Catalytic activation of glycosyl phosphates for stereoselective coupling reactions

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Glycosyl phosphates are shown to be activated to stereospecific nucleophilic substitution reactions by precisely tailored bis-thiourea catalysts. Enhanced reactivity and scope is observed with phosphate relative to chloride leaving groups. Stronger binding (K_m) to the H-bond donor and enhanced reactivity of the complex (k_{cat}) enables efficient catalysis with broad functional group compatibility under mild, neutral conditions.

glycosylation | organocatalysis | H bonding | phosphate

Phosphates are nature's leaving group of choice in the biosynthesis of nearly all biological polymers, including oligonucleotides, proteins, terpenes, and carbohydrates (Fig. 1*A*). In rather stark contrast, phosphates are very rarely employed as leaving groups in nonbiological synthesis; synthetic chemists typically resort to more intrinsically reactive leaving groups such as halides and sulphinates (1). In a classic analysis, Westheimer (2) posited that nature evolved to use phosphates as a result of their kinetic stability and strong Lewis basic character, enabling specific enzymatic recognition. In principle, these advantages could translate to abiotic chemistry, as well, if methods could be devised for activating phosphates under mild conditions to promote nucleophilic substitution reactions. Instead, existing synthetic methods using phosphates (3) rely on highly Lewis acidic, stoichiometric activating agents that are poorly functional group-tolerant (4). Herein we report the catalytic activation of phosphate leaving groups with bis-thiourea catalysts to effect stereoselective glycosylation reactions. The neutral hydrogen-bond donor catalyst promotes coupling reactions between glycosyl phosphates and complex partners including glycosides, peptides, and highly functionalized natural products.

The use of hydrogen-bond donor organocatalysts in glycosylation reactions has been reported by several groups (5–12). While such systems can provide stable, mild, and user-friendly alternatives to strongly Lewis acidic and oxidizing reagents typical of chemical glycosylation, the attainment of useful reactivity and stereoselectivity with functionally complex coupling partners has proven elusive. We reported recently that precisely linked bis-thiourea derivatives catalyze stereospecific nucleophilic substitution reactions of glycosyl chlorides (13). On the basis of experimental and computational modeling studies, the mode of catalysis was proposed to involve simultaneous activation of both the donor and acceptor reacting partners through general acid and general base activation (14), in a manner loosely reminiscent of the mechanisms by which glycosyl transferase enzymes have been postulated to function (Fig. 1*B*) (15). However, in subsequent efforts to apply and extend the synthetic methodology to targets of interest for biological studies, we have observed that severe rate suppression occurs with functionally complex coupling partners, thereby imposing severe limitations to reaction scope. Kinetic analyses reveal that complex nucleophiles bearing multiple Lewis basic groups in fact function as catalyst inhibitors, presumably due to unproductive association of the key H-bond donor motifs (*SI Appendix*, Fig. S5). We hypothesized that catalytic efficiency was limited by the low Lewis basicity of the chloride leaving group, and that leaving groups with higher affinity toward the thiourea moiety might enable superior functional group compatibility in glycosylation reactions with complex coupling partners (Fig. 1*C*).

Here we report that precisely linked bis-thiourea catalysts activate glycosyl phosphates and promote β-selective glycosylation reactions. Engagement of phosphates as leaving groups results in significant improvements in reactivity and scope compared with previous systems, enabling efficient conjugation of saccharides to a variety of functionally complex coupling partners.

Results and Discussion

Inspired by nature's selection of phosphate leaving groups, we hypothesized that our synthetic hydrogen-bond donor catalysts would be well suited to bind an organic phosphate leaving group due to its high Lewis basicity (16). We compared the binding of galactosyl phosphate **2a** to bis-thiourea catalyst **1** to that of the corresponding glycosyl chloride **2b** (Fig. 2*B*). The phosphate was observed to bind 19 times more strongly than the chloride.

While the binding study supported the straightforward idea that more Lewis basic leaving groups could result in increased concentration of the catalyst–donor complex, it was much less apparent whether this enhanced affinity would result in more facile H-bond-donor promoted nucleophilic substitution reactions, or whether catalyst turnover would be attainable. To address these questions, we evaluated a model glycosylation reaction between the galactosyl phosphate **2a** and the protected serine derivative **3a** as the acceptor. This substrate combination was poorly reactive in couplings with glycosyl chlorides in the previously reported system, providing 16% conversion (75:25 β:α) with 10 mol % catalyst after 14 h (*SI Appendix*, Scheme S2). In the presence of bis-thiourea **1**, the glycosylation product was generated in quantitative yield and with high β selectivity (97:3 β:α). In a broad survey of other potential galactosyl donors, including the acetate **2c** and trichloroacetimidate **2d**, phosphate **2a** proved to be

Significance

Despite their dominant role as electrophiles in biosynthetic pathways promoted by enzymes, organic phosphates are rarely employed in laboratory synthesis, because phosphates are generally very poor leaving groups. Here we report that a bis-thiourea catalyst promotes β-selective glycosylation reactions of phosphate donors under mild, neutral conditions. The catalytic method enables glycosylation of peptides, complex natural products, and pharmaceutical agents bearing a variety of reactive functionalities, thereby representing an important step toward enabling convenient access to glycosylated chemical matter of interest to synthetic chemists and chemical biologists.

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Fig. 1. (A) Examples of phosphate leaving groups found in nature. (B) Invertive glycosylation mechanism utilized by glycosyl transferase enzymes. (C) Previously reported work catalyzing stereospecific glycosylation reactions via dual-activation of glycosyl chloride donors and alcohol acceptors with bis-thioureas. (D) Improving catalytic efficiency through tighter binding and increased reactivity of phosphate leaving group activated by hydrogen-bond-donor catalyst.

uniquely reactive under the catalytic conditions (*SI Appendix*, Scheme S4).

A systematic evaluation of monomeric and dimeric hydrogen-bond donor derivatives revealed that linked bis-thiourea and bis-urea catalysts were the only effective catalysts for the model reaction (*SI Appendix*, Scheme S2). No reactivity was observed with macrocyclic bis-thiourea catalysts of varying size, in direct contrast to prior studies using glycosyl chlorides as donors. The relative and absolute stereochemistry and the linker length of bis-thiourea **1** were all found to be crucial parameters for effective catalysis.

We sought to establish whether the diphenylphosphoric acid generated as a stoichiometric by-product has any effect on the glycosylation reaction. In prior work by Schmidt and coworkers (6, 17–19), Fairbanks and coworkers (20), Toshima and coworkers (21), Nagorny and coworkers (22, 23), Galan and coworkers (24), Bennett and coworkers (25), and others (26, 27), phosphoric acids have been shown to be competent catalytic activators in glycosylation reactions involving different types of donors. However, in the model system, addition of exogenous diphenylphosphoric acid was shown to have no detectable effect on either reaction rate or α/β selectivity. Analysis of reaction mixtures by NMR

revealed no measurable concentration of the acid by-product in solution, suggesting that insolubility is most likely responsible for the absence of any catalytic or inhibitory activity in the thiourea-promoted glycosylation. This effect is solvent-dependent, and optimal anomeric selectivity and reactivity are obtained with nonpolar solvents such as cyclohexane and ethereal solvents (*SI Appendix*, Table S11). Notably, when the reaction is performed in a solvent in which the phosphoric acid by-product is soluble (e. g., dichloromethane), the hydrogen-bond donor catalyzed pathway is suppressed almost completely.

Independent and competitive rate data for glycosyl donors **2a** and **2b** were generated to evaluate the effect of improved leaving group recognition on the catalytic reaction. In reactions with serine **3a** as the nucleophile, the reactivity of chloride **2b** was found to be severely inhibited (from 4.0 mM·h^{-1} to 0.4 mM·h^{-1}) by the presence of equimolar concentrations of the phosphate **2a**, while the reactivity of **2a** was unaffected (32 mM·h^{-1}) by the presence of the chloride (Fig. 2*C*). Michaelis−Menten kinetic analyses of reactions catalyzed by **1** revealed both lower Michaelis constant (K_m) and a faster maximum rate (k_{cat}) for the glycosyl phosphate relative to the chloride (Fig. 2*D*). Thus, the phosphate not only binds more tightly to the catalyst but also is more labile toward substitution when bound, resulting in a 16-fold improvement in catalytic efficiency (k_{cat}/K_m) throughout the entire course of reaction (Fig. 2*F*). Saturation kinetics are observed with respect to both donor (**2a** or **2b**, Fig. 2*D*) and serine acceptor (*SI Appendix*, Figs. S10 and S13), consistent with rate-determining reaction of a ternary catalyst–donor–acceptor complex to form product (Fig. 2*E*).

The improved binding and reactivity of glycosyl phosphate donors relative to chloride prompted us to evaluate the scope of the catalytic reaction with acceptors containing multiple Lewis basic sites. The model reaction in Fig. 2*A* could now be carried out efficiently using 1 mol % of bis-thiourea **1** and 1.2 equivalents of acceptor **3a** in 12 h to afford **4a** in 94% NMR yield (>95% conversion) and a β:α selectivity of 93:7. Excellent reactivity and β:α selectivity were observed with simple hydroxylic amino acids, including serine, threonine, and hydroxyproline (Fig. 3). Dipeptides L-Ser-L-Leu and L-Leu-L-Ser as well as the tripeptide L-Ser-L-Leu-L-Phe were engaged effectively as acceptors, although the limited solubility of the tripeptide under the reaction conditions resulted in decreased reactivity. Galactosylation of compounds bearing basic nitrogen functionality such as clindamycin and quetiapine was also achieved, suggesting that elaboration of medicinally active small molecules may be possible in a general manner. Phenols and thiols were also engaged effectively as acceptors, as illustrated in the β-selective galactosylations of both tyrosine and cysteine. However, while good scope was thus observed with protic nucleophiles, carbon-centered nucleophiles such as allyl-trimethylsilane proved unreactive under the catalytic conditions (although primary and secondary amino acceptors afforded the β-product selectively under catalytic conditions, background reactivity was often competitive as well as β-selective). These observations are consistent with catalysis involving both anion abstraction from the electrophile and general base activation of the protic nucleophile (Fig. 1*B*), although substantiation of that hypothesis will require fuller mechanistic characterization. Initial efforts to expand the scope of the catalytic reaction to different phosphate donors are outlined in Fig. 4. Efficient reaction between 2-azido-galactosyl phosphate **2e** and a disaccharide acceptor was promoted by **1** to afford the corresponding β-trisaccharide **4o**, exclusively. Construction of cis-1,2 β-linkages was achieved, albeit with lower catalytic efficiency, as illustrated in the generation of β−mannosylation and β−rhamnosylation products **4p** and **4q**. While glycosylation reactions with bis-thiourea **1** were noted above to be insensitive to the absolute stereochemistry of the acceptors (e.g., **4a** and **4b**; Fig. 3), matching of the stereochemical
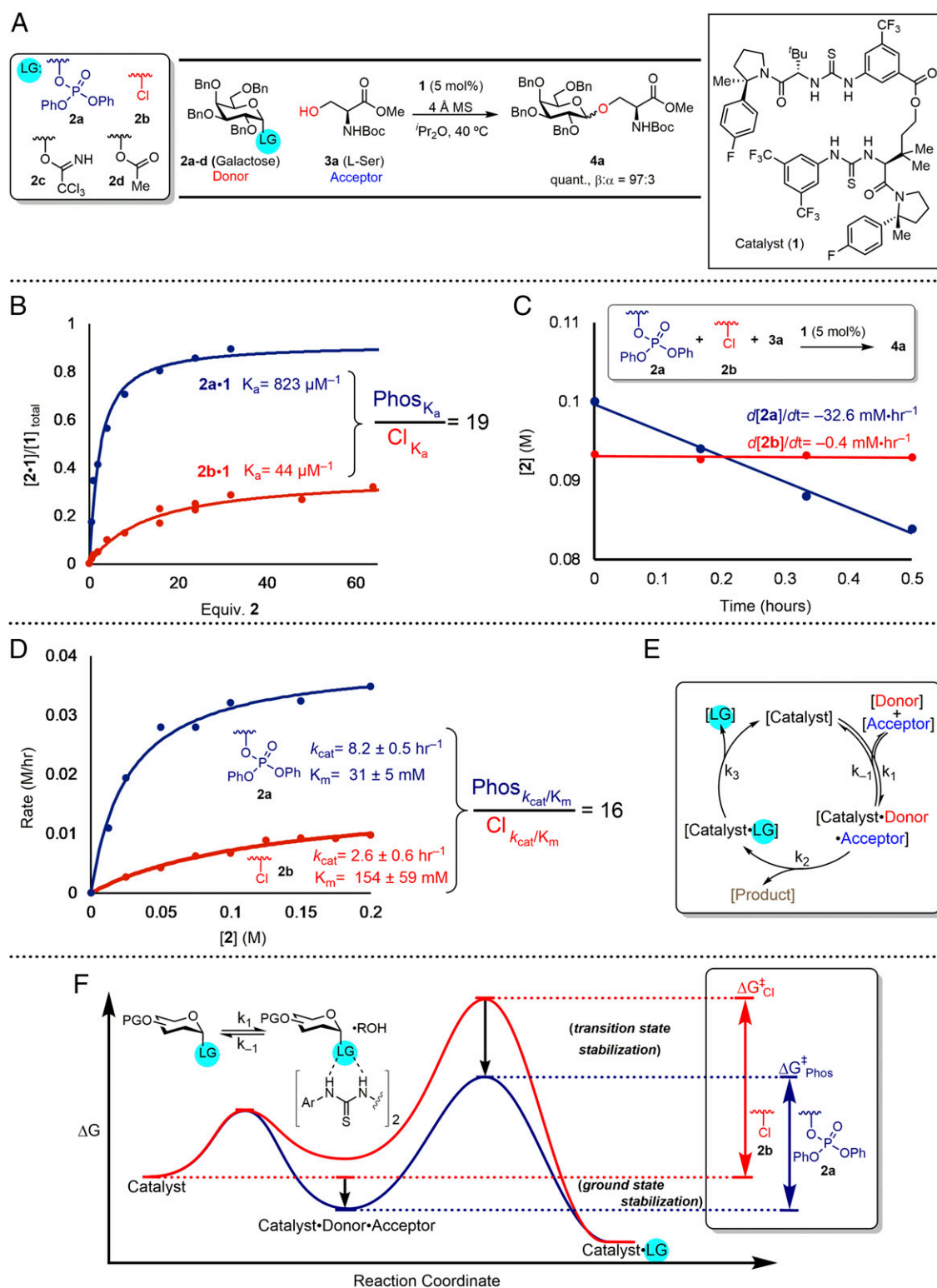
Fig. 2. (A) Model system for leaving group evaluation. (B) Binding of galactosyl phosphate **2a** and galactosyl chloride **2b** to bis-thiourea catalyst **1** as quantified by ^{19}F NMR. The curves correspond to 1:1 binding fits. (C) Competition rate kinetics between **2a** and **2b** measured by ^{1}H NMR. (D) Michaelis–Menten initial rate analysis determined by HPLC. (E) Proposed catalytic cycle. (F) Reaction coordinate diagram for phosphate and chloride leaving groups in model system.

features of the glycosyl donor and catalyst proved important. Thus, β–rhamnosylation was achieved with **1**, while effective reactions with the pseudoenantiomeric mannosyl phosphate required use of *ent*-**1**.

We have demonstrated in this study that precisely tailored neutral H-bond donor catalysts can activate organic phosphates as leaving groups, allowing stereospecific glycosylation reactions of functionally rich coupling partners under mild and neutral
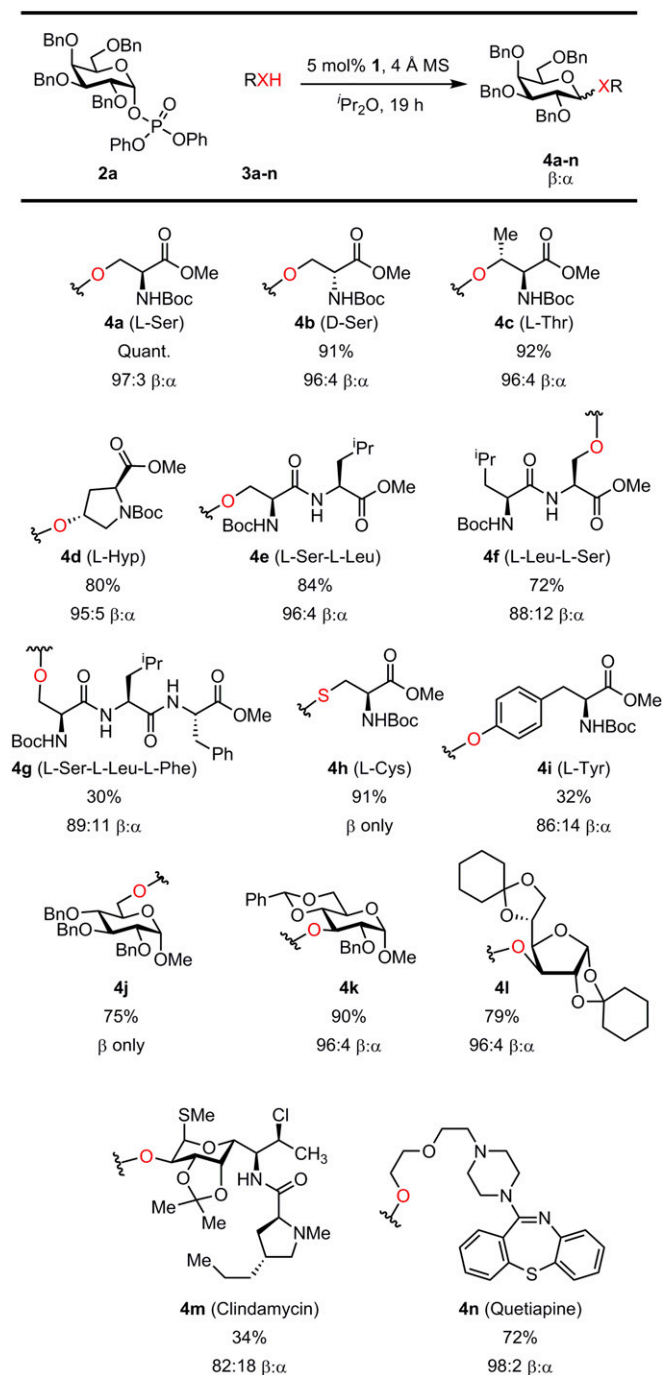
Fig. 3. Acceptor scope for galactose phosphate donor.

prone to rearrangement to the acetamide (28). Strong Lewis acid activation conditions are also known to promote poorly regioselective glycosylations of phenols, yielding mixtures of *O*- and *C*-glycosylated products (29). As a result of these limitations, reaction conditions and substrates often need to be tailored for specific reactions.

Similarly, enzymatic methods have been applied with exceptional success in the laboratory synthesis of complex polysaccharides, but there are important limitations to that approach that are avoided with the system described here. For example, access to the UDP-glycosyl donors can be limited, and the requisite glycosyltransferases are often difficult to obtain, unstable, narrow in substrate scope, challenging to manipulate, and/or prone to inhibition by the by-product nucleotide leaving group (30).

Ultimately, we anticipate that the successful development of this methodology may facilitate the synthesis and production of biomedically relevant glycans, glycopeptides, glycoproteins, glycolipids, and microbial polysaccharides and glycoconjugates.

Materials and Methods

General Procedure for Glycosylation Reaction Catalyzed by Catalyst 1. To a flame-dry 5-mL round-bottom flask with a stir bar was charged 0.250 mmol of glycosyl donor, 250 mg of flame-dry 4-Å molecular sieves, 0.0125 mmol of catalyst, and 0.500 mmol of acceptor; 2.50 mL of diisopropyl ether was added to the reaction mixture open to air, the flask was closed with a plastic cap, and parafilm was used to seal the cap tightly. The mixture was then heated with efficient stirring at 40 °C in an oil bath for 19 h, over which time the mixture thickened. After 19 h, the mixture was cooled to room temperature, and 100 µL of aliquot was diluted with diethyl ether and filtered to remove the molecular sieves. This solution was concentrated, and ¹H NMR analysis of the crude mixture
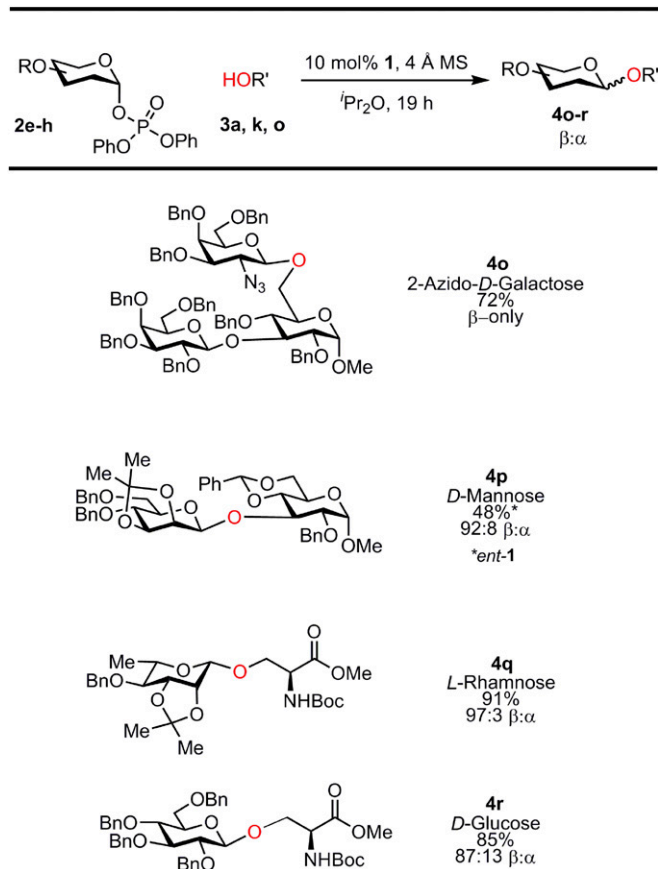
Fig. 4. Donor scope with diphenylphosphate leaving group.

conditions. The catalytic method allows glycosylation of a wide range of functionally complex acceptors with good scope and under mild, neutral conditions. We propose that this approach represents an important advance in glycosylation methodology. Prior advances in nonenzymatic glycosylation have provided enabling tools for selective glycosidic bond construction, but these approaches typically require strongly oxidizing and Lewis acidic promotors that can be incompatible with sensitive substrates. Methods engaging glycosyl phosphates, for example, require stoichiometric trimethylsilyltriflate under cryogenic temperatures (3, 4). Other leaving groups are susceptible to competing pathways: Thioglycosides undergo aglycone transfer (29), and imidates are

was used to determine the anomeric selectivity. The mixture was then chromatographed directly with 50 g of SiO₂ using an diethyl ether:hexanes gradient.

Hazard Note. Diisopropyl ether is known to form peroxides and should be handled with appropriate care. Reaction outcomes with alternative solvents are described in *SI Appendix*, Table S11.

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