Direct Addition of Amides to Glycals Enabled by Solvation-Insusceptible 2-Haloazolium Salt Catalysis

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Abstract: The direct 2-deoxyglycosylation of nucleophiles with glycals leads to biologically and pharmacologically important 2-deoxysugar compounds. Although the direct addition of hydroxyl and sulfonamide groups have been well developed, the direct 2-deoxyglycosylation of amide groups has not been reported to date. Here, we show the first direct 2-deoxyglycosylation of amide groups using a newly designed Brønsted acid catalyst under mild conditions. Through mechanistic investigations, we discovered that the amide group can inhibit acid catalysts, and the inhibition has made the 2-deoxyglycosylation reaction difficult. Diffusion-ordered two-dimensional NMR spectroscopy analysis implied that the 2-chloroazolium salt catalyst was less likely to form aggregates with amides in comparison to other acid catalysts. The chlorine atom and the extended π-scaffold of the catalyst played a crucial role for this phenomenon. This relative insusceptibility to inhibition by amides is more responsible for the catalytic activity than the strength of the acidity.

Peptide-based drugs are important in the pharmaceutical industry and in the biological and chemical sciences.[1] Peptides are often glycosylated to improve the solubility, biological stability, and lower the immunogenicity. The oligosaccharides of natural glycoproteins play a key role in molecular recognition, [2-4] and asparagine (Asn) residues are one of the major sites for glycosylation in glycoproteins.[5] However, the glycosylated amide bond of the Asn side chain can be readily hydrolyzed by glycoamidases,[6] and crystallographic analysis and site-directed mutagenesis have revealed that these enzymes require the 2-acetamide group on the Asn-linked GlcNAc for substrate recognition. [7,8] Thus, a 2-deoxyglycosylation of the Asn residues could give the peptide chain stability against hydrolysis, resulting in enhanced biological activity and selectivity[9] (Fig. 1A). Despite the utilities of the *N*-(2-deoxysugar)-amide structure in the fields of biochemistry and pharmacology, only limited synthetic methodologies for this structure have been reported.[10,11] The direct 2-deoxyglycosylation of nucleophiles with glycals is one of the most straightforward approaches to 2-deoxysugar compounds.[12] Therefore, the direct 2-deoxyglycosylation of hydroxyl groups[13,14] (Fig. 1B, eq. 1) and sulfonamide groups[13a,14e, 15] (Fig. 1B, eq. 2) has been well-developed using various transition-metal catalysts and organocatalysts, whereas the direct 2-deoxyglycosylation of simple amides has not been reported to date (Fig. 1B, eq. 3).

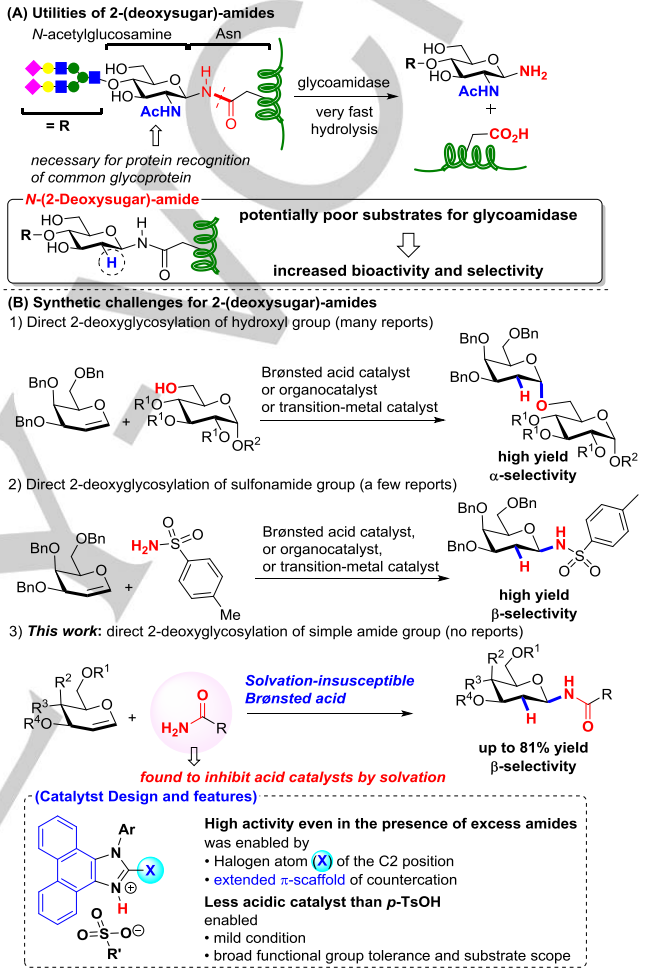

Figure 1. Summary of this work

We have recently reported a new methodology for the direct *N*-glycosylation of amide groups[16] with glycosyl trichloroacetimidates,[17] and the product yields depended greatly on the structure of the countercations of ammonium salt as a Brønsted acid catalyst. In light of this observation, we envisioned an unprecedented concept that the designed ammonium salts could exhibit superior catalytic performance for the direct transformation of amides, such as in *N*-2-deoxyglycosylation. In this paper, we report the first example of the direct 2-deoxyglycosylation of an amide group with our newly designed 2-halogenated azolium salts as Brønsted acids. The salient features of this method are as follows: (1) the formation of the azolium salt enabled a mild condition, and this atom-economical reaction was widely applied to various amides, including the asparagine residues, and (2) the 2-halogenated azolium salts improved the catalytic activity, whereas the ammonium salts

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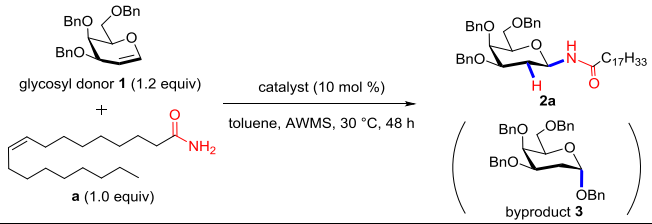
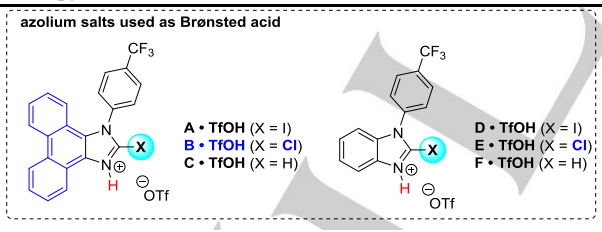
have mainly been used just for improving the selectivity in catalytic asymmetric reactions.[18] In addition, during the course of our investigations, it was found that the acid catalysts were inhibited by the amide groups. Detailed mechanistic investigations suggested that the halogen atom and the extended π-scaffold of our new catalyst played an important role for the improved catalytic activity, even in the presence of amides.

Table 1. Optimization of the reaction condition.



Entry	Catalyst	Conversion of **1** (%)[a,b]	**2a** (%)[a]	**3** (%)[a,b]
1	*p*-TsOH•H_2O (MS 4Å)[c]	0	n.d.[d]	n.d.[d]
2	*p*-TsOH•H_2O	42	15	n.d.[d]
3	*p*-TsOH•H_2O (70 ºC)	100	26	14
4	[(*p*-$CF_3C_6H_4)_3P$]AuCl + AgOTf	0	n.d.[d]	n.d.[d]
5	(4-$NO_2C_6H_4O)_2P(O)OH$	0	n.d.[d]	n.d.[d]
6	Ph_3P•HBr	72	27	n.d.[d]
7	TfOH	100	21	30
8	BF_3•OEt_2	100	24	20
9	**A•TfOH** (X = I)	74	58	n.d.[d]
10	**B•TfOH (X = Cl)**	100	**73 (74)**[e]	n.d.[d]
11	**C•TfOH** (X = H)	25	7	n.d.[d]
12	**D•TfOH** (X = I)	32	8	n.d.[d]
13	**E•TfOH** (X = Cl)	96	43	n.d.[d]
14	**F•TfOH** (X = H)	23	8	n.d.[d]
15	pyridine•TfOH	0	n.d.[d]	n.d.[d]



[a] NMR yields and conversion were calculated using 4'-nitrophenylacetophenone as an internal standard. [b] Based on the employed glycosyl donor **1**. [c] MS 4Å was used instead of AWMS (acid-washed molecular sieves). [d] Not detected. [e] Isolated yield.

We initially investigated the reaction conditions used for the direct 2-deoxyglycosylation of hydroxyl groups,[12] and tested the ability of various acid catalysts to promote the 2-deoxyglycosylation of amides with the typical galactal substrate **1** as a model reaction (Table 1). First, we employed *p*-toluenesulfonic acid (TsOH) as the Brønsted acid and MS 4Å as the desiccant agent, but the reaction did not proceed at all (entry 1). The use of acid-washed molecular sieves, instead of MS 4Å, promoted the reaction, and the desired adduct **2a** was obtained in 15% yield along with a considerable amount of unreacted glycosyl donor **1** (58%). Although the donor **1** was completely consumed at 70 °C, the yield of **2a** was not significantly improved owing to the degradation of donor **1**, affording byproduct **3** (entry 3). A cationic gold (I) catalyst, which is known to be an effective catalyst for the direct 2-deoxyglycosylation of hydroxyl groups, was not effective for this reaction (entry 4). An effective phosphoric acid catalyst for the *O*-glycosylation reaction[19] could not promote the 2-deoxyglycosylation of amides (entry 5). Ph_3P·HBr[14a,15] slightly improved the yield, while compound **1** was still recovered in 28% yield (entry 6). Strong Brønsted acid and Lewis acid catalysts, such as triflic acid (TfOH) and BF_3·OEt_2, could completely consume the glycosyl donor **1**, but the yields of **2a** were still not improved because of a considerable amount of byproducts. The benzyl alcohol adduct **3** was isolated as a major byproduct, implying that a strongly acidic or heated condition caused the degradation of the glycal **1** via *e.g.*, the Ferrier reaction (entries 3, 7, 8). From this preliminary screening, we became interested in acid-base salt-type Brønsted acids,[18] such as Ph_3P·HBr. Fortunately, the previously designed 2-iodoazolium salt **A·TfOH**, [17] which is also an acid-base salt-type Brønsted acid, promoted the reaction well to furnish **2a** in 58% yield (entry 9). Encouraged by this result, we next screened a series of azolium salts. Among them, the 2-chloroazolium salt **B·TfOH** exhibited the best performance, and **2a** was obtained in 73% yield with the full conversion of compound **1** (entry 10). Notably, the use of the catalyst **C·TfOH** without a halogen atom (entry 11) and the benzoimidazolium catalyst **E·TfOH** (entry 13) significantly decreased the yield of **2a**, strongly indicating that both the halogen atom and the azolium cation scaffold played an important role in the catalytic activity (entries 9–15). Interestingly, from the characterization by 1D and 2D NMR (Fig. S2), as well as X-ray crystallographic analysis (Fig. S1), the stereochemistry of the product **2** was unambiguously determined as the β-anomer, which was in contrast to the α-selectivity observed in the reaction between the glycals and hydroxyl groups.[12] The predominant β-selectivity could be explained by the *exo*-anomeric effect[20] (Fig. S13).

With the optimized conditions in hand, we next investigated the substrate scope for the amides (Fig. 2A). *o*-Ethoxybenzamide, a commercially available pharmaceutical compound, was suitable for this transformation (**2c**). Pivalamide, which has a quaternary carbon at the α-position, was also converted into the corresponding *N*-(2-deoxyglycosyl)-amide **2d** in 53% yield. *N*-Cbz-glycineamide could be directly converted into the desired product **2e** in 78% yield. A protected Asn derivative was effectively conjugated with the glycosyl donor to afford the desired product **2f** in 80% yield, and the scale-up synthesis did not considerably impair the reaction (71% yield). The amide residue of a protected glutamine derivative was transformed into the glycoconjugate **2g** with a good yield. Notably, the Asn residues of a dipeptide (**2h**) and tripeptide (**2i**) were also glycosylated by the **B·TfOH**. Because the glycosylated asparagines can be directly used in the solid-phase synthesis of *N*-glycopeptides, these products can be readily applied to

biochemical research.[21] Pleasingly, *N*-Cbz-benzylamine (**2k**) and *Nε*-Cbz-lysine (**2l**) gave the glycosylated compounds with moderate to good yields. These results are especially appealing for the synthesis of various *N*-(2-deoxysugar)-amines via the simple protection of amines, considering that no effective methodologies for the 2-deoxyglycosylation of amines with acid catalysts have been reported to date. An α-branched carbamate did not afford the desired adduct **2j**, similar to the HNCbz groups of compounds **2f–h**, indicating that steric factors play an important role in the reaction. Cyclohexylurea also underwent the glycofunctionalization, albeit in low yield (**2m**). When a complex alcohol substrate, which bears an amide and HNCbz groups, was employed as the substrate, **B·TfOH** showed an obviously higher reactivity than that of TsOH (**2n**).

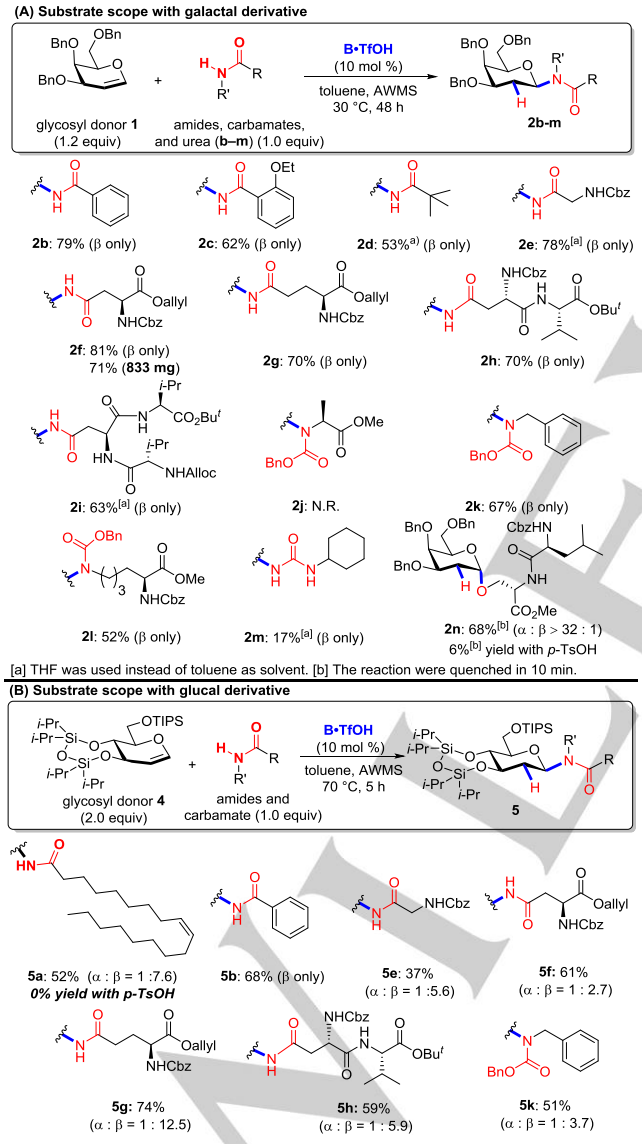
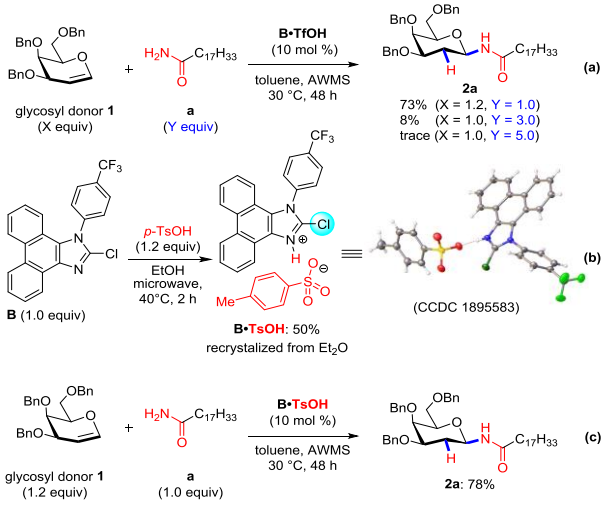


Figure 2. Substrate scope

To broaden the scope of the glycosyl donors, we then investigated the reaction of the amides with protected glucals (Fig. 2B), which are challenging substrates because most glucals are more likely to undergo a Ferrier rearrangement than the galactal derivatives. In fact, tri-*O*-benzylglucal only produced Ferrier-rearrangement compounds. After various conditions were examined using Galan's glycosyl donor[14c] **4** and oleamide as substrates, the 2-deoxyglucose derivative **5a** was obtained in 52% yield, although the reaction needed to be conducted at 70 °C. Remarkably, TsOH as an acid catalyst did not afford **5a** at all with this substrate. Surprisingly, a 2D NMR analysis of the product **5** indicated that the β-anomer was the major isomer[20] (Fig. S2), and the α-anomer was obtained as a minor product (Fig. S8-S11). These results are also in sharp contrast to the addition of alcohols to Galan's glycosyl donor, generally affording an α-anomer as a major product.[14c] Because of the decomposition of the product in heated THF (Fig. S12), all reactions with the glycosyl donor **2** were performed in toluene, and the corresponding adducts **5b–k** were obtained in 37%–74% yields.

To gain mechanistic insights, we conducted several control experiments (Scheme 1). During the course of the kinetic studies, we found that an excess amount of amide led to a dramatically decreased yield of **2a** (Scheme 1a), implying that the amide groups inhibited the acid catalyst, and that this inhibition could be responsible for the difficulty of this reaction.[22] The 2-halogenated azole precursor **B** of the **B·TfOH** formed the corresponding sulfonate salt with TsOH, and the structure was confirmed by X-ray analysis (Scheme 1b). Importantly, this salt formation clearly means that this catalyst is a weaker acid than TsOH. Remarkably, **B·TsOH** considerably increased the yield despite the weaker acidity, compared with TsOH itself (Scheme 1c). These results suggested that the chlorinated azole moieties of **B·TsOH** play an important role in the promotion of the reaction. From these results, we hypothesized that the formation of a salt with the azole **B** was responsible for the improved catalytic activity of TsOH, because of the suppression of solvation (*i.e.*, catalyst inhibition) by the amide groups.



Scheme 1. Control experiments

To support this hypothesis, diffusion-ordered two-dimensional NMR spectroscopy (DOSY) experiments[23] were performed using each catalyst and oleamide (1:10, 0.072 M) in toluene-d_8 (Fig. 3). Interestingly, the peaks of the amide itself were only observed in regions with very small diffusion coefficients (ca. 1.0 × 10^{-10} [m^2/s]), indicating that the amides should exist in a highly aggregated form through hydrogen-bonding networks (Fig. S3a). The mixture of TsOH and oleamide (1:10) showed the same small diffusion coefficient (Fig. 3A), indicating that TsOH formed an aggregate with amides. In contrast, the mixture of **B·TfOH** and oleamide (1:10) showed two different diffusion coefficients for the most part (Fig. 3B). The larger diffusion coefficient (ca. 1.2 × 10^{-9} [m^2/s]) corresponded to the peaks of **B·TfOH**, demonstrating that **B·TfOH** was less likely to form aggregated complexes[24] and therefore exhibited high catalytic performance. Further measurements of DOSY spectra (Fig. S3b–S3e) were also in accordance with the results shown in Scheme 1. For example, the peaks of **B·TsOH**, which gave 78% yield of **2a** (Scheme 1c), were observed without the complete aggregation with amides. However, only small diffusion coefficients were observed under low-yielding conditions, such as using **C·TfOH** or **E·TfOH** (Table 1), and using **B·TfOH** in the presence of 30 equivalents of amides (Scheme 1a). The computational studies further supported our hypothesis (Fig. S4). The interaction energies between the amides and each catalyst were estimated, and the best catalyst **B·TfOH** had weaker interaction energies (~3 kcal/mol) than **C·TfOH** or **E·TfOH**, possibly because of the steric repulsion of the expanded aromatic ring and less attractive electrostatic interactions derived from the chlorine atom at the C2 position, which suppressed the aggregation with amides.

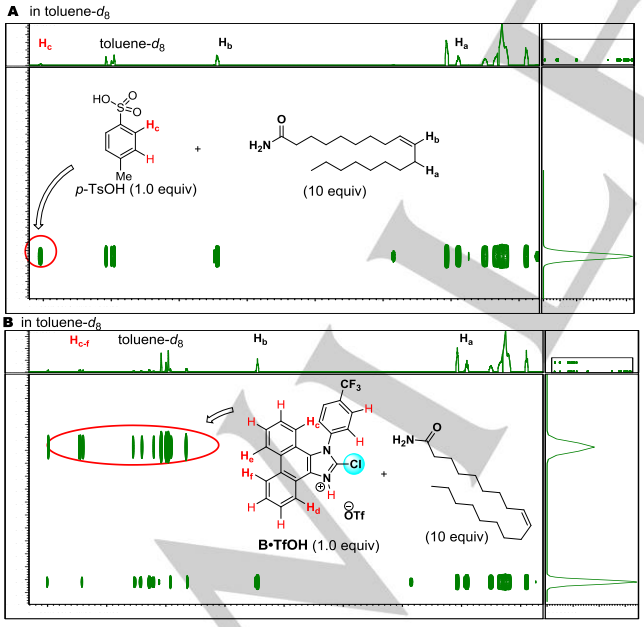


Figure 3. ^{1}H DOSY NMR experiments

In conclusion, we have developed the first direct 2-deoxyglycosylation of amide groups using a 2-halogenated azolium salt as the Brønsted acid catalyst. Our synthesized 'weaker' acid catalyst showed a higher reactivity than the other 'stronger' acid catalyst, and the relative insusceptibility to inhibition by the substrate[25] is likely to be more responsible for the catalytic activity than the strength of the acidity. This concept is by no means limited to this reaction, and should be applicable to various acid-catalyzed reactions, when the acid catalyst can be inhibited by substrates, products, or solvents. The catalyst design for this 'insusceptible' cation scaffold can be synergistically combined with existing acid catalysts, opening the way for otherwise inaccessible transformations.

Experimental Section

To a stirred solution of glycosyl donor **1** (816 mg, 1.96 mmol) and amides **f** (1.0 equiv, 1.63 mmol) in toluene (20 mL) was added activated AWMS (1.0 g), and the reaction mixture was stirred at the ambient temperature for 30 min. Then, the catalyst **B·TfOH** (89.2 mg, 0.163 mmol, 10 mol %) were added, and the resulting mixture was stirred at 30 °C for 48 hours. After the AWMS was filtered off and washed with chloroform several times, the filtrate was concentrated under reduced pressure to give crude *N*-(2-deoxyglactosyl)-amide, which was purified by silica gel column chromatography (*n*-hexane/EtOAc = 70/30 to 50/50) to afford **2f** (833 mg, 71%) as a colorless oil.

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Keywords: Brønsted acid catalysis • carbohydrates • glycosylation • organocatalysis

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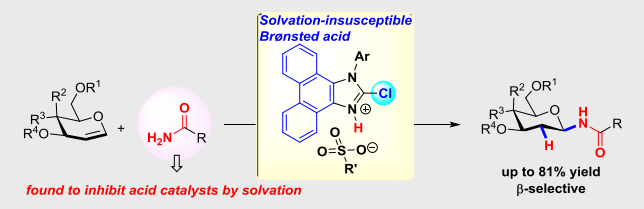

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