*Glycoproteins*

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# The Direct Formation of Glycosyl Thiols from Reducing Sugars Allows One-Pot Protein Glycoconjugation**

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Glycoconjugates have become essential tools for the investigation of many biological processes,[1] and it is now well established that protein and lipid-bound carbohydrate units play essential roles in cell signaling regulation,[2] cellular differentiation,[3] and immune response.[4] In recent years, glycosyl thiols have become useful building blocks for the synthesis of certain glycoconjugates that may be considered to be analogues of glycopeptides and glycoproteins.[5–8] Their use has allowed specific glycosylation of peptides to form S-linked glycopeptides through alkylation[6] or conjugate-addition strategies.[7] Moreover, we have demonstrated that a combi-

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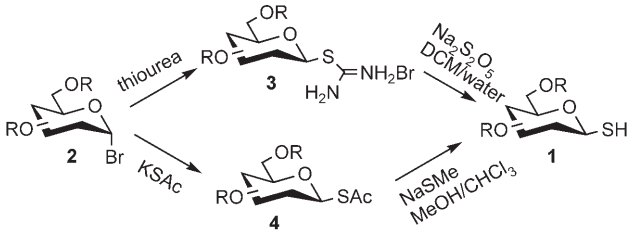
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

nation of site-directed mutagenesis followed by thiol-mediated chemoselective ligation can be used for site-selective protein glycosylation. In this two-step strategy (Glyco–SeS),[8] a cysteine residue is incorporated into the desired position within the protein backbone; the free thiol in the side chain of this cysteine is subsequently converted into a selenenylsulfide following exposure to phenylselenenyl bromide. This activated protein, upon the addition of a glycosyl thiol, is converted directly into the corresponding homogenous glycoprotein.

Given this value of such glycosyl thiols and their other potential uses, for example, as precursors[9] to widely used thioglycosides[10] and glycosyldisulfide[11] donors, it is all the more surprising that methods for producing these compounds are somewhat protracted and that until now no ready and general direct method for their preparation exists. Furthermore, thioglycosides and their resulting glycoconjugates often demonstrate increased chemo- and enzymatic stability and are tolerated by most biological systems.[12] Indeed, gold salts of thioaldoses[12b] are used in the treatment of rheumatoid arthritis and have recently been identified as potential blockers of transformed growth of lung-cancer cells.[12c]

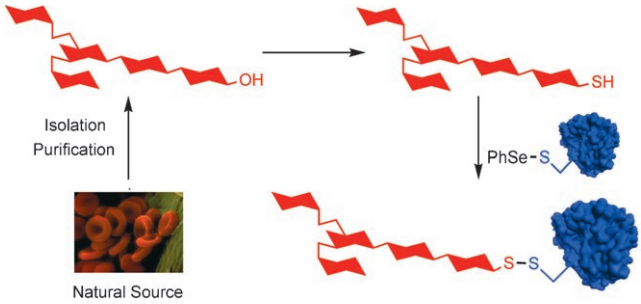
The most frequently employed method for the synthesis of glycosyl thiols **1** involves the treatment of glycosyl halides **2** with a sulphur nucleophile (either thiourea[13] or potassium thioacetate) in acetone,[14] followed by mild hydrolysis (Scheme 1).[15,16] Other methods include the use of anomeric



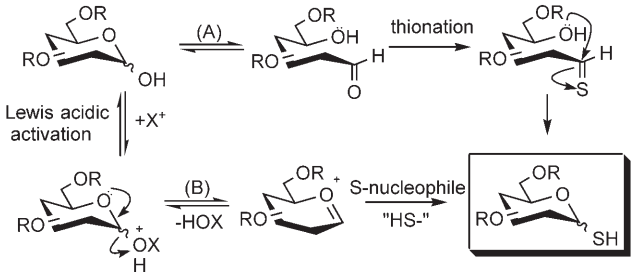

***Scheme 1.*** Current approaches for the synthesis of glycosyl thiols. DCM = dichloromethane.

acetates under Lewis acidic conditions[17] and the Birch reduction of anomeric thiobenzyl.[18] Defaye et al. have described the preparation of glucosyl thiols from D-glucose typically in yields of < 30 % by bubbling hydrogen sulfide into a solution of the deprotected sugar in hydrogen fluoride.[19] However, this procedure requires special precautions and generates a complex mixture of products.

We considered that a direct method of thiol formation in combination with Glyco–SeS[8] would allow a one-pot protein glycosylation method that could utilize sugars directly isolated from natural sources (Scheme 2).[20] With the goal of finding a more efficient strategy for the direct synthesis of glycosyl thiols, we speculated that a reagent that operated through a concerted Lewis acid activation and displacement might allow chemo- and regioselective thionation of C1 in the presence of other, perhaps unprotected, functionalities. However, to date, no such reagent or method exists. Such a C1-selective thionation might be considered mechanistically in two ways as shown in Scheme 3.



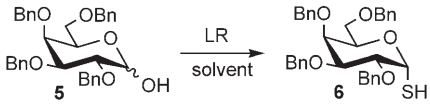

***Scheme 2.*** One-pot protein glycosylation with reducing sugars isolated from natural sources.




***Scheme 3.*** Two possible mechanisms for the direct formation of glycosyl thiols from reducing sugars through path A) open chain or path B) an oxacarbenium ion intermediate.

The mechanism of Lawesson's reagent[21] (LR) suggests that it might service either pathway, acting potentially as both an oxaphilic electrophile and a sulphur source. This reagent has been extensively used for the efficient conversion of a wide variety of carbonyl functions into their corresponding thiocarbonyl functionalities.[22] Moreover, an earlier report had highlighted a rare single use of LR in the conversion of a benzylic alcohol into the corresponding thiol,[23] thereby suggesting potential utility in $S_N1$ or $S_N1$-like pathways. Therefore, owing to the enhanced reactivity of the anomeric hydroxy group in $S_N1$-like processes, it was thought that this

***Table 1:*** Optimization of direct thionation reaction conditions.




| Entry | Equiv LR | $T$ [°C] | $t$ [h] | Solvent[a] | Yield [%] |
|---|---|---|---|---|---|
| 1A | 2 | RT | 48 | dioxane | no reaction |
| 1B | 0.6 | 80 | 5 | dioxane | 56 |
| 1C | 1.2 | 80 | 2.5 | dioxane | 83 |
| 1D | 2 | 80 | 2 | dioxane | 58 |
| 1E | 1.2 | reflux | 2 | dioxane | 84 |
| 1F | 2 | RT | 48 | toluene | no reaction |
| 1G | 0.6 | 80 | 5 | toluene | 54 |
| 1H | 1.2 | 80 | 2.5 | toluene | 70 |
| 1I | 1.2 | reflux | 2 | toluene | 75 |
| 1J | 1.2 | RT | 48 | acetonitrile | 14 |
| 1K | 0.6 | 80 | 5 | acetonitrile | 49 |
| 1L | 1.2 | 80 | 2.5 | acetonitrile | 62 |

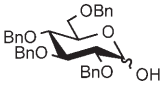
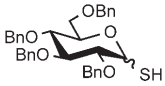
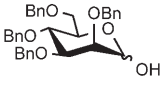
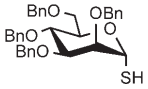
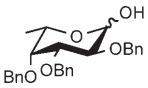
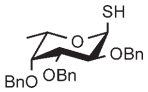
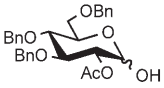
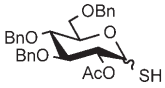
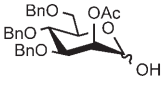
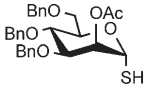
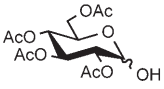
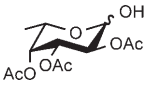
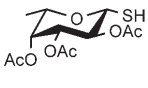
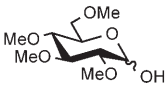
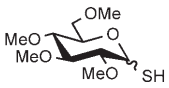
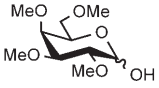
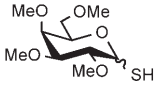
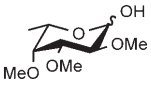
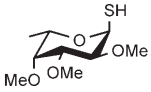
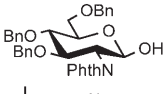
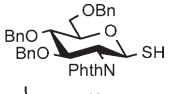
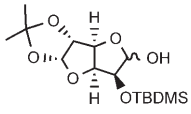
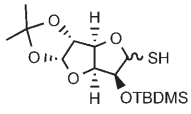
[a] Reactions conducted in anhydrous solvents (5 mL for a 200 mg scale reaction) and under an atmosphere of argon. Bn = benzyl.

procedure might proceed in an analogous fashion with sugar substrates. This approach appeared attractive for several reasons: 1) allowing direct formation of the anomeric thiosugars in one step from the corresponding anomeric alcohols; 2) application to differently protected and to unprotected sugars and 3) the possibility for direct site-selective glycosylation of proteins with sugars isolated from natural sources in just two steps and in one pot (Scheme 2).

Initial investigations (Table 1) used reducing sugar **5** as a test substrate. Encouragingly, a first attempt (Table 1, entry B) performed in anhydrous toluene at 80 °C by using 0.6 equivalents of LR for 5 h directly afforded thiogalactopyranose **6** in 54 % yield as a single α-anomer. Fortunately, 1-thiohexoses do not mutarotate under basic or neutral conditions.[24] Following this initial success, reaction conditions were optimized with respect to solvent system, temperature, reaction time, and equivalents of LR (Table 1). It was observed that prolonged reaction times and large excesses of LR were detrimental, resulting in the generation of numerous side products. Optimum conditions used anhydrous dioxane at 80 °C or above over a period of 2–3 h with 1.2 equivalents of LR affording **6** in a yield of 83–84 % (Table 1, entries C and E). Although yields in toluene were nearly comparable (Table 1, entry I), the use of more-polar dioxane opened up the exciting possibility of using deprotected sugars, which are not soluble in toluene or acetonitrile.

Next we explored the scope and limitations of this LR-mediated protocol (Table 2), demonstrating that this procedure is quite general and is applicable for the preparation of a variety of differently protected 1-thiosugars, including for the

***Table 2:*** Direct formation of glycosyl thiols using Lawesson's reagent.[a]

| Entry | Substrate[b] | | $T$ [°C] | $t$ | Equiv LR | Glycosyl thiol | | Yield[c] |
|---|---|---|---|---|---|---|---|---|
| 1 | see Table 1 | | | | | | | |
| 2 |  | **7** | 80 | 2.5 h | 1.2 |  | **8** | 82 %<br>α/β 2:1 |
| 3 |  | **9** | 80 | 2 h | 1.2 |  | **10** | 80 %<br>α anomer only |
| 4 |  | **11** | 80 | 1 h | 1.2 |  | **12** | 78 %<br>α anomer only |
| 5 |  | **13** | 80 | 3 h | 1.2 |  | **14** | 82 %<br>α/β 1:5 |
| 6 |  | **15** | 80 | 3 h | 1.2 |  | **16** | 85 %<br>α anomer only |
| 7 |  | **17** | 80<br>110<br>110 | 3 h<br>24 h<br>72 h | 1.2<br>1.5<br>2 | no product formed | **18** | –<br>32 % (63 %)<br>β anomer only<br>41 % (68 %)<br>β anomer only |
| 8 |  | **19** | 80<br>110 | 2.5 h<br>24 h | 1.2<br>1.5 |  | **20** | 43 % (65 %)<br>β anomer only<br>47 % (69 %)<br>β anomer only |
| 9 |  | **21** | 80 | 2.5 h | 1 |  | **22** | 75 %<br>α/β 2:5 |
| 10 |  | **23** | 80 | 1 h | 1 |  | **24** | 77 %<br>α/β 2:1 |
| 11 |  | **25** | 80 | 45 min | 1 |  | **26** | 85 %<br>α anomer only |
| 12 |  | **27** | 80 | 4.5 h | 1.2 |  | **28** | 82 %<br>β anomer only |
| 13 |  | **29** | 80 | 4 h | 1.2 |  | **30** | 74 %<br>3:1 |

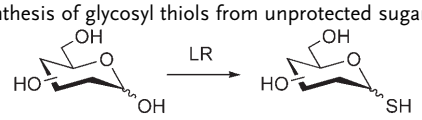
[a] Reactions conducted in anhydrous dioxane under an atmosphere of argon; see the Supporting Information for details. [b] All substrates were synthesized according to literature procedures; see the Supporting Information for details [c] Parentheses indicate yield based on recovered starting material. TBDMS = dimethyl-1,1-dimethylethyl-silyl, Phth = phthalimidyl.

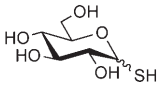
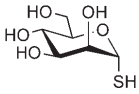
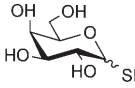
  

direct conversion of sugars fully protected with benzyl and methyl ether (78–85%). Partially *O*-benzyl-protected sugars with an *O*-acetyl function at C-2 were also converted to the corresponding glycosyl thiols in good yields (82–85%). Lower yields were observed on deactivated, so called disarmed,[25] systems such as acetylated sugars (41–43%); however, the starting material was largely recovered and could be recycled (yields based on recovered starting material 63–69%). Nitrogen functionality at C-2 in the form of phthalimide (Table 2, entry 12), and base and acid-labile protecting groups, such as silyl groups and acetonides (Table 2, entry 13), remained stable under the reaction conditions, thereby expanding the functional-group tolerance significantly.

Finally, application of this methodology to unprotected sugars was investigated (Table 3). The optimized conditions were applied to unprotected sugars, and after 48 h the formation of the desired thiol product and corresponding disulfide species was observed. Chromatographic separation of these from the LR decomposition products proved cumbersome and was exacerbated by further disulfide formation.[26] Two strategies were adopted to aid in isolation. First, an acetylation–deacetylation protocol afforded the corresponding glycosyl thiols **31a–c** in fair yields (Table 3). Alternatively, treatment with tributylphosphine (solution in

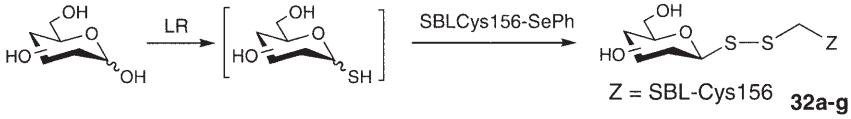
***Table 3:*** Synthesis of glycosyl thiols from unprotected sugars.

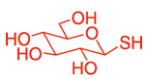
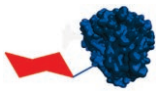
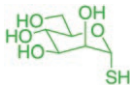
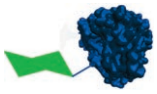
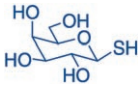
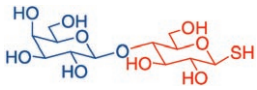
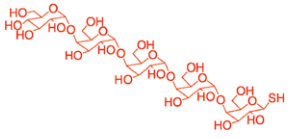
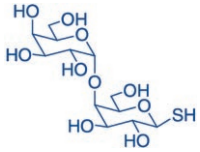
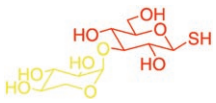



| Entry | Substrate | product | | Yield A[a] | Yield B[b] |
|---|---|---|---|---|---|
| 1 | Glc |  | **31a** | 61% α/β 1:9 | 71% α/β 1:6 |
| 2 | Man |  | **31b** | 48% α anomer | 63% α anomer |
| 3 | Gal |  | **31c** | 58% α/β 1:8 | 70% α/β 1:4 |

[a] Protocol A: 1) LR (1.5 equiv), anhydrous dioxane, 110°C. 2) $Ac_2O$, pyridine. 3) NaOMe, MeOH. [b] Protocol B: 1) LR (1.5 equiv), anhydrous dioxane, 110°C. 2) $Bu_3P$, MeOH/$CHCl_3$; see the Supporting Information for full details.

***Table 4:*** Two-step strategy for direct protein conjugation from deprotected reducing sugars through LR and Glyco–SeS.[8]




| Sugar | Thiol product[a] | Protein product[b] | | Conversion [%][c] | ESI-MS Found (calcd) |
|---|---|---|---|---|---|
| Glc |  |  | **32a** | >95 | 27066 (27064) |
| Man |  |  | **32b** | >95 | 27064 (27064) |
| Gal |  |  | **32c** | >95 | 27064 (27064) |
| Lactose (Galβ1,4Glc) |  |  | **32d** | >95 | 27234 (27225) |
| Maltopentaose (Glcα1,4)$_4$Glc |  |  | **32e** | >95 | 27722 (27711) |
| Galabiose (Gaα1,4Gal) |  |  | **32f** | >95 | 27224 (27225) |
| (Xylα1,3Glc) |  |  | **32g** | >95 | 27194 (27194) |

[a] Typically, LR (1.2 equiv) was added to a solution of the deprotected sugar in anhydrous dioxane and left to stir at 110°C for 48 h; see the Supporting Information for more details. [b] Typically, crude thiol (20–50 equiv), in water, was added to preactivated SBLS156C-SePh in CHES (70 mM), MES (5 mM), $CaCl_2$ (2 mM); pH 9.5. After 30 min at RT, the reaction was analyzed by LC–MS; [c] Conversion determined by ESI-MS.

$CHCl_3$/MeOH) reduced the resulting disulfides allowing the direct isolation of pure unprotected glycosyl thiols **31a–c** in 63–71% yields (Table 3). These results importantly demonstrated application to unprotected sugars providing a method to prepare glycosyl thiols suitable for protein glycosylation.

Following this key result, it was envisaged that our newly developed thionation procedure would offer a direct route from free sugars to glycoproteins by using our protein glycosylation strategy (Scheme 2). A variety of representative, free sugars (Table 4), including those isolated from N-(galabiose Galα1,4Gal)[27] and O-linked (Xylα1,3Glc) glycoproteins,[28] were treated with LR after the optimized conditions.[29] The subsequent addition of these sugars to a selenenylsulfide-activated single-cysteine mutant protein[8] (subtilisin *Bacillus lentus*, SBLS156C), gave complete conversion to the corresponding glycoproteins (Table 4).

In summary, we have established that Lawesson's reagent may be used in a direct and general manner for the preparation of glycosyl thiols from the corresponding anomeric lactols/reducing sugars. Notably, this procedure has also been shown to be fully compatible with unprotected sugars, the products of which can be directly used in our selenenylsulfide-mediated protein glycosylation strategy.[8] The result is a one-pot method for direct protein glycosylation. Further investigations into the use of LR are currently being explored by this laboratory.

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