Synthesis of α-GalNAc Thioconjugates from an α-GalNAc Mercaptan

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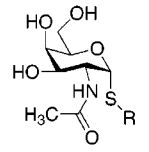
A variety of functionalized thioglycosides and other derivatives (**10**−**24**) of 2-acetamido-2-deoxy-1-thio-α-D-galactopyranose have been prepared in good yields and with high anomeric purities by S-substitution reactions of the sulfide anion or sulfur-centered radical from mercaptan **6**. Given the importance in nature of the α-GalNAc 1-*O*-linkage, and the greater chemical and biological stability of the corresponding 1-*S*-linkage, these thioconjugates may find application in studies of synthetic vaccines, enzyme inhibitors, glycomimetic scaffolds, and other complex carbohydrate systems.

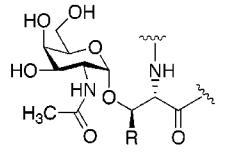
Introduction

Replacement of the linking or anomeric oxygen in oligosaccharides and other glycoconjugates with a sulfur atom leads to an important class of synthetic carbohydrates—the thioglycosides.[1] These compounds have been exploited as glycosyl donors,[2] enzyme inhibitors,[3] enzyme-resistant scaffolds,[4] and synthetic vaccines,[5] and have served as objects of study in their own right.[6] The versatility of organosulfur chemistry is reflected in the preparation of thioglycosides by forming the S-bond to the glycon or the aglycon or both,[7] with sulfur able to serve as a nucleophilic, electrophilic, or radical site, and in the option of preparing several different oxidation states at sulfur. The anomeric C−S bond is more stable than C−O under some conditions (acidic hydrolysis, enzymatic cleavage),[8] but more reactive under others, such as electrophilic activation leading to *O*-, *N*-, or *C*-glycosylation. The anomeric mercaptan of 1-thiopyranoses anomerizes much more slowly than the pyranose itself, particularly under basic conditions or when heated, and this is a property that can be exploited for the synthesis of thioglycosides.

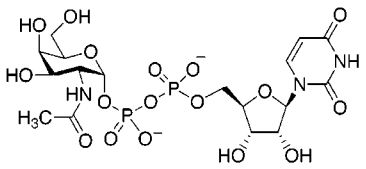
Following our development of a method for generating an α-GlcNAc mercaptan, and of reactions for linking it with β-iodoalanine and phosphoramidite,[9] we became interested in extending this chemistry to the synthesis of α-GalNAc[10] thioconjugates (**1**). Although this modification is modest in chemical terms (only the stereochemistry at C-4′ is altered), it is of great significance in the biological world. The Tn epitope (**2**), which features *N*-acetylgalactosamine α-*O*-linked to a serine or threonine residue, is one of the most common human tumor cell surface motifs, and scarcely occurs at all in normal cells. More elaborate tumor cell surface antigens (T, sialyl-T, sialyl-Tn, and 2,3-sialyl-T) also feature this linkage, and considerable effort has been expended in synthesizing these motifs and various analogues and linked versions in an effort to develop synthetic cancer vaccines.[11−16] The *S*-linked α-GalNAc-cysteine analogues[17,18] should exhibit enhanced resistance to chemical or enzymatic transformation, and thus are attractive synthetic targets. Indeed, a lipophilic thioconjugate of α-GalNAc was prepared as a Tn vaccine candidate by *S*-glycosylation of a protected cysteine, and was shown to exhibit immunostimulating activity in mice splenocytes at lower doses than the corresponding *O*-linked serine conjugate, presumably because of its greater stability toward α-glycosidases.[5]



1: α–GalNAc thioconjugate



2: Tn antigen (R = H,Me)



3: UDP–GalNAc

* To whom correspondence should be addressed.

(1) Horton, D.; Wander, J. D. In *The Carbohydrates. Chemistry and Biochemistry*; Pigman, W., Horton, D., Eds.; Academic Press: New York, 1980; Vol. IB, pp 799−842.

(2) Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015−9020 and references therein.

(3) Driguez, H. *Chembiochem* **2001**, *2*, 311−318. Driguez, H. *Top. Curr. Chem.* **1997**, *187*, 85−116 and references therein.

(4) Roy, R.; Hernandez-Mateo, F.; Santoyo-Gonzales, F. *J. Org. Chem.* **2000**, *65*, 8743−8746 and references therein.

(5) Bousquet, E.; Spadaro, A.; Pappalardo, M. S.; Bernardini, R.; Romeo, R.; Panza, L.; Ronisvalle, G. *J. Carbohydr. Chem.* **2000**, *19*, 527−541.

(6) Muñoz, J. L.; García-Herrero, A.; Asensio, J. L.; Auzennaeau, F.-I.; Cañada, F. J.; Jiménez-Barbero, J. *J. Chem. Soc., Perkin Trans. 1* **2001**, 867−872.

(7) Jobron, L.; Hummel, G. *Org. Lett.* **2000**, *2*, 2265−2267. Ibatullin, F. M.; Shabalin, K. A.; Jänis, J. V.; Selivanov, S. I. *Tetrahedron Lett.* **2001**, *42*, 4565−4567. Xu, W. Z.; Springfield, S. A.; Koh, J. T. *Carbohydr. Res.* **2000**, *325*, 169−176 and references therein.

(8) Cohen, S. B.; Halcomb, R. L. *J. Org. Chem.* **2000**, *65*, 6145−6152,

(9) Knapp, S.; Myers, D. S. *J. Org. Chem.* **2001**, *66*, 3636−3638.

(10) α-GalNAc refers to 2-acetamido-2-deoxy-α-D-galactopyranosyl as a component of glycoconjugates.

(11) Komba, S.; Meldal, M.; Werdelin, O.; Jensen, T.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1999**, 415−419.

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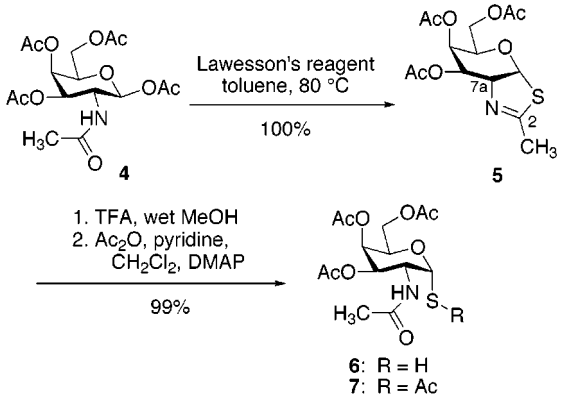
Scheme 1. Preparation of 2-Acetamido-2-deoxy-2,3,4-tri-*O*-acetyl-1-thio-α-D-galactopyranose (6)

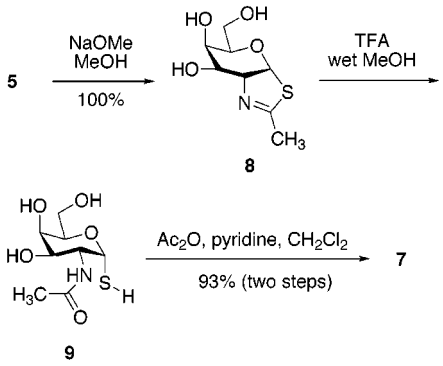


The serine/threonine α-GalNAc linkage also appears in a variety of other glycoproteins,[19] while other linked α-GalNAc units appear inter alia in the lipoteichoic acids of certain bacterial cell walls,[20] in pituitary hormones,[21] in the blood group A determinant,[22] and in the biological GalNAc donor species UDP-GalNAc (**3**).

Results and Discussion

2-Acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose (**4**; Scheme 1) was prepared as the pure β-anomer according to the literature procedure.[23] Treatment of **4** with Lawesson's reagent [2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide] in hot toluene caused conversion of the amide to the corresponding thioamide, and then cyclization with participation of the thiocarbonyl sulfur atom at the amoneric center produced the GalNAc-thiazoline **5**. The structure of **5** follows from the resemblance of its ^{1}H and ^{13}C NMR spectra to those of the isomeric GlcNAc-thiazoline,[9,24] including the five-bond coupling (J = 1.5 Hz) between *H*-7a and 2-C*H*$_3$. The thiazoline ring of **5** underwent hydrolysis with cleavage of the imine C−S bond (rather than the anomeric C−S bond) to give the acetamido β-mercaptan **6**, which was further characterized as the known *S*-acetate **7**.[25] No trace of the β-anomerof **6** or **7** was detected. Mercaptan **6** represents an attactive building block for the synthesis of α-GalNAc thioconjugates through linking reactions of the α-situated −SH with a variety of aglycon precursors and other spacers. For some linking reactions, such as those run in aqueous solution, the deprotected mercaptan **9** (Scheme 2) might be more appropriate. Therefore, **5** was deacetylated and then hydrolyzed to give **9**, whose identity was confirmed by reacetylation to generate **7**. Although **9** appeared by ^{1}H NMR analysis to be free of the β-anomer, some anomerization of **9** may have occurred during its acetylation, as the characteristic doublet at 5.16 ppm (J = 10 Hz),[25] reflecting a trace impurity, was observed in the spectrum of the crude reaction mixture.

Scheme 2. Preparation of 2-Acetamido-2-deoxy-1-thio-α-D-galactopyranose (9)



Mercaptan **6** was linked with a variety of coupling partners, resulting in the efficient preparation of a diverse family of potentially useful α-GalNAc thioconjugates (**10**−**24**) (Tables 1−3), each as single isomers. These conjugation reactions can be divided into three types: couplings under essentially neutral conditions (Table 1), couplings under basic (anionic) conditions (Table 2), and free radical couplings with alkenes (Table 3). They are the first nonglycosylative[5] examples of α-GalNAc thioconjugate synthesis.

Oxidative dimerization of **6** could be accomplished by treatment with 10% aqueous hydrogen peroxide,[26] but disulfide **10** (Table 1) was obtained in better yield and purity by treating a THF solution of **6** with iodine and pyridine. Glycosyl disulfides have previously been advanced as useful glycoconjugates.[26] Literature conditions[8,9] sufficed for conversion of **6** to the *S*-glycosyl phosphothioate **11**, a potential precursor to nucleoside diphosphate analogues (compare **3**) and other *S*-phosphorylated derivatives of 2-acetamido-2-deoxy-1-thio-α-D-galactopyranose. Benzoquinone coupled smoothly to 1 equiv of **6** in methanol solution; subsequent mild oxidation[27] gave in quantitative yield the quinone **12**, which may be viewed as a glycosylated cyclitol mimic.

Mitsunobu reactions of 1-thiopyranoses have been previously demonstrated to lead to thioconjugates.[28] In the case of **6**, coupling with 5 equiv of 2-butyne-1,4-diol resulted in the mono-Mitsunobu product **13**, a thioconjugate with the potentially useful butyne spacer. 4-Iodo-

(12) Winterfeld, G. A.; Ito, Y.; Ogawa, T.; Schmidt, R. R. *Eur. J. Org. Chem.* **1999**, *5*, 1167−1171.
(13) George, S. K.; Schwientek, T.; Holm, B.; Reis, C. A.; Clausen, H.; Kihlberg, J. *J. Am. Chem. Soc.* **2001**, *123*, 11117−11125 and references therein.
(14) Allen, J. R.; Harris, C. R.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 1890−1897 and references therein.
(15) Kihlberg, J.; Elofsson, M. *Curr. Med. Chem.* **1997**, *4*, 85−116.
(16) Peri, F.; Cipolla, L.; Rescigno, M.; La Ferla, B.; Nicotra, F. *Bioconjugate Chem.* **2001**, *12*, 325−328.
(17) Connolly, D. T.; Roseman, S.; Lee, Y. C. *Carbohydr. Res.* **1980**, *87*, 227−239.
(18) Zhu, Y.; van der Donk, W. A. *Org. Lett.* **2001**, *3*, 1189−1192.
(19) Brockhausen I. In *Glycoproteins*; Montreuil, J., Vliegenthart, J. F. G., Schachter, H., Eds.; Elsevier: Amsterdam, 1995; Vol. 29a, pp 201−259.
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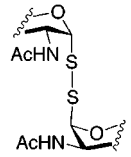
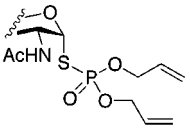
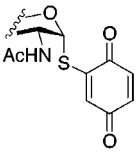
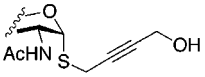
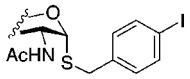
(21) Bielinska, M.; Boime, I. In *Glycoproteins*; Montreuil, J., Vliegenthart, J. F. G., Schachter, H., Eds.; Elsevier: Amsterdam, 1995; Vol. 29a, pp 565−587.
(22) Watkins, W. M. In *Glycoproteins*; Montreuil, J., Vliegenthart, J. F. G., Schachter, H., Eds.; Elsevier: Amsterdam, 1995; Vol. 29a, pp 313−390.
(23) Kim, T. Y.; Davidson, E. A. *J. Org. Chem.* **1963**, *28*, 2475−2476.
(24) Knapp, S.; Vocadlo, D.; Gao, Z.; Kirk, B.; Lou, J.; Withers, S. G. *J. Am. Chem. Soc.* **1996**, *118*, 6804−6805.
(25) Rakotomanomana N.; Lacombe, J.-M.; Pavia, A. A. *Carbohydr. Res.* **1990**, *197*, 318−323.
(26) Szilágyi, L.; Illyés, T.-Z.; Herczegh, P. *Tetrahedron Lett.* **2001**, *42*, 3901−3903 and references therein.
(27) Schnabelrauch, M.; Vasella, A.; Withers, S. G. *Helv. Chim. Acta* **1994**, *77*, 778−799.
(28) Falconer, R. A.; Jablonkai, I.; Toth, I. *Tetrahedron Lett.* **1999**, *40*, 8663−8666.

Table 1. α-GalNAc Thioconjugates Prepared from 6 under Neutral Conditions

conditions	product (yield)
I_2, pyridine, THF, 0 °C, 15 min	 **10** (100%)
i-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, CH_3CN, -40 °C, 2 h; then *t*-BuOOH, -40 °C, 2 h	 **11** (63%)
benzoquinone, MeOH, 30 min; then $PhI(OAc)_2$, 30 min	 **12** (100%)
2-butyne-1,4-diol, DMAD, Ph_3P, DMF, 24 h	 **13** (84%)
4-iodobenzyl alcohol, DMAD, Ph_3P, DMF, 24 h	 **14** (88%)

benzyl alcohol also coupled smoothly with **6** to afford **14**, a possible substrate for palladium-mediated reactions.

The sodium salt of **6** could be efficiently generated in DMF solution, and this mercaptide was coupled to several alkylating agents (Table 2). Alkylation with a *β*-iodoalanine[29] gave *S*-glycosylated cysteine derivative **15** without apparent epimerization at the α-carbon. This thioconjugate mimics the important Tn antigen (compare **2**). Reaction of sodio-**6** with *β*-propiolactone led to the 3-thiopropionic acid derivative **16**, the first product of a *β*-lactone/1-thiopyranose coupling of which we are aware. Aryl substitution with 1-fluoro-4-nitrobenzene gave the nitrophenyl thioglycoside **17** as expected,[30] and alkylation with 5-bromopentene and 4-nitrobenzyl bromide to give **18** and **19**, respectively, was also straightforward. The nitro groups of **17** and **19** could be reduced to amino groups for peptide coupling or other further transformation.

Radical additions[31] of mercaptans (even anomeric −SH[32]) to alkenes can be an efficient process for linking the two components under mild, neutral conditions, provided one of them is used in excess. Mercaptan **6** reacted smoothly with several commercially available alkenes to give the corresponding adducts (Table 3). The alkene was used in excess, with chloroform as a cosolvent, and the additions were carried out at reflux with initiation by

Table 2. α-GalNAc Thioconjugates Prepared from 6 under Basic (Anionic) Conditions

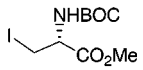
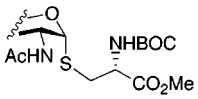
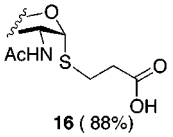
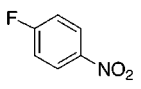
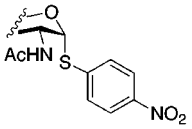
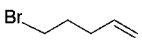
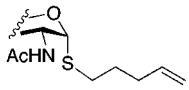
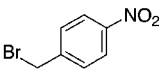
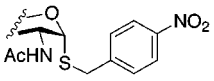
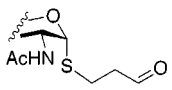
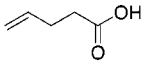
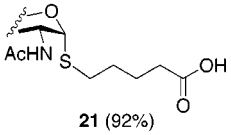
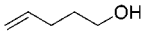
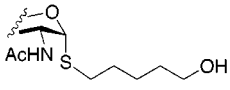
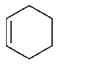
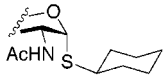
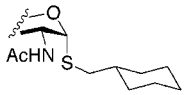
electrophile	product (yield)
	 **15** (87%)
	 **16** (88%)
	 **17** (89%)
	 **18** (93%)
	 **19** (78%)

Table 3. α-GalNAc Thioconjugates Prepared from 6 by Radical Addition to Alkenes

radical acceptor	product (yield)
	 **20** (81%)
	 **21** (92%)
	 **22** (90%)
	 **23** (81%)
	 **24** (81%)

azo(bis)isobutyronitrile. The electrophilic acceptor acrolein gave the fastest addition (15 min), whereas the more hindered cyclohexene required 3 h. The products **20**−**24** were isolated as single isomers. Three of them (**20**−**22**) possess useful linker functionality (carboxaldehyde, carboxylate, and hydroxyl, respectively) for further transformation.

(29) Stocking, E. M.; Schwarz, J. N.; Senn, H.; Salzmann, M.; Silks, L. A. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2443−2447.
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Conclusion

Mercaptan **6** is a versatile coupling partner for generating α-GalNAc thioconjugates by a variety of coupling reactions, as exemplified by **10**−**24**. Presumably other functionalized thioglycosides, including thiodisaccharides, could also be derived from **6** by using well-precedented mercaptan *S*-alkylation methods, such as triflate alkylation,[3] or conjugate addition to levoglucosenones.[33] Selective oxidation at sulfur or other modifications elsewhere in the aglycon could further extend this family. The availability of **6**, and for that matter the α-GlcNAc version as well,[9] allows for the preparation of hundred-milligram quantities of thioconjugates if required for applications involving subsequent synthetic steps, and further scaleup seems feasible. The α-GalNAc and α-GlcNAc thioconjugates can thus join other thioglycosides as useful probes of biological systems and as enzyme-stable components in biomimetic constructs.

Experimental Section

(3a*R*,5*R*,6*R*,7*R*,7a*R*)-5-(Acetoxymethyl-6,7-diacetoxy-2-methyl-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-d]thiazole (5). A stirred suspension of 360 mg (0.925 mmol) of 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranose (**4**) and 318 mg of Lawesson's reagent (0.785 mmol, 0.85 equiv) in 3.0 mL of toluene was heated at 80 °C for 1.5 h. The red, homogeneous reaction mixture was neutralized by the addition of 30 mg of sodium bicarbonate and then chromatographed directly on silica gel with 3:7 ethyl acetate/dichloromethane as the eluant to provide 319 mg (100%) of the thiazoline triacetate **5** as a yellow syrup: R_f 0.51 (1:1 ethyl acetate/dichloromethane); FAB-MS *m*/*z* 352.1 (M + Li) $^+$, 346.1 (M + H)$^+$.

2-Acetamido-2-deoxy-1-thio-3,4,6-tri-*O*-acetyl-α-D-galactopyranose (6). A solution of 319 mg of thiazoline **5** in 3.0 mL of methanol was stirred at 0 °C. Five drops of TFA and 5 drops of water were added, and the reaction was allowed to warm to room temperature and stir for 2 h. The reaction was concentrated and then chromatographed with 8:2 ethyl acetate/dichloromethane as the eluant to provide 335 mg (100%) of mercaptan **6** as a white foam: R_f 0.22 (19:1 dichloromethane/methanol); FAB-MS *m*/*z* 731.4 (2M + Li)$^+$.

2-Acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-1-thio-α-D-galactopyranose (7). A solution of 28 mg (0.0771 mmol) of **6** in 1.25 mL of a 3:2 pyridine/dichloromethane mixture was treated with 73 μL of acetic anhydride (0.771 mmol) and one crystal of DMAP and then stirred for 2 h. The reaction mixture was concentrated and then chromatographed on silica with 1:2 ethyl acetate/dichloromethane as the eluant to give 31 mg (99%) of the pentaacetate **7** as a white solid: mp 194−194.5 °C; R_f 0.38 (19:1 dichloromethane/methanol); FAB-MS *m*/*z* 412.1 (M + Li)$^+$.

(3a*R*,5*R*,6*R*,7*R*,7a*R*)-(6,7-Dihydroxy-5-(hydroxymethyl-2-methyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole (8). A stirred solution of 48 mg of **5** was dissolved in methanol and cooled to 0 °C. One drop of a 25% solution of sodium methoxide in methanol was added, and the reaction was allowed to warm to room temperature. The reaction was complete in 2 h according to TLC analysis. The reaction mixture was concentrated and then chromatographed on silica with 19:1 dichloromethane/methanol as the eluant to give 31 mg of **8** as a white solid: mp 157−158 °C; R_f 0.19 (9:1 dichloromethane/methanol); FAB-MS *m*/*z* 226.1 (M + Li)$^+$.

2-Acetamido-2-deoxy-1-thio-α-D-galactopyranose (9). A stirred solution of **8** in 1.0 mL of methanol was cooled to 0 °C, treated with 2 drops of TFA and 2 drops of water, and allowed to react for 6 h. The crude reaction mixture was concentrated to give 33 mg (100% crude yield) of **9**: R_f 0.28 (3:1 dichloromethane/methanol). The mercapto triol **9** was characterized by conversion to the known peracetate **7** (93% isolated yield) by treatment with acetic anhydride/pyridine as for **6**.

Bis-*S*-(2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl) Disulfide (10). Iodine (66 μL of a 0.79 M solution in THF, 0.052 mmol) was added in aliquots over a 15 min period to a solution of 32 mg (0.088 mmol) of mercaptan **6** in 0.75 mL of THF and 14 μL (0.176 mmol) of pyridine at 0 °C, whereupon a white precipitate formed and an orange color persisted. The reaction mixture was concentrated and then chromatographed on silica with 97:3 dichloromethane/methanol as the eluant to afford 32 mg (100%) of the disulfide **10** as a white solid: mp 115−116 °C; R_f 0.25 (19:1 dichloromethane/methanol); FAB-MS *m*/*z* 731.3 (M + Li)$^+$.

***S*-(2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl) *O,O*-Diallyl Phosphothioate (11).** Diallyl *N,N*-diisopropylphosphoramidite (37 μL, 0.140 mmol) was added by syringe to a stirred solution of 30 mg (0.083 mmol) of the mercaptan **6** and 17 mg of 1*H*-tetrazole in 0.5 mL of acetonitrile at −40 °C. After 2 h, 114 μL (0.83 mmol) of *tert*-butyl hydroperoxide was added, and the reaction was allowed to stir for an additional 2 h at −40 °C. The reaction was concentrated and then chromatographed on silica with 1:1 ethyl acetate/dichloromethane as the eluant to give 27 mg (63%) of the phosphothioester **11** as a colorless oil: R_f 0.20 (19:1 dichloromethane/methanol); FAB-MS *m*/*z* 530.0 (M + Li)$^+$.

***S*-(2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)-2-thiobenzoquinone (12).** A solution of freshly sublimed benzoquinone (12 mg, 0.110 mmol) in 0.65 mL of methanol was added to a stirred solution of 40 mg of mercaptan **6** (0.110 mmol) in 0.65 mL of methanol. After 30 min, the yellow color faded and the reaction was complete according to TLC analysis. The R_f of the presumed intermediate hydroquinone is 0.22 (19:1 dichloromethane/methanol). Iodobenzenediacetate (53 mg, 0.165 mmol) was added, and the reaction was stirred for an additional 30 min, during which time the yellow color returned. The reaction was concentrated and then chromatographed on silica with 1:1 ethyl acetate/dichloromethane as the eluant to give 52 mg (100%) of **12** as a yellow film: R_f 0.40 (19:1 dichloromethane/methanol); FAB-MS *m*/*z* 478.1 (M + 2H + Li)$^+$.

1-Hydroxy-2-butyn-4-yl 2-Acetamido-2-deoxy-1-thio-3,4,6-tri-*O*-acetyl-α-D-galactopyranoside (13). A 40% solution of dimethyl azodicarboxylate in toluene (65 μL, 0.172 mmol) was added by syringe to a stirred solution of 42 mg (0.115 mmol) of mercaptan **6**, 50 mg (0.190 mmol) of triphenylphosphine, and 50 mg (0.575 mmol, 5 equiv) of 2-butyne-1,4-diol in 1.2 mL of a 5:1 dichloromethane/DMF mixture at 0 °C. After 24 h the reaction was concentrated and then chromatographed on silica with 3:2 ethyl acetate/dichloromethane as the eluant to provide 42 mg (84%) of **13** as a colorless oil: R_f 0.21 (19:1 dichloromethane/methanol); FAB-MS *m*/*z* 438.1 (M + Li)$^+$.

(4-Iodophenyl)methyl 2-Acetamido-2-deoxy-1-thio-3,4,6-tri-*O*-acetyl-α-D-galactopyranoside (14). A solution of 50 mg (0.14 mmol) of **6** in 1 mL of dichloromethane was coupled to 42 mg (0.18 mmol) of iodobenzyl alcohol by using the Mitsunobu procedure described for **13**. Chromatography as for **13** provided 70 mg (88%) of **14** as a colorless oil: R_f 0.26 (19:1 dichloromethane/methanol); FAB-MS *m*/*z* 586.0 (M + Li)$^+$.

***S*-(2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)-*N*-(tert-butoxycarbonyl)-L-cysteine Methyl Ester (15).** A stirred solution of 56 mg (0.154 mmol) of GalNAc mercaptan **6** in 1 mL of DMF was quickly cooled with a dry ice/acetone bath and then treated with 141 μL (0.154 mmol) of a 1.09 M solution of bis(trimethylsilyl)amide in DMF followed by 44 mg (0.185 mmol, 1.2 equiv) of *N*-Boc-3-iodoalanine methyl ester in 0.1 mL of DMF. The solution was allowed to warm to room temperature and then stirred for 24 h. The reaction mixture was concentrated and then chromatographed on silica with 3:7 ethyl acetate/dichloromethane as the eluant to give 63 mg (87%) of **15** as a colorless oil: R_f 0.45 (19:1 dichloromethane/methanol); FAB-MS *m*/*z* 571.1 (M + Li)$^+$.

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***S*-(2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)-3-thiopropionic Acid (16).** A solution of 40 mg (0.110 mmol) of **6** in 0.75 mL of DMF was converted to its sodium mercaptide as described for **15**, and then 9 μL (0.143 mmol, 1.3 equiv) of β-propiolactone (*caution: highly toxic, cancer suspect agent*) was added. The reaction mixture was allowed to stir at room temperature for 24 h, concentrated, and then chromatographed on silica with 19:1 dichloromethane/methanol as the eluant to give 42 mg (88%) of **16** as a colorless oil: R_f 0.31 (9:1 dichloromethane/methanol); FAB-MS *m*/*z* 448.7 $(M + Li)^+$.

4-Nitrophenyl 2-Acetamido-2-deoxy-1-thio-3,4,6-tri-*O*-acetyl-α-D-galactopyranoside (17). A stirred solution of 37 mg (0.110 mmol) of mercaptan **6** in 0.75 mL of DMF was converted to its sodium salt as described for **15** and then treated with 14 μL (0.133 mmol) of 1-fluoro-4-nitrobenzene. The solution was allowed to stir at room temperature for 24 h, concentrated, and then chromatographed on silica with 1:2 ethyl acetate/dichloromethane as the eluant to give 47 mg (89%) of **17** as a white foam: R_f 0.59 (1:1 ethyl acetate/dichloromethane); FAB-MS *m*/*z* 491.1 $(M + Li)^+$.

4-Pentenyl 2-Acetamido-2-deoxy-1-thio-3,4,6-tri-*O*-acetyl-α-D-galactopyranoside (18). A stirred solution of 29 mg (0.080 mmol) of **6** in 0.50 mL of DMF was converted to its mercaptide as described for **15** and then treated with 12 μL (0.104 mmol, 1.2 equiv) of 4-pentenyl bromide. The solution was allowed to stir at room temperature for 24 h, concentrated, and then chromatographed on silica with 1:4 ethyl acetate/dichloromethane as the eluant to give 32 mg (93%) of **18** as a colorless oil: R_f 0.43 (7:3 dichloromethane/ethyl acetate); FAB-MS *m*/*z* 438.1 $(M + Li)^+$.

(4-Nitrophenyl)methyl 2-Acetamido-2-deoxy-1-thio-3,4,6-tri-*O*-acetyl-α-D-galactopyranoside (19). A stirred solution of 40 mg (0.110 mmol) of **6** in 0.75 mL of DMF was converted to its mercaptide as described above and then treated with 29 mg (0.132 mmol, 1.2 equiv) of *p*-nitrobenzyl bromide. The solution was allowed to stir at room temperature for 24 h, concentrated, and then chromatographed on silica with 1:2 ethyl acetate/dichloromethane as the eluant to give 43 mg (78%) of **19** as a colorless oil: R_f 0.41 (1:1 ethyl acetate/dichloromethane); FAB-MS *m*/*z* 505.7 $(M + Li)^+$.

***S*-(2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)-3-thiopropionaldehyde (20).** A solution of 46 mg (0.127 mmol) of **6** and 2 mg of AIBN in 2.4 mL of a 5:1 acrolein/chloroform mixture was heated at reflux for 15 min. The reaction mixture was cooled, concentrated, and then chromatographed on silica with 39:1 dichloromethane/methanol as the eluant to give 43 mg (81%) of **20** as a colorless oil: R_f 0.29 (19:1 dichloromethane/methanol); FAB-MS *m*/*z* 426.1 $(M + Li)^+$.

***S*-(2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)-5-thiopentanoic Acid (21).** A solution of 40 mg (0.110 mmol) of **6** and 10 mg of AIBN in 0.75 mL of a 1:2 4-pentenoic acid/chloroform mixture was heated at reflux for 1 h. The reaction mixture was cooled, concentrated, and then chromatographed on silica with 19:1 dichloromethane/methanol as the eluant to give 43 mg (92%) of **21** as a colorless oil: R_f 0.20 (19:1 dichloromethane/methanol); FAB-MS *m*/*z* 476.1 $(M + 2Li - H)^+$.

***S*-(2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)-5-thiopentanol (22).** A solution of 50 mg (0.137 mmol) of **6** and 15 mg of AIBN in 2.4 mL of a 1:1 4-pentenol/chloroform mixture was heated at reflux for 1 h. The reaction mixture was cooled, concentrated, and then chromatographed on silica with 39:1 dichloromethane/methanol as the eluant to give 55 mg (90%) of **22** as a colorless oil: R_f 0.3 (19:1 dichloromethane/methanol); FAB-MS *m*/*z* 456.7 $(M + Li)^+$.

Cyclohexyl 2-Acetamido-2-deoxy-1-thio-3,4,6-tri-*O*-acetyl-α-D-galactopyranoside (23). A solution of 50 mg (0.137 mmol) of **6** and 5 mg of AIBN in 2.2 mL of a 10:1 cyclohexene/chloroform mixture was heated at reflux. Additional 5 mg aliquots of AIBN were added after 1 and 2 h. After 3 h of total reaction time, the mixture was cooled, concentrated, and then chromatographed on silica with 1:3 ethyl acetate/dichloromethane as the eluant to give 52 mg (81%) of **23** as a colorless oil: R_f 0.50 (7:3 dichloromethane/ethyl acetate); FAB-MS *m*/*z* 452.1 $(M + Li)^+$.

Cyclohexylmethyl 2-Acetamido-2-deoxy-1-thio-3,4,6-tri-*O*-acetyl-α-D-galactopyranoside (24). A solution of 55 mg (0.151 mmol) of **6** and 2 mg of AIBN in 1.6 mL of a 3:1 methylenecyclohexane/chloroform mixture was heated at reflux. Additional 2 mg aliquots of AIBN were added after 30 min and 1 h. After 1.5 h of total reaction time, the reaction mixture was cooled, concentrated, and then chromatographed on silica with 2:1 dichloromethane/ethyl acetate as the eluant to give 56 mg (81%) of **24** as a colorless oil: R_f 0.33 (19:1 dichloromethane/methanol); FAB-MS *m*/*z* 466.2 $(M + Li)^+$.

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Supporting Information Available: Structures and numbering, tabulated ^1H and ^{13}C NMR data, and printed ^1H and ^{13}C NMR spectra for compounds **5**−**24**. This material is available free of charge via the Internet at http://pubs.acs.org.

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