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Glycal Assembly by the *in situ* Generation of Glycosyl Dithiocarbamates

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Abstract

Glycal assembly offers an expedient entry into β -linked oligosaccharides, but epoxyglycal donors can be capricious in their reactivities. Treatment with Et_3NH and CS_2 enables their *in situ* conversion into glycosyl dithiocarbamates, which can be activated by copper triflate for coupling with complex or sterically congested acceptors. The coupling efficiency can be further enhanced by *in situ* benzylation, as illustrated in an 11-step synthesis of a branched hexasaccharide from glucals in 28% isolated yield and just 4 chromatographic purifications.

General strategies for efficient and stereoselective glycosyl couplings continue to provide a challenge for synthetic method development, despite the many mechanistic insights and technological advances in glycosyl bond formation.^{1,2,3} In 1989, Halcomb and Danishefsky introduced an approach for the expedient synthesis of β -linked oligosaccharides, based on the stereoselective epoxidation of glycols followed by coupling with glycal acceptors under mild Lewis acid conditions.^{4,5} The “glycal assembly” strategy differs significantly from conventional methods of carbohydrate synthesis: the intermediate epoxyglycal donors can be used without workup or purification, and the C2 substituent of the glycosylation products can be modified at a later stage, permitting greater flexibility in synthetic design. Glycals and their epoxides have also proven amenable to solid-phase oligosaccharide synthesis.⁶

Despite its potential advantages, the efficiency of glycal assembly has been limited by the variable reactivity of the epoxyglycal donors, as well as by the range of acceptors that can serve as nucleophiles. For instance, tri-*O*-benzyl α -epoxyglucal is highly sensitive even to mild Lewis acids, and its use as a glycosyl donor has produced moderate yields at best.^{5,7,8,9,13} Efforts to improve coupling yields by optimizing the Lewis acid catalyst or by increasing the nucleophilicity of the acceptor have met with mixed success.^{10,11,12} Epoxyglycals can be converted into standard glycosyl donors for activation by stronger Lewis acid catalysts, but this requires additional steps to isolate and purify the synthetic

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Supporting Information Available. Complete experimental details and NMR spectra of enumerated compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

intermediates.^{13,14,15} Furthermore, the use of strong Lewis acids is incompatible with glycal acceptors, which defeats a primary merit of the glycal assembly method.

Here we describe coupling conditions that bring the glycal assembly strategy to its full potential. They are based on the one-pot conversion of epoxyglycals into glycosyl dithiocarbamates (DTCs), by treatment with diethylamine and CS₂, and the selective activation of the glycosyl DTCs with Cu(I) or Cu(II) triflate to initiate glycosyl coupling (Figure 1). This procedure is both highly β -selective and compatible with glycals and other sensitive glycosyl acceptors, and does not require purification of the glycosyl DTC intermediate.

The coupling reaction proceeds with a free C2 hydroxyl on the glycosyl donor, but can be enhanced further by *in situ* 2-*O*-acylation for the expeditious assembly of complex β -linked oligosaccharides. We demonstrate this with the concise synthesis of a branched hexasaccharide from the β -glucan family, whose members have strong immunomodulatory potential but whose structure–activity relationships have not been fully addressed, due to limited availability of well-characterized structures.^{16,17}

Glycosyl DTCs have been underutilized as donors compared to glycosyl sulfides or thioimides.^{1,18,19,20} However, the reduction potentials of DTCs are less than that of thiols ($E^0(\text{Et}_2\text{NCS}_2^-/\text{thiuram disulfide}) = -302 \text{ mV}$, versus $E^0(\text{PhS}^-/\text{PhSSPh}) = -541 \text{ mV}$),²¹ indicating facile ionization and mild activation conditions for glycosyl coupling.²² Furthermore, DTCs can be prepared by the simple condensation of amines with CS₂ in polar solvents,^{23,24} and are excellent nucleophiles for epoxide ring-opening. These features suggested to us that glycosyl DTCs could be ideal surrogates for glycal assembly.

The conversion of tri-*O*-benzyl glucal **1** into a glycosyl *N,N*-diethyldithiocarbamate (Et₂DTC) was achieved *in situ*, by stereoselective DMDO oxidation followed by treatment with Et₂NH and CS₂ (pre-mixed to form the Et₂DTC diethylammonium salt) to yield the desired glycosyl DTC with >95% β selectivity.²⁵ A survey of Lewis acids revealed that glycosyl DTCs were readily activated by Cu^{II}(OTf)₂ or Cu^IOTf·(C₆H₆)_{0.5} at low temperatures, using 2,4,6-(*t*Bu)₃-pyrimidine (TTBP) as an acid scavenger. We were pleased to find that Cu(OTf)_x activation could be performed using unpurified glycosyl DTCs, and was also compatible with both acid- and base-sensitive functional groups, allowing glycal donors to be coupled efficiently with a variety of acceptors without intermediate workup.

One-pot glycal couplings were performed using **1** or Glc β (1→6)glucal **2** as donors and 1.2–1.5 equiv of acceptors to produce β -glycosides **3–9** in good yields and selectivities (Table 1). The stereochemistry of all products or their 2-*O*-acetates was confirmed by ¹H NMR coupling constant or pulsed-field gradient (pfg) COSY analysis (see Supporting Information).

It is worth noting that the C2 hydroxyl of the intermediate DTC glycoside does not interfere with glycosyl coupling, as no side products corresponding to self-coupling or oligomerization could be identified. Instead, the C2 hydroxyl likely plays an active role in promoting β -glycosylation by generating a tetrahedral intermediate that hinders nucleophiles from approaching the α face (Figure 2). Complementary studies on the reactivity of glycosyl DTCs support this argument, and will be reported elsewhere.²⁶

While the scope of glycal assembly is substantially improved by the *in situ* generation of glycosyl DTCs (Table 1), this, alone, is not enough for the efficient coupling of glycal donors and acceptors into larger oligosaccharides, which is an important objective for its continued development.^{5,6} For instance, some 1,3- and 1,4-linked disaccharides could only be obtained in fair yields (Table 2, footnote d). We recognized that a 2-*O*-benzoate (Bz)

would be useful to enhance donor reactivity, as has been demonstrated with glycosyl thioimidates.^{20,27} We thus developed a chromatography-free method for preparing 2-*O*-Bz glycosides that would retain the processing advantages of glycal assembly. *In situ* conversion of glycal donors **1**, **10**, and **11** into their corresponding 2-*O*-Bz glycosyl DTCs proved to be facile and also compatible with CuOTf or Cu(OTf)₂-mediated glycosylation directly after workup. This allowed compounds **12–17** to be produced in high yields and with exclusive β selectivity (Table 2).

Benzoylation of the intermediate DTC glycoside was particularly beneficial in couplings involving less reactive glycal donors such as conformationally locked glucal **11**, with an increase in overall yields by more than 30%. Such modifications effectively remove any limitations previously associated with the efficiency of glycal assembly, including the formation of β -1,2-, β -1,3-, and β -1,4-linked oligosaccharides.^{13,28}

The modified glycal assembly conditions can be applied to a variety of naturally-occurring β -linked oligosaccharides and glycoconjugates. For example, glycal **15** is a precursor for the branched tetrasaccharide of a pregnane glycoside (Glc β (1 $\rightarrow$ 6)[Glc β (1 $\rightarrow$ 2)]Glc β (1 $\rightarrow$ 6)Glc β -) isolated from *Stelmatocrypton khasianum*.²⁹ To determine whether modified glycal assembly could provide an efficient entry into sterically congested glycans, we designed a synthetic route toward oligomers of [Glc β (1 $\rightarrow$ 3)[Glc β -(1 $\rightarrow$ 2)]Glc β (1 $\rightarrow$ 3)]_{*n*}-, a trisaccharide repeat unit of a branched β -glucan produced by probiotic strains of the *Pediococcus* genus.³⁰ The diverse biological activities of branched β -glucans have gained prominence in recent years, and include a broad range of human health benefits^{16,17,31} as well as regulatory functions in plants³² and bacterial biofilm formation.^{33,34} The crowded 1,2;1,3-linkage provides a stringent test for glycosyl coupling, as the steric congestion around the branched acceptor impedes both coupling efficiency and stereoselectivity.³⁵

The *p*-methoxybenzyl (PMB)-protected glucal donor **18** was coupled with glucal acceptor **19** using a slight variation of the previous reaction conditions. Epoxidation was performed at -30°C using a modest excess of DMDO, followed by treatment with 1.3 equivalents of Et₂DTC in MeOH (Scheme 1). In this case, the DTC salt also served as a DMDO scavenger to prevent over-oxidation of the 3-*O*-PMB ether. The crude glycosyl DTC intermediate was benzoylated as previously described, then subjected to CuOTf-mediated coupling with 1.5 equivalents of **19** to produce disaccharide glycal **20** in 71% overall yield from **18** after chromatography.

The 2'-*O*-Bz ester was saponified and then subjected to a one-pot coupling with two equivalents of donor **1** using our modified glycal assembly conditions, to produce branched trisaccharide glycal **14** in 92% yield from **20**.³⁶ Acceptor **21** (1.2 equiv) was prepared from donor **14** by oxidative cleavage of the 3'-*O*-PMB ether in 90% yield, then coupled under previously optimized conditions to produce **22** in 47% isolated yield (Scheme 1). The synthesis of hexasaccharide glycal **22** from glucals **18** and **19** was achieved in 11 steps and with only 4 chromatographic purifications, in an overall yield of 28%. Furthermore, **22** was produced as a single diastereomer despite the creation of 10 stereocenters. In this regard, the DMDO epoxidation of disaccharide glycal **20**, trisaccharide glycal **14** and hexasaccharide glycal **22** all furnished β -glycosyl DTCs with over 95% stereoselectivity, irrespective of their size.²⁵

Remarkably, the internal β -glucoside at the newly formed 1,3-linkage (ring D) of hexasaccharide **22** was significantly distorted from a chair (⁴C₁) conformation (*J*_{1,2} = 5.3 Hz), despite the torsional constraints imposed by the 4,6-benzylidene acetal. Additional NMR analysis of ring D suggested a twist-boat conformation, which may serve to alleviate

steric interactions with neighboring residues on ring B (see Supporting Information). This is in accord with earlier reports of anomalously low $J_{1,2}$ values for one or more pyranosides in protected derivatives of 1,3-linked β -glucans.³⁷ The β configuration of ring D was eventually confirmed by ^{13}C NMR analysis of its anomeric carbon ($\delta(\text{C1}_\text{D})$ 99.8 ppm; $^1J_{\text{CH}} = 166.1$ Hz),³⁸ and also by key dipolar couplings between rings B and D via 2D-ROESY analysis.

It is worth mentioning that hexasaccharide glycal **22** could also be oxidized into an epoxyglycal with DMDO, and coupled with trisaccharide acceptor **21** using modified glycal assembly conditions (see Supporting Information for partial characterization). However, the expected nonasaccharide glycal could not be isolated by chromatographic separation, possibly due to the sensitivity of the strained β -1,3-linkages.

In conclusion, glycal assembly via the *in situ* generation of 2-*O*-Bz glycosyl DTCs is competitive with standard methods of preparing β -linked oligosaccharides, while reducing the burden of isolating and purifying synthetic intermediates. Glycal donors can be coupled with sterically congested acceptors under mild conditions to enable the expedient assembly of complex β -glucans, including those whose internal linkages exhibit considerable torsional strain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

The glycal assembly approach to β -linked glycosides, aided by the *in situ* generation of a glycosyl dithiocarbamate prior to glycosyl coupling and chromatographic purification.

Figure 2.

Cu-mediated activation of the glycosyl DTC intermediate (L = coordination ligand). The participation of the C2 hydroxyl promotes the formation of β -glycosides.

Scheme 1.

Synthesis of a branched β-glucan by modified glycal assembly

Table 1

One-pot glycal coupling via *in situ* glycosyl dithiocarbamate (DTC) formation.

$\text{PO} \begin{array}{c} \diagup \text{O} \\ \diagdown \end{array} \begin{array}{c} \text{O} \\ \diagup \end{array} \xrightarrow[\text{ii) Et}_2\text{NH, CS}_2]{\text{i) DMDO}} \left[\begin{array}{c} \text{glycosyl} \\ \text{DTC} \end{array} \right] \xrightarrow[\text{R-OH (acceptor)}]{\text{iii) Cu(OTf)}_x, \text{TTBP}} \text{PO} \begin{array}{c} \diagup \text{O} \\ \diagdown \end{array} \begin{array}{c} \text{O} \\ \diagup \end{array} \text{OR}$ glycal donor			
donor	react cond ^a	coupling product	overall yield ^b
1	A	3	71% (65%) ^c
2	A	4	64%
2	A ^{d,e}	5	63%
2	B	6	70% ^f (46%) ^c
2	B	7	69% (56%) ^c
2	B	8	76% (52%) ^c
2	B ^{g,h}	9	63% ⁱ (32%) ^c

^a Standard conditions: (i) DMDO (1.5 equiv), 0 °C, CH₂Cl₂; (ii) CS₂ (1.05 equiv), Et₂NH (2.1 equiv), MeOH, rt; (iii) [Donor] = 0.15 M; Cu(OTf)_x, TTBP (2 equiv each), 4 Å mol sieves, -50 °C, 1:1 CH₂Cl₂: C₂H₄Cl₂, then acceptor (1.5 equiv); reaction quenched at 10 °C. A: Cu(OTf)₂; B: CuOTf. ^b Isolated yields of β isomer, unless stated otherwise. ^c Yields in parentheses (reported in references 4–7) are shown for comparison. ^d 1.3 equiv acceptor. ^e Reaction quenched at -30 °C. ^f Obtained as a 9:1 β:α mixture. ^g DMDO, -55 °C. ^h [Donor] = 0.2 M; 1.2 equiv acceptor. ⁱ Also isolated α isomer (7%).

Table 2

Oligosaccharide assembly via *in situ* benzylation of glycosyl DTC intermediates.

donor	react cond ^a	coupling product	overall yield ^b
1 A		12 81% ^c (48%) ^d
A		13 75% (56%) ^d
B ^e		14 82%
C		15 80%
10 ^f B		16 73% ^g
11 B		17 73% (42%) ^d

^a Standard conditions: (i) DMDO (1.5 equiv), 0 °C, CH₂Cl₂; (ii) CS₂ (1.05 equiv), Et₂NH (2.1 equiv), MeOH, rt; (iii) BzCl (4 equiv, 0.4 M), pyridine, rt; (iv) [Donor] = 0.15 M; Cu(OTf)_x, TTBP (2 equiv each), 4 Å mol sieves, -50 °C, 1:1 CH₂Cl₂:C₂H₄Cl₂; acceptor added at -50 °C; reaction quenched at 10 °C. A: Cu(OTf)₂ and 1.5 equiv acceptor; B: CuOTf and 1.5 equiv acceptor; C: CuOTf and 1.4 equiv donor. ^b Isolated yields of β isomer, unless stated otherwise. ^c Also isolated 1,2-orthobenzoate byproduct (6%). ^d Yields in parentheses for glycosyl couplings without *in situ* 2-O-benzylation, shown for comparison. ^e 1.3 equiv acceptor. ^f DMDO (1.5 equiv), -55 °C. ^g Glycosyl coupling without *in situ* 2-O-benzylation produces a 5:1 β:α mixture.