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Tautomeric Modification of GlcNAc-Thiazoline

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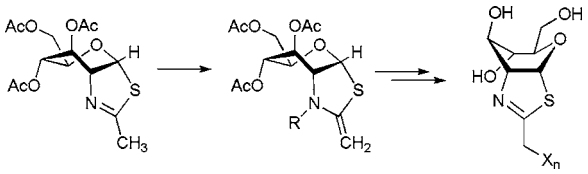
ABSTRACT



The potent *O*-GlcNAcase (OGA) inhibitor GlcNAc-thiazoline has been modified by buffer- or acylation-induced imine-to-enamine conversion and then electrophile or radical addition (X_n = D_3, F, N_3, OH, SMe, $COCF_3$, CF_3). Several functionalized GlcNAc-thiazolines show highly selective inhibition of OGA vs human hexosaminidase and thus have promise as tools for targeted investigations of OGA, an enzyme linked to diabetes and neurodegeneration. A new radical addition/fragmentation reaction of the *N*-(trifluoroacetyl)enamine has been discovered.

A wide variety of nuclear and cytoplasmic proteins are modified on serine and threonine residues by the dynamic addition and removal of β-*O*-GlcNAc units.[1−3] These diverse targets mediate important biological processes that may in turn be regulated by the β-*O*-GlcNAc cycling. *O*-GlcNAc addition and removal are catalyzed, respectively, by *O*-GlcNac transferase (OGT) and *O*-GlcNAcase (OGA, Figure 1), the study of which has acquired urgency as the importance of the *O*-GlcNAc modification to processes such as cellular signaling and regulation[4] and to disease states such as type II diabetes,[5] cancer,[6] and Alzheimer's[7] has become clear. To help sort out the mechanisms and effects of protein "*O*-GlcNAc-ylation," a significant effort has been directed toward the development of inhibitors of OGA that do not simultaneously inhibit the mechanistically related human *N*-acetylhexosaminidases HexA and HexB. In comparison

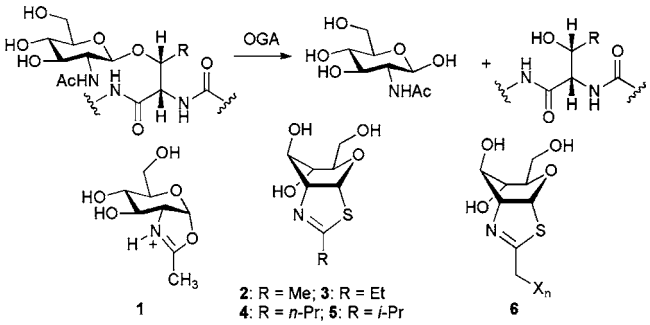


Figure 1. Hydrolysis of a serine/threonine linked β-*O*-GlcNAc catalyzed by OGA, the OGA intermediate GlcNAc-oxazolinium ion **1**, and GlcNAc-thiazoline inhibitors **2**−**6**.

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‡ National Institutes of Health.

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to the latter, OGA tolerates inhibitor groups larger than acetamido -CH_3, or its equivalent, in the acetamido binding pocket and thus provides an avenue for potential optimization of inhibitor selectivity, efficacy, solubility, transport, and metabolic stability. GlcNAc-thiazoline[8] (**2**, Figure 1) is a

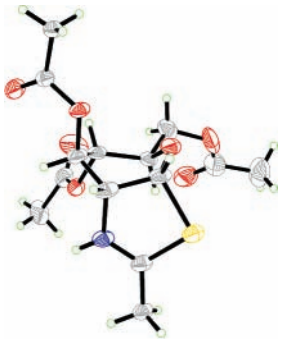
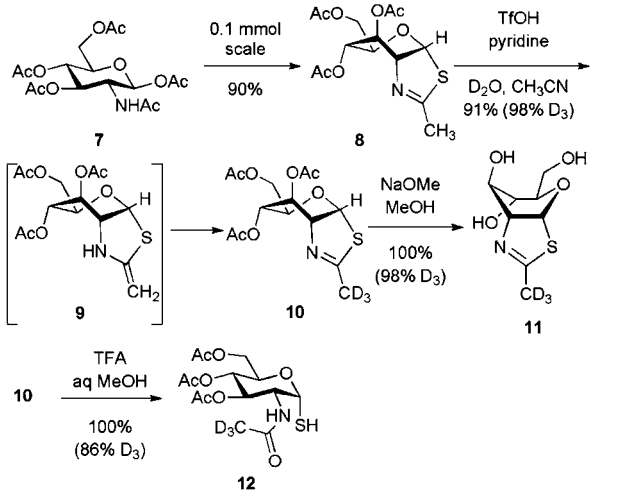


Figure 2. ORTEP view of one cation of the salt **8**·HO_3SAr (Ar = 2,4-dinitrophenyl), showing the $^{O}S_2$ pyranose conformation.

nanomolar but nonselective inhibitor of OGA, HexA,[9] and HexB by virtue of its resemblence to the transition state leading to the enzyme intermediate, oxazolinium ion **1**.[10] By increasing the size of the thiazoline ring substituent from methyl to ethyl, propyl, and isopropyl, Vocadlo and co-workers were able to increase the selectivity for inhibition of OGA (see **3**−**5**).[11,12] Analogous steric-based selectivity improvements to other OGA inhibitors have also been realized.[13−15] On the other hand, inhibitors that possess *functionalized* acetamido mimics could provide an expanded array of options for biochemical and medicinal chemistry studies.[16] We have now prepared a new series of methyl-modified GlcNAc-thiazolines **6** by exploiting the previously unrecognized propensity of GlcNAc-thiazolines to undergo buffer- and acylation-induced imine-to-enamine conversion.

The GlcNAc-thiazoline triacetate **8** is available on a multigram scale by treatment of commercial glucosamine pentaacetate **7** with P_4S_{10} (Scheme 1).[17] Whereas **2** binds in

Scheme 1. Tautomeric Deuteration of GlcNAc-Thiazoline



enzyme active sites in an apparent *pseudo*-chair ($^4C_1$) pyranose conformation,[18,19] GlcNAc-thiazoline triacetates such as **8** exist principally in a twist boat ($^{O}S_2$) in $CDCl_3$ solution.[20] In the solid state, the 2,4-dinitrobenzenesulfonic acid salt of **8** also exhibits the $^{O}S_2$ pyranose conformation (Figure 2). The corresponding thiazoline triols (e.g., **2**) are closer to a boat ($^{O,4}B$) in CD_3OD solution.[21]

The methyl protons of **8** exchange with deuterium in certain solvents in the presence of acid. As this reaction could also be used to prepare tritiated **2**, the deuteration was optimized as follows. Treatment of **8** with 2.4 equiv of pyridine, 1.2 equiv of triflic acid, and 100 equiv of D_2O in acetonitrile solution for 8 h at 23 °C and then extractive workup gave the trideuterated GlcNAc-thiazoline **10**. No C-deuteration was detected in the absence of the buffer components pyridine and triflic acid. Standard deacetylation then led to the corresponding triol **11** without significant loss of deuterium, according to integration of the methyl signal in the ^1H NMR spectrum. Alternatively, triol **2** was directly trideuterated by treatment with the same buffer system, and **11** was separated from the buffer components by partitioning between 1-butanol and saturated aqueous sodium bicarbonate (93% yield, 95% D_3). In polar solvents at acidic pH, **8**

(8) Systematic name: (3a*R*,5*R*,6*S*,7*R*,7a*R*)-6,7-dihydroxy-5-hydroxymethyl-2-methyl-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole.

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(20) Vicinal proton coupling constants for **8**: $J_{1,2} = 7$, $J_{2,3} = 3$, $J_{3,4} = 1.5$, and $J_{4,5} = 9$ Hz. See also: Foces-Foces, C.; Cano, F. H.; Bernabe, M.; Penades, S.; Martin-Lomas, M. *Carbohydr. Res.* **1984**, *135*, 1−11.

(21) Coupling constants for **2**: $J_{1,2} = 7$, $J_{2,3} = J_{3,4} = 4$, and $J_{4,5} = 9$ Hz.

hydrolyzes to the acetamido mercaptan, and thioconjugates can then be prepared by various S-alkylation and arylation reactions.[22] Hydrolysis of **10** led analogously to the trideuteroacetamido mercaptan **12**; however, ~5% of the deuterium was lost in the process. The transformations in Scheme 1 are consistent with acid-promoted tautomerization of **8** to give the enamine **9**; reprotonation leads to sequential replacement of all three methyl H's.

Would other electrophiles react with **9**? Treatment of **8** with the same buffer but in the presence of 3.2 equiv of *N*-bromosuccinimide gave the tribromide **14** (Figure 3). The

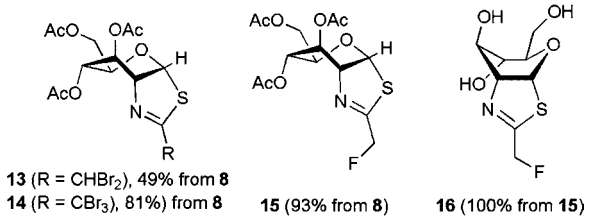
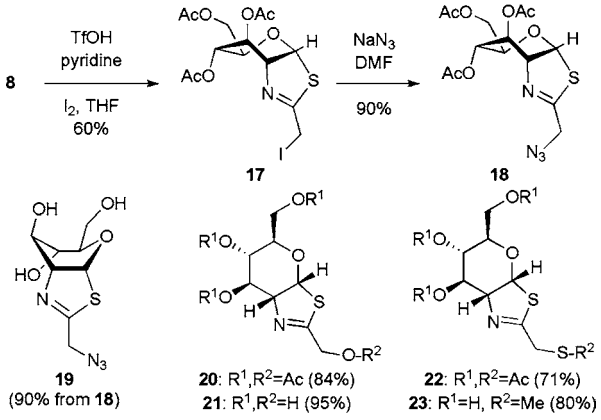


Figure 3. Tautomeric halogenation of GlcNAc-thiazoline.

dibromide **13** could be obtained (along with **14**) by reducing the amount of NBS to 2.2 equiv. The monobromide could not be prepared selectively, evidently because the second and third brominations are faster than the first. Fluorination, however, could be effectively stopped after one substitution: exposure of **8** to buffer and 1.5 equiv of Selectfluor[23] gave **15** in high yield. Standard deacetylation led to the fluoro thiazoline triol **16**.

Iodination of **8** could also be stopped after a monosubstitution (Scheme 2). The product **17** proved to be unstable

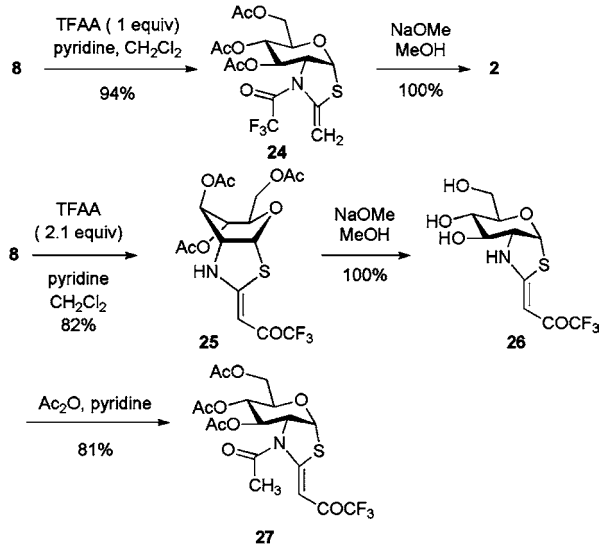
Scheme 2. Tautomeric Iodination and Displacement Reactions



to storage but could be isolated, characterized, and subsequently treated with nucleophiles. Thus, substitution of iodo by azido led to **18** and, following deacetylation, to **19**. Replacement of iodo with acetoxy and *S*-acetylthio was also successful, and the resulting thiazolines **20** and **22** were deacetylated (the latter in the presence of iodomethane) to afford **21** and **23**, respectively.

The ease of tautomerization of **8** suggested that an *N*-acylenamine might also be accessible (Scheme 3).[24] Reaction of

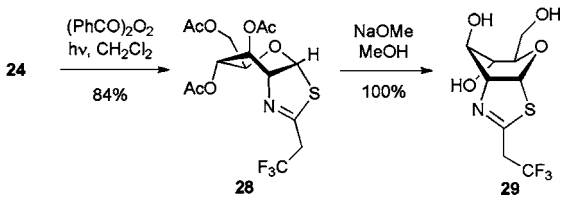
Scheme 3. Thiazoline Acylations



8 with 1 equiv of TFAA indeed gave the enamine **24**. In methanol solution, **24** reverted to **8**, and when treated with methoxide, **24** gave **2**. Upon acylation of **8** with 2.1 equiv of TFAA, the *C*-acylated product **25** formed in good yield, presumably through **24** as an intermediate. Deacetylation gave keto triol **26**, as confirmed by peracetylation to **27**. Both **24** and **27** exist as *pseudo*-chair conformers in solution, according to the vicinal proton *J* values.

During attempts to brominate **24**, we discovered a new trifluoromethylation reaction. Treatment of **24** with benzoyl peroxide and a low power UV light source gave the 2,2,2-trifluoroethyl thiazoline **28** (Scheme 4). This product

Scheme 4. Radical Trifluoromethylation



shows the diagnostic five-bond coupling[25] between the thiazoline methylene H's and the pyranose H-2. ^{19}F NMR

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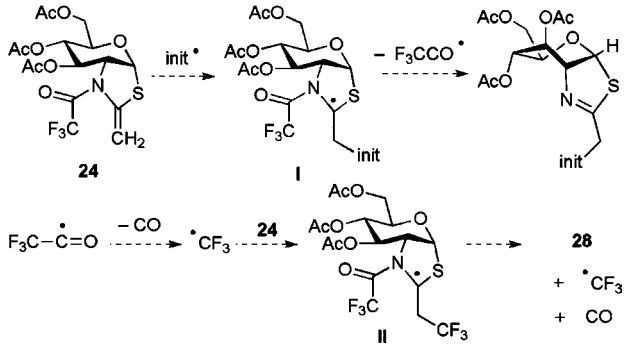
analysis (δ −63.9, t, J = 10.3 Hz) supports this structure, as do the spectra of the deacetylated product **29**.

A radical chain mechanism (Scheme 5) accounts for the formation of **28**. The initiating radical can add to the C=C

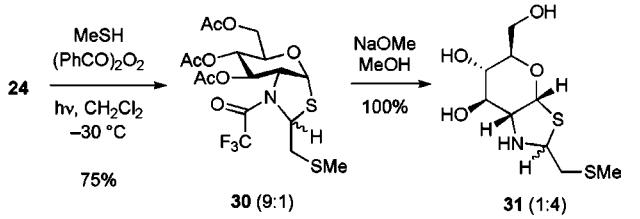
Scheme 5. Proposed Mechanism for Radical Trifluoromethylation



of **24**, leading to a tertiary thiazolidine radical **I**, fragmentation of which would give the trifluoroacetyl radical and a thiazoline product. The trifluoroacetyl radical likely fragments further to provide carbon monoxide and the trifluoromethyl radical.[26] Addition of trifluoromethyl radical to **24** again leads to a thiazolidinyl radical (**II**), and then the chain is propagated by another fragmentation, giving **28** as well as more trifluoromethyl radical.[27]

This mechanism is supported by the successful trapping of the thiazolidine radical by methyl mercaptan (Scheme 6).

Scheme 6. Radical Addition of CH_3SH



Exposure of **24** to the radical initiating conditions, but in the presence of excess mercaptan, led to the formation of thioether adduct **30** as an inseparable 9:1 mixture of stereoisomers (respective anomeric H's at 5.94 and 6.21 ppm). Kinetic hydrogen atom abstraction likely occurs preferentially from the less hindered β face[28] and is faster than loss of $CF_3CO^{\bullet}$, accounting for the formation of product still bearing this group. Deacetylation of **30** gave triol **31**, but as a 1:4 mixture of isomers (respective H-1's at 5.77 and 5.98 ppm). The change in isomeric composition upon basic hydrolysis[29] reflects thiazolidine ring opening to an imine mercaptide intermediate, which recloses to give the thermodynamic mixture of isomers of **31**.

Figure 4 shows the selective inhibition of human recombinant *O*-GlcNAcase by the new modified GlcNAc-thiazolines, relative to their inhibition of human placental β-hexosaminidase.[30] While all seven new compounds show somewhat reduced inhibition relative to the parent **2**, the azide **19** and the fluoride **16** exhibit excellent selectivity for the *O*-GlcNAcase, and **19** retains nearly all of the inhibitory ativity of **2**. These two highly selective and potent GlcNAc-thiazolines differ significantly from previously characterized selective *O*-GlcNAcase inhibitors. The fluorine and azide derivatives may prove useful for developing reagents for imaging, labeling, and interfering with *O*-GlcNAc cycling in living cells and tissues.

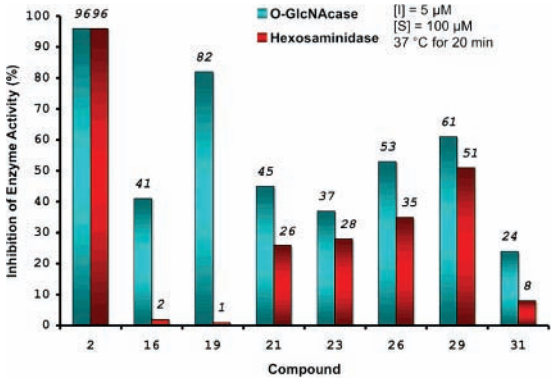


Figure 4. Inhibition by modified GlcNAc-thiazolines of *O*-GlcNAcase in comparison to human placental β-hexosaminidase.

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Supporting Information Available: Experimental details and spectroscopic characterization for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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