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Macrocyclic Bis-Thioureas Catalyze Stereospecific Glycosylation Reactions†

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Abstract

Carbohydrates are involved in nearly all aspects of biochemistry, but their complex chemical structures present long-standing practical challenges to their synthesis. In particular, stereochemical outcomes in glycosylation reactions are highly dependent on the steric and electronic properties of coupling partners, and thus, carbohydrate synthesis is not easily predictable. Here, we report the discovery of a macrocyclic bis-thiourea derivative that catalyzes stereospecific invertive substitution pathways of glycosyl chlorides. The utility of the catalyst is demonstrated in the synthesis of 1,2-trans-, 1,2-cis-, and 2-deoxy-β-glycosides. Mechanistic studies are consistent with a cooperative mechanism in which an electrophile and a nucleophile are simultaneously activated to effect a stereospecific substitution reaction.

Main Text

Carbohydrates are essential constituents of all living organisms, providing such vital functions as energy storage, structural support, and signaling capabilities (1). For well over a century, intensive effort has been directed towards the development of methods and strategies for the chemical synthesis of simple and complex sugars, culminating in many extraordinary achievements in oligo and polysaccharide synthesis (2–5). However, the lack of a unified strategy for the synthesis of complex carbohydrates presents a roadblock in the glycosciences. Stereochemical control in the construction of glycosidic linkages lies at the heart of the challenge (6–7). Here we present a catalytic method for β-selective glycosylations, providing a unified approach to the construction of 1,2-cis-, 1,2-trans-, and 2-deoxy-β-glycosidic linkages. The catalyst is shown to operate in a manner reminiscent of the cooperative mechanisms employed by glycosyltransferase enzymes.

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The stereochemical outcome of glycosylation reactions is influenced to varying degrees by the steric and electronic properties of the coupling partners, and the problem of stereoselective glycosylation has historically been addressed by tailoring the protecting groups and coupling method to achieve high diastereoselectivity (8, 9). However, such substrate-controlled methods rely by definition on the exact structure of the substrates, and as a result lack in generality. An alternative strategy has emerged wherein control over stereoselectivity in glycosylations would rely on the agency of an external, synthetic catalyst (10–19), though no general solutions have yet been identified.

We considered the two classical limiting mechanisms of nucleophilic substitution reactions, S_N1 and S_N2, as possible templates for the development of catalytic glycosylation strategies (Fig. 1) (20–23). Catalysts promoting stereoselective glycosylation via S_N1 pathways would have to bias the addition of the nucleophilic partner to either diastereotopic face of an oxocarbenium intermediate generated by ionization of the electrophilic partner (Fig. 1A). Catalyst-controlled enantioselectivity is well-precedented in additions to simple prochiral cationic intermediates including oxocarbenium ions (Fig. 1D) (24, 25). However, designing catalysts capable of overriding the stereochemical biases of chiral glycosyl electrophiles presents a formidable challenge, particularly with any degree of generality across the broad spectrum of structures of interest (26, 27).

A fundamentally different approach to catalysis of glycosylation would rely on promotion of stereospecific (S_N2) substitution, such that the stereochemistry of the product would be dictated entirely by the configuration of the electrophile at the anomeric position (Fig. 1B). A general solution to stereocontrolled glycosylation by this strategy would require facile access to stereochemically pure glycosyl electrophiles, and effective promotion of invertive substitution by the catalyst. Glycosyltransferases have been shown to operate by this principle, through cooperative activation of both nucleophiles and α-glycosyl phosphate to produce β-glycosidic linkages (Fig. 1C) (28). In either catalytic approach, competing substitution mechanistic pathways must be minimized to achieve high stereoselectivity.

Both the S_N1 and S_N2 strategies outlined above require leaving group dissociation. Our research group has developed an extensive array of chiral hydrogen-bond donor catalysts that promote stereoselective nucleophilic substitution reactions via abstraction of anionic leaving groups (29). We therefore chose to explore the effect of such catalysts on the reaction of neutral alcohols with glycosyl chlorides. The 1,2-cis-*O*-glycosylation of α-mannosyl chloride (**1**) is a prototypical case in which formation of the β-glycosidic linkage is strongly disfavored, both sterically and electronically (Fig. 2) (30). As expected, glycosylation with benzyl alcohol in the absence of a catalyst was found to afford α-product predominantly (84:16 α:β). A wide variety of chiral monomeric urea and thiourea derivatives (e.g., **3**) were evaluated as potential catalysts, but poor reactivity and α/β selectivity (*ca.* 50:50) were observed in all cases. In contrast, the bis-thiourea (**4**), which was designed to promote anion binding through cooperative hydrogen-bonding interactions and demonstrated previously to be a more reactive analog of **3** (31), overturned the intrinsic substrate bias to afford the β-product (**2**) selectively. The preference for the β-mannoside was observed in several solvents (Table S1), with reactions carried out in *o*-dichlorobenzene providing the best reactivity and selectivity at a relatively high concentration (0.5 M). The

HCl byproduct can be scavenged through the use of inorganic bases, or most conveniently using isobutylene oxide (IBO) as an electrophilic trap (Table S2).

Only catalysts bearing two precisely linked thiourea or urea groups were found to promote efficient and β-selective glycosylation in the model reaction. For example, analogs of bis-thiourea **4** bearing linker units of different length or amino acid components of opposite configuration were ineffective catalysts in the model reaction (Table S3). In contrast, the macrocyclic bis-thiourea **5** promoted the glycosylation with increased yield and selectivity relative to **4**, and a further improvement in catalyst performance resulted from replacement of the chiral arylpyrrolidine moiety with indoline as in **6** (88% yield, 8:92 α:β). The C_2-symmetric design of **6** offers additional practical advantages as it greatly simplifies catalyst synthesis: starting from known amino acid, **6** was synthesized in 7 steps and 41% yield, with homodimerization as the ultimate step (32).

The stereochemical outcome of the glycosylation reaction was found to depend almost entirely on the configuration of the electrophilic partner. Invertive substitution was observed with both α- and β-configured glucosyl chloride with methanol as a nucleophile (Fig. 2B). Consistent with a stereospecific process, neither the relative stereochemical relationship between the catalyst and electrophile nor the relationship between the reacting partners had a major influence. Both (*R*,*R*)-**6** and (*S*,*S*)-**6** catalyzed the model glycosylation of α-mannosyl chloride (**1**) with benzyl alcohol with nearly identical selectivities (Fig 2A); similarly, glycosylation of 2-deoxy-α-glucosyl chloride (**9**) with (+)-menthol or (–)-menthol proceeded with comparable preference for the β-products (Fig. 2C).

An important practical consequence of this stereochemical independence is that the reaction is compatible with many different reacting partners (Fig. 3). For example, galactosyl chloride can be efficiently and selectively coupled to a variety of glycosyl nucleophiles to afford β(1,6)- and β(1,3)-linkages (**12–14**). Methyl-protected nucleophiles were employed to facilitate NMR analysis; however, more easily cleaved benzyl protecting groups can also be used. For instance, disaccharide **14**, which contains benzylidene acetal and benzyl protecting groups, was obtained in good yield and selectivity. However, more challenging β(1,4)-linkages could only be produced if steric demand in the nucleophile was reduced as in **15**.

Glycosyl chlorides derived from a wide variety of simple carbohydrates could be used to generate β(1,6)- and β(1,3)-mannosides (**16** and **17**), 1,2-cis-β-L-rhamnosides (**18** and **19**), and 2-deoxy- and 2,6-dideoxy-β-glycosides (**20** and **21**) (30, 33). Furthermore, in all systems derived from fucose (**22** and **23**), xylose (**24** and **25**), 2-azidogalactose (**26** and **27**), glucose (**28**), 2-acetamidoglucose (**29**), and 2-acetamidogalactose (**30** and **31**), good to excellent β-selectivities were observed. No oxazolidine formation was observed in the preparation of **29–31**, allowing direct access to β-*N*-acyl disaccharides without the use of nitrogen protecting groups. Iterative glycosylations are also feasible. For example, conversion of disaccharide **28** to its corresponding chloride, followed by glycosylation under the same conditions yielded trisaccharide **32** with excellent diastereoselectivity.

Overall, this catalytic system provides a general means for the selective formation of glycosidic bonds. The above observations of stereospecific inversion, insensitivity to relative

catalyst/substrate and nucleophile/electrophile stereochemical relationships, and broad scope are best reconciled with an S_N2 mechanism. In general, leaving group activation would be expected to promote both the S_N1 and S_N2 pathways, but nucleophile activation would promote the S_N2 mechanism selectively. We considered whether the alcohol nucleophile is activated by a Lewis basic interaction with an amide carbonyl in the catalyst (Fig. 4). Replacement of the indoline amides in bis-urea **33** with less basic isopropyl esters as in **34** resulted in substantially reduced reactivity and selectivity (Fig. 4A) (34). Furthermore, DFT calculations (M06-2X/6-31G*/PCM) on the concerted glycosylation of glucosyl chloride by methanol predict a loose and asynchronous transition structure with a hydrogen bond between the indoline amide carbonyl oxygen and the methanol hydroxyl group (Fig. 4B). However, the relationship between Lewis basicity and catalytic efficiency is complex. For example, pyrrolidine amide catalyst **35**, which possesses a more Lewis basic carbonyl group than **8**, was found to be less effective (Fig. 4A). We hypothesize that catalysts bearing stronger general acid or general base components may be more prone to unproductive catalyst aggregation (35).

To probe the role of Lewis basicity of the catalyst substituents without interference from competing pathways, we measured a series of competitive secondary hydrogen/deuterium kinetic isotope effects (SDKIEs) in the glycosylation reaction (Fig. 4A) (36). For the β-products, the SDKIEs at the anomeric C-H bond were determined to be normal ($k_H/k_D > 1$) and increased with the Lewis basicity of the catalyst, reflecting an increase in the forming carbon-oxygen bond distance in the transition state as the reactivity of the nucleophile increases (37). In contrast, no such trend is observed in the case of the minor α-diastereomers. Relatively large SDKIEs with similar values for the three different catalysts ($k_H/k_D > 1.20$) and the intrinsic α-bias of the mannose oxocarbenium intermediate are consistent with a competing S_N1 mechanism.

The invertive catalytic system described here provides access to 1,2-trans-, 1,2-cis, and 2-deoxy-β-glycosides for a variety of glycosyl coupling partners. By obviating the need for specific protecting groups and reaction conditions, we anticipate that this catalyst may find broad application in the efficient synthesis of carbohydrates. The picture that emerges for glycosylation catalyzed by the macrocyclic bis-thiourea **6** involves selective promotion of a stereospecific S_N2 mechanism by the proposed simultaneous activation of the reacting partners, in a manner that is loosely reminiscent of the mechanisms employed by natural glycosyltransferases (28). In both catalytic strategies, readily accessible α-configured glycosyl electrophiles are transformed to β-*O*-glycosides linkages via invertive substitution. In many cases, the corresponding α-*O*-glycosidic linkages are already accessible through existing methods. However, the prevalence of glycosyltransferases that generate α-*O*-glycosides through putative double-inversion mechanisms (28) suggests the possibility of designing retentive small-molecule catalysts analogous to **6**.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Complete experimental and characterization data are provided as supplementary material. Financial support from the NIH (GM116249 and GM43214), the NSF (predoctoral fellowship for Y.P.), the Dreyfus Foundation (postdoctoral fellowship to K.C. H.), and the Alexander von Humboldt Foundation (postdoctoral fellowship to N.K.) are gratefully acknowledged.

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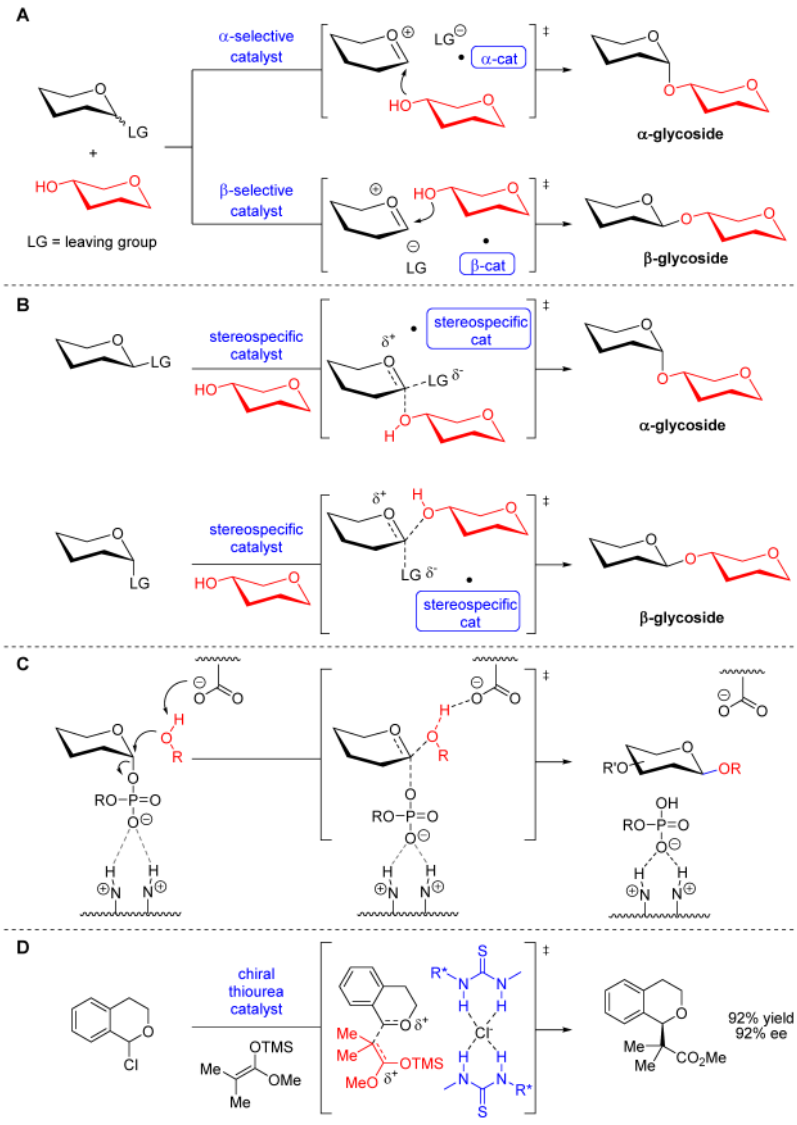

Fig. 1.

Strategies for catalytic glycosylation. (**A**) Catalyst-controlled stereoselective glycosylation. (**B**) Catalyst-mediated stereospecific glycosylation. (**C**) Glycosyltransferase-catalyzed stereospecific glycosylation. (**D**) Anion-binding catalysis with oxocarbenium intermediates.

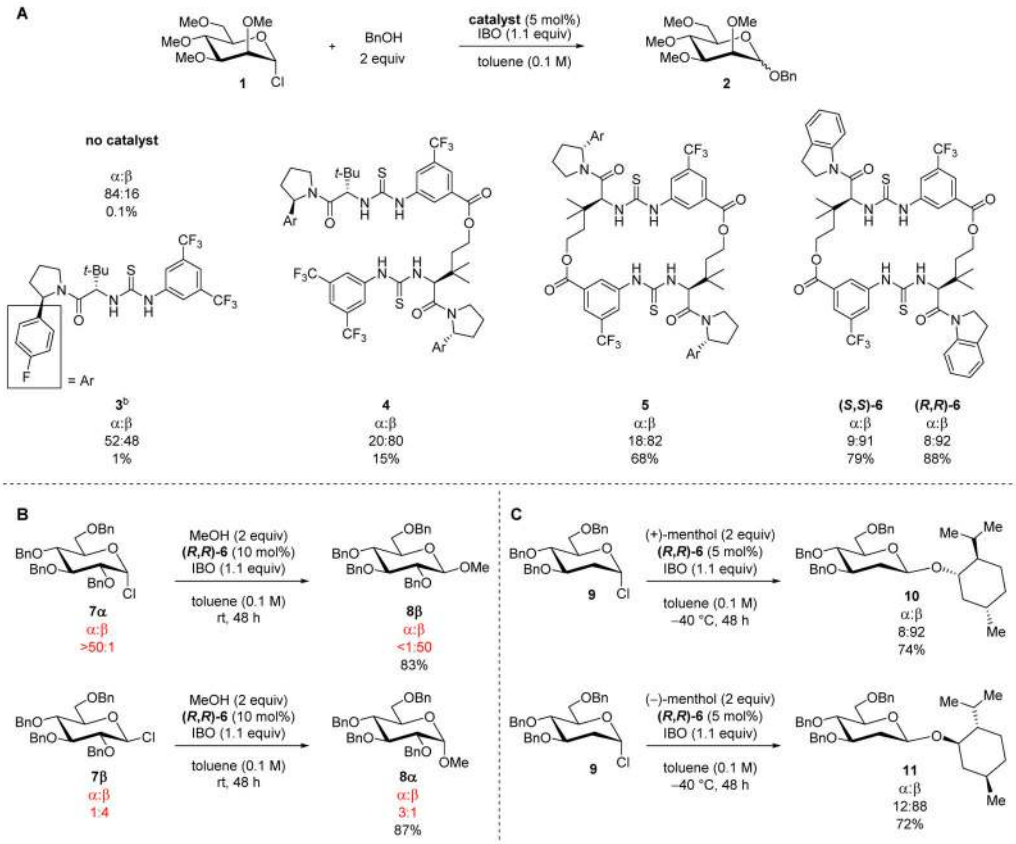

Fig. 2.

Evaluation of reaction components. (**A**) Catalyst optimization. (**B**) Effect of glycosyl chloride configuration at the anomeric center. (**C**) Effect of nucleophile chirality.

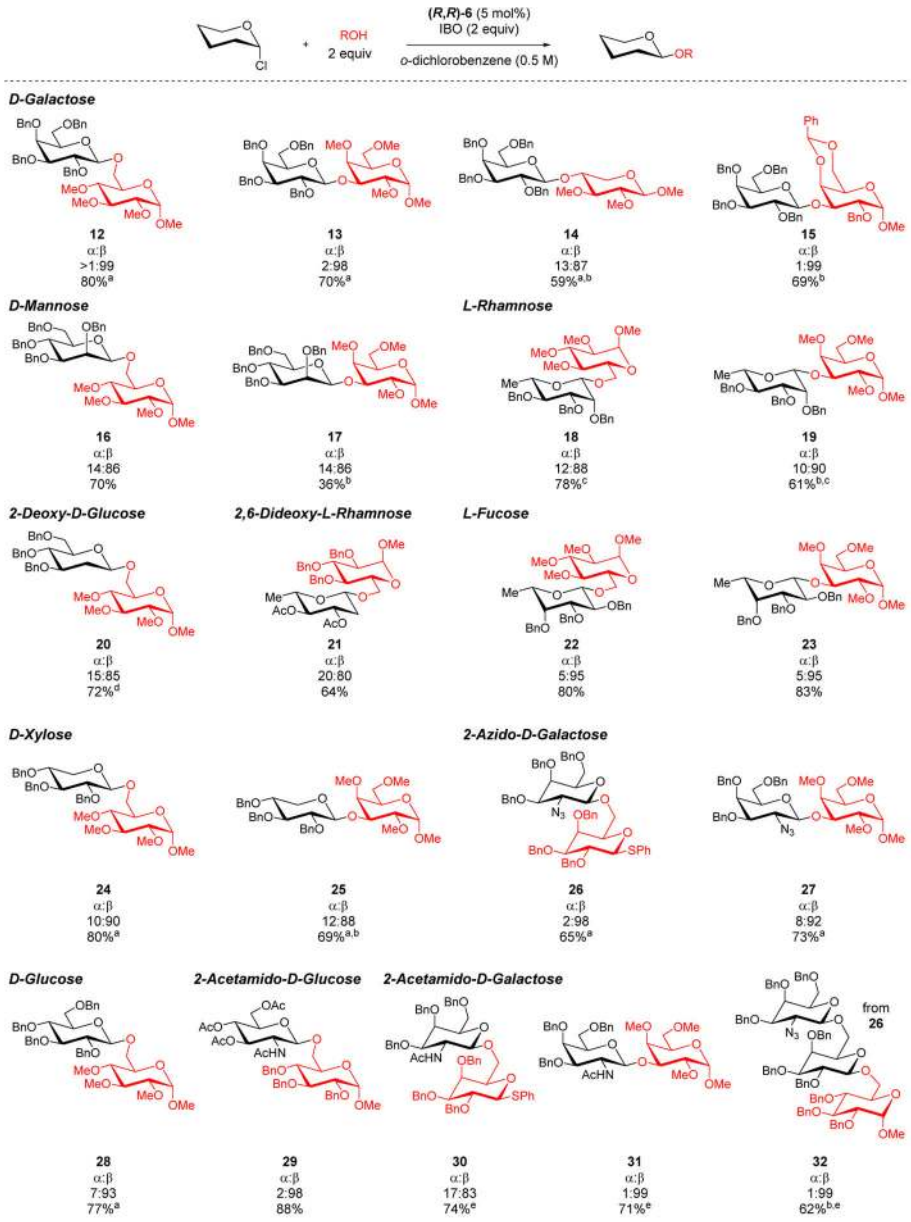

Fig. 3.

Substrate Scope. Isolated yields are reported. Diastereomeric ratios were determined by NMR or HPLC analysis of crude material. * Reaction run at 40 °C. †Reaction run with 10 mol% catalyst. ‡Reaction run at 0°C with **(S,S)-6**. §Reaction run in toluene (0.1 M) at −40 °C. ‖Reaction run in dichloromethane.

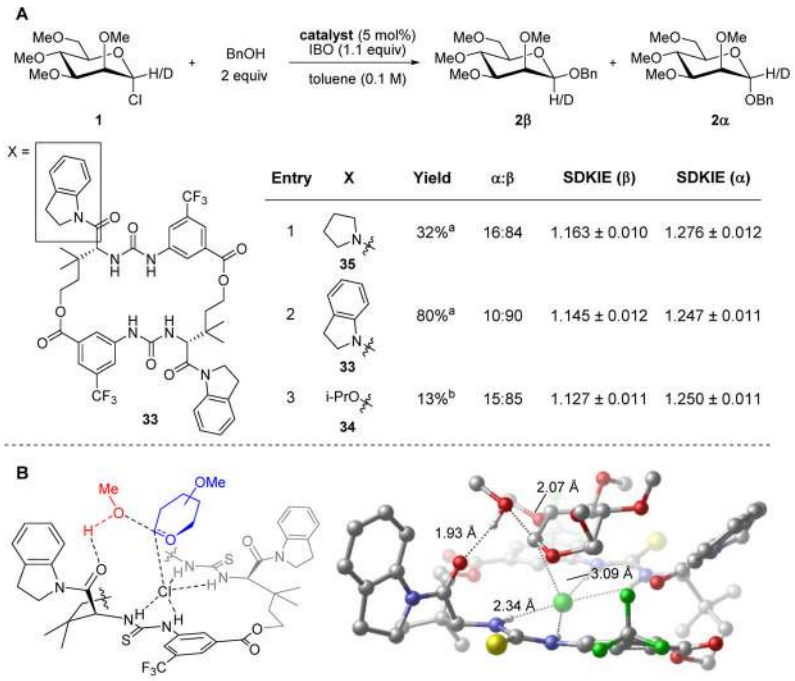

Fig. 4.

Mechanistic studies. (**A**) Amide substituent effects. * reaction run for 18 hours. † reaction run run for 48 hours. Secondary deuterium kinetic isotope effects were determined from intermolecular competition experiments at low conversion (ca. 10–20%). (**B**) Transition state calculated using M06-2X/6-31G*/PCM (polarized continuum model, benzene).