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N-Acetylhexosaminidase inhibitory properties of C-1 homologated GlcNAc- and GalNAc-thiazolines

Benjamin Amorelli,[a] Chunhua Yang,[a] Brian Rempel,[b] Stephen G. Withers[b] and Spencer Knapp[a,*]

[a]*Department of Chemistry & Chemical Biology, Rutgers, The State University of New Jersey, 610 Taylor Road, Piscataway, NJ 08854, USA*
[b]*Department of Chemistry, University of British Columbia, Vancouver, BC, Canada V6T 1Z1*

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Abstract—Several C-1 homologated GlcNAc- and GalNAc-thiazolines, as well as a related GalNAc-thiazole, have been prepared. The compounds are analogues of GlcNAc-thiazoline, a potent transition-state-mimicking inhibitor of retaining β-*N*-acetylglycosaminidases. Kinetic evaluation of these fused pyranose-heterocycles against the bacterial *N*-acetylhexosaminidase SpHex suggests active site steric restrictions around the substrate anomeric carbon.

Glycosidases mediate a variety of important cellular functions. As an example, retaining *N*-acetyl-β-hexosaminidases of families 18 and 20 are a class of glycosyl hydrolases that are responsible for the removal of 2-acetamido-2-deoxy-D-glucopyranosyl and -galactopyranosyl residues from glycoconjugates, and that utilize a mechanism featuring substrate-assisted catalysis.[1] An inherited abnormality in the human glycosidases Hex A and Hex B results in the accumulation of certain gangliosides, causing the neurodegenerative disorders Tay-Sachs and Sandoff's diseases, respectively.[2] The hydrolysis of the terminal GlcNAc/GalNAc residue from the aglycon polysaccharide features an oxazolinium intermediate held noncovalently in the active site (Fig. 1).

The remarkable binding properties of GlcNAc- and GalNAc-thiazolines **1** and their resistance to enzymatic hydrolysis has facilitated the determination of the co-crystal structure of **1a** intact and bound in the active site of the *N*-acetylhexosaminidase from *Streptomyces plicatus* (SpHex; K_i for **1a** = 20 μM and 270 nM vs SpHex and human β-hexosaminidase A, respectively; K_i for **1b** = 100 μM and 820 nM vs SpHex and human β-hexosaminidase A, respectively).[3,4] A schematic of H-bonding interactions of SpHex with **1a** indicated by the crystallographic analysis is shown in Figure 2. A hydrophobic pocket surrounds the thiazoline ring of **1a**, protecting the iminium carbon from water. Within this pocket, Tyr393 and Asp313 residues can position the 2-acetamido group of the substrate for nucleophilic participation at the anomeric center and diffuse the positive charge distributed into the oxazolinium ring as it forms. The normal role of the general acid residue Glu314 (cir-

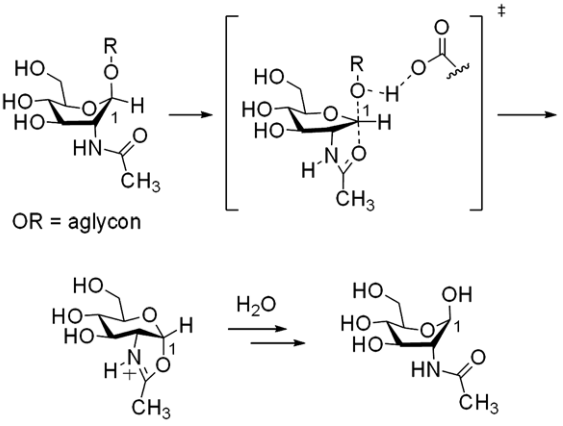

Figure 1. Mechanism for retaining *N*-acetylhexosaminidases that use substrate assistance.

Keywords: Glycosidase; *O*-GlcNAcase; *Streptomyces plicatus*; Pyranose conformation; Transition state mimic.
* Corresponding author. Tel.: +1 732 445 2627; e-mail: spencer.knapp@rutgers.edu

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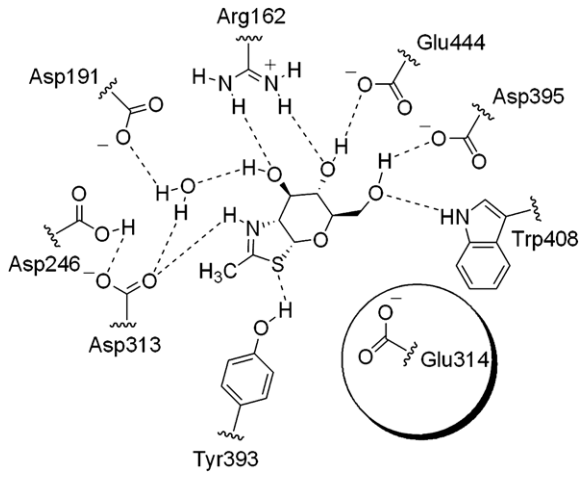
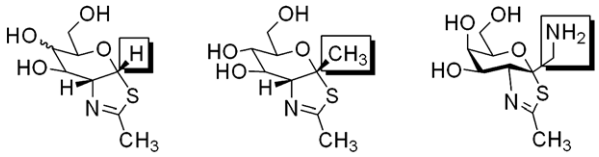


Figure 2. Schematic showing interactions between **1a** and the active site of SpHex.

cled) is to assist binding and departure of the aglycon oxygen from C-1. However, Glu314 does not interact with **1a**, as the latter lacks a hydrogen bond donor in this position.

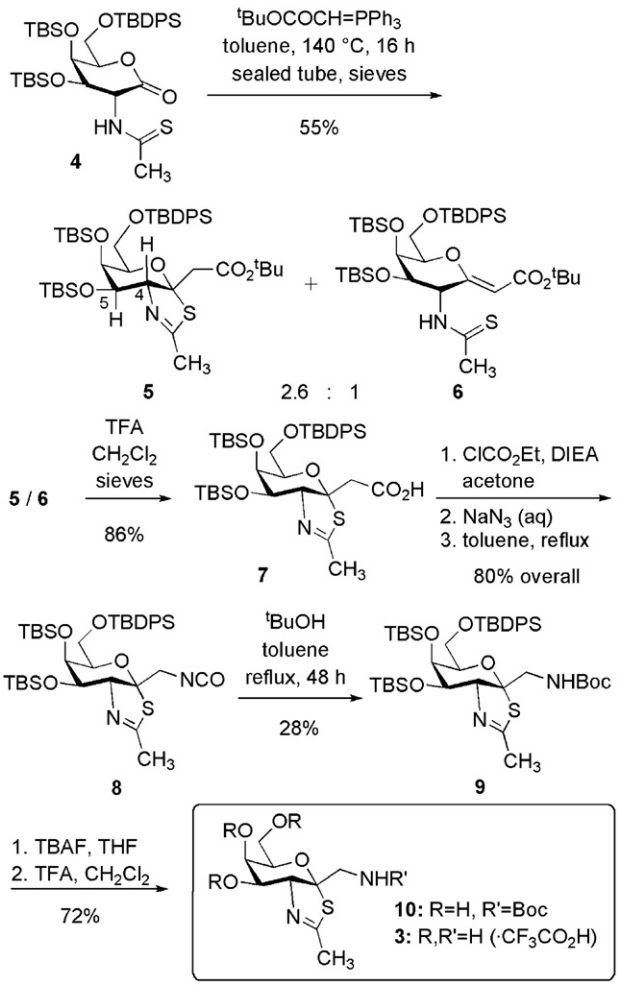
Human *O*-GlcNAcase (OGA) is a critical enzyme for post-translational modification of intracellular proteins.[5] OGA is a family 84 glycoside hydrolase responsible for the cleavage of GlcNAc from β-*O*-GlcNAc-modified serine and threonine residues. The aforementioned polysaccharide glycosidases exhibit little sequence similarity to OGA; however, GlcNAc-thiazoline **1a** has also been shown to inhibit OGA (K_i = 70 nM),[4] implicating this enzyme as a hydrolase with the 2-acetamido substrate-assisted catalytic mechanism as well.[6]



Past efforts towards glycosidase inhibitor design have included the synthesis and evaluation of inhibitors that superficially resemble posited electrostatic and conformational changes within the transition state, including a flattened substrate ($^4H_3$) pyranose ring and a much debated glycosyl cation-like structure (sp^2 hybridized and partially positively charged at C-1). Free energy correlation studies[7] have recently demonstrated, however, that strong adventitious binding by many glycosidase inhibitors does not in itself indicate transition state mimicry.[8] Vocadlo and coworkers have homologated the acetamido group of **1a**, and shown that this family of inhibitors [sp^3 hybridized at C-1 (pyranose numbering), $^4C_1$ pyranose pseudo-chair conformation when bound in the active site] displays excellent correlation of free energy of activation calculated from enzymatic substrate hydrolysis relative to inhibitor binding, suggesting that **1a** is a true transition state mimic for OGA and presumably also for the mechanistically related hexosaminidases.[9]

Unlike OGA, the hexosaminidases do not tolerate substrate or inhibitor amide groups much larger than acetamido in the binding pocket.[3,9,10] Whether groups at C-1 (see methylated analogue **2**) would be tolerated has not yet been established. Crystallographic studies of the piperidine carbohydrate analogue GalNAc-isofagomine (K_i = 21 μM vs SpHex)[11] bound in the active site of SpHex show a key noncovalent interaction between the equatorial piperidine N–H and the general acid residue Glu314, an interaction absent[3] in the case of the SpHex·**1a** complex. A thiazoline related to **1**, but which might additionally possess a group capable of hydrogen bonding or other interaction with Glu314, ought to be an even more effective inhibitor. Therefore, we also set out to enable this additional enzyme(Glu314)–inhibitor interaction through the preparation of functionally elaborated thiazolines such as **3**. The evaluation of **2** and **3** as a means to probe glycosidase tolerance of substitutions (shown in boxes) for –H at the anomeric center of parent inhibitors GlcNAc- and GalNac-thiazolines **1** is the subject of this report. The route to **2** was described earlier,[12] and an unusual fragmentation leading to the fused GalNAc-thiazole **11** was also reported.[13]

Lactone **4** (Scheme 1),[13] treated with *tert*-butoxycarbonylmethylenetriphenylphosphorane, reacted slowly under reflux, but at 140 °C in a sealed reaction tube gave a mixture of thiazoline ester **5** and the chain extended vinylogous carbonate **6** in 55% combined isolated yield (respective ratio 2.6:1). The ^{1}H NMR signal for the thiazoline CH_3 of **5** at 2.17 ppm (br s, long range coupled to H-2)[14] and the downfield thioacetamide CH_3 singlet (2.47 ppm) of **6** are indicative of their structures. Deprotection of the mixture of **5** and **6** with trifluoroacetic acid in dichloromethane gave **7** in 86% yield (Scheme 1). Compounds **5** and **6** were both converted to **7** in this reaction, as the amount of either alone is insufficient to account for the isolated yield. Some hydrolysis of **7** to the mercapto amide under these conditions might be expected.[14,15] However, with exclusion of moisture, **7** was stable to the reaction conditions, concentration without workup, and spectroscopic characterization in chloroform solution. Installation of nitrogen was carried out by first converting **7** to the acyl azide. Attempts to convert **7** directly by using diphenylphosphoryl azide (DPPA)[16] were unsuccessful. The conversion of **7** to the mixed anhydride was followed by addition of aqueous sodium azide. After concentration of the organic layer, the acyl azide was verified by infrared spectroscopic analysis, as this intermediate is not stable to chromatography on silica gel. The product shows a strong stretch at 2135 cm^{-1} due to the azide, but there was also partial rearrangement to isocyanate **8** (2257 cm^{-1}). Heating the crude azide for 2 h in toluene solution completed the rearrangement to **8**. The isocyanate was also unstable to silica gel, although ^{1}H and ^{13}C NMR spectra of the crude concentrated reaction mixture provide evidence for an efficient and reliable conversion to **8**.



Scheme 1. Synthesis of elaborated GaINAc-thiazolines **10** and **3**.

Trapping **8** with *tert*-BuOH to form the stable carbamate was extremely slow, giving **10** in only 28% yield after 48 h.

Thiazoline **9** was deprotected with *n*-Bu_4NF to carbamate triol **10**. After treatment of **10** with TFA in dichloromethane solution, aminomethyl thiazoline triol **3** was isolated as the trifluoroacetate salt. TFA salt **3** is somewhat unstable in acidic methanol; therefore,**3** was characterized by ^1H NMR in dry CD_3CN: the diagnostic ABq (3.42 and 3.57 ppm, $J = 14$ Hz each) for the $–CH_2NH_3^+$ unit, the broad singlet at 2.21 ppm for the thiazoline CH_3, and the electrospray mass spectrum (*m/z* 249 MH^+) confirm the structure.

The parent GalNAc-thiazoline **1b** adopts a pseudo-chair $^4C_1$ conformation in D_2O solution according to ^1H NMR analysis, with $J_{2,3} = 7.8$ Hz.[14] In the single crystal, **1b** also exists as a $^4C_1$ chair conformation, as shown in Figure 3. Likewise, the homologated analogue *N*-Boc-aminomethyl GalNAc-thiazoline **10** has a chair-like pyranose conformation in CD_3OD solution, according to its ^1H NMR spectrum ($J_{2,3} = 8.4$ Hz). In contrast, the GlcNAc-thiazoline **1a** exists primarily in a pseudo-boat conformation in CD_3OD solution ($J_{2,3} = 4.0$ Hz).[10] Both GlcNAc- and (presumably) Gal-

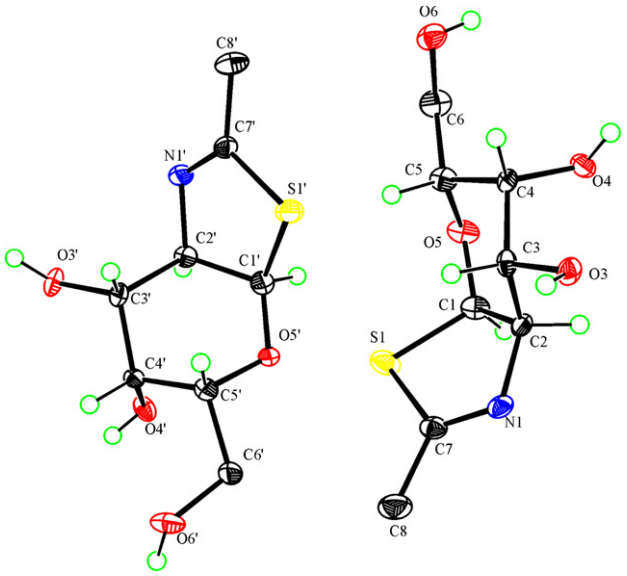


Figure 3. Unit cell of crystalline GalNAc-thiazoline **1b**, showing the $^4C_1$ pseudo-chair pyranose conformation.

NAc-thiazolines bind in hexosaminidase active sites as pyranose pseudo-chairs,[3,4,9] which conformations most closely match the substrate-derived transition state (see Fig. 1). Thus, more conformational change upon enzyme binding is required of **1a** compared with **1b**, which is already in a chair.

Inhibition studies[3] of the new C-1 homologated thiazolines (**2**, **10**, and **3**) and thiazole **11** against SpHex reveal greatly reduced affinities relative to the parent thiazolines **1a** and **1b** (Chart 1). The relatively small C-1 methyl group of **2** reduces binding by a factor of 10 relative to **1a**, but larger groups (i.e., $–CH_2NH_3^+$ and –CH_2NHBoc) reduce the binding affinity by factors of at least 100-fold. Thus, the kinetic studies on the aminomethyl thiazoline **3** show no evidence of a binding interaction with the general acid Glu314. The decrease in inhibition might be due to changes in inhibitor size, shape, conformation, or, in the case of **3**, a possibly

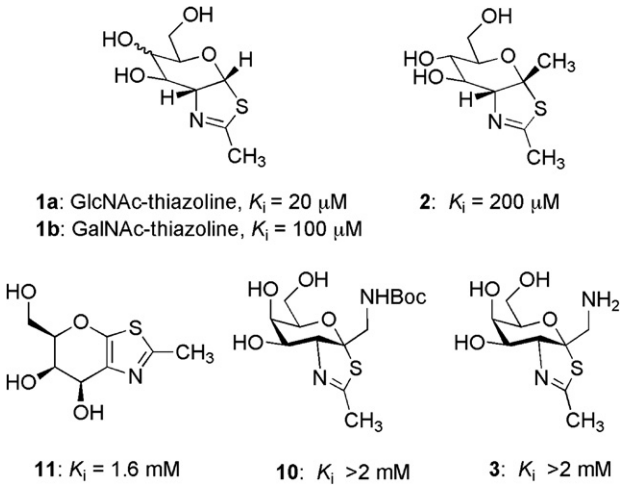


Chart 1. Inhibition studies of the new C-1 homologated thiazolines (**2**, and **10** and **3** and thiazole **11** against SpHeX.

unfavorable polar interaction. In particular, the portion of the enzyme active site near the anomeric carbon and the scissile bond, which must normally accommodate the aglycon upon initial binding of the substrate, evidently cannot accommodate bulky substituents at the anomeric carbon when introduced in the context of the GalNAc-thiazoline inhibitors. This is presumably a consequence of the tightness of the enzyme binding pocket surrounding the pseudo-equatorial C-1 β-substituent, which is H in the case of the best inhibitors **1a** and **1b**.

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Supplementary data

Experimental procedures and copies of spectra for new compounds. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as Supplementary Publication No. CCDC 678775. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.03.067.

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