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Glycal Assembly by the in Situ Generation of Glycosyl Dithiocarbamates

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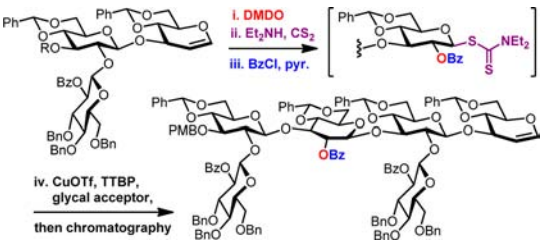
ABSTRACT



Glycal assembly offers an expedient entry into β-linked oligosaccharides, but epoxyglycal donors can be capricious in their reactivities. Treatment with Et_2NH 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 benzoylation, as illustrated in an 11-step synthesis of a branched hexasaccharide from glucals in 28% isolated yield and just four 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.[1,2a,b] In 1989, Halcomb and Danishefsky introduced an approach for the expedient synthesis of β-linked oligosaccharides, based on the stereoselective epoxidation of glycals followed by coupling with glycal acceptors under mild Lewis acid conditions.[3a,b] 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.[4]

Despite its potential advantages, the efficiency of glycal assembly has been limited by the variable reactivity of the epoxyglycal donors, and 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 only moderate yields.[3b,5−7,10] Efforts to improve coupling yields by optimizing the Lewis acid catalyst or the nucleophilicity of the acceptor have met with mixed success.[8,9a,b] Epoxyglycals can be converted into standard glycosyl donors for activation by stronger Lewis acid catalysts, but extra steps are needed to isolate and purify the synthetic intermediates.[10,11a,b] Furthermore, the use of strong Lewis acids is incompatible with

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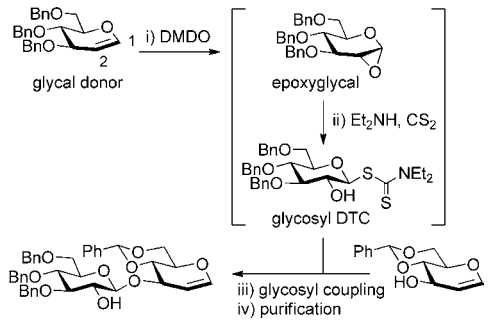

Figure 1. The glycal assembly approach to β-linked glycosides, aided by the in situ generation of a glycosyl dithiocarbamate prior to glycosyl coupling and chromatographic purification.

glycal acceptors, defeating 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 CS_2, 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, because of limited availability of well-characterized structures.[12,13]

Glycosyl DTCs have been underutilized as donors compared to glycosyl sulfides or thioimidates.[1,14–16] However, the reduction potentials of DTCs are less than that of thiols ($E^0(Et_2NCS_2^-$/thiuram disulfide) = −302 mV, versus $E^0(PhS^-$/PhSSPh) = −541 mV),[17] indicating facile ionization and mild activation conditions for glycosyl coupling.[18] Furthermore, DTCs can be prepared by the simple condensation of amines with CS_2 in polar solvents[19a,b] 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 DTC was achieved in situ, by stereoselective DMDO oxidation followed by treatment with Et_2NH and CS_2 (premixed to form the Et_2DTC diethylammonium salt) to yield the desired glycosyl DTC with >95% β selectivity.[20] A survey of Lewis acids revealed that glycosyl DTCs were readily activated by $Cu(OTf)_2$ or $CuOTf\cdot(C_6H_6)_{0.5}$ at low temperatures, using 2,4,6-(tBu)$_3$-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 Glcβ(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 ^{1}H NMR coupling constant or pulsed-field gradient (pfg) COSY analysis (see the 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.[21]

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.[3b,4] 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.[16,22] We thus developed a chromatography-free method of benzoylation that retains 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 both facile and compatible with CuOTf or $Cu(OTf)_2$-mediated

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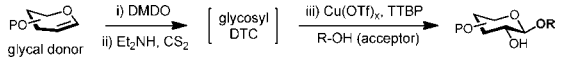
Table 1. One-Pot Glycal Coupling via in Situ Glycosyl Dithiocarbamate (DTC) Formation



donor	react cond[a]	coupling product	overall yield[b]
1	A	3	71% (65%)[c]
	A	4	64%
	A[d,e]	5	63%
	B	6	70%[f] (46%)[c]
	B	7	69% (56%)[c]
	B	8	76% (52%)[c]
2	B[g,h]	9	63%[i] (32%)[c]

[a] Standard conditions: (i) DMDO (1.5 equiv), 0 °C, CH_2Cl_2; (ii) CS_2 (1.05 equiv), Et_2NH (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 $CH_2Cl_2/C_2H_4Cl_2$, 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 refs 3−5) are shown for comparison. [d] 1.3 equiv of 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 of acceptor. [i] Also isolated α isomer (7%).

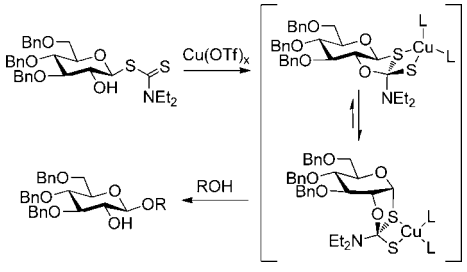
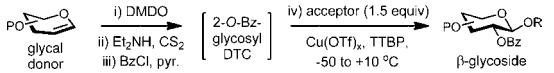


Figure 2. Cu-mediated activation of the glycosyl DTC intermediate (L = coordination ligand). The participation of the C2 hydroxyl promotes the formation of β-glycosides.

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**,

Table 2. Oligosaccharide Assembly via in Situ Benzoylation 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, CH_2Cl_2; (ii) CS_2 (1.05 equiv), Et_2NH (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 $CH_2Cl_2/C_2H_4Cl_2$; acceptor added at −50 °C; reaction quenched at 10 °C. (A) $Cu(OTf)_2$ and 1.5 equiv of acceptor; (B) CuOTf and 1.5 equiv of 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*-benzoylation, shown for comparison. [e] 1.3 equiv of acceptor. [f] DMDO (1.5 equiv), −55 °C. [g] Glycosyl coupling without in situ 2-*O*-benzoylation produces a 5:1 β:α mixture.

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.[10,23]

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 (Glcβ(1→6)[Glcβ(1→2)]Glcβ-(1→6)Glcβ-) isolated from *Stelmatocrypton khasianum*.[24] To determine whether modified glycal assembly could provide an efficient entry into sterically congested glycans, we designed a synthetic route toward oligomers of [Glcβ(1→3)[Glcβ-(1→2)]Glcβ(1→3)]$_n$-, a trisaccharide repeat unit of a branched β-glucan produced by probiotic strains of the *Pediococcus* genus.[30] The diverse biological

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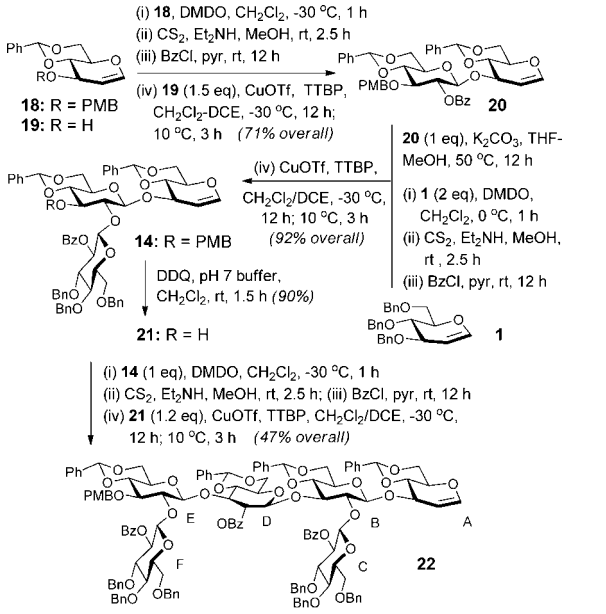
activities of branched β-glucans have gained prominence in recent years and include a broad range of human health benefits[12,13,26] as well as regulatory functions in plants[27] and bacterial biofilm formation.[28,29] The crowded 1,2;1,3-linkage provides a stringent test for glycosyl coupling, as the steric congestion around the branched acceptor impedes both coupling efficiency and stereoselectivity.[30]

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 using a modest excess of DMDO, followed by treatment with 1.3 equiv of Et_2DTC in MeOH (Scheme 1). In this case, the DTC salt also served as a DMDO scavenger to prevent overoxidation of the 3-*O*-PMB ether. The crude glycosyl DTC intermediate was benzoylated, then subjected to CuOTf-mediated coupling with 1.5 equiv of **19** to produce disaccharide glycal **20** in 71% overall yield from **18** after chromatography.

The 2′-*O*-Bz ester was saponified and subjected to a one-pot coupling with 2 equiv of **1** using our modified glycal assembly conditions, to produce branched trisaccharide glycal **14** in 92% yield from **20**.[31] Acceptor **21** (1.2 equiv) was prepared from donor **14** by oxidative cleavage of the 3′-*O*-PMB ether in 90% yield and then subjected to CuOTf-mediated coupling 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 four 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, we note that the DMDO epoxidation of disaccharide glycal **20**, trisaccharide glycal **14**, and hexasaccharide glycal **22** all furnished glycosyl DTCs with $>95\%$ β selectivity.[20]

Remarkably, the internal β-glucoside at the newly formed 1,3-linkage (ring D) of hexasaccharide **22** was significantly distorted from a chair ($^4C_1$) 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 the 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.[32] The β configuration of ring D was eventually confirmed by ^{13}C NMR analysis of its anomeric carbon ($\delta(C1_D)$ 99.8 ppm; $^1J_{CH}$ = 166.1 Hz)[33]

Scheme 1. Synthesis of a Branched β-Glucan by Modified Glycal Assembly



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 the Supporting Information for partial characterization). However, the expected nonasaccharide glycal could not be isolated by chromatographic separation, possibly because of 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.

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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.

The authors declare no competing financial interest.

(31) We note that glycal coupling with donor as the limiting agent is also high-yielding (Table 2, entry 3) and permits recovery of excess acceptor, but limiting the acceptor is more practical in this case because of ease of purification.

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