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A divergent synthesis to generate targeted libraries of inhibitors for *endo*-*N*-acetylglucosaminidases

Isaac K. Seo, Esther H. Woo, Samy Cecioni, and David J. Vocadlo

Abstract: Cell active inhibitors of glycoside processing enzymes are valuable research tools that help us understand the physiological roles of this diverse class of enzymes. *endo*-*N*-Acetylglucosaminidases have gained increased attention for their important roles in both mammals and human pathogens; however, metabolically stable cell active inhibitors of these enzymes are lacking. Here, we describe a divergent synthetic strategy involving elaboration of a thiazoline core scaffold. We illustrate the potential of this approach by using the copper catalysed azide-alkyne click (CuAAC) reaction, in combination with a suitable catalyst to avoid poisoning by the thiazoline moiety, to generate a targeted panel of candidate inhibitors of *endo*-*N*-acetylglucosaminidases and chitinases.

Key words: enzyme inhibitor, glucosaminidase, substrate-assisted catalysis, thiazoline.

Résumé : Les inhibiteurs d'enzymes de transformation des glycosides actifs en milieu cellulaire constituent des outils de recherche précieux qui aident à comprendre les rôles physiologiques de cette classe diversifiée d'enzymes. On a découvert que les *endo*-*N*-acétylglucosaminidases jouaient un rôle important chez les pathogènes affectant tant les mammifères que les hu-mains. Cependant, on ne dispose pas d'inhibiteurs de ces enzymes qui soient stables et actifs en milieu cellulaire. Dans le présent article, nous décrivons une stratégie de synthèse divergente faisant intervenir l'élaboration d'une structure centrale à base de thiazoline. Au moyen d'une réaction catalysée par le cuivre de type « clic » entre un azoture et un alcyne dans laquelle nous avons employé un catalyseur compatible afin d'éviter qu'il soit empoisonné par le fragment thiazoline, nous illustrons le potentiel de cette approche pour générer une série ciblée de composés susceptibles d'inhiber les *endo*-*N*-acétylglucosaminidases et les chitinases. [Traduit par la Rédaction]

Mots-clés : inhibiteur enzymatique, glucosaminidase, catalyse assistée par le substrat, thiazoline.

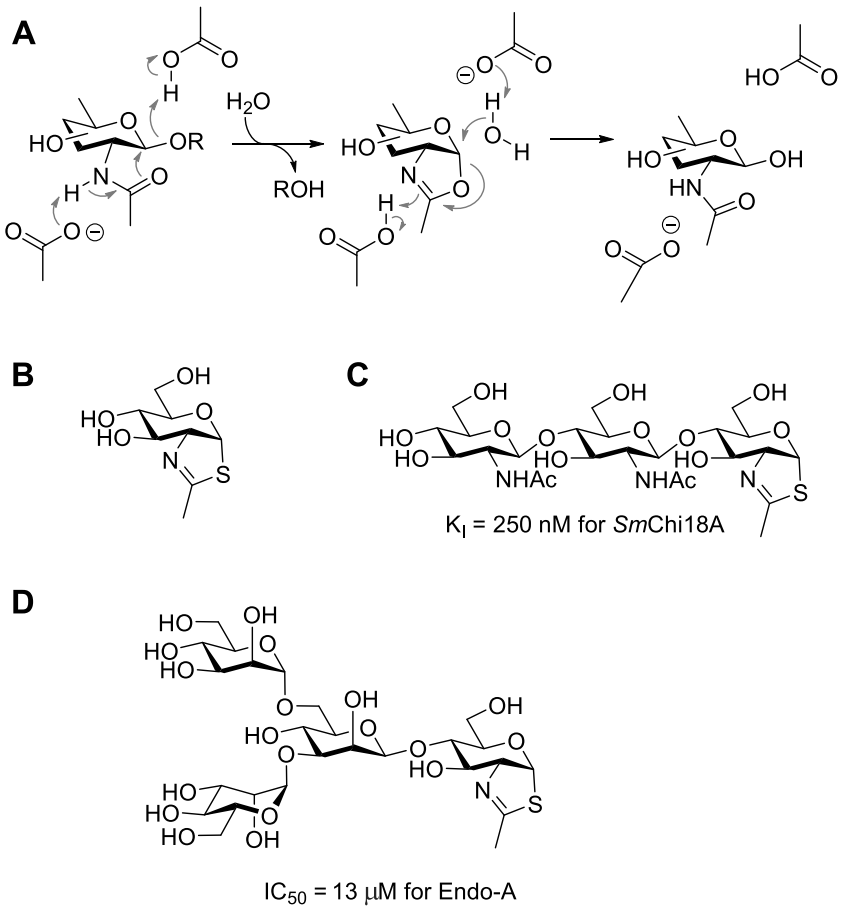
Introduction

endo-*N*-Acetylglucosaminidases, including chitinases and hyaluronidases, are responsible for hydrolyzing the glycosidic bonds of 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc) residues found within oligosaccharides and polysaccharides.[1] These enzymes, along with muramidases, which process structurally related 2-acetamido-2-deoxy-3-*O*-lactyl-β-D-glucopyranose (MurNAc) residues found within the peptidoglycan of bacterial cell walls, play diverse roles in biology, e.g., remodeling of the ECM in metazoans, controlling bacterial cell wall structure, and regulating insect and parasite maturation.[2,3] All of these families of enzymes are structurally distinctive and are accordingly distributed among distinct families of glycoside hydrolases (GHs), including GH18,[4,5] GH22,[6,7] GH25,[8] GH56,[9] GH73,[10,11] and GH85[12,13] of the CAZy classification system.[14] Of these families of enzymes, GH18, GH56, GH73, and GH85 use a catalytic mechanism involving neighbouring group participation of the 2-acetamido group. This substrate assisted catalytic mechanism has been well defined, particularly for *exo*-acting β-glucosaminidases from families GH20[15–17] and GH84.[18–22] In this two-step double displacement mechanism (Fig. 1), the amide carbonyl of the 2-acetamido group is oriented and polarized by a hydrogen bond between the amide nitrogen and an enzymic carboxylate residue. The first step of this mechanism involves attack of the carbonyl group on the anomeric carbon, which aids concomitant expulsion of the leaving group alcohol in a process that is facilitated by an enzymic general acid catalytic residue. The resulting oxazoline intermediate is hydrolyzed in a second enzyme catalyzed step that is the near microscopic reverse of the first step, differing principally in that a molecule of water takes the place of the leaving group. Such oxazoline intermediates have recently been observed directly within the active site of GH84 enzymes,[21] and stable thiazoline-based analogues of these oxazolines, including 1,2-dideoxy-2′-ethyl-α-D-glucopyranoso-[2,1-d]-Δ2′-thiazoline (NAG-thiazoline)[18,23] and more potent derivatives such as Thiamet-G,[24,25] have been found to be potent inhibitors of GH84 enzymes. Such inhibitors have been found to be transition state analogues,[26] which contributes to their generally high inhibitor potencies, which show picomolar K_i values in some cases.[27]

Despite the potency of such thiazoline-based inhibitors, limited effort has focused on creating such inhibitors for endo-acting enzymes from these families. Notably, NAG-thiazoline containing oligosaccharide inhibitors of GH20 chitinases[28] and GH85 *endo*-*N*-acetylglucosaminidases[29] show strong inhibition of these enzymes (Fig. 1). These oligosaccharide-derived inhibitors are, however, susceptible to processing by other glycoside hydrolases that can

I.K. Seo,* E.H. Woo, and S. Cecioni. Department of Chemistry, Simon Fraser University, 8888 University Drive, Burnaby, BC V5A 1S6, Canada.
D.J. Vocadlo. Department of Molecular Biology and Biochemistry, Simon Fraser University, 8888 University Drive, Burnaby, BC V5A 1S6, Canada.
Corresponding author: David J. Vocadlo (email: dvocadlo@sfu.ca).
*Current affiliations: Department of Chemistry, Kwantlen Polytechnic University, 12666 72 Avenue, Surrey, BC V3W 2M8, Canada; Geering Up, Faculty of Applied Sciences, University of British Columbia, 2329 West Mall, Vancouver, BC V6T 1Z4, Canada.

Fig. 1. (A) Mechanism of substrate-assisted catalysis, (B) NAG-thiazoline **1**, (C) chitotriose-thiazoline inhibitor of chitinase, and (D) Man3GlcNAc-thiazoline inhibitor of *endo*-beta-glucosamindase-A from *Arthrobacter protophormiae*. [Colour online.]



trim off the nonreducing residues. Because they are also comprised of natural saccharide units, it is difficult to improve their physicochemical properties to make them more suitable for use in cells or in vivo. We were stimulated by these observations to develop a strategy enabling parallel synthesis to generate a panel of inhibitors of *endo*-acting enzymes that use a substrate assisted catalytic mechanism. Notably, the structures of GH18,[30,31] GH56,[9] and GH85[32–34] enzymes reveal cleft-shaped active site architectures in which aromatic amino acid side chains act to bind saccharide residues at the nonreducing end within a series of subsites. Therefore, we reasoned that appending aryl substituents to the 4-hydroxyl group of the thiazoline-containing residue bound in the –1 subsite could enable these aryl groups to take the position of sugar residues of the oligosaccharide chain bound within the –2 and –3 subsites.

Relatively limited chemistry has been applied to derivatization of NAG-thiazoline at its various hydroxyl groups[29,35] The similar reactivity of the 3- and 4-hydroxyl positions of gluco-configured saccharides,[36] coupled with the sensitivity of the thiazoline moiety towards oxidative, reductive, radical, and acidic conditions,[37–39] has likely hindered the development of such chemistries. Accordingly, to advance on our goal, we describe here a method to selectively alkylate NAG-thiazoline at the 4-position. Late stage propargylation of a common deprotected intermediate, in particular, allowed us to use the copper catalysed azide-alkyne click (CuAAC) reaction to rapidly assemble a targeted panel of inhibitors for GH18 and GH85 enzymes.

Experimental

General

All commercially available chemicals were purchased from Sigma-Aldrich, Alfa Aesar, Carbosynth, or TCI and used without further purification. Noncommercial azides were purchased from Peng Wu at the Scripps Research Institute. All dry solvents were purchased from Sigma-Aldrich or VWR and used without further purification. The progress of all reactions was monitored via thin layer chromatography on Merck pre-coated silica gel plates using ethyl acetate/hexanes, ethyl acetate/dichloromethane, methanol/dichloromethane, or acetone/hexanes solvent elution systems. Flash chromatography was performed under positive pressure using Fisher Scientific silica gel (230–400 mesh) where spots were visualized under ultraviolet light (254 nm) and staining with $KMnO_4$ and gentle heating. Proton (^{1}H) and carbon (^{13}C) spectra were obtained on either a Bruker AVANCE 500 (500 MHz for ^{1}H, 125 MHz for ^{13}C) or a Bruker AVANCE 400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) unless otherwise specified. NMR samples were dissolved in deuterated chloroform, methanol, or DMSO from Cambridge Isotope Laboratories. High resolution mass spectroscopy data was obtained using a Bruker maXis TOF LC/MS/MS instrument.

Procedure for the synthesis of 3,6-*O*-dipivaloyl-NAG-thiazoline 2

NAG-thiazoline (1.80 g, 8.66 mmol), prepared from glucosamine hydrochloride,[23] was added to a 250 mL flame-dried, round-bottom flask under an atmosphere of argon. NAG-thiazoline, along with DMAP (100 mg, 0.9 mmol), was dissolved in pyridine

(100 mL), and pivaloyl chloride (2.1 mL, 17.0 mmol) was added dropwise to the resulting solution, which had been cooled to 0 °C. Once this addition was complete, the reaction mixture was allowed to warm to room temperature and left stirring for 16 h. Upon completion, the solution was concentrated under vacuum to one-fifth of the original volume by co-evaporation with toluene. Ethyl acetate (200 mL) was added, and the organic layer was washed five times with water. The resulting organic layer was further washed with a saturated aqueous solution of potassium bisulfate until no pyridinium bisulfate precipitates were observed. The organic fraction was then dried over sodium sulfate before being filtered and concentrated in vacuo. Purification by silica gel flash column chromatography (2:3 ethyl acetate:hexanes) yielded equimolar amounts of 1,2-dideoxy-2′-ethyl-3,6-di-*O*-pivaloyl-α-D-glucopyranoso-[2,1-d]-Δ2′-thiazoline (3,6-*O*-dipivaloyl-NAG-thiazoline) **2** and 1,2-dideoxy-2′-ethyl-4,6-di-*O*-pivaloyl-α-D-glucopyranoso-[2,1-d]-Δ2′-thiazoline (4,6-*O*-dipivaloyl-NAG-thiazoline) **3** as clear colourless gums (1.10 g for each, 33%) along with 1,2-dideoxy-2′-ethyl-6-*O*-pivaloyl-α-D-glucopyranoso-[2,1-d]-Δ2′-thiazoline (6-*O*-pivaloyl-NAG-thiazoline) **4** (762 mg, 29%).

Procedure for recycling 4,6-*O*-dipivaloyl-NAG-thiazoline 3

The undesired 4,6-*O*-dipivaloyl-NAG-thiazoline **3** (1.00 g, 2.58 mmol) was added to a 100 mL flame-dried, round-bottom flask under an atmosphere of argon. The compound was dissolved in methanol (50 mL), and the mixture was cooled on ice before adding sufficient solid sodium methoxide such that the pH value of the solution reached 10. The mixture was left stirring at room temperature for 16 h. Upon completion of the reaction, acetic acid was added dropwise until the pH value of the solution was neutral. The solvent was removed in vacuo, and dichloromethane (400 mL) was added to the residue, which was triturated. The resulting suspension was filtered, and the solid was washed with dichloromethane. The filtrate was concentrated and purified by silica gel flash column chromatography (1:9 methanol:dichloromethane) to yield NAG-thiazoline (413 mg, 73%).

Procedure for synthesis of 3,6-*O*-dipivaloyl-4-*O*-propargyl-NAG-thiazoline 5

3,6-*O*-Dipivaloyl-NAG-thiazoline **2** (200 mg, 0.516 mmol) was added to a 100 mL flame-dried, round-bottom flask under an atmosphere of argon. The compound was dissolved in anhydrous DMF (25 mL) and cooled in a dry ice – brine bath at –20 °C. Sodium hydride (42 mg, 1.04 mmol, 60% dispersion in mineral oil) was slowly added in small portions over 5 min, and the resulting mixture was left to stir for 10 min. Propargyl bromide (974 μL, 1.04 mmol, 80% in toluene) was then added dropwise by syringe, and the resulting reaction mixture was stirred at –20 °C for 6 h after which it was warmed to 0 °C and allowed to then stir for 4 h. Ethyl acetate was next added to the reaction mixture, and the solution was washed five times with water, followed by back extraction of the aqueous layer with ethyl acetate. The combined organic layers were washed eight times with water and brine to remove residual DMF. The remaining solvent was dried over Na_2SO_4, filtered, and concentrated in vacuo to yield a gummy residue. The residue was subjected to silica gel flash column chromatography (1:4 ethyl acetate:hexanes) to afford 1,2-dideoxy-2′-ethyl-3,6-di-*O*-pivaloyl-4-*O*-propynyl-α-D-glucopyranoso-[2,1-d]-Δ2′-thiazoline (3,6-*O*-dipivaloyl-4-*O*-propargyl-NAG-thiazoline) **5** as a clear, colourless gum (129 mg, 59%). A small amount of pivaloyl migration was observed with the formation of the 3-*O*-propargyl derivative (26 mg, 10%).

Procedure for synthesis of 4-*O*-propargyl-NAG-thiazoline 6

3,6-*O*-Dipivaloyl-4-*O*-propargyl-NAG-thiazoline **5** was added to a 50 mL flame-dried, round-bottom flask under an atmosphere of argon. The compound was dissolved in methanol, after which sufficient 10% w/w methanolic solution of sodium methoxide was added to render the pH value of the reaction mixture basic (pH = 10), and the mixture was then allowed to stir for 16 h at room temperature. The mixture was cooled to 0 °C, and then, dilute acetic acid, in methanol, was added to neutralize the mixture. The solvent was then removed under vacuum, the resulting residue triturated using DCM, and filtered. The filtrate was concentrated in vacuo and purified via silica gel flash column chromatography (1:19 methanol:DCM) to afford 1,2-dideoxy-2′-ethyl-4-*O*-propynyl-α-D-glucopyranoso-[2,1-d]-Δ2′-thiazoline (4-*O*-propargyl-NAG-thiazoline) **6** as a white foam (quantitative).

Table 1. Synthesis of a targeted panel of 4-(alkyl/aryl-1-H-1,2,3-triazol-4-yl)methyl-NAG-thiazoline derivatives via CuAAC reaction in the presence of BTTAA catalyst.

Compound	Isolated yield (%)
7	59
8	63
9	42
10	60
11	71
12	64
13	82
14	68
15	81
16	41
17	38
18	47
19	56
20	73
21	39
22	54
23	53
24	91

General procedure for synthesis of 4-*O*-(aryl-1-H–1,2,3-triazol-4-yl)methyl-NAG-thiazolines 7–14 and 4-*O*-(alkyl-1-H–1,2,3-triazol-4-yl)methyl-NAG-thiazolines 15–24

4-*O*-propargyl-NAG-thiazoline **6** (13 mg, 0.0505 mmol), copper sulfate (8 mg, 0.032 mmol), sodium ascorbate (10 mg, 0.05 mmol), 2-(4-((bis((1-(tert-butyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetic acid (BTTAA) (1.0 mg, 2.3 μmol), and the desired azide (0.056 mmol) were added to a 50 mL round-bottom flask and dissolved in a mixture of *t*-butanol (2 mL) and water (500 μL). The reaction mixture was stirred for 3 h. Upon completion, the solvent was removed in vacuo by azeotrope with ethanol, and the desired material was purified from the residue using silica gel flash column chromatography (1:19 methanol:DCM) to afford 4-*O*-(aryl-1-H–1,2,3-triazol-4-yl)methyl-NAG-thiazolines **7–14** and 4-*O*-(alkyl-1-H–1,2,3-triazol-4-yl)methyl-NAG-thiazolines **15–24** as a clear white foams (47%–91%). For details on yields, please see Table 1, and for the corresponding structures of all compounds, please see the Supplementary data.

General procedure for synthesis of 4-azido benzamides 25–29

4-azidobenzoic acid (20 mg, 0.123 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (26 mg, 0.135 mmol) were added to a 50 mL flame-dried round-bottom flask under an atmosphere of argon. The compounds were dissolved in chloroform (10 mL), then TEA (14 μL, 0.135 mmol) and amine (200 mmol) were added. The reaction mixture was heated and maintained at refluxed for 16 h. Upon completion of the reaction, the solvent was removed in vacuo, and the title compound was purified by silica gel flash column chromatography (3:7 ethyl acetate:hexanes) to afford 4-azido-*N*-alkylbenzamides **25–29** as a clear colourless liquid (54%–89%). For details on yields please see

Table 2. Synthesis of 4-azido benzamides.

Compound	Isolated yield (%)
25	76
26	89
27	76
28	54
29	67

Table 2 and for the corresponding structures of all compounds please see the Supplementary data.

Spectral data analysis for compounds

For structures of numbered compounds, please see the Supplementary data.

Compound 2

^{1}H NMR (400 MHz, Chloroform-*d*) δ_H 6.33 (d, *J* = 7.3 Hz, 1H), 4.75 (ddd, *J* = 9.1, 2.7, 0.9 Hz, 1H), 4.48 (m, 1H), 4.33 (t, *J* = 3.1 Hz, 1H), 4.16 (dd, *J* = 12.0, 2.7 Hz, 1H), 4.11 (dd, *J* = 12.0, 6.1 Hz, 1H), 3.65 (ddd, *J* = 8.8, 6.0, 2.7 Hz, 1H), 2.26 (d, *J* = 2.0 Hz, 3H), 1.19 (s, 18H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ_c 178.23, 178.21, 167.29, 89.43, 79.31, 71.93, 70.56, 69.43, 63.39, 38.97, 38.83, 36.78, 27.29, 27.09, 24.81, 20.79. HRMS (ESI$^+$) m/z: [M + H]$^+$ calcd for $C_{18}H_{30}NO_6S$, 388.1794; found, 388.1817.

Compound 5

^{1}H NMR (400 MHz, Chloroform-*d*) δ_H 6.27 (d, *J* = 7.3 Hz, 1H), 4.82 (d, *J* = 8.0 Hz, 1H), 4.54 (m, 1H), 4.41 (d, *J* = 2.7 Hz, 2H), 4.30 (m, 1H), 4.14 (dd, *J* = 12.1, 2.5 Hz, 1H), 4.03 (dd, *J* = 12.0, 6.5 Hz, 1H), 3.53 (ddd, *J* = 8.9, 6.6, 2.3 Hz 1H), 2.42 (t, *J* = 2.1 Hz, 1H), 2.27 (d, *J* = 2.4 Hz, 1H), 1.20 (s, 9H), 1.10 (s, 9H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ_c 178.25, 177.28, 88.78, 79.24, 75.01, 74.76, 69.28, 63.36, 57.21, 38.96, 39.91, 27.35, 27.15, 20.72. HRMS (ESI$^+$) m/z: [M + H]$^+$ calcd for $C_{21}H_{32}NO_6S$, 426.1950; found, 426.1942.

Compound 6

^{1}H NMR (400 MHz, Chloroform-*d*) δ_H 6.34 (d, *J* = 6.0 Hz, 1H), 4.45–4.40 (m, 2H), 4.31 (dd, *J* = 16.1, 2.4 Hz, 1H), 4.25 (dd, *J* = 16.0, 2.4 Hz, 1H), 3.73 (dd, *J* = 12.2, 2.0 Hz, 1H), 3.60–3.52 (m, 2H), 3.22 (m, 1H), 2.87 (t, *J* = 3.1 Hz, 1H), 2.27 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR (126 MHz, Methanol-*d*) δ 170.81, 89.98, 89.83, 80.59, 80.54, 80.48, 80.43, 76.22, 75.99, 73.98, 69.47, 69.41, 63.61, 57.76, 57.64, 20.22, 20.18. HRMS (ESI$^+$) m/z: [M + H]$^+$ calcd for $C_{11}H_{16}NO_4S$, 258.0800; found, 258.0789.

Compound 7

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.24 (s, 1H), 7.80–7.70 (m, 4H), 6.34 (d, *J* = 6.4 Hz, 1H), 5.49 (s, 0H), 4.47–4.39 (m, 2H), 4.35–4.20 (m, 2H), 3.73 (dd, *J* = 12.1, 2.4 Hz, 1H), 3.64–3.50 (m, 2H), 3.22 (ddd, *J* = 9.1, 6.6, 2.3 Hz, 1H), 2.27 (d, *J* = 1.9 Hz, 3H). ^{13}C NMR (125 MHz, Methanol-d_4) δ 167.07, 148.19, 134.15, 130.76, 130.22, 121.16, 119.75, 78.64, 76.41, 75.12, 75.00, 70.66, 62.12, 59.29, 19.67. HRMS (ESI$^+$) m/z: [M + H]$^+$ calcd for $C_{17}H_{20}ClN_4O_4S$, 411.0894; found, 411.0891.

Compound 8

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.39 (s, 1H), 7.82–7.69 (m, 1H), 7.69–7.48 (m, 3H), 6.38 (d, *J* = 7.0 Hz, 1H), 4.97 (d, *J* = 12.4 Hz, 1H), 4.79 (d, *J* = 12.4 Hz, 1H), 4.57 (dd, *J* = 3.7, 2.0 Hz, 1H), 4.52–4.45 (m, 1H), 3.68–3.58 (m, 2H), 3.57–3.45 (m, 1H), 3.32–3.27 (m, 1H), 2.28 (d, *J* = 2.1 Hz, 3H). ^{13}C NMR (125 MHz, Methanol-d_4) δ 167.07, 148.31, 136.54, 131.86, 127.91, 127.88, 127.67, 120.89, 120.34, 78.64, 76.41, 75.12, 75.00, 70.66, 62.12, 59.51, 19.67. HRMS (ESI$^+$) m/z: [M + H]$^+$ calcd for $C_{17}H_{20}ClN_4O_4S$, 411.0894; found, 411.0891.

Compound 9

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.35 (s, 1H), 7.96–7.81 (m, 2H), 7.79–7.59 (m, 2H), 6.16 (d, *J* = 2.4 Hz, 1H), 4.80–4.62 (m, 2H), 4.54 (d, *J* = 12.1 Hz, 1H), 4.45 (tdd, *J* = 8.0, 6.9, 1.9 Hz, 1H), 4.10–3.96 (m, 2H), 3.86 (dt, *J* = 12.1, 5.3 Hz, 2H), 3.81 (s, 3H), 3.70–3.61 (m, 1H), 3.57–3.50 (m, 3H), 2.34 (d, *J* = 1.5 Hz, 3H), 1.98 (t, *J* = 5.3 Hz, 4H). ^{13}C NMR (125 MHz, Chloroform-*d*) δ 171.03, 167.07, 148.19, 138.02, 134.47, 129.00, 119.75, 119.19, 106.05, 78.64, 76.41, 75.12, 75.00, 70.66, 64.33, 62.12, 59.29, 41.44, 35.38, 19.67. HRMS (ESI$^+$) *m/z*: [M + H]$^+$ calcd for $C_{25}H_{32}N_5O_7S$, 546.2022; found, 546.2021.

Compound 10

^{1}H NMR (500 MHz, Methanol-*d*) 7.87 (dd, *J* = 8.5, 1.6 Hz, 2H), 6.90 (dd, *J* = 8.5, 1.6 Hz, 2H), 6.15 (d, *J* = 7.5 Hz, 1H), 4.36 (q, *J* = 4.6, 3.2 Hz, 2H), 4.30–4.12 (m, 2H), 3.71 (dd, *J* = 14.2, 2.7 Hz, 2H), 3.62–3.46 (m, 2H), 3.22 (m, 1H), 2.33 (d, *J* = 2.2 Hz, 3H). ^{13}C NMR (125 MHz, Methanol-*d*) δ 167.07, 166.17, 148.19, 138.65, 130.97, 128.15, 119.75, 118.98, 78.64, 76.41, 75.12, 75.00, 70.66, 62.12, 59.29, 52.17, 19.67. HRMS (ESI$^+$) m/z: [M + H]$^+$ calcd for $C_{19}H_{23}N_4O_6S$, 435.1338; found, 435.1339.

Compound 11

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.92–7.73 (m, 2H), 7.45–7.25 (m, 5H), 7.18–7.02 (m, 2H), 6.45 (s, 1H), 6.36 (d, *J* = 6.3 Hz, 1H), 4.66 (d, *J* = 5.6 Hz, 2H), 4.51–4.40 (m, 2H), 4.35–4.18 (m, 2H), 3.75 (dd, *J* = 12.1, 2.3 Hz, 1H), 3.64–3.50 (m, 2H), 3.24 (ddd, *J* = 9.0, 6.6, 2.3 Hz, 1H), 2.29 (d, *J* = 1.9 Hz, 3H). ^{13}C NMR (125 MHz, Chloroform-*d*) δ 167.66, 167.07, 148.19, 138.71, 137.75, 132.77, 128.89, 128.40, 127.31, 127.17, 119.75, 119.13, 78.64, 76.41, 75.12, 75.00, 70.66, 62.12, 59.29, 43.79, 19.67. HRMS (ESI$^+$) m/z: [M + H]$^+$ calcd for $C_{25}H_{28}N_5O_5S$, 510.1811; found, 510.1818.

Compound 12

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.87–7.73 (m, 5H), 6.55 (t, *J* = 4.4 Hz, 1H), 6.26 (d, *J* = 2.4 Hz, 1H), 4.74 (d, *J* = 12.1 Hz, 1H), 4.69–4.54 (m, 2H), 4.21 (tdd, *J* = 8.3, 6.9, 2.0 Hz, 1H), 3.98 (ddd, *J* = 11.9, 7.1, 5.7 Hz, 1H), 3.90 (qd, *J* = 5.7, 2.0 Hz, 1H), 3.77–3.72 (m, 1H), 3.55–3.38 (m, 4H), 2.36 (s, 3H), 1.23 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (125 MHz, Chloroform-*d*) δ 167.70, 167.07, 148.19, 137.75, 132.37, 128.89, 119.75, 119.13, 78.64, 76.41, 75.12, 75.00, 70.66, 62.12, 59.29, 35.96, 19.67, 14.66. HRMS (ESI$^+$) m/z: [M + H]$^+$ calcd for $C_{20}H_{26}N_5O_5S$, 448.1655; found, 448.1641.

Compound 13

^{1}H NMR (400 MHz, Methanol-*d*) δ 7.90–7.68 (m, 2H), 7.21–6.97 (m, 2H), 6.36 (d, *J* = 6.4 Hz, 1H), 6.15 (s, 1H), 4.52–4.37 (m, 2H), 4.37–4.17 (m, 2H), 3.75 (dd, *J* = 12.1, 2.3 Hz, 1H), 3.60–3.51 (m, 2H), 3.47 (td, *J* = 7.2, 5.7 Hz, 2H), 3.24 (dd, *J* = 15.8, 2.3 Hz, 1H), 2.29 (d, *J* = 1.8 Hz, 3H), 1.62 (tt, *J* = 7.8, 6.4 Hz, 2H), 1.53–1.35 (m, 2H), 0.98 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (125 MHz, Methanol-*d*) δ 168.77, 167.07, 148.19, 137.75, 128.89, 126.87, 119.75, 119.13, 78.64, 76.41, 75.12, 75.00, 70.66, 62.12, 59.29, 40.10, 30.74, 20.43, 19.67, 13.74. HRMS (ESI$^+$) m/z: [M + H]$^+$ calcd for $C_{22}H_{30}N_5O_5S$, 476.1968; found, 476.1964.

Compound 14

^{1}H NMR (400 MHz, Methanol-*d*) δ 7.83–7.70 (m, 2H), 7.32–7.19 (m, 5H), 7.10–7.00 (m, 2H), 6.36 (d, *J* = 6.4 Hz, 1H), 6.31 (d, *J* = 7.7 Hz, 1H), 4.96 (qt, *J* = 7.2, 4.3 Hz, 1H), 4.45 (dp, *J* = 6.0, 2.0 Hz, 2H), 4.37–4.23 (m, 2H), 3.75 (dd, *J* = 12.1, 2.3 Hz, 1H), 3.66–3.52 (m, 2H), 3.44 (dd, *J* = 16.3, 7.1 Hz, 2H), 3.24 (ddd, *J* = 9.0, 6.6, 2.3 Hz, 1H), 2.95 (dd, *J* = 16.2, 4.4 Hz, 2H), 2.28 (d, *J* = 2.0 Hz, 3H). ^{13}C NMR (100 MHz, Methanol-*d*) δ 170.79, 167.07, 148.19, 138.30, 137.84, 134.58, 128.70, 127.77, 127.41, 119.75, 119.22, 78.64, 76.41, 75.12, 75.00, 70.66, 62.12, 59.29, 51.48, 35.93, 19.67. HRMS (ESI$^+$) m/z: [M + H]$^+$ calcd for $C_{26}H_{30}N_5O_5S$, 524.1968; found, 524.1966.

Compound 15

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.57 (s, 1H), 7.40 (t, *J* = 5.4 Hz, 5H), 6.46 (s, 1H), 5.54 (s, 2H), 4.85 (d, *J* = 6.8 Hz, 1H), 4.54 (s, 1H), 4.45 (s, 1H), 3.85 (d, *J* = 7.8 Hz, 2H), 3.73 (dd, *J* = 23.2, 5.1 Hz, 3H), 3.46 (s, 1H), 2.34 (s, 3H). ^{13}C NMR (125 MHz, Chloroform-*d*) δ 169.07, 149.50, 139.18, 132.31, 132.07, 124.76, 120.53, 78.64, 76.41, 75.12, 75.00,

70.66, 62.12, 59.23, 52.53, 19.67. HRMS (ESI+) m/z: [M + H]+ calcd for $C_{18}H_{23}N_4O_4S$, 391.1440; found, 391.1438.

Compound 16

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.59–7.48 (m, 2H), 7.41 (s, 1H), 7.19–7.09 (m, 2H), 6.05 (d, *J* = 2.4 Hz, 1H), 5.62 (d, *J* = 11.2 Hz, 1H), 5.44 (d, *J* = 11.2 Hz, 1H), 4.71 (d, *J* = 12.1 Hz, 1H), 4.54 (d, *J* = 11.9 Hz, 1H), 4.28 (dd, *J* = 8.2, 2.4 Hz, 1H), 4.18 (m, 1H), 3.91 (m, 1H), 3.80–3.71 (m, 1H), 3.68–3.54 (m, 2H), 2.37 (d, *J* = 1.5 Hz, 3H). ^{13}C NMR (125 MHz, Chloroform-*d*) δ 167.07, 148.88, 145.00, 140.68, 136.38, 123.42, 122.16, 120.27, 116.57, 114.38, 78.64, 76.41, 75.12, 75.00, 70.66, 62.12, 59.23, 45.87, 19.67. HRMS (ESI+) m/z: [M + H]+ calcd for $C_{19}H_{23}N_6O_4S$, 431.1502; found, 431.1509.

Compound 17

^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.30 (dd, *J* = 1.5, 0.7 Hz, 1H), 8.37 (s, 1H), 7.58–7.47 (m, 2H), 5.87 (d, *J* = 2.4 Hz, 1H), 5.71 (d, *J* = 11.3 Hz, 1H), 5.44 (d, *J* = 11.4 Hz, 1H), 4.56 (d, *J* = 11.9 Hz, 1H), 4.32 (d, *J* = 11.9 Hz, 1H), 4.11 (dd, *J* = 8.3, 2.4 Hz, 1H), 4.06–3.96 (m, 1H), 3.71 (m, 1H), 3.61 (m, 1H), 3.53–3.39 (m, 2H), 2.21 (d, *J* = 1.6 Hz, 3H), 1.29 (s, 9H). ^{13}C NMR (125 MHz, Chloroform-*d*) δ 167.07, 152.71, 152.58, 146.50, 142.46, 133.79, 125.34, 124.31, 120.61, 79.88, 78.64, 76.41, 75.12, 75.00, 70.66, 62.12, 59.23, 51.98, 28.11, 19.67. HRMS (ESI+) m/z: [M + H]+ calcd for $C_{22}H_{31}N_6O_6S$, 507.2026; found, 507.2022.

Compound 18

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.65 (s, 1H), 7.87–7.75 (m, 1H), 7.53–7.40 (m, 2H), 6.74 (m, 1H), 5.17 (d, *J* = 2.4 Hz, 1H), 4.35 (d, *J* = 12.1 Hz, 1H), 4.21 (m, 1H), 4.19–4.04 (m, 3H), 4.04–3.95 (m, 1H), 3.42 (m, 1H), 3.36–3.27 (m, 1H), 3.27–3.10 (m, 2H), 2.54 (dd, *J* = 14.1, 7.7 Hz, 1H), 2.51–2.39 (m, 1H), 1.97 (d, *J* = 1.5 Hz, 3H). ^{13}C NMR (125 MHz, Chloroform-*d*) δ 170.12, 167.07, 153.15, 147.71, 142.95, 137.86, 122.68, 118.60, 114.33, 78.64, 76.41, 75.12, 75.00, 70.66, 62.12, 59.23, 45.14, 37.18, 19.67. HRMS (ESI+) m/z: [M + H]+ calcd for $C_{19}H_{25}N_6O_5S$, 449.1607; found, 449.1599.

Compound 19

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.83 (d, *J* = 8.4 Hz, 1H), 7.69–7.63 (m, 1H), 7.59 (s, 1H), 7.55 (d, *J* = 2.2 Hz, 1H), 7.38 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.18 (dd, *J* = 8.4, 2.2 Hz, 1H), 5.57 (d, *J* = 2.4 Hz, 1H), 5.44 (d, *J* = 15.2 Hz, 1H), 5.17 (d, *J* = 15.2 Hz, 1H), 4.70 (d, *J* = 11.9 Hz, 1H), 4.51 (dd, *J* = 8.2, 2.4 Hz, 1H), 4.39 (d, *J* = 11.9 Hz, 1H), 4.34 (m, 1H), 3.84–3.71 (m, 3H), 3.65 (m, 1H), 3.55 (dd, *J* = 7.9, 6.0 Hz, 1H), 2.38 (d, *J* = 1.6 Hz, 3H). ^{13}C NMR (125 MHz, Chloroform-*d*) δ 168.96, 167.07, 155.75, 144.84, 137.75, 129.18, 128.67, 127.25, 125.81, 124.60, 121.19, 117.00, 114.90, 105.99, 78.64, 76.41, 75.12, 75.00, 70.66, 62.12, 59.23, 52.48, 19.67. HRMS (ESI+) m/z: [M + H]+ calcd for $C_{23}H_{26}N_5O_6S$, 500.1604; found, 500.1611.

Compound 20

^{1}H NMR (500 MHz, Chloroform-*d*) 7.69–7.63 (m, 2H), 7.37–7.31 (m, 1H), 7.18 (dd, *J* = 8.0, 1.1 Hz, 1H), 7.11 (m, 1H), 7.01 (d, *J* = 2.6 Hz, 1H), 5.57 (d, *J* = 2.4 Hz, 1H), 4.78–4.65 (m, 2H), 4.59 (m, 1H), 4.55–4.45 (m, 2H), 4.40 (m, 1H), 3.82 (m, 1H), 3.75–3.67 (m, 1H), 3.67–3.55 (m, 2H), 3.41 (m, 1H), 3.25 (m, 1H), 2.37 (d, *J* = 1.6 Hz, 3H). ^{13}C NMR (125 MHz, Chloroform-*d*) δ 167.07, 142.95, 136.16, 127.32, 123.25, 122.96, 121.24, 119.31, 118.29, 111.72, 111.33, 78.64, 76.41, 75.12, 75.00, 70.66, 62.12, 59.23, 50.08, 26.33, 19.67. HRMS (ESI+) m/z: [M + H]+ calcd for $C_{21}H_{26}N_5O_4S$, 444.1706; found, 444.1720.

Compound 21

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.09–7.93 (m, 2H), 7.50–7.35 (m, 3H), 5.54 (d, *J* = 2.4 Hz, 1H), 5.46 (m, 1H), 5.24 (m, 1H), 4.73 (d, *J* = 11.9 Hz, 1H), 4.55–4.42 (m, 2H), 4.39 (m, 1H), 3.82–3.72 (m, 1H), 3.67 (m, 1H), 3.64–3.48 (m, 2H), 2.37 (d, *J* = 1.5 Hz, 3H). ^{13}C NMR (125 MHz, Chloroform-*d*) δ 167.07, 146.50, 146.37, 137.08, 129.62, 129.23, 120.53, 120.46, 78.64, 76.41, 75.12, 75.00, 70.66, 62.12, 59.23, 52.24, 19.67. HRMS (ESI+) m/z: [M + H]+ calcd for $C_{19}H_{23}N_8O_5S$, 475.1512; found, 475.1516.

Compound 22

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.89 (d, *J* = 5.7 Hz, 1H), 8.51 (s, 1H), 8.10 (d, *J* = 5.7 Hz, 1H), 8.02 (d, *J* = 1.7 Hz, 1H), 7.92 (d, *J* = 8.6 Hz, 1H), 7.64 (dd, *J* = 8.4, 1.8 Hz, 1H), 5.70–5.61 (m, 1H), 4.73 (d, *J* = 12.1 Hz, 1H), 4.64–4.49 (m, 4H), 4.08–3.95 (m, 1H), 3.85–3.69 (m, 2H), 3.72–3.60 (m, 1H), 3.52 (m, 1H), 2.38 (d, *J* = 1.5 Hz, 3H). ^{13}C NMR (125 MHz, Chloroform-*d*) δ 167.07, 149.26, 147.59, 147.29, 137.07, 133.72, 129.58, 127.71, 125.90, 123.65, 121.10, 108.47, 78.64, 76.41, 75.12, 75.00, 70.66, 62.12, 59.51, 19.67. HRMS (ESI+) m/z: [M + H]+ calcd for $C_{20}H_{21}ClN_5O_4S$, 462.1003; found, 462.1007.

Compound 23

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.51–7.39 (m, 3H), 7.34–7.30 (m, 2H), 6.04 (d, *J* = 2.5 Hz, 1H), 5.56 (s, 2H), 4.67 (d, *J* = 11.9 Hz, 1H), 4.62–4.51 (m, 2H), 4.35 (m, 1H), 4.11 (ddd, *J* = 11.9, 7.1, 5.7 Hz, 1H), 3.96 (qd, *J* = 5.7, 1.9 Hz, 1H), 3.79–3.67 (m, 1H), 3.61 (ddd, *J* = 11.9, 7.0, 5.6 Hz, 1H), 2.38 (d, *J* = 1.5 Hz, 3H). ^{13}C NMR (125 MHz, Chloroform-*d*) δ 164.19, 147.72, 146.66, 140.07, 137.07, 131.45, 122.61, 118.44, 117.66, 112.58, 77.14, 76.40, 75.00, 70.75, 62.13, 59.44, 47.15, 19.51. HRMS (ESI+) m/z: [M + H]+ calcd for $C_{19}H_{22}ClN_6O_4S$, 465.1112; found, 465.1122.

Compound 24

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.49 (s, 1H), 5.54 (d, *J* = 2.4 Hz, 1H), 4.63 (d, *J* = 12.1 Hz, 1H), 4.56–4.44 (m, 2H), 4.37 (tdd, *J* = 8.1, 6.9, 2.0 Hz, 1H), 4.07 (t, *J* = 6.3 Hz, 2H), 4.03–3.94 (m, 1H), 3.75–3.67 (m, 1H), 3.58 (dd, *J* = 7.9, 5.9 Hz, 1H), 3.56–3.41 (m, 3H), 2.35 (d, *J* = 1.5 Hz, 3H), 2.26–2.18 (m, 2H). ^{13}C NMR (125 MHz, Chloroform-*d*) δ 167.07, 142.95, 122.65, 78.64, 76.41, 75.12, 75.00, 70.66, 62.12, 59.59, 59.23, 47.50, 31.54, 19.67. HRMS (ESI+) m/z: [M + H]+ calcd for $C_{14}H_{23}N_4O_5S$, 359.1389; found, 359.1386.

Compound 25

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.50–7.30 (m, 2H), 7.11–6.90 (m, 2H), 3.98 (s, 4H), 3.81 (s, 2H), 3.50 (s, 2H), 1.72 (m, 4H). ^{13}C NMR (125 MHz, Chloroform-*d*) δ 170.77, 142.59, 133.16, 129.68, 119.40, 105.83, 64.40, 41.43, 35.21. HRMS (ESI+) m/z: [M + H]+ calcd for $C_{14}H_{17}N_4O_3$, 289.1301; found, 289.1289.

Compound 26

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.84–7.71 (m, 2H), 7.44–7.26 (m, 5H), 7.11–7.00 (m, 2H), 6.42 (s, 1H), 4.63 (d, *J* = 5.6 Hz, 2H). ^{13}C NMR (125 MHz, Chloroform-*d*) δ 167.51, 142.34, 138.68, 131.26, 128.98, 128.40, 127.31, 127.17, 119.27, 43.74. HRMS (ESI+) m/z: [M + H]+ calcd for $C_{14}H_{13}N_4O$, 253.1090; found, 253.1094.

Compound 27

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.99–7.79 (m, 2H), 7.35 (t, *J* = 4.3 Hz, 1H), 7.25–7.22 (m, 2H), 3.47 (qd, *J* = 7.1, 4.3 Hz, 2H), 1.23 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (125 MHz, Chloroform-*d*) δ 167.51, 142.45, 130.86, 128.98, 119.27, 35.96, 14.66. HRMS (ESI+) m/z: [M + H]+ calcd for $C_9H_{11}N_4O$, 191.0933; found, 191.0925.

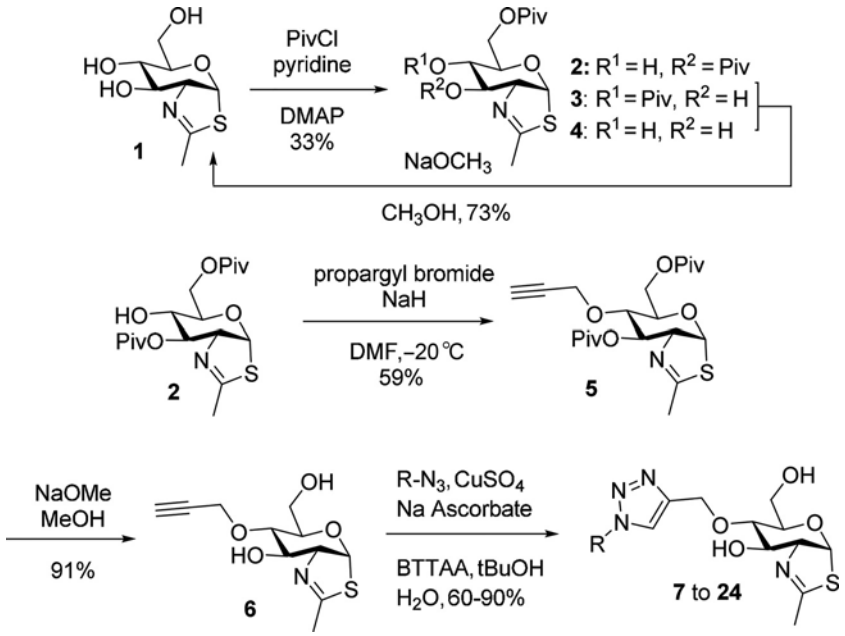
Compound 28

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.92–7.71 (m, 2H), 7.22–6.98 (m, 2H), 6.15 (s, 1H), 3.47 (td, *J* = 7.2, 5.7 Hz, 2H), 1.69–1.59 (m, 2H), 1.60–1.34 (m, 2H), 0.98 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (125 MHz, Chloroform-*d*) δ 168.71, 142.34, 130.79, 128.98, 119.27, 40.10, 30.74, 20.43, 13.74. HRMS (ESI+) m/z: [M + H]+ calcd for $C_{11}H_{15}N_4O$, 219.1246; found, 219.1239.

Compound 29

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.82–7.66 (m, 2H), 7.27 (d, *J* = 3.2 Hz, 2H), 7.26–7.17 (m, 3H), 7.11–6.98 (m, 2H), 6.28 (d, *J* = 7.5 Hz, 1H), 4.94 (qt, *J* = 7.2, 4.3 Hz, 1H), 3.42 (dd, *J* = 16.2, 7.1 Hz, 2H), 2.92 (dd, *J* = 16.2, 4.4 Hz, 2H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 170.53, 142.40, 138.27, 133.26, 129.34, 128.70, 127.77, 127.41, 119.43, 51.72, 35.93. HRMS (ESI+) m/z: [M + H]+ calcd for $C_{15}H_{15}N_4O$, 267.1246; found, 267.1256.

Scheme 1. Synthesis of divergent intermediate **6** and generation of a targeted panel of inhibitors.



Results and discussion

The sensitivity of the thiazoline ring of NAG-thiazoline limits the conditions to which this molecule can be subjected, which in turn circumscribes those chemical transformations that can be used to derivatize this compound. For example, in acidic conditions (pH < 6.0), thiazolines are protonated to form an easily hydrolyzed thiazolinium;[38] in oxidative conditions, the sulfur can be oxidized; and in reductive or radical conditions, the N=C double bond can be degraded.[37] Thus, mild protection and deprotection conditions are required. Based on these considerations, we developed a convenient route using selective pivaloylation of NAG-thiazoline at the 3- and 6-hydroxyl groups (Scheme 1). Notably, we ultimately settled on this bulky protecting group after a range of silyl- and ether-based protecting groups were explored, none of which proved suitable. We were surprised by the complete lack of regioselectivity between the 3- and 4-positions during selective pivaloylation despite our exploring a range of different conditions. Nevertheless, we could readily recycle undesired by-products back to NAG-thiazoline using methanolic sodium methoxide. This approach allowed for large-scale synthesis of selectively protected **2**, which we propargylated through the addition of base at low temperatures to yield **5**. The regioselectivity of pivaloylation was confirmed by analysis of HMBC NMR data. Specifically, the quaternary pivaloyl ^{13}C signals (178.21 and 178.23 in **2**, 178.25 and 177.28 in **5**) were found to strongly correlate with the H-3 and H-6 ^{1}H resonances. Finally, global deprotection of the pivaloyl protecting groups yielded the advanced common intermediate **6**. The structure of **6**, as opposed to other regioisomers, was supported by comparison of the ^{1}H NMR spectra of compound **6** with the NAG-thiazoline, which showed the by far most pronounced chemical shits in the resonances for H-4 (0.24 ppm) and C-4 (4 ppm), whereas all other resonances of the pyranose ring differed by approximately 0.1 ppm for ^{1}H data and 2 ppm for ^{13}C. Last step derivatization of this intermediate enabled us to rapidly prepare a targeted panel of candidate inhibitors via click CuAAC chemistry.[40] Although CuAAC chemistry has been well studied and shown to be compatible with a wide range of functional groups, we observed that the thiazoline moiety of NAG-thiazoline greatly impaired this coupling reaction. For this reason, we resorted to stabilizing the cuprous ion using added ligands, which we expected would protect against poisoning of the catalyst by the thiazoline moiety. In this regard, we found that BTTAA, a tris-triazole ligand used to catalyze the CuAAC reaction in conditions of low copper (I) in vivo and live cell environments[41,42] worked efficiently in our hands for this purpose (Scheme 1; Table 1). Using this strategy, we were able to conveniently prepare a panel of 18 compounds as potential GH18 and GH85 inhibitors. The success of targeted libraries of inhibitors in which carbohydrate core structures have been derivatized in various ways has proven to be of use in finding high affinity ligands for Siglecs[43] and hexosaminidases.[44] Accordingly, we anticipate this small panel of compounds, as well as larger libraries generated using our strategy, should prove useful for those interested in identifying inhibitors of various classes of enzymes using substrate-assisted catalysis. Testing these compounds against various enzymes is a future topic being pursued, and results from these studies will be reported in due course.

Conclusion

We have developed a route to create targeted panels of derivatized NAG-thiazolines as potential inhibitors of GH18 and GH85 enzymes. This strategy relied of selective protection of NAG-thiazoline followed by propargylation and deprotection to yield a late stage common intermediate. CuAAC reaction, using BTTAA as a protective ligand and catalyst, enabled the rapid generation of a library of potential inhibitors. These compounds, as hybrid transition state medicinal chemistry like compounds, may serve as leads to obtain useful inhibitors that could be used to both further our fundamental understanding regarding the roles of these enzymes, as well as help validate inhibition of these enzymes as a viable strategy to treat diseases. Examples of enzymes of high interest for which inhibitors may have therapeutic potential include human ENGase, inhibition of which may be valuable for protecting against the deleterious effects associated with loss of *NGLY1*,[45,46] as well as several known bacterial virulence factors, including Endohexosaminidase D (GH85),[47,32] Auto (GH73),[48] and peptidoglycan hydrolase FlgJ (GH73).[49,50]

Supplementary data

Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjc-2017-0461.

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