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Phosphate Chemical Probes Designed for Location Specific Inhibition of Intracellular Carbonic Anhydrases

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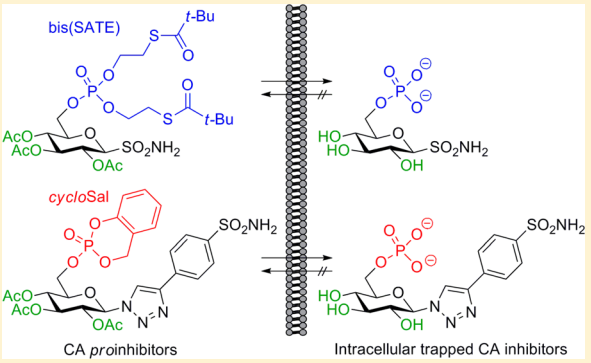
Supporting Information

ABSTRACT: Chemical probes are small molecules designed to bind to a specific protein and disrupt the proteins function. Although many inhibitors are reported for human carbonic anhydrase (CA) enzymes, few may be considered useful as chemical probes as they exhibit broad action against the 12 catalytically active CA isozymes. In addition, most do not possess an appropriate physicochemical profile to discriminate intracellular CA activity from either global or extracellular CA activity. We report herein the synthesis of three monophosphate CA *pro*inhibitors (compounds **2**, **3**, and **5**) that are derived from cyclosaligenyl (*cyclo*Sal) phosphate and *S*-acyl-2-thioethyl (SATE) phosphate as protecting groups. The *pro*inhibitors are designed as neutral, membrane-permeable compounds that once inside the cell may be hydrolyzed by pH-driven or enzymatic-driven mechanisms to release a negatively charged monophosphate. The resulting monophosphate compound is trapped intracellularly and available for locality specific inhibition of intracellular CAs.



INTRODUCTION

There are 12 catalytically active isozymes of carbonic anhydrase (CA, EC 4.2.1.1) that have been characterized in humans; seven have their active site domain in the intracellular space (CA I, II, III, VII, XIII – cytosolic; CA VA and VB, mitochondrial), while the remaining five have an extracellular active site domain (CA IV, IX, XII, and XIV – membrane anchored; CA VI, secreted).[1] CA enzymes catalyze the reversible hydration of carbon dioxide to bicarbonate and a proton: $CO_2 + H_2O \leftrightarrows HCO_3^- + H^+$. The active site of the different CA isozymes are structurally similar, such that small molecule inhibitors with CA isozyme selectivity are challenging to design.[2] While there has been some success to address selectivity, inhibitors with exceptional isozyme selectivity for the most part remain elusive. The association of CA IX and/or CA XII with cancer invasion, metastasis, and drug resistance provides the driving force for the development of compounds with selectivity for extracellular CAs.[3] Our group has contributed to the development of inhibitors with this inhibition profile, compound **1**, a polar glycoconjugate with predicted poor membrane permeability, is exemplary in this respect.[4] Compound **1** has >1000-fold selectivity for CA IX and >100-fold selectivity for CA XII over both intracellular CA I and CA II. Owing to the combination of physicochemical properties (poor membrane permeability) with preferential isozyme selectivity for CA IX and XII, compound **1** has provided a useful chemical tool to explore the action of extracellular CA inhibition. Acetazolamide (AZA), a commercially available and clinically used CA inhibitor, is commonly employed as the "go to" CA inhibitor in studies to explore the biology surrounding CA inhibition (Figure 1). AZA delivers broad CA inhibition (except for hCA III) and is membrane permeable,[5] hence it acts to provide a global readout of CA inhibition (i.e., intracellular + extracellular), Table 1. If undertaking studies with cell-based models of disease, employing a panel of CA inhibitors with properties that enable them to discriminate global, intracellular and extracellular CA inhibition effects, is desirable. This panel could substantially value add to the correlation of phenotypic assay readout with localization of the CA(s) responsible for the phenotype. Inhibitors with

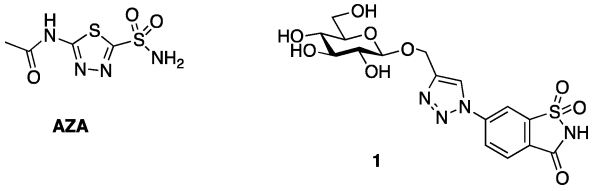


Figure 1. Known chemical probes for the study of human carbonic anhydrases (CAs): acetazolamide (AZA) provides global CA inhibition, and saccharin glycoconjugate **1** provides extracellular CA inhibition.

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Table 1. Human CA Inhibition Profile of the "Go To" Global CA Inhibitor, Acetazolamide (AZA)

	intracellular CAs							extracellular CAs				
	isozyme I	isozyme II	isozyme III	isozyme VA	isozyme VB	isozyme VII	isozyme XIII	isozyme IV	isozyme VI	isozyme IX	isozyme XII	isozyme XIV
K_i (nM)	250	12	> 25000	63	54	2.5	17	74	11	25	5.7	41

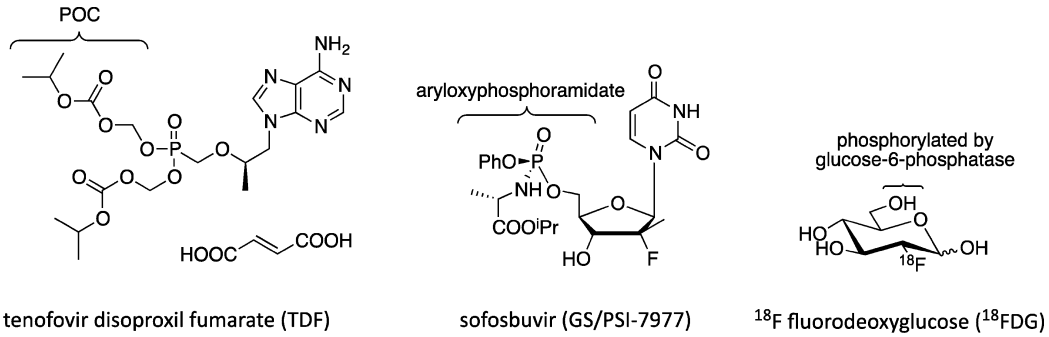

Figure 2. FDA approved prodrug compounds with activity that is dependent on the intracellular formation and subsequent trapping of a negatively charged monophosphate.

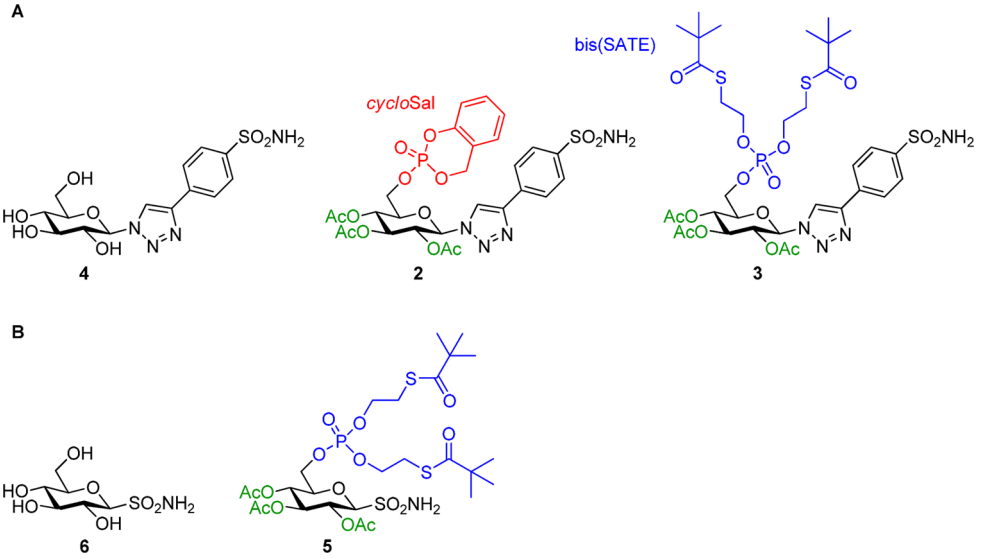

Figure 3. (A) *Cyclo*Sal and bis(SATE) glucosyl-6-phosphate *pro*inhibitors **2** and **3**, based on the structure of known glucose-based CA inhibitor **4**. (B) Bis(SATE) glucosyl-6-phosphate *pro*inhibitor **5** based on the structure of known glucose-based CA inhibitor **6**.[9]

physicochemical properties that permit them to localize predominantly intracellularly or predominantly extracellularly (such as compound **1**) are potentially powerful chemical probes to complement studies investigating the impact of CA in disease that otherwise employ AZA in isolation.[6] While the development of extracellular CA chemical probes has advanced, the concomitant development of locality specific intracellular acting CA chemical probes has not been forthcoming. This paper focuses on the development of such intracellular acting CA inhibitors to provide for a comprehensive panel of chemical probes, alongside AZA (global acting) and compound **1** (extracellular acting), to specifically modulate intracellular CA activity if employed in cell models.

RESULTS AND DISCUSSION

Compound Design. The inspiration for the design of intracellular targeting CA inhibitors is derived from a combination of two classes of phosphorus-based compounds that are used in clinical medicine, first, nucleoside monophosph(on)ate prodrugs, and second, ^{18}F-fluorodeoxyglucose. The success of phosphorus prodrug technology is evidenced by the FDA approval of the antiviral nucleoside analogues tenofovir disoproxil fumarate (TDF) and sofosbuvir (GS/PSI-7977), Figure 2.[7] TDF has two bis-(isopropyloxymethyl) carbonate (POC) phosphate protecting groups, while GS/PSI-7977 is an aryloxyphosphoramidate prodrug. The prodrugs can freely enter cells by passive membrane diffusion, and once inside the cell, their phosphate groups are unmasked via enzymatic or chemical hydrolysis (or a combination of both) to generate the active nucleoside monophosphate.[7] The monophosphate is thus delivered and trapped intracellularly, and the second and third enzymatic-mediated phosphorylation to a nucleoside triphosphate is usually efficient,[7] allowing for nucleoside prodrugs to elicit their eventual pharmacological response. The second inspirational compound for the target compound design of this study is ^{18}F-Fluorodeoxyglucose (FDG), a PET radiopharmaceutical that is used to image tumors. Like glucose, FDG is actively

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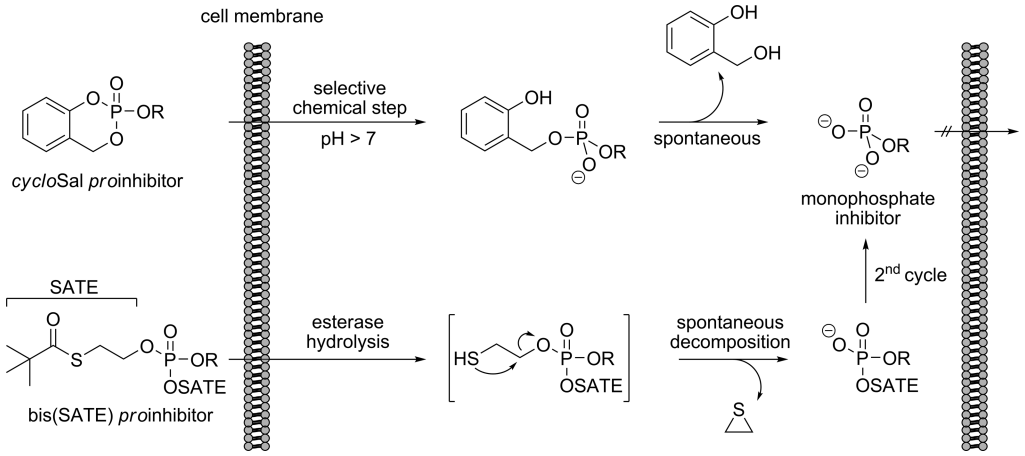
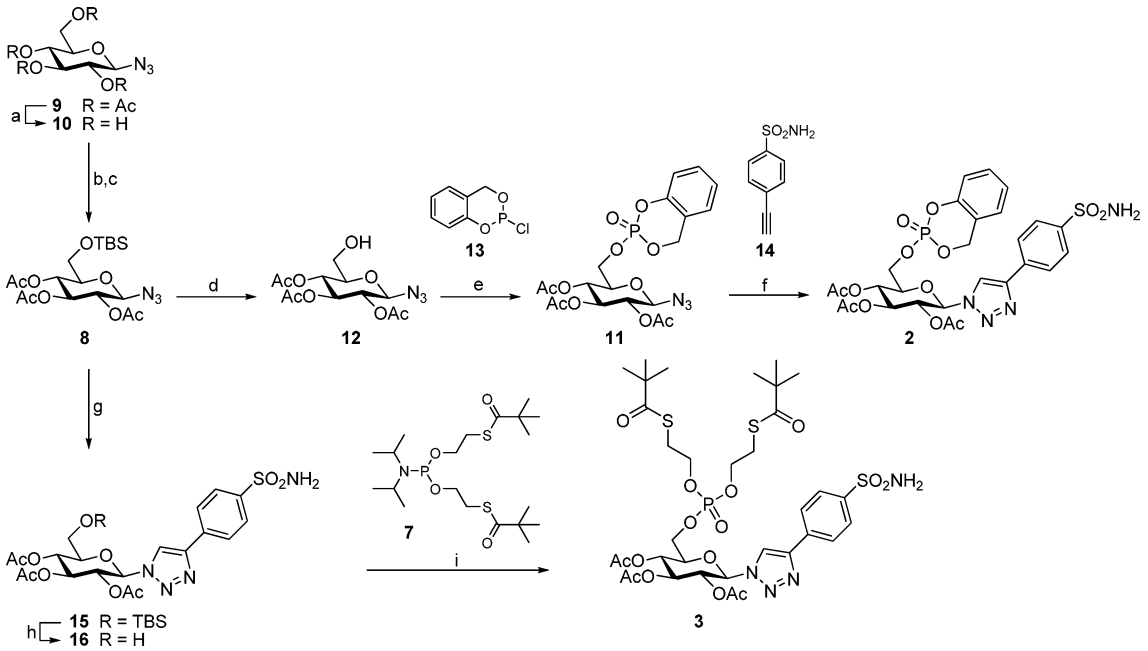


Figure 4. Intracellular hydrolysis mechanisms for *cyclo*Sal and bis(SATE) protected monophosphate *pro*inhibitors.

Scheme 1. Synthesis of *Cyclo*Sal *Pro*inhibitor 2 and Bis(SATE) *Pro*inhibitor 3[a]



[a]Reagents and conditions: (a) NaOMe, CH_2Cl_2/MeOH, quant; (b) TBSCl, imidazole, DMF; (c) py, Ac_2O, 70%, 2 steps; (d) AcCl, CH_2Cl_2, MeOH, 97%; (e) (i) **13**, DIPEA, CH_3CN, −20 °C, (ii) *t*-BuOOH, −20 °C to rt, 55%; (f) **14**, $CuSO_4.H_2O$, sodium ascorbate, EtOH/H_2O 3:1, 60 °C, 76%; (g) **14**, $CuSO_4{\cdot}H_2O$, sodium ascorbate, *tert*-butyl alcohol/H_2O 1:1, 60 °C, 93%; (h) AcCl, CH_2Cl_2, MeOH, 90%; (i) (i) **7**, 1*H*-tetrazole, CH_3CN, (ii) *m*-CPBA, CH_2Cl_2, −30 °C to rt, 51%.

transported into cells by glucose transport proteins (GLUT). Once intracellular, glucose and FDG are monophosphorylated by the enzyme glucose-6-phosphatase. Glucose-6-phosphate continues along the glycolytic pathway for energy production, however, ^{18}FDG-6-phosphate is not suited for glycolysis and instead is effectively trapped intracellularly as a monophosphate that is deprotonated at physiological pH.[8]

We have selected two phosphate prodrug approaches in the design of CA *pro*inhibitors for intracellular CA targeting, cyclosaligenyl (*cyclo*Sal) phosphate and *S*-acyl-2-thioethyl (SATE) phosphate, Figure 3. The *pro*inhibitors consist of two glucose-based glycoconjugate CA inhibitors previously reported by us. *Pro*inhibitors **2** and **3** stem from modification of the C-6 hydroxyl moiety of the glucoconjugate benzenesulfonamide CA inhibitor **4** and comprise *cyclo*Sal and bis(SATE) moieties, respectively.[9] Bis(SATE) *pro*inhibitor **5** is based on modification of the C-6 hydroxyl moiety of the anomeric glucose sulfonamide CA inhibitor **6**.[9] All three *pro*inhibitors additionally comprise acetyl esters on the 2, 3, and 4 glucose hydroxyl groups. The three acetyl groups increase the lipophilicity of *pro*inhibitors **2**, **3**, and **5**; to aid cell membrane permeability, these groups are hydrolyzed by intracellular esterases to generate a compound with free 2, 3, and 4 hydroxyls.[10] We recently reported that the acetyl groups had a half-life of <1 h in plasma when presented on a glucose scaffold, consistent with esterase processing.[10] The mechanisms of hydrolysis that operates to cleave the *cyclo*Sal and SATE moieties to release the free monophosphate of prodrugs/*pro*inhibitors inside the cell are described next.

*Cyclo*Sal groups are one of the most extensively explored moieties for the masking of monophosphates, particularly in the development of antiviral nucleotides.[11] The *cyclo*Sal com-

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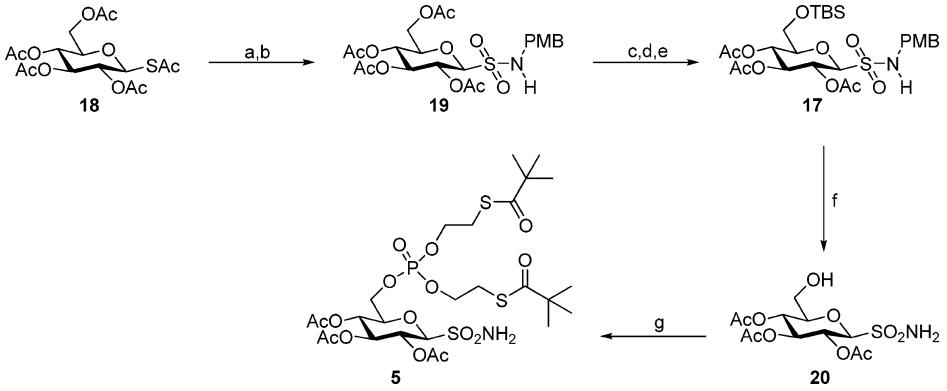
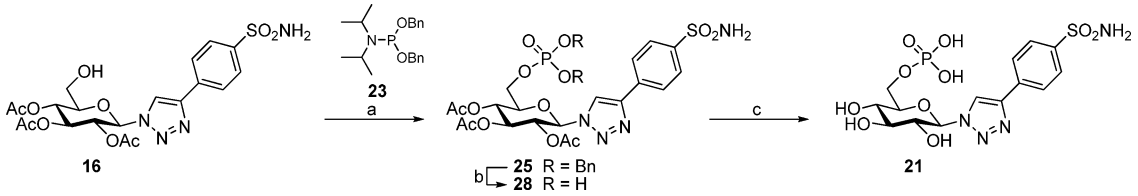
Scheme 2. Synthesis of Bis(SATE) *Pro*inhibitor 5[a]



[a]Reagents and conditions: (a) $BrCH(CO_2Et)_2$, NH_2PMB, MeOH; (b) $KMnO_4/CuSO_4$, CH_3CN/H_2O, 40% 2 steps; (c) NaOMe, CH_2Cl_2/MeOH, quant; (d) TBSCl, imidazole, DMF; (e) py, Ac_2O, 60% 2 steps; (f) CAN, CH_3CN/H_2O, 90%; (g) (i) **7**, 1*H*-tetrazole, CH_3CN, (ii) *m*-CPBA, CH_2Cl_2, −30 °C to rt, 89%.

Scheme 3. Synthesis of Monophosphate CA Inhibitor 21[a]



[a]Reagents and conditions: (a) (i) **23**, 1*H*-tetrazole, CH_3CN, (ii) *m*-CPBA, CH_2Cl_2, −30 °C to rt, 82%; (b) $Pd(OH)_2$, H_2, THF/MeOH, 78%; (c) NaOMe, MeOH, quant.

pounds are designed as neutral, membrane-permeable compounds that once inside the cell are hydrolyzed by pH-driven mechanisms to release the negatively charged monophosphate, which is then trapped intracellularly, Figure 4.[12] Under slightly basic pH conditions, the aryl ester P−O bond is first cleaved to generate a benzyl phosphate diester intermediate, and this is followed by spontaneous cleavage of the C−O benzyl ester bond to release the free phosphorylated drug and salicyl alcohol.[11a] The half-life of *cyclo*Sal phosphates may be tuned via substitution on the salicyl alcohol.

In contrast to the pH-mediated delivery of the free phosphate from the parent *cyclo*Sal ester, hydrolysis of the bis(SATE) phosphotriester moiety is dependent on esterase activity for activation.[7,13] First, esterase hydrolysis of the thioester generates an unstable thioethyl phosphotriester intermediate. Next, this intermediate decomposes spontaneously via an intramolecular nucleophilic displacement mechanism to release ethylene sulfide with concomitant formation of the corresponding phosphodiester. The second SATE group is similarly hydrolyzed to afford the free monophosphorylated compound.

Chemical Synthesis. The introduction of the *cyclo*Sal and bis(SATE) masked monophosphate moieties occurs by modification of the primary hydroxyl group on the foundation inhibitor structure. For *cyclo*Sal, this is commonly achieved using P(III) chemistry,[11b,14] while for bis(SATE), it is achieved with a 1*H*-tetrazole mediated coupling of a bis(*S*-pivaloyl-2-thioethyl) *N*,*N*-diisopropylphosphoramidite (**7**) followed by in situ oxidation.[15] For our target compounds, each route requires a precursor 2,3,4-triacetylated glucosyl moiety with the C-6 hydroxyl group available for the introduction of the masked monophosphate moiety. 2,3,4-Tri-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-*β*-D-glucopyranosyl azide (**8**) is a common intermediate for the synthesis of *pro*inhibitors based on triazole CA inhibitor **4**. Azide **8** was prepared by deacetylation of *β*-D-glucopyranosyl azide (**9**) under Zemplén conditions[16] to give **10**, followed by silylation at *O*-6 with *tert*-butyldimethylsilyl chloride and subsequent reacetylation of the remaining hydroxyl groups (*O*-2, *O*-3, and *O*-4) using acetic anhydride in pyridine, Scheme 1.

To continue to *cyclo*Sal *pro*inhibitor **2** required the preparation of *cycol*Sal glycosyl azide **11**. Deprotection of the *tert*-butyldimethylsilyl group of **8** using mild acidic conditions afforded **12**, with no acetyl group migration observed. Compound **11** was prepared in a "one-pot" procedure by coupling alcohol **12** and saligenyl chlorophosphite **13**,[11a,17] followed by in situ oxidation with *t*-BuOOH. The reaction of the *cycol*Sal glycosyl azide **11** with 4-ethynylbenzenesulfonamide (**14**)[18] using Cu(I)-catalyzed azide alkyne cycloaddition (CuAAC) provided *cyclo*Sal prodrug **2** in 76% yield, as a diastereoisomeric mixture about the phosphorus center, Scheme 1.

The synthesis of bis(SATE) *pro*inhibitor **3** was achieved in three steps from glucosyl azide **8**. First, CuAAC of azide **8** and alkyne **14** gave triazole **15** in high yield. Subsequent acidic cleavage of the silyl ether of **15** afforded alcohol **16** in 90% yield. The 1*H*-tetrazole mediated coupling of phosphoramidite **7**[15] with **16**, followed by in situ oxidation with *m*-CPBA, furnished the target bis(SATE) *pro*inhibitor **3**, Scheme 1.

The bis(SATE) protected phosphate *pro*inhibitor **5** was prepared via an adaptation of the synthesis developed for the foundation anomeric sulfonamide **6**.[19] The key modification of the synthesis to allow for installation of the protected phosphate moiety is the selective orthogonal protection of

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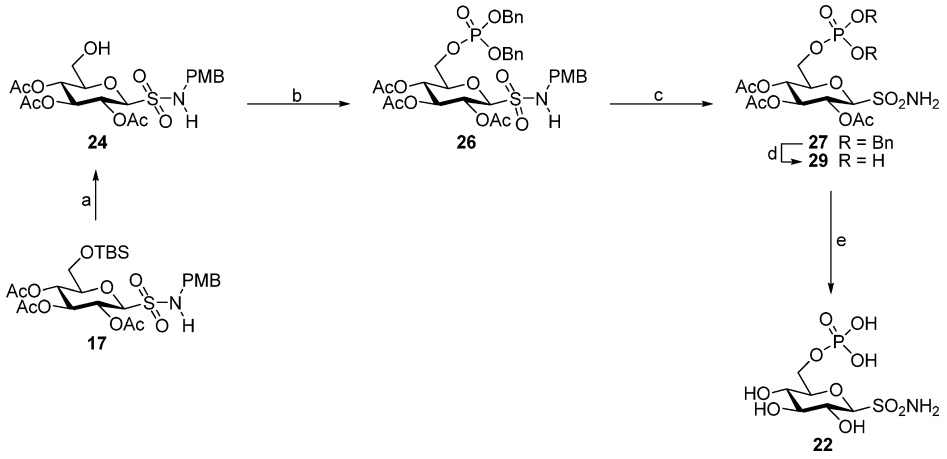
Scheme 4. Synthesis of Monophosphate CA Inhibitor 22[a]



[a]Reagents and conditions: (a) AcCl, CH_2Cl_2, MeOH, 96%; (b) (i) **23**, 1*H*-tetrazole, CH_3CN, (ii) *m*-CPBA, CH_2Cl_2, −30 °C to rt, 90%; (c) CAN, CH_3CN/H_2O, 90%; (d) $Pd(OH)_2$, H_2, THF/MeOH, 79%; (e) NaOMe, MeOH, quant.

the glucosyl C-6 primary hydroxyl group as a silyl ether (**17**). The reaction of glucosyl thioacetate **18** with diethyl bromomalonate and 4-methoxybenzylamine and subsequent oxidation afforded **19**.[19] A sequence of standard carbohydrate protecting group manipulations of **19**, specifically deacetylation, silylation of the C-6 primary hydroxyl group, and reacetylation, afforded **17** in 60% yield over three steps. Oxidative cleavage of the PMB protecting group of **17** using ceric ammonium nitrate removed the silyl ether to afford **20** in high yield. Phosphorylation of **20** with phosphoramidite **7**[15] and subsequent oxidation with *m*-CPBA gave the target bis(SATE) *pro*inhibitor **5** in 89% yield, Scheme 2.

With the three target prodrugs in hand, our attention turned toward the synthesis of the corresponding fully deprotected monophosphate inhibitors **21** and **22**, Schemes 3 and 4, respectively. These control compounds are needed in order to establish the CA inhibition profile of inhibitors that would follow cell-mediated hydrolysis of the *cyclo*Sal, bis(SATE), and acetyl groups of *pro*inhibitors **2**, **3**, and **5**. Our approach toward the synthesis of **21** and **22** was to use the reactive dibenzyl *N*,*N*-diisopropylphosphoramidite (**23**) to install the phosphate moiety. The phosphate triester compounds formed from **23** are lipophilic and amenable to straightforward purification by normal phase flash chromatography. The benzyl groups may be conveniently removed when needed by hydrogenolysis. 1*H*-Tetrazole mediated coupling of dibenzyl *N*,*N*-diisopropylphosphoramidite (**23**) with the C-6 primary hydroxyl group of precursor glucosyl compounds, **16** and **24**, was followed by in situ oxidation with *m*-CPBA to provide **25** and **26**, respectively. Oxidative cleavage of the PMB protecting group of anomeric sulfonamide **26** using ceric ammonium nitrate gave **27**. Hydrogenolysis of the benzyl groups of **25** and **27** afforded debenzylated monophosphates **28** and **29**, respectively. Finally, hydrolysis of the acetate groups of **28** and **29** under Zemplén conditions[16] furnished the target monophosphate inhibitors **21** and **22** in quantitative yield.

CA Inhibition. The CA enzyme inhibition activity for *pro*inhibitors (**2**, **3**, and **5**) and the corresponding unmasked inhibitors (**21** and **22**) was measured against cytosolic hCA I and II, and extracellular hCA IX and XII, using an assay that measures the hydration activity of carbonic anhydrase,[20] Table 2.

Table 2. Human CA Inhibition Profile and cLogP for *Pro*inhibitors (2, 3, and 5) and the Corresponding Unmasked Inhibitors (21 and 22)

			K_i (nM)[b]			
			intracellular		extracellular	
compd	cLogP[a]	masking groups	CA I	CA II	CA IX	CA XII
2	+0.9	(i) *cyclo*Sal (ii) 3× acetyl	437	3.7	84.3	51.3
3	+3.7	(i) bis(SATE) (ii) 3× acetyl	528	6.3	135	75.1
5	+2.6	(i) bis(SATE) (ii) 3× acetyl	860	74.8	154	630
21	−2.8	unmasked	564	1.8	248	76.8
22	−3.9	unmasked	922	52.3	213	90.4

[a]Calculated using ChemDraw Ultra 12. [b]Errors in the range of ±5% of the reported value from three determinations.

The cLogP values for *pro*inhibitor compounds **2**, **3**, and **5** are indicative of compounds that are likely to have good membrane permeability, while the negative cLogP values for the deprotected compounds **21** and **22** are consistent with expected poor cell membrane permeability, Table 2.[21] All test compounds have strongest inhibitory activity against intracellular hCA II, with the unmasked inhibitors **21** and **22** experiencing further enhanced inhibition of hCA II compared to their parent *pro*inhibitors. For example, the bis(SATE) *pro*inhibitors, **3** and **5**, had K_is of 6.3 and 74.8 nM, respectively, while their unmasked counterparts, **21** and **22**, had K_is of 1.8 and 53.3 nM, respectively. This confirms that the *pro*inhibitor compounds are able to successfully target intracellular CAs. It is common for primary sulfonamide compounds to experience up to 3 orders of magnitude weaker inhibition at CA I than at CA II. This relationship was observed for the test compounds of this study, with both masked and unmasked inhibitors having K_i values 437−922 nM. When considered together, these structure−activity and structure−property relationships establish that *pro*inhibitors are likely destined for intracellular localization. The *pro*inhibitors **2**, **3**, and **5** should readily enter cells by passive membrane diffusion, unmask intracellularly with a combination of pH and/or enzyme driven removal of the acetyl, bis(SATE), and *cyclo*Sal protecting groups, with the

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resulting inhibitors **21** and **22** trapped intracellularly. Furthermore, as CA I and CA II are two of the most prevalent intracellular CAs in humans, and the free monophosphate inhibitors **21** and **22** show good inhibition of these isozymes, the approach has been successful in providing chemical probes for location specific inhibition of intracellular CAs. While **21** and **22** are not directly viable as chemical probes to study intracellular acting CAs, their *pro*inhibitor form, with a combination of phosphate and hydroxyl protecting groups and complementary unmasking mechanism (chemical or enzyme activated), provide suitable characteristics to be employed as chemical probes.

CONCLUSION

It is noteworthy to acknowledge that the "go to" global CA inhibitor, AZA, entered clinical use several decades in advance of the discovery and characterization of most human CA isozymes. As a chemical probe, AZA exhibits global CA inhibition, with broad action against the 12 catalytically active human CA isozymes, seven of which are intracellular and five of which have an extracellular active site domain. A panel of three CA inhibitors, one acting intracellularly only, one acting extracellularly only (e.g., compound **1**), and a third global acting probe (e.g., AZA), may provide the needed chemical probes to interrogate more sophisticated biological hypotheses associated with CA inhibition. This panel may enhance the extrapolation from in vitro CA inhibition constants to the more complex biology of whole cells. In this study, we have successfully designed and synthesized monophosphate protected CA *pro*inhibitors **2**, **3**, and **5**. The *pro*inhibitors are destined for intracellular trapping as the corresponding monophosphates **4** and **6**. The *pro*inhibitors present the required structure−property and structure−activity profiles with potential to fulfill the criteria for location specific chemical probes for intracellular CAs with predicted excellent location fidelity. In addition to human CA studies, these proinhibitors may be useful for the study of CAs role in pathogenic organisms such as bacteria and protozoa.[22]

EXPERIMENTAL SECTION

General Chemistry. All starting materials and reagents were purchased from commercial suppliers. All solvents were available commercially dried or dried prior to use. Reaction progress was monitored by TLC using silica gel-60 F254 plates with detection by short wave UV fluorescence (λ = 254 nm) and staining with 5% w/v dodecamolybdophosphoric acid in ethanol or vanillin stain (5 g of vanillin in a mixture of EtOH:H_2O:H_2SO_4 = 85:10:2.75) with subsequent heating. Silica gel flash chromatography was performed using silica gel 60 Å (230−400 mesh). NMR (^{1}H, ^{13}C, ^{31}P, gCOSY, and HSQC) spectra were recorded on either a 400 or 500 MHz spectrometer at 30 °C. ^{1}H NMR spectra were obtained at 500 MHz and referenced to the residual solvent peak ($CDCl_3$ δ 7.26 ppm, DMSO-d_6 δ 2.50 ppm). ^{13}C NMR spectra were recorded at 125 MHz and referenced to the internal solvent ($CDCl_3$ δ 77.0 ppm, DMSO-d_6 δ 39.5 ppm). ^{31}P NMR spectra were recorded at 162 MHz with H_3PO_4 (δ 0.0 ppm) as the external reference. Multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), m (multiplet), dd (doublet of doublet), ddd (doublet of doublet of doublet), b (broad). Coupling constants are reported in hertz (Hz). Melting points are uncorrected. Mass spectra (low and high resolution) were recorded using electrospray as the ionization technique in positive ion and/or negative ion modes as stated. All MS analysis samples were prepared as solutions in methanol. Optical rotations were measured at 25 °C and reported as an average of 10 measurements. Purity of all compounds was ≥95% as determined by HPLC with UV. ^{1}H, ^{13}C, and ^{31}P NMR spectra of all novel compounds are provided in the Supporting Information. Glycoconjugates are named in accordance with the recommendations of the IUPAC-IUBMB Joint Commission on Biochemical Nomenclature: "Nomenclature of Carbohydrates (Recommendations 1996)" (http://www.chem.qmul.ac.uk/iupac/2carb/).

*β-D-Glucopyranosyl Azide (**10**).*[10a,23] Sodium methoxide (prepared from reacting Na (0.300 g, 13.5 mmol) with MeOH (10 mL)) was added dropwise to a stirred solution of *β*-azido-2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (**9**)[10a,23] (3.02 g, 8.08 mmol) in MeOH:CH_2Cl_2 (9:1, 30 mL) until the pH of the solution remained greater than 11. The reaction mixture was stirred overnight then neutralized by the addition of Amberlite IR-120 H^+ ion-exchange resin. The solution was filtered, washed with MeOH (50 mL), and the solvent removed in vacuo to give the title compound **10** (1.70 g, quant) as a colorless oil which was used without further purification. ^{1}H NMR (500 MHz, D_2O) δ 3.33 (t, J = 9.0 Hz, 1H, H-2), 3.41−3.63 (m, 3H, H-3, H-4, H-5), 3.81 (dd, J = 5.6, 12.4 Hz, 1H, H-6), 3.98 (dd, J = 2.2, 12.5 Hz, 1H, H-6). LRMS-ESI $[M + Na]^+$ m/z = 228. ^{1}H NMR was in agreement with the data reported in the literature.

*2,3,4-Tri-O-acetyl-6-O-tert-butyldimethylsilyl-β-D-glucopyranosyl Azide (**8**).*[24] *tert*-Butyldimethylsilyl chloride (1.47 g, 9.78 mmol) was added to a stirred solution of *β*-D-glucopyranosyl azide (**10**) (1.67 g, 8.15 mmol) and imidazole (1.66 g, 24.5 mmol) in anhydrous DMF (20 mL) at rt. The reaction mixture was left to stir overnight. The reaction was quenched by the addition of H_2O (2 mL) at 0 °C and the solvent removed in vacuo to afford 6-*O*-*tert*-butyldimethylsilyl-*β*-D-glucopyranosyl azide as a pale-yellow oil. The residue was then dissolved in anhydrous pyridine (15 mL). To this was added acetic anhydride (10.0 mL, 106 mmol) and the solution stirred at rt for 3 h. The reaction mixture was quenched by the addition of a few drops of MeOH and the solvent removed under reduced pressure. The residue was dissolved into CH_2Cl_2 (100 mL) and washed with water (40 mL). The aqueous fraction was re-extracted with CH_2Cl_2 (2 × 50 mL), then the combined organic fractions were washed with brine (50 mL), dried ($MgSO_4$), filtered, and the solvent removed in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 1:9 to 1:2) to give the title compound **8** (2.52 g, 70%, 2 steps) as a white solid; mp 158−160 °C (EtOAc/hexane); $[\alpha]_D^{24}$ −17.4 (c = 0.13, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.06 (s, 3H, $SiCH_3$), 0.07 (s, 3H, $SiCH_3$), 0.90 (s, 9H, $SiC(CH_3)_3$), 2.01 (s, 3H, $OCOCH_3$), 2.02 (s, 3H, $OCOCH_3$), 2.07 (s, 3H, $OCOCH_3$), 3.61−3.65 (m, 1H, H-5), 3.71 (dd, J = 5.0, 11.7 Hz, 1H, H-6), 3.77 (dd, J = 2.0, 11.7 Hz, 1H, H-6), 4.58 (d, J = 8.8 Hz, 1H, H-1), 4.92 (t, J = 9.2 Hz, 1H, H-2), 5.09 (t, J = 9.7 Hz, 1H, H-4), 5.21 (t, J = 9.5 Hz, 1H, H-3). ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.34, −5.33 ($Si(CH_3)_2$), 18.4 ($SiC(CH_3)_3$), 20.6 ($OCOCH_3$), 20.7 (2 × $OCOCH_3$), 25.8 ($SiC(CH_3)_3$), 62.1 (C-6), 68.4 (C-4), 70.9 (C-2), 73.1 (C-3), 77.0 (C-5), 87.8 (C-1), 169.2, 169.3, 170.3 ($OCOCH_3$). LRMS-ESI $[M + Na]^+$ m/z = 468.

*2,3,4-Tri-O-acetyl-β-D-glucopyranosyl Azide (**12**).*[25] Acetyl chloride (0.05 mL, 0.70 mmol) was added to a solution of 2,3,4-tri-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-*β*-D-glucopyranosyl azide (**8**) (0.555 g, 1.25 mmol) in CH_2Cl_2/MeOH (2:1, 30 mL) and stirred at rt for 30 min. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with $NaHCO_3$ (20 mL). The aqueous layer was re-extracted with CH_2Cl_2 (2 × 30 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and the solvent removed in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 1:2 to 1:1) to give the title compound **12** (0.40 g, 97%) as a white solid; mp 125−127 °C (EtOAc/hexane); $[\alpha]_D^{24}$ −33.2 (c = 0.14, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.02 (s, 3H, $OCOCH_3$), 2.06 (s, 3H, $OCOCH_3$), 2.08 (s, 3H, $OCOCH_3$), 2.22 (dd, J = 5.5, 8.1 Hz, 1H, OH), 3.60−3.67 (m, 2H, H-5, H-6), 3.77−3.82 (m, 1H, 6-H), 4.67 (d, J = 8.9 Hz, 1H, H-1), 4.93 (t, J = 9.3 Hz, 1H, H-2), 5.07 (t, J = 9.6 Hz, 1H, H-4), 5.27 (t, J = 9.5 Hz, 1H, H-3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.61, 20.62, 20.7 ($OCOCH_3$), 61.2 (C-6), 68.3 (C-4), 70.9 (C-2), 72.6 (C-3), 76.5 (C-5), 88.0 (C-1), 169.3, 170.1, 170.2 ($OCOCH_3$). LRMS-ESI $[M + Na]^+$ m/z = 354. HRMS-ESI $[M + Na]^+$ calcd for $C_{12}H_{17}N_3NaO_8$, 354.0908; found, 354.0909.

*CycloSal-(1-azido-2,3,4-tri-O-acetyl-β-D-glucopyranosyl-6)-phosphate (**11**).* To a solution of alcohol **12** (0.200 g, 0.604 mmol) in

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anhydrous CH_3CN (5 mL) at −20 °C was added DIPEA (0.263 mL, 1.51 mmol). To this solution was added saligenyl chlorophosphite **13**[17] (0.228 g, 1.21 mmol) dissolved in anhydrous CH_3CN (1.0 mL) dropwise over 5 min. The reaction was left to stir for 30 min. *tert*-Butylhydroperoxide (5−6 M in *n*-decane, 0.252 mL, 1.51 mmol) was added dropwise at −20 °C and stirred for 30 min before warming to rt and stirred for 1.5 h. Na_2SO_3 10% in H_2O (0.10 mL) was added to destroy the excess peroxide. The reaction was diluted with CH_2Cl_2 (50 mL) and washed with brine (20 mL). The aqueous layer was re-extracted with CH_2Cl_2 (3 × 20 mL), and the combined organic fractions were dried ($MgSO_4$), filtered, and the solvent removed in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 2:3 to 3:2) to give the title compound **11** (0.165 g, 55%, diastereomeric ratio β_1/β_2 = 1:1) as a white foam; $[\alpha]_D^{26}$ −7.1 (*c* = 0.10, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.96 (s, 1.5H, $OCOCH_3$), 1.99 (s, 3H, 2 × $OCOCH_3$), 2.04 (s, 1.5H, $OCOCH_3$), 2.06 (s, 1.5H, $OCOCH_3$), 2.07 (s, 1.5H, $OCOCH_3$), 3.81−3.89 (m, 1H, H-5), 4.21−4.31 (m, 1H, H-6), 4.33−4.40 (m, 1H, 6-H), 4.61 (d, *J* = 8.7 Hz, 0.5H, H-1), 4.63 (d, *J* = 9.2 Hz, 0.5H, H-1), 4.87 (t, *J* = 9.3 Hz, 0.5H, H-2), 4.92 (t, *J* = 9.2 Hz, 0.5H, H-2), 5.00 (t, *J* = 9.9 Hz, 0.5H, H-4), 5.04 (t, *J* = 10.2 Hz, 0.5H, H-4), 5.17−5.23 (m, 1H, 3-H), 5.30−5.38 (m, 1H, $POCH_2$), 5.40−5.47 (m, 1H, $POCH_2$), 7.06−7.11 (m, 2H, Ar-H), 7.13−7.17 (m, 1H, Ar-H), 7.31−7.36 (m, 1H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.5, 20.57, 20.6 ($OCOCH_3$), 66.0 (d, *J* = 5.0 Hz, C-6), 66.1 (d, *J* = 5.1 Hz, C-6), 67.9, 68.0 (C-4), 68.9 (d, *J* = 5.5 Hz, $POCH_2$), 69.0 (d, *J* = 5.4 Hz, $POCH_2$), 70.61, 70.62 (C-2), 72.59, 72.6 (C-3), 74.4 (d, *J* = 7.3 Hz, C-5), 74.5 (d, *J* = 7.0 Hz, C-5), 87.9, 88.0 (C-1), 118.78 (d, *J* = 9.2 Hz, $POCCH_2$), 118.82 (d, *J* = 9.1 Hz, $POCCH_2$), 120.6, 120.7 (Ar-C), 124.5, 124.52, 125.4, 125.4, 129.92, 129.93, 129.95 (Ar-CH), 150.1, 150.2 (Ar-C), 169.19, 169.21, 169.3, 169.4, 170.10, 170.11 ($OCOCH_3$). ^{31}P NMR (162 MHz, $CDCl_3$) δ −9.31, −9.34. LRMS-ESI $[M + Na]^+$ *m*/*z* = 522. HRMS-ESI $[M + Na]^+$ calcd for $C_{19}H_{22}N_3NaO_{11}P$, 522.0884; found, 522.0892.

*CycloSal-(1-(4-[4-(aminosulfonyl)phenyl]-1H-1,2,3-triazole)-2,3,4-tri-O-acetyl-β-D-glucopyranosyl-6)-phosphate (**2**).* β-D-Glucopyranosyl azide **11** (0.040 g, 0.080 mmol) and 4-ethynylbenzenesulfonamide (**14**)[18] (0.015 g, 0.080 mmol) were suspended in an EtOH/H_2O mixture (3:1, 4 mL). To the reaction mixture was added a solution of sodium ascorbate (0.013 g, 0.064 mmol) in water (0.25 mL), followed by a solution of $CuSO_4{\cdot}5H_2O$ (0.008 g, 0.032 mmol) in water (0.25 mL). The bright-yellow suspension was stirred vigorously at 60 °C for 2 h. The solvent was removed in vacuo, and the residue was purified by column chromatography on silica gel (EtOAc/hexane = 3:2 to 4:1) to give the title compound **2** (0.042 g, 76%, diastereomeric ratio β_1/β_2 = 1:0.8) as a white solid; mp 203−205 °C (EtOAc/hexane); $[\alpha]_D^{26}$ 9.3 (*c* = 0.11, MeOH). ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 1.81 (s, 3H, 2 × $OCOCH_3$), 1.94 (s, 1.5H, $OCOCH_3$), 1.95 (s, 1.5H, $OCOCH_3$), 1.97 (s, 1.5H, $OCOCH_3$), 2.04 (s, 1.5H, $OCOCH_3$), 4.14−4.34 (m, 2H, CH_2-6), 4.41−4.48 (m, 1H, H-5), 5.13−5.19 (m, 1H, H-4), 5.38−5.45 (m, 2H, $POCH_2$), 5.57−5.62 (m, 1H, H-3), 5.64−5.69 (m, 1H, H-2), 6.40 (d, *J* = 9.1 Hz, 0.55H, H-1), 6.42 (d, *J* = 9.3 Hz, 0.45H, H-1), 7.00−7.08 (m, 1.5H, Ar-H), 7.13−7.17 (m, 1H, Ar-H), 7.20−7.24 (m, 0.5H, Ar-H), 7.25−7.32 (m, 1H, Ar-H), 7.40 (s, 2H, SO_2NH_2), 7.93 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.99−8.04 (m, 2H, Ar-H), 8.96 (s, 0.55H, $CH_{triazole}$), 9.06 (s, 0.45H, $CH_{triazole}$). ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 19.8, 20.2, 20.4 ($OCOCH_3$), 65.6 (d, *J* = 4.1 Hz, C-6), 65.7 (d, *J* = 5.5 Hz, C-6), 67.0, 67.1 (C-4), 68.37, 68.43 ($POCH_2$), 70.1, 70.2 (C-2), 72.03, 72.07 (C-3), 73.4 (d, *J* = 7.8 Hz, C-5), 73.5 (d, *J* = 7.4 Hz, C-5), 83.9, 84.0 (C-1), 117.9, 118.0, 118.1 (Ar-CH), 120.8, 120.87, 120.89, 120.97 (Ar-C), 121.47, 121.5 ($CH_{triazole}$), 124.2, 124.3, 125.5, 125.8, 126.0, 126.4, 126.5, 129.54, 129.55, 129.58, 129.59 (Ar-CH), 133.1 ($C_{triazole}$), 143.5, 143.6, 145.6, 145.7, 149.2, 149.27 149.33 (Ar-C), 168.5, 169.1, 169.2, 169.5 ($OCOCH_3$). ^{31}P NMR (162 MHz, $(CD_3)_2SO$) δ −9.08, −9.39. LRMS-ESI $[M + H]^+$ *m*/*z* = 681, $[M + Na]^+$ *m*/*z* = 703.0949. HRMS-ESI $[M + Na]^+$ calcd for $C_{27}H_{29}N_4NaO_{13}PS$, 703.1082; found, 703.1088.

*4-[4-(Aminosulfonyl)phenyl]-1-(2,3,4-tri-O-acetyl-6-O-tert-butyldimethylsilyl-β-D-glucopyranosyl)-1H-1,2,3-triazole (**15**).* Azide **8** (0.500 g, 1.12 mmol) and alkyne **14**[18] (0.203 g, 1.12 mmol) were suspended in an EtOH/H_2O mixture (5:1, 12 mL). To the reaction mixture was added a solution of sodium ascorbate (0.089 g, 0.449 mmol) in water (0.5 mL), followed by a solution of $CuSO_4{\cdot}5H_2O$ (0.056 g, 0.224 mmol) in water (0.5 mL). The bright-yellow suspension was stirred vigorously at 60 °C for 3 h. The solvent was removed in vacuo, and the residue was purified by column chromatography on silica gel (EtOAc/hexane = 2:3 to 1:1) to give the title compound **15** (0.653 g, 93%) as a white solid; mp 163−165 °C (EtOAc/hexane); $[\alpha]_D^{26}$ −41.6 (*c* = 0.13, MeOH). ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ −0.07 (s, 3H, $SiCH_3$), −0.04 (s, 3H, $SiCH_3$), 0.81 (s, 9H, $SiC(CH_3)_3$), 1.82 (s, 3H, $OCOCH_3$), 1.97 (s, 3H, $OCOCH_3$), 2.03 (s, 3H, $OCOCH_3$), 3.66 (dd, *J* = 2.3, 12.3 Hz, 1H, H-6), 3.74 (dd, *J* = 5.2, 12.1 Hz, 1H, H-6), 4.18−4.23 (m, 1H, H-5), 5.16 (t, *J* = 9.5 Hz, 1H, H-4), 5.57 (t, *J* = 9.3 Hz, 1H, H-3), 5.62 (t, *J* = 9.1 Hz, 1H, H-2), 6.39 (d, *J* = 8.7 Hz, 1H, H-1), 7.38 (s, 2H, SO_2NH_2), 7.92 (d, *J* = 8.2 Hz, 2H, Ar-H), 8.03 (d, *J* = 8.2 Hz, 2H, Ar-H), 9.05 (s, 1H, $CH_{triazole}$). ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ −5.53, −5.49 ($Si(CH_3)_2$), 17.9 ($SiC(CH_3)_3$), 19.9, 20.2, 20.4 ($OCOCH_3$), 25.6 ($SiC(CH_3)_3$), 61.8 (C-6), 67.7 (C-4), 70.4 (C-2), 72.5 (C-3), 75.9 (C-5), 84.0 (C-1), 121.6 ($CH_{triazole}$), 125.4, 126.4 (Ar-CH), 133.2 ($C_{triazole}$), 143.5, 145.5 (Ar-C), 168.5, 169.0, 169.5 ($OCOCH_3$). LRMS-ESI $[M + Na]^+$ *m*/*z* = 649. HRMS-ESI $[M + Na]^+$ calcd for $C_{26}H_{38}N_4NaO_{10}SSi$, 649.1970; found, 649.1975.

*4-[4-(Aminosulfonyl)phenyl]-1-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-1H-1,2,3-triazole (**16**).* Acetyl chloride (0.05 mL, 0.70 mmol) was added to a solution of **15** (0.400 g, 0.638 mmol) in CH_2Cl_2/MeOH (2:1, 15 mL), and the solution was stirred at rt for 30 min. The reaction mixture was quenched with Et_3N (100 μL), stirred for 5 min, and the solvent removed in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 3:2 to 4:1) to give the title compound **16** (0.294 g, 90%) as a white solid; mp 224−227 °C (EtOAc/hexane); $[\alpha]_D^{26}$ −72.1 (*c* = 0.12, MeOH). ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 1.82 (s, 3H, $OCOCH_3$), 1.97 (s, 3H, $OCOCH_3$), 2.04 (s, 3H, $OCOCH_3$), 3.41−3.47 (m, 1H, H-6), 3.52−3.57 (m, 1H, H-6), 4.12−4.16 (m, 1H, H-5), 4.90 (t, *J* = 5.7 Hz, 1H, OH), 5.12 (t, *J* = 9.5 Hz, 1H, H-4), 5.54−5.62 (m, 2H, H-3, H-2), 6.39 (d, *J* = 8.5 Hz, 1H, H-1), 7.38 (s, 2H, SO_2NH_2), 7.92 (d, *J* = 8.4 Hz, 2H, Ar-H), 8.03 (d, *J* = 8.4 Hz, 2H, Ar-H), 9.09 (s, 1H, $CH_{triazole}$). ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 19.9, 20.2, 20.4 ($OCOCH_3$), 59.9 (C-6), 67.9 (C-4), 70.5 (C-2), 72.4 (C-3), 76.4 (C-5), 84.1 (C-1), 121.6 ($CH_{triazole}$), 125.4, 126.4, (Ar-CH), 133.2 ($C_{triazole}$), 143.5, 145.6 (Ar-C), 168.5, 169.2, 169.5 ($OCOCH_3$). LRMS-ESI $[M + H]^+$ *m*/*z* = 513, $[M + Na]^+$ *m*/*z* = 535. HRMS-ESI $[M + Na]^+$ calcd for $C_{20}H_{24}N_4NaO_{10}S$, 535.1105; found, 535.1106.

*1-(4-[4-(Aminosulfonyl)phenyl]-1H-1,2,3-triazole)-2,3,4-tri-O-acetyl-6-O-[[(bis((2-((2,2-dimethylpropionyl)sulfanyl)ethoxy)]-phosphoryloxy)-ethyl)]-β-D-glucopyranoside (**3**).* A solution of alcohol **16** (0.100 g, 0.195 mmol) and phosphoramidite 7[14,26] (0.177 g, 0.390 mmol) in CH_2Cl_2 (20 mL) was coevaporated to dryness and dried under high vacuum for 30 min. The mixture was azeotroped from anhydrous CH_3CN (2 × 30 mL) to dryness, then anhydrous CH_3CN (30 mL) was added and the solution reduced to approximately half its volume. To the stirred reaction mixture was added 1*H*-tetrazole (0.45 M in CH_3CN, 1.30 mL, 0.585 mmol), and the mixture was stirred at rt for 1.5 h. Anhydrous CH_2Cl_2 (10 mL) was added, and the reaction mixture was stirred for a further 10 min before being cooled to −30 °C. *m*-CPBA (60%, 0.112 g, 0.390 mmol) was added to the cooled solution and stirred for 10 min, then warmed to rt and stirred for a further 1 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL), quenched with solid $NaHCO_3$ (0.1 g), and diluted with H_2O (30 mL) and 10% Na_2SO_3 (20 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 40 mL), and the combined organic phases were dried ($MgSO_4$), filtered, and the solvent removed in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 1:0 to 19:1) to give the title compound **3** (0.088 g, 51%) as a white solid; mp 145−147 °C (CH_2Cl_2/MeOH); $[\alpha]_D^{28}$ −31.3 (*c* = 0.10, MeOH). ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 1.127, 1.132 (2 × s, 18H, $C(CH_3)_3$), 1.82 (s, 3H, $OCOCH_3$), 1.98 (s, 3H, $OCOCH_3$), 2.06 (s, 3H, $OCOCH_3$), 3.00−3.07 (m, 4H, 2 × CH_2S), 3.92−4.00 (m, 4H, 2

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× CH_2O), 4.04−4.10 (m, 1H, H-6), 4.13−4.18 (m, 1H, H-6), 4.42−4.47 (m, 1H, H-5), 5.20 (t, J = 9.7 Hz, 1H, H-4), 5.62 (t, J = 9.4 Hz, 1H, H-3), 5.69 (t, J = 9.3 Hz, 1H, H-2), 6.45 (d, J = 9.0 Hz, 1H, H-1), 7.39 (s, 2H, SO_2NH_2), 7.91 (d, J = 8.2 Hz, 2H, Ar-H), 8.02 (d, J = 8.2 Hz, 2H, Ar-H), 9.06 (s, 1H, $CH_{triazole}$). ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 19.8, 20.2, 20.4 ($OCOCH_3$), 26.8 ($C(CH_3)_3$), 27.9 (d, J = 7.6 Hz, CH_2S), 28.0 (d, J = 7.6 Hz, CH_2S), 45.9 ($C(CH_3)_3$), 65.1 (d, J = 5.1 Hz, C-6), 65.5 (d, J = 5.5 Hz, CH_2O), 65.6 (d, J = 5.5 Hz, CH_2O), 67.1 (C-4), 70.2 (C-2), 72.1 (C-3), 73.7 (d, J = 7.8 Hz, C-5), 84.0 (C-1), 121.4 ($CH_{triazole}$), 125.5, 126.4 (Ar-CH), 133.1 ($C_{triazole}$), 143.6, 145.7 (Ar-C), 168.5, 169.1, 169.5 ($OCOCH_3$), 204.9, 204.9 ($SCOC(CH_3)_3$). ^{31}P NMR (162 MHz, $(CD_3)_2SO$) δ −1.34. LRMS-ESI $[M + Na]^+$ m/z = 903. HRMS-ESI $[M + Na]^+$ calcd for $C_{34}H_{49}N_4NaO_{15}PS_3$, 903.1986; found, 903.1989.

*N-(4-Methoxybenzyl)-1-S-(2,3,4,6-tetra-O-acetyl)-D-glucopyranosylsulfonamide (**19**).*[19] To a solution of thioacetate derivative **18**[19] (5.00 g, 12.3 mmol) in anhydrous MeOH (100 mL) under a nitrogen atmosphere was added diethyl bromomalonate (5.03 mL, 29.5 mmol), and the solution was stirred at rt for 20 min. 4-Methoxybenzylamine (8.20 mL, 62.7 mmol) was then added and the reaction mixture stirred at rt overnight. The solvent was removed in vacuo. The residue was dissolved into EtOAc (100 mL) and washed with brine (2 × 50 mL). The aqueous layers were combined and re-extracted with EtOAc (2 × 30 mL), and the combined organic fractions were dried ($MgSO_4$), filtered, and the solvent removed in vacuo. The residue was semipurified by column chromatography on silica gel (EtOAc/hexane = 1:2 to 2:3, 1% Et_3N) to give *N*-(4-methoxybenzyl)-1-*S*-(2,3,4,6-tetra-*O*-acetyl)-D-glucopyranosylsulfenamide (6.15 g, 12.3 mmol) as a pale-yellow oil. To the residue dissolved in CH_3CN (60 mL), was added a finely ground mixture of the catalytic oxidative system ($KMnO_4$/$CuSO_4$·$5H_2O$; 1:1; w/w) (14.3 g, 51.7/12.3 mmol) in H_2O (10 mL), and the solution was stirred at rt for 2 h. The reaction mixture was filtered through Celite and washed with EtOAc (300 mL). The organic layer was washed with H_2O (100 mL) and brine (100 mL), dried ($MgSO_4$), and the solvent removed in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 1:4 to 1:2) to give the title compound **19** (2.63 g, 40%) as a white solid; mp 140−142 °C (EtOAc/hexane); $[\alpha]_D^{26}$ −27.6 (c = 0.12, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.01 (s, 3H, $OCOCH_3$), 2.03 (s, 3H, $OCOCH_3$), 2.06 (s, 3H, $OCOCH_3$), 2.08 (s, 3H, $OCOCH_3$), 3.63−3.67 (m, 1H, H-5), 3.82 (s, 3H, OCH_3), 4.16 (dd, J = 2.4, 12.6 Hz, 1H, H-6), 4.22−4.32 (m, 3H, H-6, H-1, N-CH_2), 4.37 (dd, J = 6.0, 14.5 Hz, 1H, N-CH_2), 4.98 (t, J = 6.0 Hz, 1H, NH), 5.05 (t, J = 9.7 Hz, 1H, H-4), 5.25 (t, J = 9.3 Hz, 1H, H-3), 5.31 (t, J = 9.5 Hz, 1H, H-2), 6.91 (d, J = 8.6 Hz, 2H, Ar-H), 7.29 (d, J = 8.6 Hz, 2H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.6 (2 × $OCOCH_3$), 20.71, 20.73 ($OCOCH_3$), 47.5 (N-CH_2), 55.4 (OCH_3), 61.5 (C-6), 67.7 (C-4), 68.0 (C-2), 73.0 (C-3), 76.4 (C-5), 87.9 (C-1), 114.3 (2 × Ar-CH), 129.1 (Ar-C), 129.3 (2 × Ar-CH), 159.6 (Ar-C), 169.3, 170.0, 170.2, 170.5 ($OCOCH_3$). LRMS-ESI $[M - H]^-$ m/z = 530. HRMS-ESI $[M + Na]^+$ calcd for $C_{22}H_{29}NNaO_{12}S$, 554.1303; found, 554.1301.

*N-(4-Methoxybenzyl)-1-S-(2,3,4-tri-O-acetyl-6-O-tert-butyldimethylsilyl)-D-glucopyranosylsulfonamide (**17**).* Sodium methoxide (25% w/v, 0.50 mL, 2.21 mmol) was added dropwise to a stirred solution of **19** (0.400 g, 0.753 mmol) in MeOH (10 mL), and the resulting pH of the solution was greater than 11. The reaction mixture was stirred at rt for 2 h then neutralized by the addition of Amberlite IR-120 H^+ ion-exchange resin. The solution was filtered, washed with MeOH (50 mL), and the solvent removed in vacuo to give *N*-(4-methoxybenzyl)-1-*S*-D-glucopyranosylsulfonamide as a colorless oil. To the residue dissolved in anhydrous DMF (5 mL) was added *tert*-butyldimethylsilyl chloride (0.131 g, 0.870 mmol) and imidazole (0.148 g, 2.18 mmol) and the solution stirred at rt overnight. The reaction was quenched by the addition of H_2O (0.5 mL) at 0 °C, and the solvent was removed in vacuo to afford *N*-(4-methoxybenzyl)-1-*S*-6-*O*-*tert*-butyldimethylsilyl-D-glucopyranosylsulfonamide as a pale-yellow oil, which was used without further purification. Acetic anhydride (1.5 mL, 15.9 mmol) was added to the residue dissolved in anhydrous pyridine (4 mL) and stirred at rt for 3 h. The reaction mixture was quenched by the addition of a few drops of MeOH and the solvent removed under reduced pressure. The residue was dissolved into EtOAc (50 mL) and washed with water (30 mL). The aqueous fraction was re-extracted with EtOAc (3 × 30 mL), then the combined organic fractions were washed with brine (50 mL), dried ($MgSO_4$), filtered, and the solvent removed in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 1:9 to 1:2) to give the title compound **17** (0.264 g, 60%, 3 steps) as a white foam; $[\alpha]_D^{25}$ −7.9 (c = 0.13, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.04 (s, 3H, $SiCH_3$), 0.05 (s, 3H, $SiCH_3$), 0.90 (s, 9H, $SiC(CH_3)_3$), 2.02 (s, 3H, $OCOCH_3$), 2.03 (s, 3H, $OCOCH_3$), 2.06 (s, 3H, $OCOCH_3$), 3.56−3.61 (m, 1H, H-5), 3.71 (dd, J = 4.0, 11.7 Hz, 1H, H-6), 3.82 (dd, J = 1.6, 11.5 Hz, 1H, H-6), 3.82 (s, 3H, OCH_3), 4.28 (dd, J = 5.0, 14.3 Hz, 1H, N-CH_2), 4.36 (d, J = 8.8 Hz, 1H, H-1), 4.41 (dd, J = 6.8, 14.3 Hz, 1H, N-CH_2), 4.89 (t, J = 6.0 Hz, 1H, NH), 5.18 (t, J = 9.3 Hz, 1H, H-4),5.25−5.34 (m, 2H, H-2, H-3), 6.90 (d, J = 8.4 Hz, 2H, Ar-H), 7.27 (d, J = 8.6 Hz, 2H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.5, −5.4 ($Si(CH_3)_2$), 18.4 ($SiC(CH_3)_3$), 20.64 ($OCOCH_3$), 20.65 ($OCOCH_3$), 20.8 ($OCOCH_3$), 25.9 ($SiC(CH_3)_3$), 47.6 (N-CH_2), 55.4 (OCH_3), 61.6 (C-6), 67.9 (C-4), 68.2 (C-2), 73.5 (C-3), 79.3 (C-5), 88.4 (C-1), 114.3 (2 × Ar-CH), 129.1 (Ar-C), 129.3 (2 × Ar-CH), 159.5 (Ar-C), 169.1, 170.2, 170.6 ($OCOCH_3$). LRMS-ESI $[M + Na]^+$ m/z = 626. HRMS-ESI $[M + Na]^+$ calcd for $C_{26}H_{41}NNaO_{11}SSi$, 626.2062; found, 626.2059.

*1-S-(2,3,4-Tri-O-acetyl)-D-glucopyranosylsulfonamide (**20**).* To a solution of sulfonamide **17** (0.500 g, 0.828 mmol) in CH_3CN (30 mL) and H_2O (10 mL) was added ceric ammonium nitrate (1.82 g, 3.31 mmol), and the reaction mixture was stirred at rt for 3 h. Water (30 mL) was added, and the reaction mixture was extracted into EtOAc (3 × 40 mL). The organic extract was washed with brine (40 mL), dried ($MgSO_4$), and the solvent removed in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 1:1 to 4:1) to give the title compound **20** (0.274 g, 90%) as a white solid; mp 190−191 °C (EtOAc/hexane); $[\alpha]_D^{26}$ −10.1 (c = 0.10, MeOH). ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 1.93 (s, 3H, $OCOCH_3$), 1.94 (s, 3H, $OCOCH_3$), 1.99 (s, 3H, $OCOCH_3$), 3.39−3.46 (m, 1H, H-6), 3.49−3.55 (m, 1H, H-6), 3.87−3.92 (m, 1H, H-5), 4.69−4.73 (m, 2H, H-1, OH), 4.85 (t, J = 9.8 Hz, 1H, H-4), 5.17 (t, J = 9.5 Hz, 1H, H-2), 5.37 (t, J = 9.4 Hz, 1H, H-3), 7.07 (s, 2H, SO_2NH_2). ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 20.3, 20.36, 20.41 ($OCOCH_3$), 60.2 (C-6), 67.9 (C-2), 68.0 (C-4), 73.1 (C-3), 77.4 (C-5), 86.2 (C-1), 168.6, 169.2, 169.5 ($OCOCH_3$). LRMS-ESI $[M - H]^-$ m/z = 368. HRMS-ESI $[M + Na]^+$ calcd for $C_{12}H_{19}NNaO_{10}S$, 392.0622; found, 392.0618.

*2,3,4-Tri-O-acetyl-6-O-[[(bis((2-((2,2-dimethylpropionyl)sulfanyl)-ethoxy)]-phosphoryloxy)-ethyl)]-β-D-glucopyranosylsulfonamide (**5**).* A solution of alcohol **20** (0.025 g, 0.068 mmol) and phosphoramidite **7**[14,26] (0.080 g, 0.176 mmol) in CH_2Cl_2 (10 mL) was coevaporated to dryness and dried under high vacuum for 30 min. The mixture was azeotroped from anhydrous CH_3CN (2 × 10 mL) to dryness, then anhydrous CH_3CN (10 mL) was added and the solution reduced to approximately half its volume. To the stirred reaction mixture was added 1*H*-tetrazole (0.45 M in CH_3CN, 0.602 mL, 0.271 mmol), and the mixture was stirred at rt for 1.5 h. Anhydrous CH_2Cl_2 (5 mL) was added, and the reaction mixture was stirred for a further 10 min before being cooled to −30 °C. *m*-CPBA (60%, 0.049 g, 0.169 mmol) was added to the cooled solution and stirred for 10 min, then warmed to rt and stirred for a further 1 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL), quenched with solid $NaHCO_3$ (0.05 g), then diluted with H_2O (30 mL) and 10% Na_2SO_3 (20 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 40 mL), and the combined organic phases were dried ($MgSO_4$), filtered, and the solvent removed in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 1:1 to 4:1) to give the title compound **5** (0.044 g, 89%) as a white foam; $[\alpha]_D^{26}$ −8.5 (c = 0.12, MeOH). ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 1.19, 1.20 (2 × s, 18H, $C(CH_3)_3$), 1.94 (s, 3H, $OCOCH_3$), 1.95 (s, 3H, $OCOCH_3$), 2.01 (s, 3H, $OCOCH_3$), 3.09−3.14 (m, 4H, 2 × CH_2S), 4.01−4.16 (m, 6H, 2 × CH_2O, CH_2-6), 4.19−4.24 (m, 1H, H-5), 4.78 (d, J = 9.7 Hz, 1H, H-1), 4.95 (t, J = 9.8 Hz, 1H, H-4), 5.21 (t, J = 9.5 Hz, 1H, H-2), 5.42 (t, J = 9.4 Hz, 1H, H-3), 7.15 (brs, 2H, NH_2). ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 20.2, 20.3, 20.4 ($OCOCH_3$), 26.8 ($C(CH_3)_3$), 28.10 (d,

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J = 7.3 Hz, SCH_2), 28.14 (d, J = 7.3 Hz, SCH_2), 46.0 ($\underline{C}(CH_3)_3$), 65.2 (d, J = 5.2 Hz, C-6), 65.7 (d, J = 5.7 Hz, CH_2O), 65.8 (d, J = 5.7 Hz, CH_2O), 67.2 (C-4), 67.7 (C-2), 72.9 (C-3), 74.8 (d, J = 7.4 Hz, C-5), 86.3 (C-1), 168.6, 169.0, 169.5 ($O\underline{C}OCH_3$), 204.5, 205.0 ($S\underline{C}OC(CH_3)_3$). ^{31}P NMR (162 MHz, $(CD_3)_2SO$) δ −1.28. LRMS-ESI $[M - H]^-$ m/z = 736. HRMS-ESI $[M - H]^-$ calcd for $C_{26}H_{43}NO_{15}PS_3$, 736.1527; found, 736.1531.

*1-(4-[4-(Aminosulfonyl)phenyl]-1H-1,2,3-triazole)-2,3,4-tri-O-acetyl-β-D-glucopyranosyl-6-O-dibenzyl Phosphate (**25**).* A solution of alcohol **16** (0.120 g, 0.234 mmol) and dibenzyl *N*,*N*-diisopropylphosphoramidite **23** (0.121 g, 0.351 mmol) in anhydrous CH_2Cl_2 (10 mL) was coevaporated to dryness and dried under high vacuum for 30 min. The mixture was azeotroped from anhydrous CH_3CN (2 × 10 mL) to dryness, then anhydrous CH_3CN (20 mL) was added and the solution reduced to approximately half its volume. To the stirred reaction mixture was added 1*H*-tetrazole (0.45 M in CH_3CN, 1.04 mL, 0.468 mmol), and the mixture was stirred at rt for 1.5 h. Anhydrous CH_2Cl_2 (10 mL) was added, and the reaction mixture was stirred for a further 10 min before being cooled to −30 °C. *m*-CPBA (60%, 0.168 g, 0.585 mmol) was added to the cooled solution and stirred for 10 min, then warmed to rt and stirred for a further 1 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL), H_2O (30 mL), and 10% Na_2SO_3 (20 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 40 mL), and the combined organic phases were dried ($MgSO_4$), filtered, and the solvent removed in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 3:2 to 4:1) to give the title compound **25** (0.149 g, 82%) as a white solid; mp 211−213 °C (EtOAc/hexane); $[\alpha]_D^{29}$ −25.7 (c = 0.12, MeOH). ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 1.82 (s, 3H, $OCOCH_3$), 1.99 (s, 3H, $OCOCH_3$), 2.02 (s, 3H, $OCOCH_3$), 4.06−4.12 (m, 1H, H-6), 4.13−4.18 (m, 1H, H-6), 4.43−4.48 (m, 1H, H-5), 4.92−4.99 (m, 4H, 2 × $PhCH_2$), 5.23 (t, J = 9.7 Hz, 1H, H-4), 5.63 (t, J = 9.5 Hz, 1H, H-3), 5.71 (t, J = 9.3 Hz, 1H, H-2), 6.47 (d, J = 9.1 Hz, 1H, H-1), 7.24−7.32 (m, 10H, Ph), 7.39 (brs, 2H, SO_2NH_2), 7.89 (d, J = 8.6 Hz, 2H, Ar-H), 7.94 (d, J = 8.5 Hz, 2H, Ar-H), 9.05 (s, 1H, $CH_{triazole}$). ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 19.9, 20.2, 20.3 ($OCO\underline{C}H_3$), 65.1 (d, J = 5.1 Hz, C-6), 67.1 (C-4), 68.5 (d, J = 5.1 Hz, $Ph\underline{C}H_2$), 68.6 (d, J = 5.3 Hz, $Ph\underline{C}H_2$), 70.2 (C-2), 72.1 (C-3), 73.7 (d, J = 7.7 Hz, C-5), 84.0 (C-1), 121.5 ($CH_{triazole}$), 125.4, 126.4, 127.6, 127.7, 128.2, 128.26, 128.31, 128.32 (Ar-CH), 133.0 ($C_{triazole}$), 135.7 (d, J = 7.1 Hz, i-Ph), 135.8 (d, J = 6.9 Hz, i-Ph), 143.5, 145.7 (Ar-C), 168.5, 169.2, 169.5 ($O\underline{C}OCH_3$). ^{31}P NMR (162 MHz, $(CD_3)_2SO$) δ −0.67. LRMS-ESI $[M + H]^+$ m/z = 773, $[M + Na]^+$ m/z = 795. HRMS-ESI $[M + Na]^+$ calcd for $C_{34}H_{37}N_4NaO_{13}PS$, 795.1708; found, 795.1724.

*1-(4-[4-(Aminosulfonyl)phenyl]-1H-1,2,3-triazole)-2,3,4-tri-O-acetyl-β-D-glucopyranosyl-6-O-phosphate (**28**).* $Pd(OH)_2$ (20%, 20 mg) was added to a solution of **25** (0.113 g, 0.146 mmol) in THF/MeOH (2:3, 10 mL). The reaction mixture was stirred under an atmosphere of hydrogen for 2 h at rt, then filtered through Celite and washed with THF (20 mL) and MeOH (40 mL). The solvent was removed in vacuo and the residue purified by RP-18 column chromatography (MeOH/H_2O 5:95 to 3:2, product eluting at 1:4) to give the title compound **28** (0.067 g, 78%) as a white solid; mp 182−183 °C (MeOH/H_2O); $[\alpha]_D^{26}$ −16.0 (c = 0.18, MeOH). ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 1.82 (s, 3H, $OCOCH_3$), 1.98 (s, 3H, $OCOCH_3$), 2.05 (s, 3H, $OCOCH_3$), 3.80−3.93 (m, 2H, CH_2−6), 4.35−4.40 (m, 1H, H-5), 5.12 (t, J = 9.4 Hz, 1H, H-4), 5.58−5.67 (m, 2H, H-2, H-3), 6.42 (d, J = 8.3 Hz, 1H, H-1), 7.39 (s, 2H, SO_2NH_2), 7.91 (d, J = 8.4 Hz, 2H, Ar-H), 8.03 (d, J = 8.4 Hz, 2H, Ar-H), 9.08 (s, 1H, $CH_{triazole}$). ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 19.9, 20.2, 20.4 ($OCO\underline{C}H_3$), 63.7 (d, J = 4.0 Hz, C-6), 67.8 (C-4), 70.3 (C-2), 72.1 (C-3), 74.5 (d, J = 8.6 Hz, C-5), 84.0 (C-1), 121.6 ($CH_{triazole}$), 125.5, 126.4, (Ar-CH), 133.1 ($C_{triazole}$), 143.5, 145.7 (Ar-C), 168.6, 169.3, 169.5 ($O\underline{C}OCH_3$). ^{31}P NMR (162 MHz, $(CD_3)_2SO$) δ −0.67. LRMS-ESI $[M - H]^-$ m/z = 591. HRMS-ESI $[M + Na]^+$ calcd for $C_{20}H_{25}N_4NaO_{13}PS$, 615.0769; found, 615.0761.

*1-(4-[4-(Aminosulfonyl)phenyl]-1H-1,2,3-triazole)-β-D-glucopyranosyl-6-O-phosphate (**21**).* Sodium methoxide (25% w/v, 0.150 mL, 2.69 mmol) was added dropwise to a stirred solution of **28** (0.020 g, 0.034 mmol) in MeOH (5 mL), and the resulting pH of the solution was greater than 11. The reaction mixture was stirred at rt for 2 h then neutralized by the addition of Amberlite IR-120 H^+ ion-exchange resin. The solution was filtered, washed with MeOH (50 mL), and the solvent removed in vacuo to give the title compound **21** (0.016 g, quant) as a colorless oil; $[\alpha]_D^{28}$ −13.3 (c = 0.13, MeOH). ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 3.32 (t, J = 9.4 Hz, 1H, H-4), 3.47 (t, J = 8.9 Hz, 1H, H-3), 3.66−3.71 (m, 1H, H-5), 3.82 (t, J = 9.1 Hz, 1H, H-2), 3.85−3.92 (m, 1H, H-6), 4.05−4.11 (m, 1H, H-6), 5.64 (d, J = 9.3 Hz, 1H, H-1), 7.36 (s, 2H, SO_2NH_2), 7.90 (d, J = 8.4 Hz, 2H, Ar-H), 8.08 (d, J = 8.4 Hz, 2H, Ar-H), 8.98 (s, 1H, $CH_{triazole}$). ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 64.4 (d, J = 3.7 Hz, C-6), 69.3 (C-4), 72.2 (C-3), 76.4 (C-2), 78.0 (d, J = 7.5 Hz, C-5), 87.6 (C-1), 121.6 ($CH_{triazole}$), 125.4, 126.4, (Ar-CH), 133.7 ($C_{triazole}$), 143.2, 145.2 (Ar-C). ^{31}P NMR (162 MHz, $(CD_3)_2SO$) δ 0.51. LRMS-ESI $[M - H]^-$ m/z = −465. HRMS-ESI $[M + Na]^+$ calcd for $C_{14}H_{19}N_4NaO_{10}PS$, 489.0452; found, 489.0443.

*N-(4-Methoxybenzyl)-1-S-(2,3,4-tri-O-acetyl)-D-glucopyranosylsulfonamide (**24**).* Acetyl chloride (0.05 mL, 0.70 mmol) was added to a solution of **17** (0.190 g, 0.315 mmol) in CH_2Cl_2/MeOH (2:1, 15 mL) and stirred at rt for 1 h. The reaction mixture was quenched with Et_3N (100 μL) and stirred for 2 min which neutralized the solution. The solvent was removed in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 1:1 to 7:3) to give the title compound **24** (0.148 g, 96%) as a white solid; mp 157−159 °C (EtOAc/hexane); $[\alpha]_D^{25}$ −14.2 (c = 0.13, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.01 (s, 3H, $OCOCH_3$), 2.03 (s, 3H, $OCOCH_3$), 2.05 (s, 3H, $OCOCH_3$), 2.42−2.50 (m, 1H, OH), 3.42 (ddd, J = 2.1, 5.0, 10.3 Hz, 1H, H-5), 3.52 (dd, J = 4.9, 12.9 Hz, 1H, H-6), 3.69 (dd, J = 2.2, 13.1 Hz, 1H, H-6), 3.81 (s, 3H, OCH_3), 4.24 (d, J = 9.1 Hz, 1H, H-1), 4.26−4.39 (m, 2H, N-CH_2), 4.91 (t, J = 9.5 Hz, 1H, H-4), 5.22−5.29 (m, 2H, H-2, H-3), 5.56−5.62 (m, 1H, NH), 6.90 (d, J = 8.5 Hz, 2H, Ar-H), 7.29 (d, J = 8.5 Hz, 2H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.59, 20.6, 20.7 ($OCO\underline{C}H_3$), 47.3 (N-CH_2), 55.4 (OCH_3), 60.9 (C-6), 68.0 (C-4), 68.1 (C-2), 73.0 (C-3), 78.8 (C-5), 87.5 (C-1), 114.3 (2 × Ar-CH), 129.26 (Ar-C), 129.34 (2 × Ar-CH), 159.5 (Ar-C), 170.0, 170.1, 170.2 ($O\underline{C}OCH_3$). LRMS-ESI $[M - H]^-$ m/z = 488. HRMS-ESI $[M + Na]^+$ calcd for $C_{20}H_{27}NNaO_{11}S$, 512.1197; found, 512.1196.

*N-(4-Methoxybenzyl)-1-S-(2,3,4-tri-O-acetyl-6-O-dibenzylphosphate)-D-glucopyranosylsulfonamide (**26**).* A solution of alcohol **24** (0.173 g, 0.353 mmol) and dibenzyl diisopropylphosphoramidite **23** (0.244 g, 0.706 mmol) in CH_2Cl_2 (10 mL) was coevaporated to dryness and dried under high vacuum for 30 min. The mixture was azeotroped from anhydrous CH_3CN (2 × 10 mL) to dryness, then anhydrous CH_3CN (20 mL) was added and the solution reduced to approximately half its volume. To the stirred reaction mixture was added 1*H*-tetrazole (0.45 M in CH_3CN, 1.57 mL, 0.706 mmol), and the mixture was stirred at rt for 1.5 h. Anhydrous CH_2Cl_2 (5 mL) was added, and the reaction mixture was stirred for a further 10 min before being cooled to −30 °C. *m*-CPBA (60%, 0.254 g, 0.883 mmol) was added to the cooled solution and stirred for 10 min, then warmed to rt and stirred for a further 1 h. The reaction mixture was diluted with CH_2Cl_2 (40 mL), H_2O (20 mL), and 10% Na_2SO_3 (20 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 40 mL), and the combined organic phases were dried ($MgSO_4$), filtered, and the solvent removed in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 1:1 to 3:2) to give the title compound **26** (0.317 g, 90%) as a white foam; $[\alpha]_D^{25}$ −3.4 (c = 0.29, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.98 (s, 3H, $OCOCH_3$), 2.00 (s, 3H, $OCOCH_3$), 2.05 (s, 3H, $OCOCH_3$), 3.43 (ddd, J = 2.4, 5.5, 10.2 Hz, 1H, H-5), 3.79 (s, 3H, OCH_3), 3.92−3.99 (m, 1H, H-6), 4.00−4.06 (m, 2H, H-1, H-6), 4.19−4.29 (m, 2H, NCH_2), 4.87 (t, J = 9.7 Hz, 1H, H-4), 4.97−5.11 (m, 4H, 2 × $PhC\underline{H}_2$), 5.14 (t, J = 9.4 Hz, 1H, H-3), 5.28 (t, J = 9.6 Hz, 1H, H-2), 6.08 (t, J = 6.4 Hz, 1H, NH), 6.85 (d, J = 8.5 Hz, 2H, Ph), 7.28 (d, J = 8.5 Hz, 2H, Ph), 7.30−7.38 (m, 10H, Ph). ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.5, 20.6, 20.7 ($OCO\underline{C}H_3$), 47.3 (NCH_2), 55.4 (OCH_3), 65.4 (d, J = 5.4 Hz, C-6), 67.7 (C-4), 67.8 (C-2), 69.7 (d, J = 5.7 Hz, $PhCH_2$), 70.0 (d, J = 5.8 Hz, $PhCH_2$), 73.1 (C-3), 76.3 (d, J = 5.4 Hz, C-5), 87.0 (C-1), 114.1 (2 × Ar-CH), 128.1 (Ar-C), 128.2, 128.7,

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128.72, 128.75 (Ar-CH), 129.4 (Ar-C), 129.6 (2 × Ar-CH), 135.6 (d, J = 6.6 Hz, i-Ph), 135.7 (d, J = 7.2 Hz, i-Ph), 159.4 (Ar-C), 169.2, 169.7, 170.1 ($O\underline{C}OCH_3$). ^{31}P NMR (162 MHz, $CDCl_3$) δ −0.73. LRMS-ESI $[M - H]^-$ m/z = 748. HRMS-ESI $[M + Na]^+$ calcd for $C_{34}H_{40}NNaO_{14}PS$, 772.1799; found, 772.1803.

*1-S-(2,3,4-Tri-O-acetyl-6-O-dibenzylphosphate)-D-glucopyranosylsulfonamide (**27**).* To a solution of **26** (0.100 g, 0.133 mmol) in CH_3CN (5 mL) and H_2O (1.5 mL) was added ceric ammonium nitrate (0.292 g, 0.534 mmol), and the reaction mixture was stirred at rt for 2 h. H_2O (30 mL) was added, and the reaction mixture was extracted into EtOAc (3 × 40 mL). The organic extract was washed with brine (40 mL), dried ($MgSO_4$), and the solvent removed in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 1:1 to 7:3) to give the title compound **27** (0.076 g, 90%) as a colorless oil; $[\alpha]_D^{28}$ +4.8 (c = 0.16, MeOH). ^1H NMR (500 MHz, $CDCl_3$) δ 2.02 (s, 3H, $OCOCH_3$), 2.03 (s, 3H, $OCOCH_3$), 2.07 (s, 3H, $OCOCH_3$), 3.77 (ddd, J = 2.0, 4.0, 10.3 Hz, 1H, H-5), 4.01 (td, J = 4.1, 12.9, 12.8 Hz, 1H, H-6), 4.16−4.23 (m, 1H, H-6), 4.39 (d, J = 9.6 Hz, 1H, H-1), 4.98−5.15 (m, 5H, H-4, 2 × $PhC\underline{H}_2$), 5.27−5.33 (m, 2H, H-2, H-3), 5.69 (brs, 2H, SO_2NH_2), 7.31−740 (m, 10H, Ph). ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.55, 20.6, 20.7 ($OCO\underline{C}H_3$), 65.5 (d, J = 6.0 Hz, C-6), 67.5 (C-4), 67.8 (C-2), 70.02 (d, J = 5.8 Hz, $PhCH_2$), 70.08 (d, J = 5.7 Hz, $PhCH_2$), 72.7 (C-3), 76.1 (d, J = 3.4 Hz, C-5), 87.1 (C-1), 128.1, 128.2, 128.7, 128.75, 128.77 (Ph), 135.62 (d, J = 6.5 Hz, i-Ph), 135.63 (d, J = 6.7 Hz, i-Ph), 169.2, 170.0, 170.3 ($O\underline{C}OCH_3$). ^{31}P NMR (162 MHz, $CDCl_3$) δ −1.41. LRMS-ESI $[M + H]^+$ m/z = 630, $[M - H]^-$ m/z = 628. HRMS-ESI $[M + Na]^+$ calcd for $C_{26}H_{32}NNaO_{13}PS$, 630.1405; found, 630.1406.

*1-S-(2,3,4-Tri-O-acetyl-6-O-phosphate)-D-glucopyranosylsulfonamide (**29**).* $Pd(OH)_2$ (20%, 25 mg) was added to a solution of **27** (0.120 g, 0.191 mmol) in THF/MeOH (2:3, 10 mL). The reaction mixture was stirred under an atmosphere of hydrogen for 2 h at rt, then filtered through Celite and washed with THF (20 mL) and MeOH (40 mL). The solvent was removed in vacuo and the residue purified by RP-18 column chromatography (MeOH/H_2O 5:95 to 3:2, product eluting at 1:9) to give the title compound **29** (0.068 g, 79%) as a white solid; mp 182−184 °C (MeOH/H_2O); $[\alpha]_D^{26}$ +8.8 (c = 0.15, MeOH). ^1H NMR (500 MHz, $(CD_3)_2SO$) δ 1.936 (s, 3H, $OCOCH_3$), 1.943 (s, 3H, $OCOCH_3$), 1.99 (s, 3H, $OCOCH_3$), 3.84−3.89 (m, 2H, H-6), 4.09−4.14 (m, 1H, H-5), 4.74 (d, J = 9.7 Hz, 1H, H-1), 4.86 (t, J = 9.8 Hz, 1H, H-4), 5.18 (t, J = 9.5 Hz, 1H, H-2), 5.39 (t, J = 9.4 Hz, 1H, H-3), 7.20 (brs, 2H, SO_2NH_2). ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 20.26, 20.3, 20.4 ($OCO\underline{C}H_3$), 63.4 (d, J = 3.9 Hz, C-6), 67.8 (C-2 or C-4), 67.9 (C-2 or C-4), 73.0 (C-3), 75.6 (d, J = 7.9 Hz, C-5), 86.3 (C-1). ^{31}P NMR (162 MHz, $CDCl_3$) δ −0.15. LRMS-ESI $[M - H]^-$ m/z = 448; HRMS-ESI $[M + Na]^+$ calcd for $C_{12}H_{20}NNaO_{13}PS$, 472.0285; found, 472.0281.

*1-S-(6-O-Phosphate)-D-glucopyranosylsulfonamide (**22**).* Sodium methoxide (25% w/v, 0.150 mL, 2.69 mmol) was added dropwise to a stirred solution of **29** (0.020 g, 0.034 mmol) in MeOH (5 mL), and the resulting pH of the solution was greater than 11. The reaction mixture was stirred at rt for 2 h then neutralized by the addition of Amberlite IR-120 H^+ ion-exchange resin. The solution was filtered, washed with MeOH (50 mL), and the solvent removed in vacuo to give the title compound **22** (0.016 g, quant) as a colorless oil; $[\alpha]_D^{28}$ +8.5 (c = 0.13, MeOH). ^1H NMR (500 MHz, $(CD_3)_2SO$) δ 3.12 (t, J = 9.4 Hz, 1H, H-4), 3.30 (t, J = 8.8 Hz, 1H, H-3), 3.42−3.48 (m, 2H, H-2, H-5), 3.87−3.94 (m, 1H, H-6), 4.09−4.14 (m, 2H, H-1, H-6), 6.72 (brs, 2H, SO_2NH_2). ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 64.9 (d, J = 5.0 Hz, C-6), 69.1 (C-4), 70.7 (C-2), 76.9 (C-3), 79.2 (d, J = 7.4 Hz, C-5), 90.6 (C-1). ^{31}P NMR (162 MHz, $CDCl_3$) δ 0.70. LRMS-ESI $[M - H]^-$ m/z = 322. HRMS-ESI $[M - H]^-$ calcd for $C_6H_{13}NO_{10}PS$, 322.0003; found, 322.0001.

CA Inhibition Assay. An Applied Photophysics stopped-flow instrument was used for assaying the CA-catalyzed CO_2 hydration activity as previously reported.[5,20] IC_{50} values were obtained from dose−response curves working at seven different concentrations of test compound by fitting the curves using PRISM (www.graphpad.com) and nonlinear least-squares methods, and values represent the mean of at least three different determinations. The inhibition constants (K_i) were then derived by using the Cheng−Prusoff equation,[23] as follows: $K_i = IC_{50}/(1 + [S]/K_m)$ where [S] represents the CO_2 concentration at which the measurement was carried out and K_m the concentration of substrate at which the enzyme activity is at half-maximal.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01228.

^1H and ^{13}C NMR spectra for compounds **8**, **12**, **15**−**17**, **19**, **20**, and **24**; ^1H, ^{31}P and ^{13}C NMR spectra for compounds **2**, **3**, **5**, **11**, **21**, **22**, **25**, **26**, **27**, **28**, and **29** (PDF)

Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

CA, carbonic anhydrase; AZA, acetazolamide; CuAAC, copper-catalyzed azide alkyne cycloaddition; SAR, structure−activity relationship; K_i, inhibition constant; *cyclo*Sal, cyclosaligenyl; SATE, *S*-acyl-2-thioethyl; POC, bis(isopropyloxymethyl) carbonate

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