[13] Destabilization of the *anti* conformation due to steric interactions has also been reported: a) S. N. Rao, P. A. Kollman, *J. Am. Chem. Soc.* **1986**, *108*, 3048 – 3053; b) S. H. Krawczyk, N. Bischofberger, L. C. Griffin, V. S. Law, R. G. Shea, S. Swaminathan, *Nucleosides Nucleotides* **1995**, *14*, 1109 – 1116; c) F. Seela, Y. Chen, C. Mittelbach, *Helv. Chim. Acta* **1998**, *81*, 570 – 583, and references therein.

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[15] In these previous systems both *syn* and *anti* ribose conformers were observed as implied in the text. Proper comparison was thus made to the signals assigned to the *syn* form.

[16] a) D. E. Burge, *Am. Lab.* **1977**, *9*, 41 – 51; b) D. E. Burge, *J. Appl. Polym. Sci.* **1979**, *24*, 293 – 299.

[17] In contrast to various previously reported lipophilic deoxyguanosine derivatives that were all found to form gels in chlorinated organic solvents in the absence of cations (that is, self-assembled ribbonlike aggregates)[4a], compound **1** was not found to form a gel even at concentrations as high as 0.2 M. As discussed in the text, we rationalize this in terms of **1**, but not these other compounds, being present in a *syn* conformation. The observation that the less sterically hindered compound, (2′,3′,5′-tri-*O*-isobutyrylribofuranosidyl)-2-aminopurin-6-one, which lacks an aryl substituent and which should be present mostly in *anti* conformation, forms a gel at concentrations as low as 0.02 M is considered consistent with this theory.

Discovery of Carbohydrate Sulfotransferase Inhibitors from a Kinase-Directed Library**

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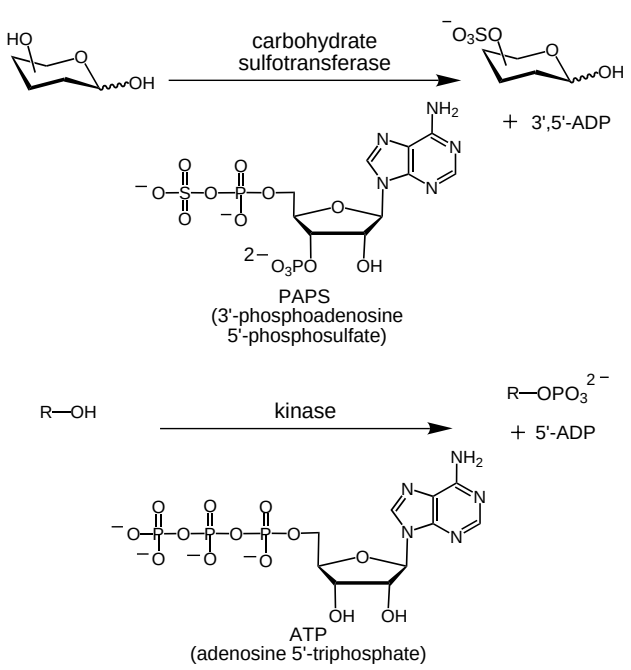
Carbohydrate sulfotransferases have recently emerged as an important and relatively unexplored class of therapeutic targets.[1] For example, the seminal discovery that sulfated sialyl Lewis^X mediates the adhesion of leukocytes to inflamed endothelium established carbohydrate sulfotransferases as potential targets for anti-inflammatory therapy.[2] Ongoing genome sequencing projects have uncovered numerous carbohydrate sulfotransferase genes in the past few years.[1, 3] It is now apparent that carbohydrate sulfotransferases comprise a large family of enzymes with overlapping tissue distribution and substrate specificities. To deconvolute the precise role of each sulfotransferase gene product and elucidate its contribution to normal and pathological processes, cell-permeable and highly specific small-molecule antagonists need to be identified. Surprisingly, no inhibitor of a carbohydrate sulfotransferase has been reported to date. At present, the limited structural and mechanistic information about this class of enzymes impedes rational approaches to inhibitor design. To identify lead inhibitors of carbohydrate sulfotransferases, we therefore adopted a strategy that involved the screening of small-molecule libraries.

To narrow our search, we focused on the similarities between the substrates utilized by sulfotransferases and kinases, a widely studied family of enzymes for which diverse and potent inhibitors are available.[4] Carbohydrate sulfotransferases catalyze the transfer of a sulfonyl group from the universal sulfate donor 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to a hydroxy (or amino) group of the acceptor oligosaccharide (Scheme 1).



Scheme 1. Reactions catalyzed by carbohydrate sulfotransferases and kinases.

Kinases catalyze a similar anionic group transfer reaction using adenosine 5′-triphosphate (ATP) as a phosphoryl donor. Thus, both enzyme classes recognize adenosine-based substrates, PAPS and ATP. Furthermore, the hydrophobic adenine binding pockets of the recently crystallized estrogen sulfotransferase,[5] and heparin N-sulfotransferase[6] are similar to those of several kinases. On the basis of these parallels, we chose to screen a panel of previously reported kinase-

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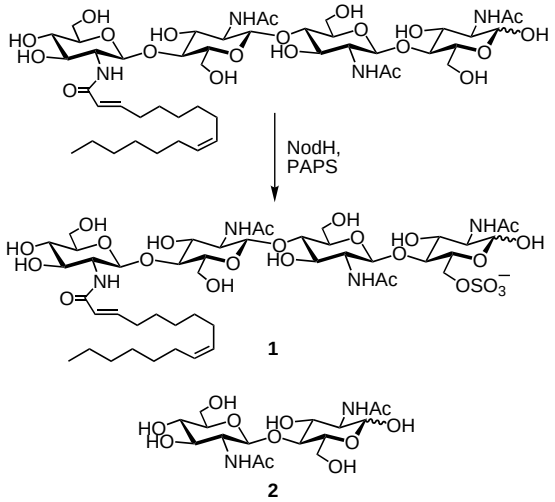
directed inhibitors that are competitive with ATP for cross-reactivity with carbohydrate sulfotransferases. Here we report the discovery of the first carbohydrate sulfotransferase inhibitors from a kinase-directed library.

We selected the well-characterized GlcNAc-6-sulfotransferase NodH from *Rhizobium meliloti* as an initial enzymatic target.[7] NodH is involved in the biosynthesis of the sulfated nodulation factor **1** (Scheme 2) which induces root nodulation in the symbiotic host plant alfalfa.[8] NodH can also utilize the simple disaccharide chitobiose (**2**) as a substrate.[9]



Scheme 2. The NodH sulfotransferase catalyzes the sulfonation of a lipochitooligosaccharide, but can use chitobiose (**2**) as a substrate.

We developed a medium-throughput radiolabel transfer assay similar to that of Bourdineaud et al.[10] that involves thin-layer chromatography separation of the product, ^{35}S-labeled 6-sulfo-chitobiose from excess substrate, and ^{35}S-labeled PAPS. Using this assay we measured a K_M value for PAPS of 4.3 ($\pm$0.3) μM and a K_I value for the product 3′,5′-ADP of 1.36($\pm$0.08) μM (data not shown).

Our initial inhibitor screen included 139 compounds comprising selected structures from purine libraries (**3** and **4**),[4b, 11] as well as commercially available protein kinase inhibitors (16 tyrphostins (**5**) and four representatives from other kinase-inhibitor families) (Figure 1). One set of purines

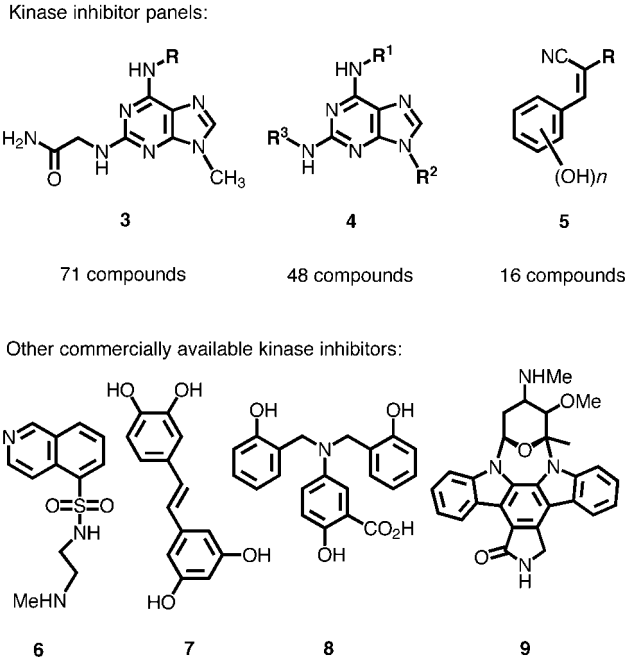


Figure 1. Initial inhibitor panel.

(**3**) possessed conserved N9-methyl and N2-carboxamide groups, but varied in structure at N6. The second set of purines (**4**) contained diverse substituents at all three positions. These libraries were originally directed toward cyclin-dependent kinases (CDKs).[4b, 11]

Enzyme assays in the presence of the test compounds (200 μM) identified several purine analogues and one tyrphostin analogue (compound **10**, Figure 2) as lead inhibitors;

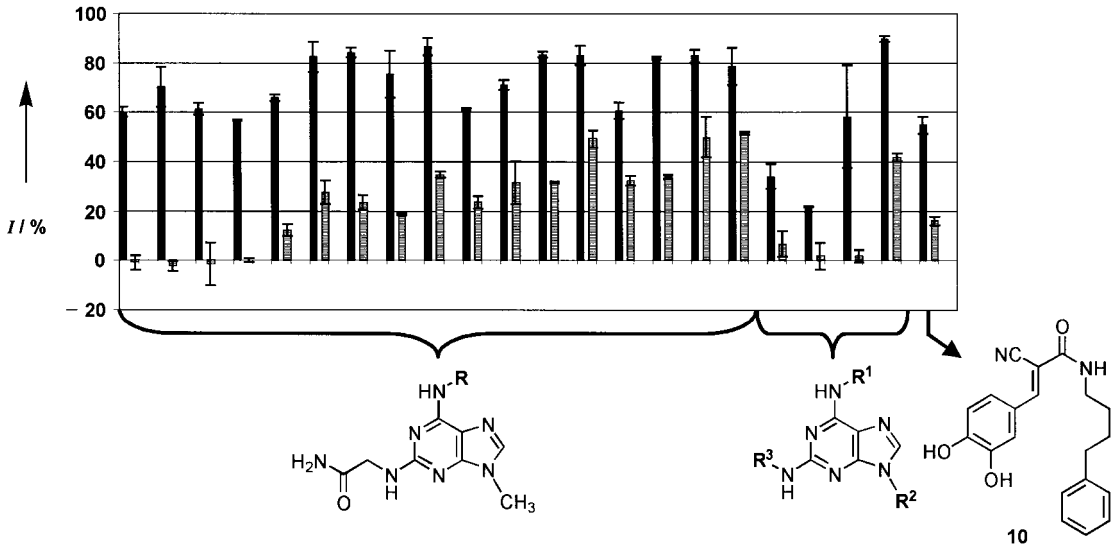


Figure 2. Inhibitor rescreen at 50 μM. I = Inhibition; black bars: 50 μM inhibitor, 2.4 μM PAPS; gray bars: 50 μM inhibitor, 8.04 μM PAPS.

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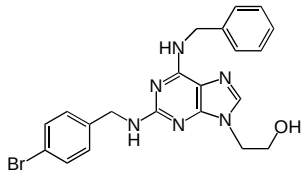
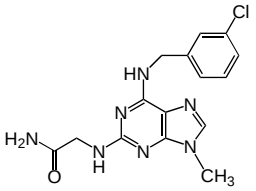
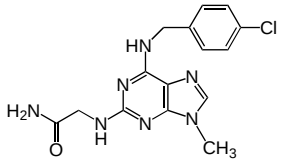
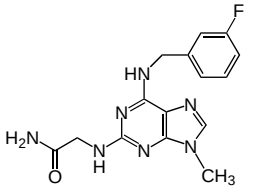
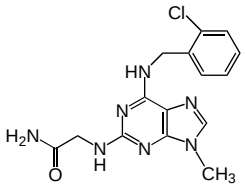
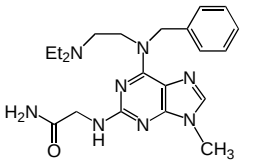
these compounds displayed over 80% inhibition of enzyme activity. To assess the relative inhibitory activity more accurately, we next rescreened the chosen compounds under more stringent conditions (Figure 2). Even at 50 μM, several compounds still displayed high levels of inhibition. As expected for a PAPS-competitive inhibitor, the inhibitory potency was highly dependent on the concentration of PAPS used in the assay. Increasing the concentration of PAPS from 2.4 to 8.0 μM (0.5 to 2 times the K_M) triggered a dramatic decrease in the inhibitory activity of most compounds. However, several of the purine analogues still maintained modest activity under these conditions. We chose the most potent six purines and determined their IC_{50} values (Table 1).

Table 1. IC_{50} [μm] values against NodH sulfotransferase.[a]

Inhibitor	IC_{50}	Inhibitor	IC_{50}
	20		40
	40		35
	25		40

[a] Serial dilutions of each inhibitor were tested in the presence of 2.4 μM PAPS, 5 mM chitobiose, and 1 μU NodH.

These ranged from 20 to 40 μM—modest yet impressive activity considering the limited pool of compounds initially screened. Interestingly, five of the six most potent inhibitors were derived from the same purine class (**3**) and possessed benzylic substituents at N6. This preliminary structure–activity information will form the basis for future sulfotransferase-directed libraries.

Selectivity has been difficult to achieve with kinase inhibitors that are competitive with ATP since all kinases possess an ATP binding pocket. Therefore, we were concerned with the selectivity of our lead compounds against other carbohydrate sulfotransferases, particularly GlcNAc-6-O-sulfotransferases. When evaluated against a GlcNAc-6-sulfotransferase expressed in human high endothelial venules[3a] and a widely expressed keratan sulfate sulfotransferase,[12] none of the six purines showed measurable inhibitory activity at 200 μM, indicating that these modest inhibitors are selective among these sulfotransferases (data not shown).

As the library above comprises known kinase inhibitors, a major concern is the possible cross-reactivity of the identified "hits" with kinases. We tested the compounds for inhibitory activity against two serine/threonine kinases, p38/MAP kinase, and cyclin-dependent kinase 2 (CDK2), the original target of the library we had screened.[4b, 13] Against the former, the compounds displayed IC_{50} values greater that 60 μM. However, not surprisingly, they were more active against CDK2, with IC_{50} values in the range of 15 to 40 μM. Our next goal is to explore structural modifications that enhance potency against the sulfotransferases while simultaneously reducing kinase inhibitory activity.

Experimental section

Purine analogues were prepared as previously described.[14] All other kinase inhibitors were purchased from Calbiochem (La Jolla, CA).

Preparation of [^{35}S]PAPS: Carrier-free [^{35}S]PAPS was synthesized in vitro using a procedure similar to that described by Long and co-workers.[15] Carrier-free [^{35}S]Na_2SO_4 (0.2 mCi) was incubated for 24 h with 0.12 U ATP sulfurylase (Sigma), 8.3 U inorganic pyrophosphatase (Sigma), and 210 mg APS Kinase in buffer A (50 mM Tris-HCl pH 8.0, 30 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 10% glycerol) containing 30 mM ATP. The incorporation of ^{35}S into [^{35}S]PAPS was quantified by TLC on PEI-cellulose plates (eluting with 0.9 M LiCl) followed by phosphorimaging analysis (Model 445 SI, Molecular Dynamics). The reaction yield typically exceeded 90% based on [^{35}S]Na_2SO_4.

NodH sulfotransferase assay: Inhibitors were screened at 200 μM with 1 μU NodH (Calbiochem) 2.4 μM [^{35}S]PAPS (1.0 μCi) and 5 mM *N,N′*-diacetylchitobiose (Calbiochem) in buffer A. After preincubation of the enzyme and inhibitors for 20 min at room temperature, the enzymatic reaction was initiated by addition of [^{35}S]PAPS and, after 6 min at room temperature, terminated by dilution with MeOH (120 μL). A 25-μL aliquot of each reaction was spotted on Whatman LKTLC plates and eluted with 6:3:2 BuOH:EtOH:H_2O. The plates were dried and analyzed by phosphorimaging. For IC_{50} determinations, serial dilutions of the inhibitors were prepared and tested in the presence of 2.4 μM [^{35}S]PAPS (1.0 μCi) and 5 mM *N,N′*-diacetylchitobiose in buffer A (100 μL total volume). Aliquots (5 μL) were removed and diluted with MeOH (30 μL) after 3, 6, 9, and 12 min. Reaction rates were determined by calculating the incorporation of radioactive sulfate into *N,N′*-diacetylchitobiose-6-OSO_3 over time.

Supporting information contains a full list of compounds from libraries **3**–**5** as well as kinetic data for K_m and K_I measurements (6 pages).

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Facile and Clean Oxidation of Alcohols in Water Using Hypervalent Iodine(III) Reagents**

Hirofumi Tohma, Shinobu Takizawa, Tomohiro Maegawa, and Yasuyuki Kita*

Oxidation of alcohols to carbonyl compounds is a pivotal reaction in organic chemistry and numerous methods using a variety of reagents and conditions have been developed. Recent demand for eco-conscious chemical processes has encouraged the development of several clean and practical oxidation reactions,[1] and awaits further development of high-yielding, clean, safe, and economical methods for the oxidation of alcohols. Hypervalent iodine reagents have been used extensively in organic syntheses as a result of their low toxicity, ready availability, and easy handling.[2] For example, the highly utilized pentavalent iodine reagents such as Dess–Martin periodinane (DMP)[3] and *o*-iodoxybenzoic acid (IBX)[4] oxidize alcohols mildly and efficiently to carbonyl compounds in high yields in organic solvents such as CH_2Cl_2, DMSO, and acetone. However, despite their utility, iodine(V) reagents are potentially explosive, cannot be stocked, and the generated iodine(III) species are usually not utilized (only iodine(V) species can be used for the oxidation of alcohols). Therefore, a facile and efficient use of the readily available and relatively stable iodine(III) reagents in place of iodine(V) reagents has been long desired. In contrast to oxidations with iodine(V)-based reagents, only a few examples of the oxidation of alcohols using iodine(III) reagents have been described.[5] Existing methods are limited to organic solvents and usually involve activation of Ru or Yb catalysts[5a,b] or of 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO)[5c] by an iodine(III) co-oxidant. As an exception, readily oxidizable benzylic alcohol derivatives do not require catalysts.[6, 13a] We have been studying the activation of hypervalent iodine reagents using several additives and solvents,[7] and report herein an efficient oxidation of alcohols using iodosobenzene (PhI=O) with an inexpensive inorganic salt, KBr, in water under neutral conditions. This method was extended to an environmentally benign polymer-supported (diacetoxyiodo)benzene (PSDIB) oxidation procedure.

Various aqueous oxidation reactions have been developed since economic and environmental concerns encourage the use of water as a reaction medium.[8] Very recently we have achieved a novel catalytic activation of PhI=O using a cationic surfactant, cetyltrimethylammonium bromide (CTAB), under neutral conditions. Its low solubility in water and in most organic solvents normally limits the reactivity of PhI=O, however, our micellar and reversed micellar systems have expanded the choice of solvents (from nonpolar solvents to water) for hypervalent iodine oxidation.[9] In this study we first examined the oxidation of alcohols using PhI=O–CTAB in water, by the CTAB-catalyzed (20 mol%) oxidation of 2-octanol (**1e**) in water to give 2-octanone (**2e**) in 67% yield. Thus, we re-examined the activation of PhI=O in this reaction with a variety of additives including alkali metal salts. The addition of bromide salts such as NaBr, KBr, and LiBr was found to activate PhI=O remarkably to give **2e** in good yields (94% yield (KBr)), while salts other than bromide (NaX: X = F, Cl, I, HSO_4, BF_4, HCO_3, ClO_4, OAc, NO_3) did not catalyze the reaction effectively.[10] We therefore chose KBr, the most economical alkali metal bromide, for further studies. The oxidation of activated alcohols such as benzylic or allylic alcohols proceeds quantitatively just by activating PhI=O with a small amount of water (10 equiv.), and without addition of KBr (Table 1, entries 1–3). With benzyl alcohol (**1a**), the reaction rate is enhanced remarkably by adding a catalytic amount of KBr. In contrast, a catalytic amount of KBr is indispensable for the oxidation of saturated primary and secondary alcohols. This PhI=O–KBr system is applicable to the oxidation of a variety of primary and secondary alcohols bearing functional groups such as ether, ester, sulfonamide, and azido groups (see Table 1). Oxidation of primary alcohol **1k** only yields carboxylic acid **2k** (52% yield) and none of the corresponding aldehyde when using 1.1 equivalents of PhI=O.

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