J* = 4.2, 2.8 Hz, 1H, H-3), 5.18 (d, *J* = 7.4, 1H, H-1), 5.00 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.81 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.70 (dd, *J* = 7.5, 4.2, 1H, H-2), 4.46 (dd, *J* = 10.2, 5.0 Hz, 1H, H-6_a), 4.22 (dd, *J* = 9.7, 2.8 Hz, 1H, H-4), 4.03 (td, *J* = 9.8, 5.0 Hz, 1H, H-5), 3.90 (t, *J* = 10.2 Hz, 1H, H-6_b), 3.44 (s, 3H, SO_2CH_3). ^{13}C NMR (101 MHz, acetone-d_6) δ 138.2, 137.9, 130.0, 129.2, 129.0, 128.7, 128.6, 127.2, 103.1, 101.1, 81.0, 75.5, 71.9, 69.2, 64.3, 63.9, 41.4, 32.4.

Benzyl 2-acetamido-3-*S*-acetyl-4,6-*O*-benzylidene-2-deoxy-3-thio-β-D-glucopyranoside (26)

Compound **24** (560 mg, 1.2 mmol) was dissolved in dry DMF (40 mL) and KSAc (5 eq, 700 mg, 6.13 mmol) was added. The mixture was stirred at rt under Ar(g) for 1 h then solvent removed *in vacuo*. The resulting solid was suspended in THF (15 mL) and treated with a solution of THF/H_2SO_4/H_2O (5 mL, 70:20:1) for 0.5 h. The mixture was extracted with DCM and washed with sodium bicarbonate solution and brine. Removal of solvent afforded a yellow residue which was purified by silica chromatography (solvent: 20:1 DCM/acetone), yielding the product as a white solid (420 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 – 6.93 (m, 10H, Ph), 5.54 (d, *J*= 10.9 Hz, 1H, NH), 5.51 (s, 1H, CHPh), 4.88 (d, *J* = 12.4 Hz, 1H, CH_2Ph), 4.57 (d, *J* = 12.4 Hz, 1H, CH_2Ph), 4.50 (d, *J* = 8.2 Hz, 1H, H-1), 4.37 (dd, *J* = 10.5, 3.9 Hz, 1H, H-6_a), 4.17 (ddd, *J* = 11.7, 9.5, 8.0 Hz, 1H, H-2), 3.84 – 3.68 (m, 2H, H-3 and H-6_b), 3.62 – 3.42 (m, 2H, H-4 and H-5), 2.33 (s, 3H, $COCH_3$), 1.90 (s, 3H, $COCH_3$). ^{13}C NMR (101 MHz, $CDCl_3$-insert) δ 196.9, 170.1, 137.3, 137.1, 129.2, 128.5, 128.4, 128.0, 127.9, 126.2, 102.1, 101.8, 77.9, 70.7, 69.9, 68.9, 54.4, 47.5, 30.9, 23.3.

Benzyl 2-acetamido-3-azido-4,6-*O*-benzylidene-2,3-dideoxy-β-D-glucopyranoside (27)

Compound **25** (140 mg, 0.3 mmol) was dissolved in dry DMF (10 mL) and NaN_3 was added (5 eq, 97.5 mg). The solution was stirred at rt for 2 h under Ar(g). DMF was evaporated under reduced pressure, yielding a yellow residue. It was suspended in THF (4 mL) and treated with THF/H_2SO_4/H_2O (1.2 mL, 70:20:1). The reaction was stirred for 1 h. The mixture was diluted with a large volume of DCM and washed with water and brine. The organic layer was concentrated to a residue and triturated with 1:4 acetone/pentane. Filtration afforded the highly insoluble product as a white solid (109.8 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 – 7.29 (m, 10H, Ph), 5.68 – 5.54 (m, 2H, NH and CHPh), 5.09 (d, *J* = 8.2 Hz, 1H, H-1), 4.89 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.58 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.51 – 4.30 (m, 2H, H-3 and H-6_a), 3.81 (t, *J* = 10.1 Hz, 1H, H-6_b), 3.66 – 3.50 (m, 2H, H-4 and H-5), 3.19 (dt, *J* = 10.7, 7.9 Hz, 1H, H-2), 1.96 (s, 3H, $COCH_3$). ^{13}C NMR (101 MHz, 2:1 $CDCl_3$/MeOD) δ 171.9, 137.6, 137.1, 128.3, 128.1, 128.0, 127.5, 127.5, 126.3, 103.3, 99.7, 77.3, 76.6, 70.5, 69.3, 66.2, 61.2, 54.2, 22.2.

Benzyl 2-methylsulfonamido-3-*S*-acetyl-4,6-*O*-benzylidene-2-deoxy-3-thio-β-D-glucopyranoside (28)

Compound **25** (140 mg, 0.281 mmol) was dissolved in dry DMF (7 mL) and KSAc was added (6.2 eq, 170 mg, 1.73 mmol). The mixture was stirred for 2 h under Ar(g). The product was subject to decomposition upon heating. Therefore, instead of rotary evaporation, DMF was removed by blowing air over the solution at room temperature for 5 h. The resulting brown residue was suspended in THF (5 mL) and treated with THF/H_2SO_4/H_2O (1.2 mL, 70:20:1). The mixture was stirred for 1 h and then diluted with DCM. The organic layer was washed with aqueous sodium bicarbonate and brine. The solution was concentrated and the product purified by silica chromatography (20:1 DCM/acetone), yielding the product as a white solid (88 mg, 64%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.78 – 7.06 (m, 10H, Ph), 6.14 (d, *J* = 9.3 Hz, 1H, NH), 5.65 (s, 1H, CHPh), 4.95 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.86 (d, *J* = 8.0 Hz, 1H, H-1), 4.73 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.33 (dd, *J* = 10.3, 4.9 Hz, 1H, H-6_a), 3.83 (t, *J* = 10.2 Hz, 1H, H-6_b), 3.80 – 3.69 (m, 2H, H-3 and H-4), 3.67 – 3.49 (m, 2H, H-2 and H-5), 2.80 (s, 3H, $COCH_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.9, 136.9, 136.3, 129.3, 128.8, 128.6, 128.5, 128.4, 126.3, 102.8, 101.9, 77.4, 71.5, 70.1, 68.8, 59.90, 47.80, 42.90, 31.0, 29.8. ESI-MS calc for $C_{23}H_{31}N_2O_7S_2$ $[M+NH_4]^+$: 511.16; found 511.16.

General procedure for benzylidene deprotection.

A solution of benzylidene protected sugar (70 mM) in 4:1 acetic acid/water was stirred for 4-6 h at 60 °C. Solvent was removed *in vacuo*. The residue was dissolved in 1:1 pyridine/acetic anhydride and stirred for 16 h at rt. The mixture was then poured into icy water and stirred for 0.5 h. The solution was extracted with DCM, then washed with 1 M HCl, saturated sodium bicarbonate, and brine. The product was purified by silica column chromatography.

Benzyl 2-acetamido-3-*S*-acetyl-4,6-di-*O*-acetyl-2-deoxy-3-thio-β-D-glucopyranoside (30)

Compound **26** (420 mg, 0.918 mmol) was deprotected according to the general procedure. Silica column chromatography (solvent gradient: 1:1 to 3:1 EtOAc/pentane) afforded the product as a white solid (340 mg, 82%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.26 (m, 5H, Ph), 5.55 (d, *J* = 9.4 Hz, 1H, NH), 5.02 (dd, *J* = 10.9, 9.6 Hz, 1H, H-4), 4.87 (d, *J* = 12.3 Hz, 1H, CH_2Ph), 4.60 (d, *J* = 12.3 Hz, 1H, CH_2Ph), 4.53 (d, *J* = 8.1 Hz, 1H, H-1), 4.24 (dd, *J* = 12.2, 4.8 Hz, 1H, H-6_a), 4.16 – 4.03 (m, 2H, H-6_b and H-

2), 3.76 (dd, *J* = 11.9, 10.9 Hz, 1H, H-3), 3.68 (ddd, *J* = 9.5, 4.8, 2.6 Hz, 1H, H-5), 2.31 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 1.88 (s, 3H, $COCH_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 196.2, 170.8, 170.0, 169.4, 137.1, 128.5, 128.1, 128.0, 101.1, 74.7, 70.4, 67.2, 62.5, 54.1, 48.1, 30.8, 23.3, 20.9, 20.7.

Benzyl 2-acetamido-3-azido-4,6-di-*O*-acetyl-2,3-dideoxy-β-D-glucopyranoside (32)

Compound **27** (87.6 mg, 0.206 mmol) was deprotected according to the general procedure and purified by silica column chromatography (solvent gradient: 100% DCM to 20:1 DCM/acetone), yielding the product as a white solid (83.3 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.14 (m, 5H, Ph), 5.83 (d, *J* = 7.3 Hz, 1H, NH), 5.13 (d, *J* = 8.2 Hz, 1H, H-1), 5.03 – 4.76 (m, 2H, H-4 and CH_2Ph), 4.57 (d, *J* = 11.7 Hz, 1H, CH_2Ph), 4.48 (dd, *J* = 11.0, 9.7 Hz, 1H, H-3), 4.27 (dd, *J* = 12.3, 4.9 Hz, 1H, H-6$_a$), 4.11 (dd, *J* = 12.3, 2.5 Hz, 1H, H-6$_b$), 3.72 (ddd, *J* = 9.9, 4.9, 2.5 Hz, 1H, H-5), 3.08 (ddd, *J* = 11.0, 8.2, 7.3 Hz, 1H, H-2), 2.11 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$), 1.96 (s, 3H, $COCH_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.1, 170.9, 169.7, 137.0, 128.7, 128.3, 128.3, 98.5, 72.6, 71.6, 69.7, 62.3, 61.8, 57.6, 23.6, 20.9, 20.8.

Benzyl 2-methylsulfonamido-3-*S*-acetyl-4,6-di-*O*-acetyl-2-deoxy-3-thio-β-D-glucopyranoside (33)

Compound **28** (88.0 mg, 0.178 mmol) was deprotected according to the general procedure. It was purified by silica column chromatography (solvent gradient: 100% DCM to 10:1 DCM/EtOAc), giving the product as a white solid (56.1 mg, 64%).^{1}H NMR (400 MHz, Acetone-d_6) δ 7.55 – 7.25 (m, 5H, Ph), 6.20 (d, *J* = 8.8 Hz, 1H, NH), 5.09 (dd, *J* = 10.5, 9.7 Hz, 1H, H-4), 4.95 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.78 (d, *J* = 7.6 Hz, 1H, H-1), 4.73 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.26 (dd, *J* = 12.1, 5.4 Hz, 1H, H-6$_a$), 4.11 (dd, *J* = 12.1, 2.6 Hz, 1H, H-6$_b$), 3.85 (ddd, *J* = 9.7, 5.4, 2.6 Hz, 1H, H-5), 3.72 – 3.56 (m, 2H, H-2 and H-3), 2.82 (s, 3H, CSO_2CH_3), 2.32 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$). ^{13}C NMR (101 MHz, Acetone-d_6) δ 195.0, 170.8, 170.0, 138.0, 129.5, 129.3, 128.9, 103.1, 103.0, 75.3, 71.9, 68.8, 63.4, 58.4, 58.3, 50.7, 50.6, 43.1, 30.8, 20.8, 20.7. ESI-MS calc for $C_{20}H_{31}N_2O_9S_2$ $[M+NH_4]^+$: 507.15; found 507.15.

General procedure for thioacetamido synthesis.

The starting acetamido sugar was suspended in dry toluene (0.2 M) under Ar(g). It was reacted with Lawesson's reagent (0.6 eq) at 80 °C for 4 h. Evaporation of solvent give a white foam which was purified by silica chromatography (20:1 DCM/EtOAc).

Benzyl 2-thioacetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (29)

Compound **19** (100 mg, 0.229 mmol) was thionated according to the general procedure. The product was obtained as viscous oil (52.9 mg, 51%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.03 (d, *J* = 9.3 Hz, 1H, NH), 7.19 – 7.00 (m, 5H, Ph), 5.09 (t, *J* = 9.8 Hz, 1H, H-3), 5.00 – 4.85 (m, 2H, H-2, H-4), 4.76 (d, *J* = 8.3 Hz, 1H, H-1), 4.68 (d, *J* = 12.2 Hz, 1H, CH_2Ph), 4.46 (d, *J* = 12.2 Hz, 1H, CH_2Ph), 4.10 (dd, *J* = 12.3, 4.9 Hz, 1H, H-6$_a$), 3.96 (dd, *J* = 12.3, 2.5 Hz, 1H, H-6$_b$), 3.68 (ddd, *J* = 10.0, 4.9, 2.5 Hz, 1H, H-5), 2.22 (s, 3H, $CSCH_3$), 1.84 (s, 3H, $COCH_3$), 1.80 (s, 3H, $COCH_3$), 1.72 (s, 3H, $COCH_3$).^{13}C NMR (101 MHz, Acetone-d_6) δ 203.2, 170.8, 170.7, 170.0, 138.5, 129.2, 129.0, 128.3, 128.3, 100.7, 74.0, 72.5, 71.2, 69.3, 62.8, 59.7, 34.1, 20.6, 20.6, 20.6.

Benzyl 2-thioacetamido-3-*S*-acetyl-4,6-di-*O*-acetyl-2-deoxy-3-thio-β-D-glucopyranoside (31)

Compound **30** (100 mg, 0.213 mmol) was was thionated according to the general procedure. The product was obtained as a white solid (54.1 mg, 53%). ^{1}H NMR (500 MHz, Acetone-d_6) δ 9.06 (d, J = 9.7 Hz, 1H, NH), 7.38 – 7.24 (m, 5H, Ph), 5.20 – 5.13 (m, 1H, H-2), 5.07 (dd, *J* = 10.8, 9.6 Hz, 1H, H-4), 4.95 – 4.90 (m, 1H, H-1), 4.87 (d, *J* = 12.3 Hz, 1H, CH_2Ph), 4.66 (d, *J* = 12.4 Hz, 1H, CH_2Ph), 4.27 (dd, *J* = 12.2, 5.0 Hz, 1H, H-6$_a$), 4.11 (dd, *J* = 12.2, 2.6 Hz, 1H, H-6$_b$), 4.00 – 3.91 (m, 1H, H-3), 3.88 (ddd, *J* = 9.6, 5.0, 2.6 Hz, 1H, H-5), 2.42 (s, 3H, $CSCH_3$), 2.29 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 1.97 (s, 3H, $COCH_3$).^{13}C NMR (126 MHz, Acetone-d_6) δ 203.1, 194.5, 170.7, 169.9, 138.6, 129.0, 128.3, 128.2, 75.3, 70.9, 68.9, 63.2, 58.4, 49.2, 34.0, 30.6, 20.7, 20.6.

Benzyl 2-acetamido-2-deoxy-β-D-glucopyranoside (20)

Compound **19** (7.85 g, 22.1 mmol) was deprotected according to the general Zemplén deacetylation procedure, giving a white solid (5.50 g, 96%). ^{1}H NMR (400 MHz, MeOD) δ 7.45 – 7.21 (m, 5H, Ph), 4.91 (d, *J* = 12.2 Hz, 1H, CH_2Ph), 4.64 (d, *J* = 12.1 Hz, 1H, CH_2Ph), 4.50 (d, *J* = 8.4 Hz, 1H, H-1), 3.94 (dd, *J* = 11.9, 2.3 Hz, 1H, H-6$_a$), 3.82 – 3.65 (m, 2H, H-2 and H-6$_b$), 3.47 (dd, *J* = 10.3, 8.5 Hz, 1H, H-3), 3.37 (t, *J* = 6.0 Hz, 1H, H-4), 3.30 (m, 1H, H-5), 1.98 (s, 3H, $COCH_3$).^{13}C NMR (101 MHz, MeOD) δ 173.7, 139.2, 129.3, 128.8, 128.6, 101.8, 78.1, 76.0, 72.2, 71.5, 62.9, 57.4, 23.0.

Benzyl 2-thioacetamido-2-deoxy-β-D-glucopyranoside (34)

Compound **29** (10.5 mg, 0.024 mmol) was deprotected according to the general Zemplén deacetylation procedure, giving a white solid (7.3 mg, 98%). ^{1}H NMR (400 MHz, D_2O) δ 7.45 – 7.24 (m, 5H, Ph), 4.82 (d, *J* = 12.3 Hz, 1H, CH_2Ph), 4.65 – 4.55 (m, 3H, H-1, H-2 and CH_2Ph), 3.89 (dd, *J* = 12.4, 2.1 Hz, 1H, H-6$_a$), 3.72 (dd, *J* = 12.4, 5.5 Hz, 1H, H-6$_b$), 3.52 (t, *J* = 9.1 Hz, 1H, H-3), 3.45 (t, *J* = 9.0 Hz, 1H, H-4), 3.42 – 3.37 (m, 1H, H-5), 2.39 (s, 3H, $CSCH_3$). ^{13}C NMR (101 MHz, D_2O) δ 204.3, 136.7, 128.7, 128.5, 128.4, 99.5, 76.0, 74.1, 71.4, 69.7, 61.1, 60.7, 32.9. DART-MS calc for $C_{15}H_{22}NO_5S$ $[M+H]^+$: 328.1; found 328.0.

Benzyl 2-acetamido-2-deoxy-3-thio-β-D-glucopyranoside (35)

Compound **30** (340.0 mg, 0.750 mmol) was deprotected according to the general Zemplén deacetylation procedure. The reaction was conducted using Ar(g) purged MeOH to prevent disulfide formation. The product was obtained as a white solid (242.1 mg, 99%). ^{1}H NMR (400 MHz, MeOD) δ 7.39 – 7.22 (m, 5H, Ph), 4.87 (d, *J* = 12.1 Hz, 1H, CH_2Ph), 4.61 (d, *J* = 12.2 Hz, 1H, CH_2Ph), 4.46 (d, *J* = 8.2 Hz, 1H, H-1), 3.90 (dd, *J* = 11.9, 1.9 Hz, 1H, H-6$_a$), 3.80 – 3.65 (m, 2H, H-2 and H-6$_b$), 3.32 – 3.28 (m obscured by MeOD, H-4 and H-5), 2.89 (dt, *J* = 10.4, 4.7 Hz, 1H, H-3), 1.95 (s, 3H, $COCH_3$).^{13}C NMR (101 MHz, MeOD) δ 173.3, 139.1, 129.3, 128.8, 128.7, 102.6, 80.6, 73.1, 71.4, 63.0, 57.3, 48.6, 22.8. ESI-MS calc for $C_{15}H_{22}NO_5S$ $[M+H]^+$: 328.1; found 328.1.

Benzyl 2-thioacetamido-2-deoxy-3-thio-β-D-glucopyranoside (36)

Compound **31** (33.0 mg, 0.066 mmol) was deprotected according to the general Zemplén deacetylation procedure. The reaction was conducted using Ar(g) purged MeOH to prevent disulfide formation. The product was obtained as a white solid (23.3 mg, 100%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.98 (d, *J* = 9.4 Hz, 1H, NH), 7.63 – 7.01 (m, 5H, Ph), 5.46 (d, *J* = 6.0 Hz, 1H, OH), 4.79 (d, *J* = 12.6 Hz, 1H, CH_2Ph), 4.76 – 4.61 (m, 2H, H-1 and H-2), 4.54 (d, *J* = 12.6 Hz, 1H, CH_2Ph), 4.50 – 4.39 (m, 1H, OH), 3.75 – 3.66 (m, 1H, H-6$_a$), 3.53 (dd, *J* = 11.9, 4.8 Hz, 1H, H-6$_b$), 3.25 – 3.18 (m, 2H, H-4 and H-5), 2.99 – 2.92 (m, 1H, H-3), 2.41 (s, 3H, $CSCH_3$).^{13}C NMR (126 MHz, DMSO) δ 200.7, 137.9, 128.2, 127.3, 127.0, 100.9, 79.6, 71.0, 69.4, 60.9, 59.9, 33.4. ESI-MS calc for $C_{15}H_{22}NO_4S_2$ [M+H]$^+$: 344.10; found 344.10.

Benzyl 2-methylsulfonamido-2-deoxy-3-thio-β-D-glucopyranoside (37)

Compound **33** (10.0 mg, 0.020 mmol) was deprotected according to the general Zemplén deacetylation procedure. However, some degradation of the sugar occurred under these conditions. The mixture was stirred overnight in neutral MeOH open to the air, allowing complete oxidation of the product to the highly insoluble disulfide. Subsequent recrystallization from MeOH/pentane afforded pure product as white crystals (4.0 mg, 54%). ^{1}H NMR (400 MHz, 3:1 MeOD/$CDCl_3$) δ 7.39 – 7.24 (m, 5H, Ph), 4.93 (d, *J* = 11.3 Hz, 1H, CH_2Ph), 4.61 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.41 (d, *J* = 8.1 Hz, 1H, H-1), 3.90 (dd, *J* = 12.0, 2.5 Hz, 1H, H-6$_a$), 3.77 (dd, *J* = 12.0, 5.2 Hz, 1H, H-6$_b$), 3.62 (t, *J* = 9.8 Hz, 1H, H-4), 3.44 (dd, *J* = 11.5, 8.0 Hz, 1H, H-2), 3.37 – 3.32 (m, 1H, H-5), 2.87 (s, 3H, SO_2CH_3), 2.83 (dd, *J* = 11.6, 10.1 Hz, 1H, H-3). ^{13}C NMR (100 MHz, 3:1 MeOD/$CDCl_3$) δ 137.7, 129.2, 129.0, 128.6, 102.8, 79.4, 71.7, 68.3, 62.5, 61.6, 58.8, 43.3. ESI-MS calc for $C_{28}H_{44}N_3O_{12}S_4$ [M+NH_4]$^+$: 742.18; found 742.18.

Benzyl 2-acetamido-2-deoxy-3-azido-β-D-glucopyranoside (38)

Compound **32** (75.0 mg, 0.178 mmol) was deprotected according to the general Zemplén deacetylation procedure. The product was obtained as a white solid (57.6 mg, 96%). ^{1}H NMR (400 MHz, MeOD) δ 7.39 – 7.24 (m, 5H, Ph), 4.87 (d, *J* = 12.2 Hz, 1H, CH_2Ph), 4.61 (d, *J* = 12.2 Hz, 1H, CH_2Ph), 4.53 (d, *J* = 8.3 Hz, 1H, H-1), 3.89 (dd, *J* = 12.0, 2.2 Hz, 1H, H-6$_a$), 3.73 – 3.63 (m, 2H, H-2 and H-6$_b$), 3.49 (dd, *J* = 10.9, 8.8 Hz, 1H, H-3), 3.38 (t, *J* = 9.2 Hz, 1H, H-4), 3.34 (dd, *J* = 5.4, 2.2 Hz, 1H, H-5), 1.95 (s, 3H, $COCH_3$). ^{13}C NMR (101 MHz, MeOD) δ 173.5, 139.0, 129.4, 128.9, 128.8, 101.4, 78.7, 71.6, 70.8, 68.5, 62.5, 55.6, 22.9. DART-MS calc for $C_{15}H_{21}N_4O_5$ [M+H]$^+$: 337.1507; found 337.1520.

Benzyl 2-acetamido-2-deoxy-3-amino-β-D-glucopyranoside (39)

Compound **38** (15.5 mg, 0.0446 mmol) was dissolved in THF/H_2O (4:1, 3.75 mL) under Ar(g). Triphenylphosphine (2.8 eq, 33.4 mg, 0.125 mmol) was added and the mixture stirred at rt for 72 h. Solvent was removed *in vacuo*. The resulting residue was suspended in water, filtered, and passed through a short plug of C18 resin. The product was eluted with 100% H_2O and lyophilized, yielding the product as a white powder (13.1 mg, 94% yield). ^{1}H NMR (399 MHz,D_2O) δ 7.53 – 7.35 (m, 5H, Ph), 4.90 (d, *J* = 12.2 Hz, 1H, CH_2Ph), 4.69 (d, *J* = 12.1 Hz, 1H, CH_2Ph), 4.56 (d, *J* = 8.4 Hz, 1H, H-1), 3.96 (dd, J = 12.3, 1.3 Hz, 1H, H-6$_a$), 3.78 (dd, *J* = 12.2, 5.9 Hz, 1H, H-6$_b$), 3.66 (dd, *J* = 10.8, 8.6 Hz, 1H, H-2), 3.49 (ddd, *J* = 9.6, 5.8, 2.1 Hz, 1H, H-5), 3.36 (t, *J* = 9.4 Hz, 1H, H-4), 2.77 (t, *J* = 10.2 Hz, 1H, H-3), 1.95 (s, 3H, $COCH_3$). ^{13}C NMR (100 MHz, D_2O) δ 174.5, 136.7, 128.7, 128.5, 128.4, 100.2, 77.0, 71.3, 69.8, 60.8, 56.0, 54.6, 22.1. DART-MS calc for $C_{15}H_{23}N_2O_5$ [M+H]$^+$: 311.1602; found 311.1610.

Acetoxymethyl-4-umbelliferone (40)

To a stirred solution of 4-methylumbelliferone (1.00 g, 5.7 mmol) and DIPEA (4 eq, 4.0 mL, 22.7 mmol) in dry DMF (35 mL) under Ar(g) was added (bromomethoxy)methyl acetate (1.67 mL, 3 eq, 17.0 mmol) dropwise. The reaction was stirred for 16 h at rt. The reaction was then diluted with EtOAc and washed twice with water and brine. The organic layer was concentrated *in vacuo* and NMR spectroscopy was used to determine the molar ratio of AcMU to AMMU.

The AcMU/AMMU mixture was dissolved in $CHCl_3$ (0.1 M to AcMU) and reacted with benzylamine (1.2 eq to AcMU). The reaction's progress was monitored by NMR every 4 hours and left for 8-18 h. After complete AcMU hydrolysis the solvent was evaporated and the residue purified by silica gel chromatography (gradient: $CHCl_3$ to 100:1 $CHCl_3$/acetone). Fractions containing product were pooled and recrystallized from $CHCl_3$/hexanes, giving the product as white needles (254.2 mg, 18%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.8 Hz, 1H), 7.02 (d, *J* = 2.5 Hz, 1H), 6.97 (dd, *J* = 8.7, 2.5 Hz, 1H), 6.19 (d, *J* = 1.3 Hz, 1H), 5.80 (s, 2H, OCH_2O), 2.41 (d, *J* = 1.2 Hz, 3H, CH_3), 2.14 (s, 3H, $COCH_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.8, 161.0, 159.6, 155.1, 152.3, 125.9, 115.3, 113.3, 113.1, 103.6, 84.9, 21.0, 18.8. DART-MS calc for $C_{13}H_{13}O_5$ [M+H]$^+$: 249.0763; found 249.062.

Acknowledgments

This work was supported by grants from the Canadian Institutes of Health Research (CIHR) and Natural Sciences and Engineering Research Council (NSERC).

Supplementary Material

Supplementary material includes spectra of new compounds and supplementary figures.

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