α-GlcNAc Thioconjugates

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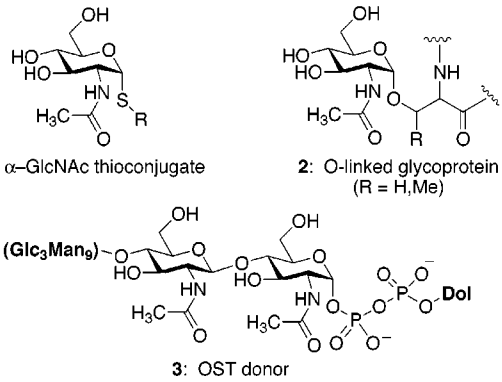
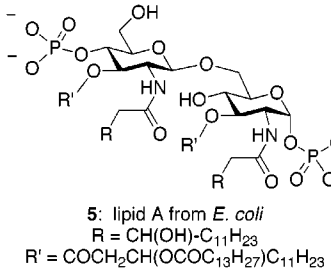
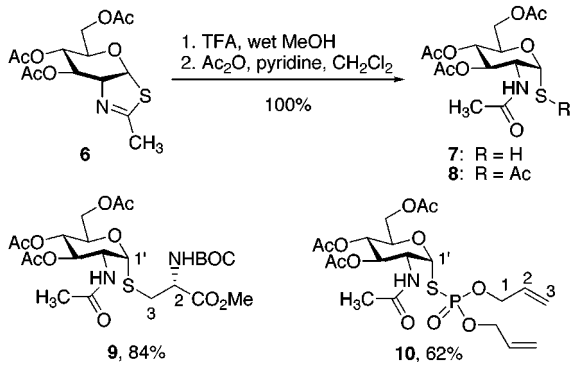
One of the most important naturally occurring glycosidic linkages features an α-GlcNAc[1] glycon and a variety of aglycons, including serine- or threonine-containing peptides and proteins, various pyranosides, cyclitols, ribitols, and glycerols, and various phosphates and pyrophosphates.[2] For example, *O*-linked glycopeptides can have structure **2**.[3] The donor for oligosaccharyl transferase, which promotes formation of *N*-linked glycoproteins, is the dolichyl oligosaccharyl pyrophosphate **3**.[4] UDP-GlcNAc **4** serves as a donor in biological glycosylations,[5] and the structure of lipid A (**5**) from the *E. coli* outer membrane endotoxin likewise contains an α-GlcNAc-like phosphate linkage.[6]





Structural modification of α-GlcNAc glycoconjugates by replacement of the linking oxygen with a sulfur atom would provide the corresponding thioglycosides **1**. These analogues can be expected to exhibit enhanced chemical[7] and biological[8] stability compared with the naturally occurring compounds. Chemical stability is a particular issue for α-GlcNAc phosphates resembling **5**, the *O*-phosphate of which has been described as "readily cleaved even by its own acidic property".[6] The prospect of using the α-GlcNAc thioglycoside analogues **1** as enzyme inhibitors[9] or as enzyme-resistant scaffolds[10] provides additional incentive for investigating methods for their preparation. As far as we can determine, however, no generally applicable literature methods are presently available, and simple α-GlcNAc mercaptans such as **7** (Scheme 1) are unknown or undescribed. In this Note we report the discovery of a simple preparation of **7** and also further modification of **7** to afford the α-GlcNAc *S*-glycosyl amino ester **9** and the α-GlcNAc thiophosphate **10**.

Scheme 1



The thiazoline **6** (Scheme 1) was previously prepared in one step from commercially available 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranose.[11] Hydrolysis of **6** under acidic conditions occurred exclusively at the iminium carbon[12] (rather than C-1) and led in quantitative yield to the α-GlcNAc mercaptan **7**. That **7** is the mercapto amide rather than a hydroxy thioamide

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was demonstrated by its acetylation to give the crystalline pentaacetate **8**. The thioacetyl of **8** exhibits the diagnostic chemical shifts in the ^{1}H and ^{13}C NMR spectra (S$\underline{C}$O$\underline{C}$H$_3$ at 2.43 and 190.3 ppm, respectively), and the downfield doublet for H-1 (J = 4.8 Hz) confirms the α anomeric stereochemistry.

S-Alkyation of **7** with *N*-Boc-3-iodoalanine methyl ester[13] was carried out on the derived sodium salt[14] in DMF solution and provided the *S*-glycosyl amino ester **9** in good yield. The NMR spectra of **9** confirm the formation of a single isomer without epimerization[15] at the cysteine α-carbon. *S*-Phosphorylation[7] of **7** was carried out by treatment with diallyl *N*,*N*-diisopropylphosphoramidite and tetrazole in acetonitrile solution followed by oxidation (aqueous *tert*-butylhydroperoxide) and likewise gave a thioconjugate, the thiophosphate **10**. The two allyl methylenes (C-1) of **10** proved to be diastereotopic according to the ^{1}H and ^{13}C NMR spectra, and coupling[16] of ^{31}P to C-1′, C-1, and C-2 was also observed.

The synthesis of 2-acetamido-2-deoxy-α-D-glucopyranosides by direct glycosylation at C-1[17] is challenging for a number of reasons, including the tendency of the acetamido carbonyl oxygen to participate at C-1 and also to complex with glycosyl donors, activating reagents, and other Lewis acids. For example, stereoselective *S*-glycosylation of an *O*,*O*-dialkylphosphorodithioate with a series of anomeric acetates and boron trifluoride etherate as the promoter gave *S*-glycosyl phosphorodithioates,[18] which are potential precursors to anomeric mercaptans. However, the reaction was not successful for 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose. While a number of avenues have been demonstrated for the preparation of β-GlcNAc thiols and thioglycosides,[19] the present method featuring intramolecular delivery of the sulfur atom and then *S*-coupling offers unique stereoselective access to two new α-GlcNAc thioconjugates.[20] Possibly other thioconjugates of **7** could be made by analogous coupling reactions.

Experimental Section

(3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(Acetoxymethyl-6,7-diacetoxy-2-methyl-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole (6).[11] A solution of 2.00 g (5.14 mmol) of 2-amino-2-deoxy-1,2,3,4,6-penta-*O*,*N*,*O*,*O*,*O*-acetyl-β-D-glucopyranose in 20 mL of toluene was treated with 1.75 g (4.37 mmol) of Lawesson's reagent and allowed to stir at 80 °C for 1.5 h. The reaction mixture was allowed to cool to room temperature, was neutralized by the addition of 200 mg of sodium bicarbonate, and then was chromatographed directly on silica with 3:7 ethyl acetate/dichloromethane as the eluant to provide 1.78 g (100%) of **6** as a yellow syrup: R_f 0.65 (19:1 dichloromethane/methanol); ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.14 (d, J = 7.2, 1 H), 5.44 (dd, J = 1.7, 3.1, 1 H), 4.83 (dd, J = 1.7, 3.1, 1 H), 4.32−4.42 (m, 1 H), 4.00 (app d, J = 4.4, 2 H), 3.43 (td, J = 4.4, 9.2, 1 H), 2.20 (d, J = 2.6, 3 H), 2.02, 1.97, and 1.97 (s, 3 H each); ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.2, 169.2, 168.9, 167.7, 88. 6, 76.4, 70.4, 69.07, 68.2, 63.0, 20.6, 20.6, 20.4, 20.4; ESI-MS m/z 346 $(M + H)^+$.

2-Acetamido-2-deoxy-3,4,6-tri-*O*,*O*,*O*-acetyl-1-thio-α-D-glucopyranose (8). A solution of 95 mg (0.275 mmol) of GlcNAc-thiazoline triacetate **6** in 1 mL of methanol was cooled to 0 °C and treated with 2 drops of trifluoroacetic acid and 2 drops of water. The reaction was allowed to warm to room temperature over 2 h period and then was concentrated to provide 100 mg (100%) of the mercaptan **7** as a colorless syrup: R_f 0.36 (19:1 dichloromethane/methanol); ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.93 (d, J = 7.9, 1 H), 5.75 (dd, J = 5.5, 6.6, 1 H), 5.11 (overlapped app t, J = 7.7, 1 H), 5.07 (overlapped app t, J = 7.7, 1 H), 4.39−4.52 (m, 1 H), 4.25−4.33 (m, 1 H), 4.20 (d, J = 12.3, 1 H), 4.07 (br d, J = 12.3, 1 H), 2.07, 2.02, 2.01, and 1.99 (s, 3 H each); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.7, 171.4, 170.8, 169.3, 78.5, 70.5, 68.9, 67.9, 61.7, 52.6, 22.9, 20.6, 20.5. The mercaptan **7** was *S*-acetylated for characterization as follows. A solution of 50 mg (0.137 mmol) of **7** in 2.5 mL of a (3:2) pyridine/dichloromethane mixture was treated with 140 μL (1.37 mmol) of acetic anhydride and a crystal of 4-(*N*,*N*-dimethylamino)pyridine and was allowed to stir for 2 h at room temperature. The solution was concentrated and chromatographed with 39:1 dichloromethane/methanol as the eluant to afford 56 mg (100%) of the pentaacetate **8** as a white solid, mp 140.5−141 °C: R_f 0.40 (19:1 dichloromethane/methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.11 (d, J = 4.8, 1 H), 5.61 (d, J = 8.7, 1 H), 5.14 (dd, J = 9.3, 9.9, 1 H), 4.86 (dd, J = 9.0, 10.8, 1 H), 4.63 (ddd, J = 4.8, 8.7, 10.8, 1 H), 4.23 (dd, J = 4.2, 12.6, 1 H), 4.02 (dd, J = 2.1, 12.3, 1 H), 3.88 (ddd, J = 2.1, 4.2, 10.2, 1 H), 2.43, 2.06, 2.02, 2.01, and 1.89 (s, 3 H each); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.3, 171.5, 170.5, 169.7, 168.9, 82.3, 72.0, 71.8, 67.5, 61.7, 51.6, 31.7, 23.1, 20.7 (2 C's), 20.6; LC-ESI-MS m/z 406 $(M + H)^+$.

***S*-(2-Acetamido-2-deoxy-1-thio-3,4,6-tri-*O*,*O*,*O*-acetyl-α-D-glucopyranosyl)-*N*-(*tert*-butoxycarbonyl)-L-cysteine Methyl Ester (9).** A stirred solution of 56 mg (0.154 mmol) of mercaptan **7** in 0.9 mL of DMF was quickly cooled with a −78 °C cooling bath and then treated with 141 μL (0.154 mmol) of a 1.09 M solution of sodium bis(trimethylsilyl)amide in DMF, followed by 44 mg (0.185 mmol) of *N*-Boc-3-iodoalanine methyl ester[13] in 0.1 mL of DMF. The solution was allowed to stir at room temperature for 24 h. The reaction mixture was concentrated and then chromatographed on silica with 3:7 ethyl acetate/dichloromethane as the eluant to give 61 mg (84%) of the glycosyl amino ester **9** as an oil: R_f 0.45 (19:1 dichloromethane/methanol); ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.72 (br d, J = 8.7, 2 H), 5.33 (d, J = 5.1, 1 H), 5.10 (t, J = 10.8, 1 H), 4.97 (dd, J = 9.3, 10.8, 1 H), 4.64 (br dt, J = 3.6, 7.2, 1 H), 4.49 (ddd, J = 5.4, 8.7, 11.1, 1 H), 4.25−2.33 (m, 2 H), 4.12−4.18 (m, 1 H), 3.75 (s, 3 H), 3.24 (dd, J = 4.8, 14.4, 1 H), 3.03 (dd, J = 3.6, 14.4, 1 H), 2.11, 2.03, 2.02, and 1.96 (s, 3 H each), 1.42 (s, 9 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.4, 170.6, 170.5, 169.7, 169.1, 155.2, 86.5, 80.3, 71.0, 69.0, 67.9, 62.0, 53.8, 52.7, 52.5, 35.8, 28.3, 23.3, 20.8, 20.8, 20.7; LC-ES-MS m/z 571.1 $(M + Li)^+$.

***S*-(2-Acetamido-2-deoxy-3,4,6-tri-*O*,*O*,*O*-acetyl-α-D-glucopyranosyl)-*O*,*O*-diallylthiophosphate (10).** Diallyl *N*,*N*-diisopropylphosphoramidite (117 μL, 0.445 mmol) was added by syringe to a stirred solution of 95 mg (0.262 mmol) of the mercaptan **7** and 55 mg (0.786 mmol) of tetrazole in 1.5 mL of acetonitrile at −40 °C. After 2 h, 380 μL (2.62 mmol) of *tert*-butyl hydroperoxide was added, and the reaction was allowed to stir an additional 2 h. The reaction was concentrated and then chromatographed with 1:1 ethyl acetate/dichloromethane as the eluant to give 85 mg (62%) of the thiophosphate **10** as a colorless oil: R_f 0.45 (39:1 dichloromethane/methanol, eluted twice); ^{1}H NMR (300 MHz, $CDCl_3$, assignments based on COSY analysis) δ 6.01−5.86 (m, 2 diastereotopic H-2's), 5.38 (tdd, J = 1.2, 1.5, 16.8, 2 H-3$_Z$'s), 5.30 (dd, J = 0.6, 11.1, 2 H-3$_E$'s), 5.18 (t, J = 9.6, H-4′), 5.03 (t, J = 9.6, H-3′), 4.50−4.70 (m, 4 H-1's, H-2), 4.16−4.29 (m, H-1′, H-5′, H-6′), 4.09 (dd, J = 1.6, 11.7, H-6′), 2.08, 2.05, 2.05 and 2.00 (s, 3 H each); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5, 170.5, 169.7, 167.0, 131.7 (d, J = 6), 131.6 (d, J = 7.1), 119.3, 85.6 (d, J = 3), 70.9, 70.6, 68.6 (d, J = 5.9), 68.5 (d, J = 6.3), 67.4, 61.6, 52.7, 23.3, 20.8, 20.8, 20.7; FAB-MS m/z 524 $(M + H)^+$.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **6**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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