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Chemoselective and Site-Selective Reductions Catalyzed by a Supramolecular Host and a Pyridine−Borane Cofactor

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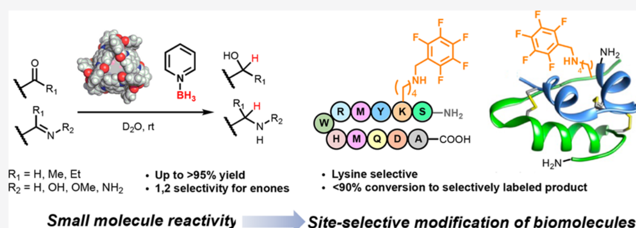
ABSTRACT: Supramolecular catalysts emulate the mechanism of enzymes to achieve large rate accelerations and precise selectivity under mild and aqueous conditions. While significant strides have been made in the supramolecular host-promoted synthesis of small molecules, applications of this reactivity to chemoselective and site-selective modification of complex biomolecules remain virtually unexplored. We report here a supramolecular system where coencapsulation of pyridine−borane with a variety of molecules including enones, ketones, aldehydes, oximes, hydrazones, and imines effects efficient reductions under basic aqueous conditions. Upon subjecting unprotected lysine to the host-mediated reductive amination conditions, we observed excellent ε-selectivity, indicating that differential guest binding within the same molecule is possible without sacrificing reactivity. Inspired by the post-translational modification of complex biomolecules by enzymatic systems, we then applied this supramolecular reaction to the site-selective labeling of a single lysine residue in an 11-amino acid peptide chain and human insulin.



INTRODUCTION

Supramolecular host−guest catalysis exerts precise molecular control over a reaction through a network of noncovalent interactions within the host cavity, shielded from competing, or even overwhelming, interactions with solvent and other reagents.[1−11] For decades, chemists have sought to utilize these hosts as synthetic enzyme mimics to harness the high selectivity and reactivity of enzymatic processes under mild and aqueous conditions. Terpene cyclization reactions are one such example, where Tiefenbacher and co-workers utilized a resorcinarene host to cyclize geranyl acetate, a process typically catalyzed by terpene synthase enzymes including 1,8-cineole synthase (Figure 1a).[12−15] Site-selective and chemoselective transformations of small molecules are another hallmark of enzymatic catalysis, such as the selective reduction/oxidation reactions of cortisol and corticosterone by 11β-hydroxysteroid dehydrogenase (11β-HSD) (Figure 1b).[16] More recently, site-selective transformations promoted by supramolecular hosts have also been demonstrated, such as the prenyl functionalization of geranyllinalool by Fujita and co-workers as well as selective Rh-catalyzed hydrogenation of a linolenic acid derivative by Toste and co-workers.[17,18] These examples represent important advances in the practical applicability of these biomimetic hosts in small molecule synthesis.

In contrast to small molecule synthesis, however, the application of supramolecular host−guest chemistry to the selective modification of larger and significantly more complex biomolecules such as proteins remains extremely rare. Protein post-translational modification, on the other hand, is an integral function of many enzymes including lysine acetyltransferases (KATs) and methyltransferases (KMTs) as well as tyrosinases,[19,20] which has been exploited in the recent years for site-selective protein bioconjugation.[21−25] We thus sought to develop a supramolecular host system that would emulate not only the mechanism of action of enzymes but also their remarkable breadth of reactivity ranging from small molecules to complex protein scaffolds. Drawing inspiration from the pyridine-based cofactor of ketoreductase enzymes (KREDs),[26−28] we report here a supramolecular host-mediated pyridine-borane reduction that proceeds under basic, aqueous conditions and allows reactivity with a range of small molecules including enones, ketones, aldehydes, oximes, hydrazones, and imines (Figure 1c). We then demonstrate the ability of this reaction to withstand unprecedented increases in substrate complexity, as we investigate applications in the site-selective reductive amination of lysine in peptides and proteins (Figure 1d).

RESULTS AND DISCUSSION

The highly anionic Ga_4L_6 host-**1** has been shown to catalyze a number of Lewis and Bronsted acid-activated organic transformations such as the Nazarov cyclization,[29] and more

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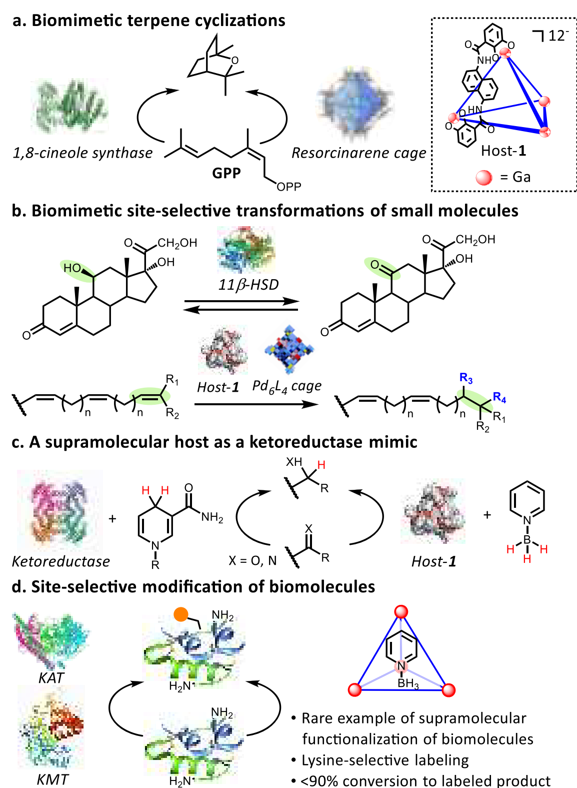

Figure 1. (a) Cyclization of GPP to 1,8-cineol catalyzed by 1,8-cineol synthase and a resorcinarene cage. (b) Site-selective oxidation/reduction by 11β-HSD and selective alkene functionalization by host-**1** and a Pd_6L_4 cage. (c) This work: host-**1**-mediated reduction with a pyridine−borane cofactor, which mimics the reactivity of ketoreductase enzymes. (d) Site-selective post-translation modification of biomolecules by various enzymes and a rare example of lysine-selective biomolecule functionalization by a supramolecular host.

recently an aza-Darzens reaction,[30] through hydrophobic-effect-driven binding and electrostatic stabilization of cationic intermediates and transition states. In view of these previous results, it was hypothesized that a hydride reduction reaction such as that illustrated in Figure 1c could be mediated by host-**1** via protonation of the substrate upon encapsulation. We first sought to identify a reducing agent that would not only be compatible with the host but would also result in minimal background reactivity in the absence of Lewis or Bronsted acid activators. A screen of various organic reducing agents identified pyridine-borane as a suitable candidate due to its inertness under basic aqueous conditions and hydrophobic-effect-driven binding to host-**1** (see Figure S1 and Table S1 in the Supporting Information).

While pyridine-borane has been used as a mild reducing agent in reductive aminations with formaldehyde under near-neutral conditions, acidic media such as acetic acid are generally required for more challenging reductions including those of ketones and enones.[31−33] In the presence of catalytic loadings of host-**1** and 1 equiv of borane, reduction of aliphatic and cyclic ketones **2a−i** to the corresponding alcohol products **3a−i** was observed, despite pD 8 water as the solvent (Table 1). Upon performing control experiments where the reaction was run in the presence of stoichiometric NEt_4^+ (added as the NEt_4Cl salt), a strongly binding inhibitor of host-**1**, only traces to low yields of alcohol product were observed in each case with the exception of cyclohexanone (Table 1, entry 9). As a

Table 1. Host-1-Catalyzed Pyridine−Borane Reduction of Aliphatic Ketones

2a-i + pyridine-BH_3 (1 eq) → (15 mol% host; pD 8, 100 mM K_3PO_4; RT, 21 h) → 3a-i

Entry	Substrate	Yield[a] [%]	$NEt_4^+ \subset$ host Yield [%]
1	R_1=R_2=Me **(2a)**	64	<5
2	R_1=Me, R_2=Et **(2b)**	98	<5
3	R_1=Me, R_2=Pr **(2c)**	67	<5
4	R_1=Me, R_2=Bu **(2d)**	15	<5
5	R_1=Me, R_2=Pe **(2e)**	<5	<5
6	R_1=Me, R_2=Pr-OH **(2f)**	68	<5
7	R_1=Me, R_2=Bu-OH **(2g)**[b]	59	11
8	cyclopentanone **(2h)**	70	<5
9	cyclohexanone **(2i)**	100	49

[a]NMR yields representing an average of two consecutive runs. [b]Reaction heated to 50 °C.

general trend, shorter alkyl ketones resulted in significantly higher yields (Table 1, entries 1−3) in the presence of catalytic loadings of host-**1**, with reactivity decreasing as the hydrophobic alkyl group grows longer (Table 1, entries 4 and 5). While a similar trend is observed for ketones with pendant alcohol groups (Table 1, entries 6 and 7), the decline in reactivity is less dramatic, particularly upon gentle heating of the reaction mixture. These observations imply that while hydrophobicity is required for the substrate to be encapsulated within the host, disproportionately strong binding of the substrate (as in the case for **2d** and **2e**) may be preventing coencapsulation with the pyridine−borane reductant, which is necessary for the reaction to proceed.

Encouraged by these initial results, α, β-unsaturated ketones were also investigated. In addition to potential acceleration in the rates of reduction, enones could provide insight into the inherent selectivity of the host-catalyzed reaction for the two reducible sites at the 1,2- and 1,4-positions. Upon subjecting mono- and disubstituted enones (**4a−e**) to host-**1**-mediated reduction conditions, the corresponding allyl alcohols **5a−e** were consistently observed as the major products in moderate yields. Control experiments with NEt_4^+-blocked host-**1** demonstrated unexpectedly high selectivities for the corresponding saturated ketone products **6a−c** for β-monosubstituted enones (Table 2, entries 1−3), yielding only trace amounts of the allyl alcohol in each case. Disubstitution at the β-position led to a marked decrease in background reactivity (Table 2, entries 4 and 5), whereas the host-catalyzed reaction preferentially yielded the allyl alcohol products **5d,e**. The high 1,2-selectivity observed in the presence of the host may be attributed to several factors: one possibility is that coencapsulation of the enone substrate with pyridine−borane enforces a conformation where the borane is oriented closer to the carbonyl group than the more distal 1,4-site.[34] Alter-

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Table 2. Host-1-Catalyzed Pyridine−Borane Reduction of α,β-Unsaturated Ketones

4a-e → (Pyridine-borane (1 eq), 15 mol%, pD 8, 100 mM K_3PO_4, RT, 21 h) → 5a-e + 6a-e + 7a-e

Entry	Substrate	Catalyst	5 Yield[a] [%]	5 : (6+7)
1	(4a)	Host-1	41	2.4 : 1
		$NEt_4^+ \subset$ 1	<5	1 : 39
2	(4b)[b]	Host-1	80	9 : 1
		$NEt_4^+ \subset$ 1	<5	1 : 16
3	(4c)[b,c]	Host-1	9	3.6 : 1
		$NEt_4^+ \subset$ 1	<5	1 : 3
4	(4d)[b]	Host-1	75	14 : 1
		$NEt_4^+ \subset$ 1	<5	n.d.
5	(4e)	Host-1	45	5 : 1
		$NEt_4^+ \subset$ 1	<5	n.d.

[a]NMR yields representing an average of two consecutive runs. [b]Selectivities determined by extracting the reaction with $CDCl_3$ and comparing to known spectral data. [c]Reaction run in 15% methanol due to low solubility of starting material under aqueous conditions.

natively, protonation of the carbonyl group, which is stabilized within the host cavity, may accelerate the 1,2-reduction pathway over the 1,4-pathway that occurs via polarization of the C−O bond. These results thus demonstrate that confinement within the host cavity not only accelerates the rate of the reaction but also overrides the innate selectivity of the uncatalyzed pathway.

We next turned our attention to oximes, which are more resistant toward reduction, even by stronger reductants such as $NaBH_4$ under pH neutral conditions. Addition of metal Lewis acid additives such as $CuSO_4$, $TiCl_4$, and $InCl_3$ or Bronsted acids such as acetic acid are typically required, which often leads to over-reduction of the hydroxylamine product to the amine.[35−37] Pyridine−borane has been shown to exclusively yield the corresponding hydroxylamine but again required strongly acidic conditions for the reaction to take place at an observable rate.[32] We thus decided to investigate whether host-**1** could be used to facilitate mild and selective reductions of oximes to the corresponding hydroxylamines.

Upon subjecting aryl and aliphatic aldoximes and ketoximes to host-**1**-mediated reduction conditions, moderate to high yields of the hydroxylamine products were observed, with no over-reduction to the amine. Aryl hydrazone **8b** (Table 3, entry 2) also underwent rapid reduction to the corresponding benzyl hydrazine product, albeit at lower yields due to heterogeneous reaction conditions. Under the mild reaction conditions acid-sensitive acetal protecting groups are also well tolerated, leading to high yields of the protected hydroxylamine product **9d** (Table 3, entry 4). Further experiments demonstrated that substituted hydroxylamine formation can also occur in a one-pot fashion, where the ketoxime is formed *in situ* by its ketone and hydroxylamine components to form **9e** (Scheme 1a). Notably, alcohol byproducts are not observed, presumably due to rapid oxime formation outcompeting ketone reduction. Blocked-host control experiments did not yield any observable conversion to the desired hydroxylamine product for any of the oxime substrates.

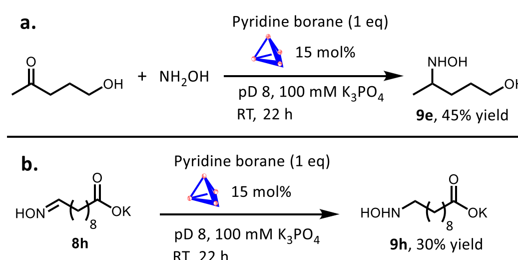
Table 3. Host-1-Catalyzed Pyridine−Borane Reduction of Oximes and Hydrazones

Pyridine-borane (1 eq), 15 mol%, pD 8, 100 mM K_3PO_4, RT, 22 h

Entry	Substrate	Product	Host Yield[a] [%]	$NEt_4^+ \subset$ Host Yield[a] [%]
1	(8a)	(9a)	100	<5
2	(8b)	(9b)	34[b]	<5
3	(8c)	(9c)	79	<5
4	(8d)	(9d)	81	<5
5	(8e)	(9e)	54	<5
6	(8f)	(9f)	67	<5
7	(8g)	(9g)	15	<5

[a]Yields represent an average of two consecutive runs. [b]Yield obtained from $CDCl_3$ extract due to lower water solubility of the product.

Scheme 1. (a) Host-1-Mediated One-Pot Synthesis of Substituted Hydroxylamines; (b) Reduction of a Decanoic Acid Oxime, Which Proceeds Despite Partial Encapsulation of the Substrate



O-Alkylation of oxime **8e** with an allyl group (**8g**), however, led to a marked decrease in yield, even under host-mediated conditions (Table 3, entry 7). In a 1:1 competition experiment with *O*-methyl oxime **8f**, this disparity in reactivity is evident, as a 15:1 selectivity for **8f** over **8g** is observed after 3 h. This result was attributed to size exclusion, where the increased steric bulk and hydrophobicity of *O*-allyl oxime **8g** inhibit coencapsulation within the host cavity with pyridine−borane.

We hypothesized that functionalization of the substrate with an anionic carboxylate group could attenuate the hydrophobicity of the substrate and encourage partial encapsulation, facilitated by unfavorable Coulombic interactions between the carboxylate group and the host. Maintaining reactivity for

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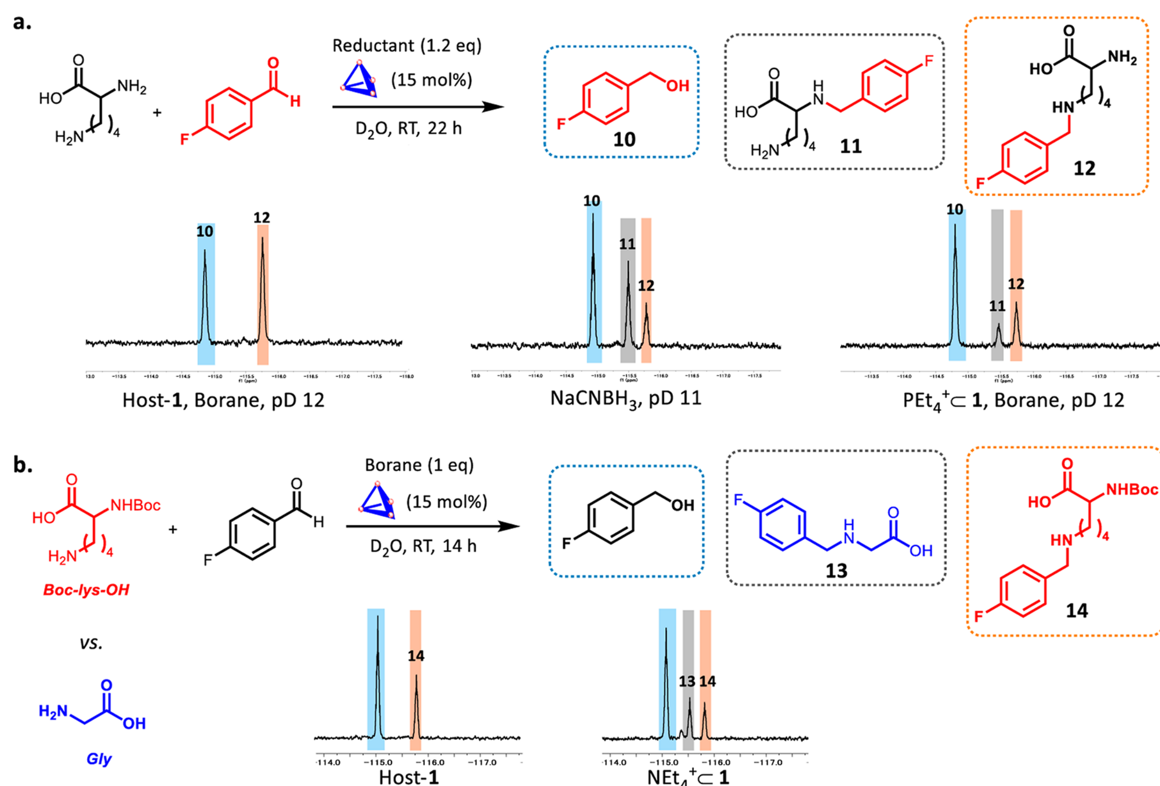

Figure 2. (a) ^{19}FNMR data depicting selective generation of ε-functionalized **12** under 1-catalyzed conditions and a mixture of α-functionalized **11** and **12** for $NaCNBH_3$ and blocked-host conditions. (b) Competition reaction between Gly and BocLysOH under **1**-catalyzed and blocked-host conditions.

partially encapsulated substrates would be imperative for site-selective applications of this host-catalyzed reduction to more complex substrates. To test this hypothesis, decanoic acid oxime **8h** was synthesized and subjected to **1**-catalyzed conditions (Scheme 1b). Despite being two carbons longer than **8g**, hydroxylamine product **9h** was observed in moderate yield (30%), whereas the NEt_4^+-blocked host afforded only trace yields (<5%).

Given the ability of host-**1** to maintain reactivity for larger, partially encapsulated substrates, we next explored lysine as a potential amphiphilic substrate for site-selective reductive amination due to the presence of two reactive primary amine sites: the α-N-terminus as well as the more basic, sterically accessible ε-terminus. Under host-mediated pD 8 conditions, however, 4-fluorobenzaldehyde undergoes rapid reduction to benzyl alcohol **10**, leading to low conversions to the reductive amination product. Increasing the pD of the solution mitigates this unproductive reaction pathway, and a catalytic loading of host-**1** at pD 12 yields ε-functionalized **12** in 65% yield with ~3% of α-functionalized product **11** (by ^{19}F NMR, Figure 2a). Upon addition of a stoichiometric amount of PEt_4I (a strongly binding guest) to host-**1**, a mixture of products is clearly observed, with a 28% yield of the ε-functionalized **12** and 15% yield of **11**. Furthermore, control reactions with $NaCNBH_3$ as the reducing agent conducted in differently buffered solutions (pD 5−11) consistently yielded a mixture of reductive amination products, with α-functionalized **11** being the major product in each case. To further determine whether host-**1** can distinguish between small differences in lipophilicity and steric accessibility, a competition experiment was performed with glycine and Nα-Boc-lysine substrates (Figure 2b). Only the lysine-functionalized product **14** was detected under host-**1** mediated conditions, whereas a mixture of both modified lysine (**14**) and glycine (**13**) products was observed upon blocking the host cavity with an equivalent of NEt_4Cl.

The high selectivity of host-**1** for the reductive alkylation of the ε-terminus of lysine led us to extend this strategy to the site-selective modification of lysine over a reactive N-terminus in peptide chains. While selective reductive amination of the N-terminus with benzaldehyde has been shown to proceed by using $NaCNBH_3$ under acidic conditions, selective alkylation of lysine is still relatively limited.[38,39] A notable strategy by Rai and co-workers involves addition of a Cu-acetylide into an *in situ* generated iminium on lysine, where site selectivity derives from a transient imidazolidinone protecting group on the N-terminus.[40] Alternatively, a recent report by Bernardes and co-workers utilized careful kinetic control with a sulfonyl acrylate electrophile to target the more nucleophilic lysine residue.[41] In developing a supramolecular bioconjugation strategy, one important consideration is the feasibility of postmodification of the functionalized peptide, given the size restrictions of the host cavity. To this end, we turned our attention to pentafluorobenzaldehyde (PFBA) as an alternative electrophile because of its ability to undergo facile S_NAr reactions with thiols after it is appended to the peptide.[42−44] We first subjected lysine and PFBA to aqueous $NaCNBH_3$ conditions and observed low levels of conversion (<15%) to a mixture of α-functionalized product **15** and ε-functionalized product **16**, presumably due to the destabilization of the iminium intermediate by the more electron-deficient arene (see Table S2 and Figures S34 and S41 in the Supporting Information). Under host-**1** catalyzed reduction conditions, however, >90% selectivity for ε-functionalized **16** was observed under a range of basic conditions (pD 10−12) (Figures S34−S38), whereas

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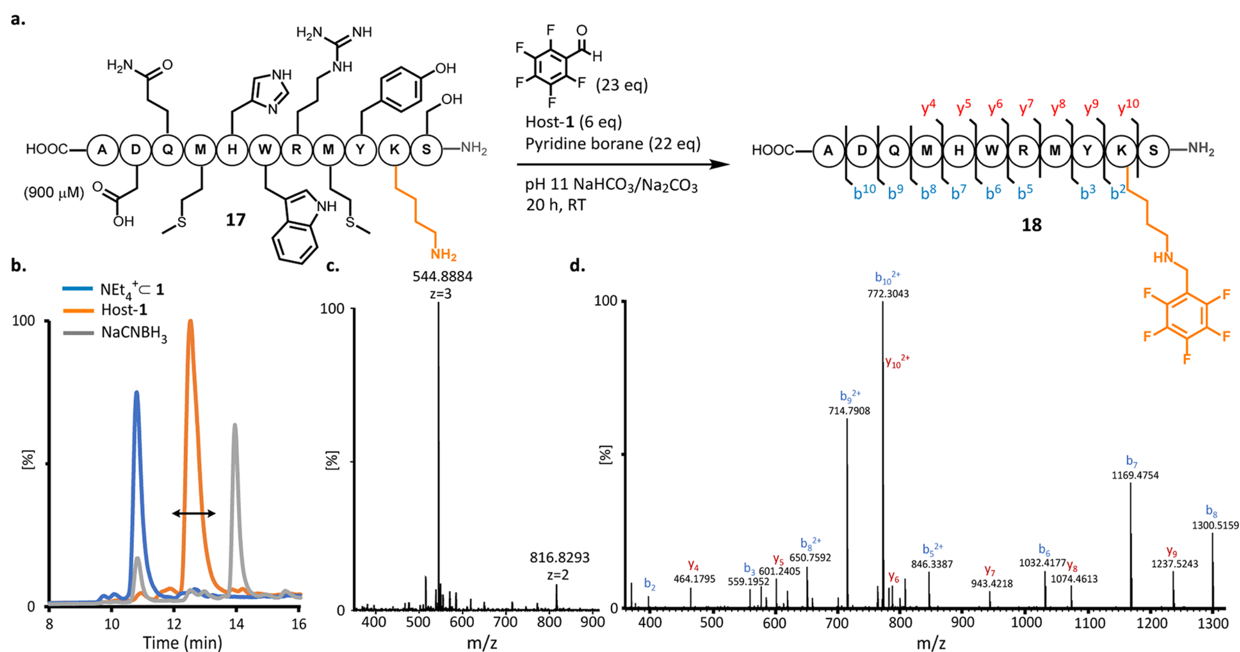

Figure 3. (a) Host-**1**-mediated reductive amination of peptide **17**. (b) LCMS trace of the host-**1**-mediated reaction, NEt_4^+-blocked host reaction, and $NaCNBH_3$ reaction. (c) HRMS of the host-**1**-mediated reaction. (d) Tandem MS/MS analysis of modified peptide **18**.

the PEt_4^+-blocked host afforded low conversions to an almost 1:1 mixture of **15** and **16** (Figures S39 and S40). The site of modification was further confirmed by subjecting Nα-Boc-lysine to the host-**1**-catalyzed conditions followed by Boc deprotection, which again yielded **16** (Figure S34).

We then subjected the 11-amino acid peptide chain **17** with a single lysine residue and serine N-terminus to the optimized supramolecular conditions and analyzed the results using an LCMS-TOF method (Figure 3a). Gratifyingly, 95% conversion to a major product **18** with a mass corresponding to a single modification was detected in the presence of host-**1** (Figure 3b,c). Blocking the host cavity with NEt_4Cl resulted in low conversion of the starting peptide, indicating that this was a host-mediated process. Control reactions with $NaCNBH_3$ at lower pH yielded almost exclusively a single product with the same m/z as the host-mediated product but with a different retention time, which was assigned as the N-terminus-modified product. Modified peptide **18** was sequenced by tandem MS/MS analysis, and the modification was determined to be exclusively at the lysine residue, with no N-terminus modification detected (Figure 3d).

To further assess the scope of this host-mediated reaction, we next turned our attention to human insulin as a potential substrate for selective lysine modification. Insulin consists of A and B peptide chains, containing 21 and 30 amino acids, respectively, with a single lysine residue in the B chain. In addition to representing a substantial increase in complexity in comparison to peptide **17** due to the larger number of amino acid residues as well as tertiary interactions, insulin contains two potentially reactive N-termini. While there is precedent for some selective N-acylation strategies, the lysine-selective reductive amination of insulin remains relatively rare.[45,46] Indeed, under reductive alkylation conditions with sodium borohydride, Feeney and co-workers observed comparable levels of modification at both the lysine residue and the glycine N-terminus.[47]

We initially subjected insulin (100 μM) to an excess of PFBA (20 mM), pyridine−borane (20 mM), and host-**1** (20 mM) under basic conditions at room temperature. Analysis of the crude reaction mixture by mass deconvoluted LCMS demonstrated high conversion (<90%) to a singly modified adduct **19**, in addition to small amounts of the doubly modified adduct. Upon blocking the host cavity with stoichiometric PEt_4I to assess background reactivity,[48] we observed only trace amounts of the singly and doubly modified product, with nearly quantitative recovery of unmodified insulin, suggesting that the modification was occurring exclusively within the cavity of host-**1**. Further optimization of the reaction conditions revealed that the concentrations of aldehyde, borane, and host-**1** could be lowered without impacting the conversion or selectivity of the reaction (Figure 4).

To determine the site of modification on **19**, the crude product was subjected to an excess of tris(2-carboxyethyl)-phosphine (TCEP) to reduce the disulfide bonds present in the molecule.[49] By HRMS, the reduced product mixture consisted primarily of modified B chain and unmodified A chain, indicative of lysine modification rather than the glycine N-terminus (Figure S51). While trace amounts of modified A chain and unmodified B chain were also detected, this was largely consistent with the presence of small quantities of the doubly modified product (resulting from low levels of background reactivity), as well as unmodified insulin. Finally, tandem MS/MS analysis of the modified B chain and unmodified A chain confirmed the site of modification to be on the desired lysine residue.

■ CONCLUSIONS

In conclusion, we have developed a supramolecular host-promoted pyridine−borane reduction, which proceeds under mild, fully aqueous conditions with a substrate scope that includes enones, ketones, aldehydes, oximes, hydrazones, and imines. In the presence of catalytic amounts of host-**1**, lysine

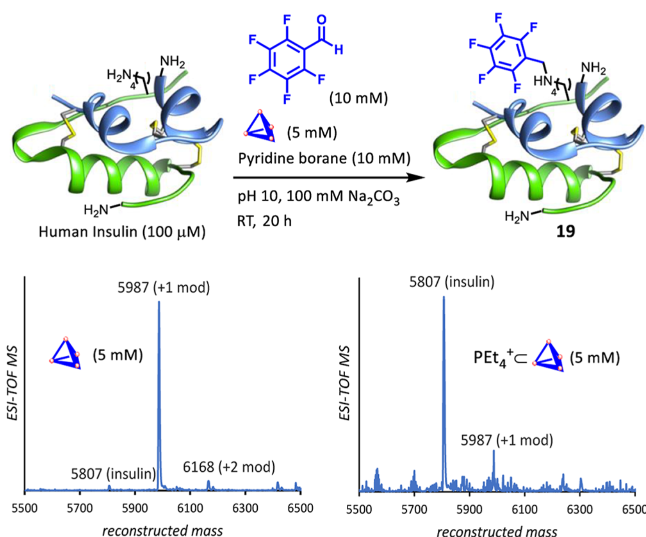

Figure 4. Host-**1**-mediated lysine-selective reductive amination of human insulin under optimized conditions; ESI-TOF data for the host-**1**-mediated and PEt_4^+-blocked host reaction.

was site-selectively functionalized at the ε-terminus via reductive amination with various fluorobenzaldehydes. We then successfully extended this method to lysine-selective alkylation of a peptide as well as a protein, demonstrating a rare example of site-selective supramolecular functionalization of complex biomolecules. The ability of the reaction to proceed at micromolar substrate concentrations under fully aqueous conditions shows promise for future applications of supramolecular methods in protein bioconjugation.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.0c12479.

General synthetic procedures, ESI-MS data, and characterization of new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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