Title

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

Journal

Journal of the American Chemical Society, 143(4)

ISSN

0002-7863

Authors

Morimoto, Mariko
Cao, Wendy
Bergman, Robert G
et al.

Publication Date

2021-02-03

DOI

10.1021/jacs.0c12479

Peer reviewed

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

Mariko Morimoto, Wendy Cao, Robert G. Bergman*, Kenneth N. Raymond*, F. Dean Toste*

Chemical Sciences Division, Lawrence Berkeley National Laboratory and Department of Chemistry, University of California, Berkeley, California 94720, United States.

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 chemo- and site-selective modification of complex biomolecules remain virtually unexplored. We report here a supramolecular system where co-encapsulation 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, excellent ε-selectivity was observed, 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 non-covalent 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- and chemo-selective 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-

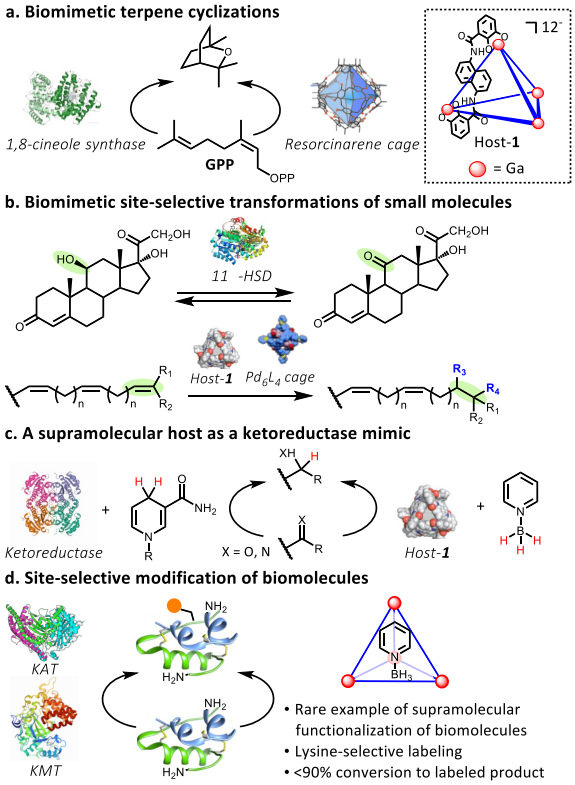


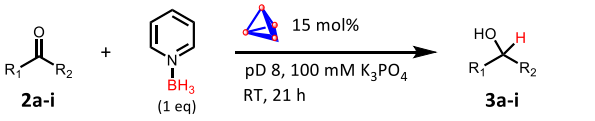
Figure 1. a) Cyclization of GPP to 1,8-cineol catalyzed by 1,8-cineol synthase and a resorcinarene cage; **b)** Site-selective oxidation/reduction by 11β-HSD and selective alkene functionalization by host-**1** and a Pd_6L_4 cage; **c)** This work: host-**1** mediated reduction with a pyridine borane 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

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 their remarkable breadth of reactivity ranging from small molecules to complex protein scaffolds. Drawing inspiration from the pyridine-based co-factor of ketoreductase enzymes (KREDs),[26–28] we report here a supramolecular host-mediated pyridine borane reduction that proceeds under basic, aqueous conditions, and reacts 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

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 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 Supporting Information).

Table 1. Host-**1** catalyzed pyridine borane reduction of aliphatic ketones



Entry	Substrate	Yield[a] [%]	NEt_4^+ 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	 **(2h)**	70	<5
9	 **(2i)**	100	49

[a] NMR yields representing an average of 2 consecutive runs

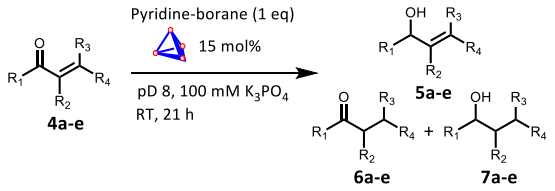
[b] Reaction heated to 50°C

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 one equivalent of borane, reduction of aliphatic and cyclic ketones **2a–i** to the corresponding alcohol products **3a–i** were observed, despite pD 8 water as 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 trace to low yields of alcohol product were observed in each case with the exception of cyclohexanone (Table 1, entry 9). As a 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, 5). While a similar trend is observed for ketones with pendant alcohol groups (Table 1, entries 6, 7), the decline in reactivity is less dramatic, particularly upon gentle heating of the reaction. These observations imply that while hydrophobicity is required for the substrate to encapsulate within the host, disproportionately strong binding of the substrate (as in the case for **2d** and **2e**) may be preventing co-encapsulation 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. Di-substitution at the β-position led to a marked decrease in background reactivity (Table 2, entries 4, 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 co-encapsulation 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] Alternatively, protonation of the carbonyl group, which is stabilized within the host cavity, may accelerate the 1,2-reduction pathway over the 1,4 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 lead 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 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.

Table 2. Host-**1** catalyzed pyridine borane reduction of α, β-unsaturated ketones



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 2 consecutive runs

[b] Selectivities determined by extracting the reaction with $CDCl_3$ and comparing to known spectral data

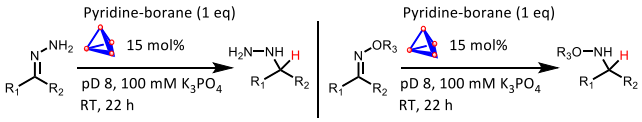
[c] Reaction run in 15% methanol due to low solubility of starting material under aqueous conditions

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 by-products are not observed, presumably due to rapid oxime formation out-competing ketone reduction. Blocked-host control experiments did not yield any observable conversion to the desired hydroxylamine product for any of the oxime substrates.

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 3h. This result was attributed to size-exclusion, where the increased steric bulk and hydrophobicity of O-allyl oxime **8g** inhibits co-encapsulation 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 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%).

Table 3. Host-**1** catalyzed pyridine borane reduction of oximes and hydrazones

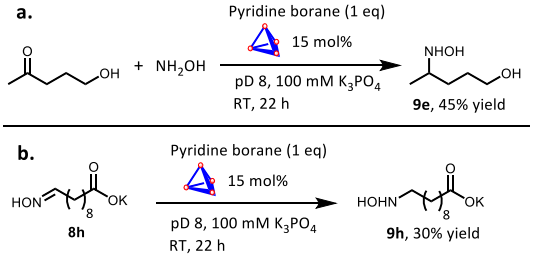


Entry	Substrate	Product	Yield[a] [%]	NEt_4^+ Yield[a] [%]
1	(8a)	(9a)	100	<5
2	(8b)	(9b)	34[b]	<5
3	(8c)	(9c)	79	<5
4	(8d)	(9d)	81	<5
5	(8e)	(9e)	54	<5
6	(8f)	(9f)	67	<5
7	(8g)	(9g)	15	<5

[a] Yields represent an average of 2 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



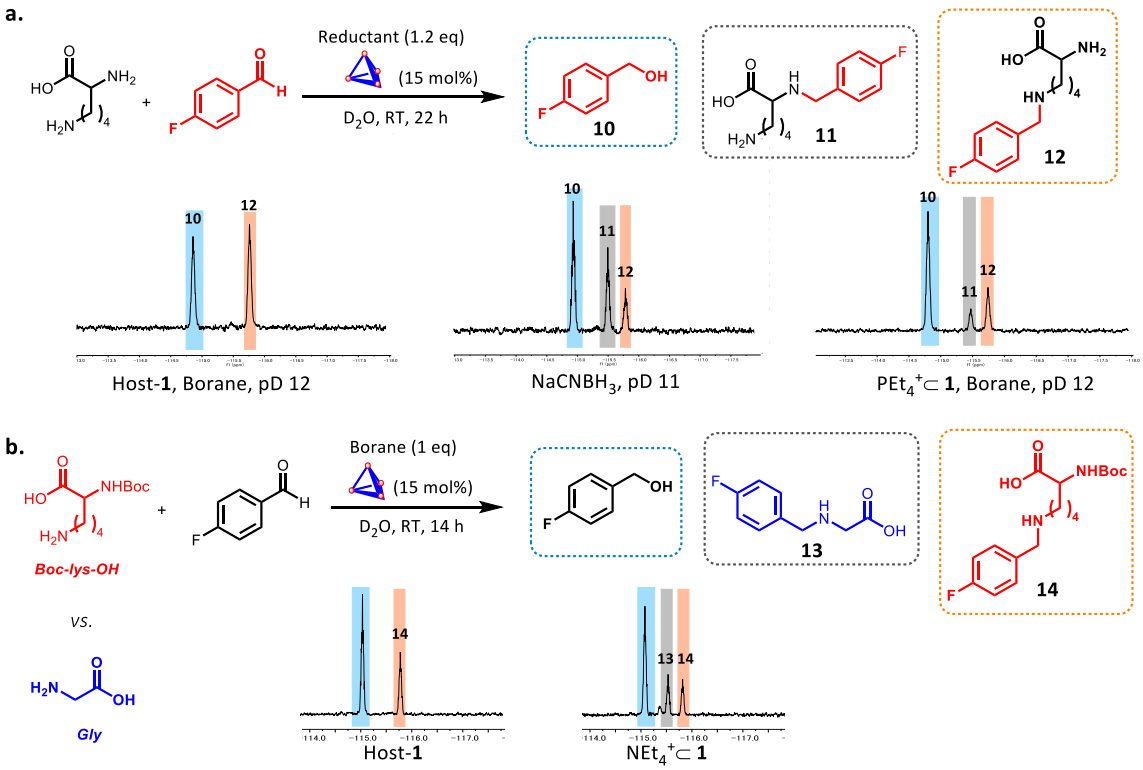


Figure 2. a) [19]FNMR data depicting selective generation of ε-functionalized **12** under 1-catalyzed conditions, and a mixture of α-functionalized **11** and **12** for $NaCNBH_3$ and blocked-host conditions; **b)** competition reaction between Gly and BocLysOH under **1**-catalyzed and blocked-host conditions

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]FNMR, 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 were 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 using $NaCNBH_3$ under acidic conditions, selective alkylation of lysine is still relatively limited.[38,39] A notable strategy by Rai and coworkers 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 coworkers utilizes careful kinetic control with a sulfonylacrylate electrophile to target the more nucleophilic lysine residue.[41] In developing a supramolecular bioconjugation strategy, one important consideration is the feasibility of post-modification 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, due to 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 Figure S34, S41 in 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) (Figure S34–38), whereas PEt_4^+-blocked host afforded low conversions to an almost 1:1 mixture of **15** and **16** (Figure S39, 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).

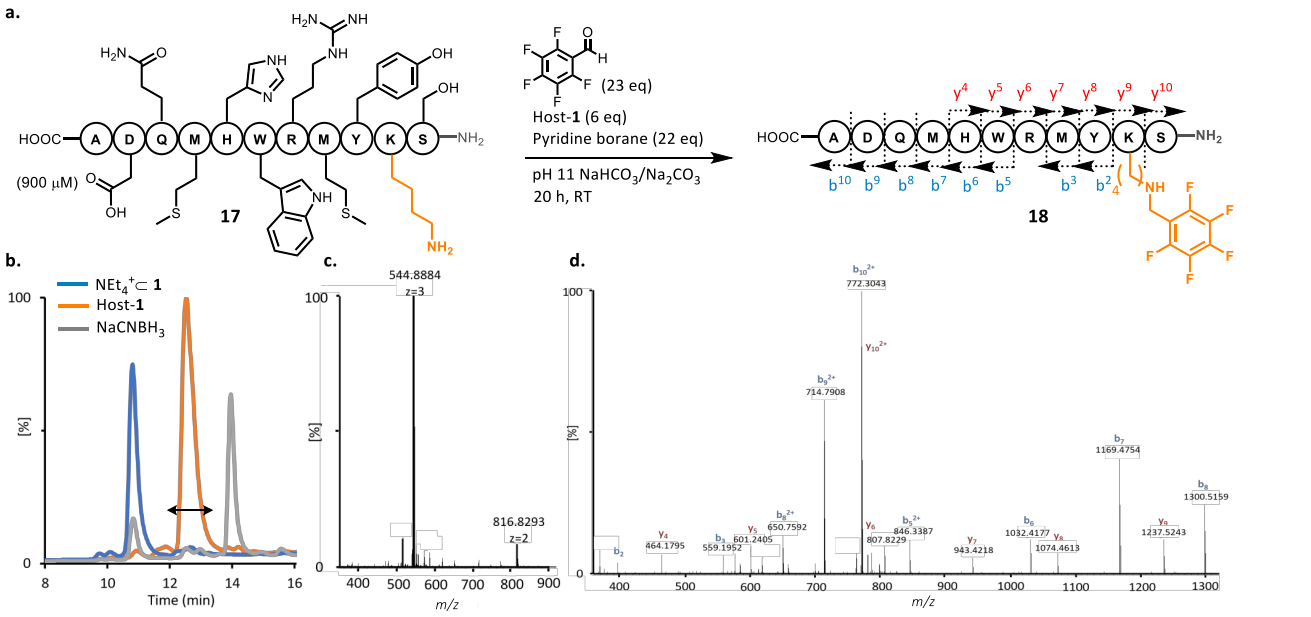


Figure 3. a) Host-**1** mediated reductive amination of peptide **17**; **b)** LCMS trace of the host-**1** mediated reaction, NEt_4^+-blocked host reaction, and $NaCNBH_3$ reaction; **c)** HRMS of the host-**1** mediated reaction; **d)** Tandem MS/MS analysis of modified peptide **18**

We then subjected 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 coworkers observed comparable levels of modification at both the lysine residue as well as 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).

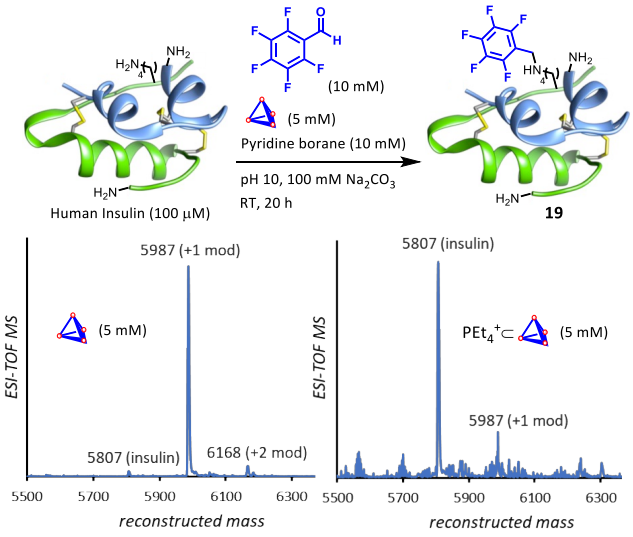


Figure 4. Host-**1** mediated lysine-selective reductive amination of human insulin under optimized conditions; ESI-TOF data for the host-**1** mediated and PEt_4^+-blocked host reaction

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 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

General synthetic procedures, ESI-MS data, and characterization of new compounds are available in the Supporting Information. The Supporting Information is available free of charge on the ACS Publications website at DOI:

AUTHOR INFORMATION

Corresponding Authors

*rbergman@berkeley.edu
*raymond@socrates.berkeley.edu
*fdtoste@berkeley.edu

Notes

The authors declare no competing financial interests.

ACKNOWLEDGMENT

This research was supported by the Director, Office of Science, Office of Basic Energy Sciences, and the Division of Chemical Sciences, Geosciences, and Bioscience of the U.S. Department of Energy at Lawrence Berkeley National Laboratory (Grant No. DE-AC02-05CH11231) and NSF GRFP to W. C. (DGE 1752814). We gratefully thank Dr. Anthony T. Iavarone for his expertise and guidance in electrospray mass spectrometry experiments and analysis. We also thank Dr. Hasan Celik and UC Berkeley's NMR facility in the College of Chemistry (CoC-NMR) for spectroscopic assistance. Instruments in the CoC-NMR are supported in part by NIH S10OD024998.

REFERENCES

(1) Yoshizawa, M.; Klosterman, J. K.; Fujita, M. Functional Molecular Flasks: New Properties and Reactions within Discrete, Self-Assembled Hosts. *Angew. Chem. Int. Ed.* **2009**, *48* (19), 3418–3438.

(2) Brown, C. J.; Toste, F. D.; Bergman, R. G.; Raymond, K. N. Supramolecular Catalysis in Metal–Ligand Cluster Hosts. *Chem. Rev.* **2015**, *115* (9), 3012–3035.

(3) Morimoto, M.; Bierschenk, S. M.; Xia, K. T.; Bergman, R. G.; Raymond, K. N.; Toste, F. D. Advances in Supramolecular Host-Mediated Reactivity. *Nat. Catal.* **2020**, 1–16.

(4) Yu, Y.; Rebek, J. Reactions of Folded Molecules in Water. *Acc. Chem. Res.* **2018**, *51*, 3031–3040.

(5) Roland, S.; Suarez, J. M.; Sollogoub, M. Confinement of Metal-N-heterocyclic Carbene Complexes to Control Reactivity in Catalytic Reactions. *Chem. Eur. J.* **2018**, *24*, 12464–12473.

(6) Jongkind, L. J.; Caumes, X.; Hartendorp, A. P. T.; Reek, J. N. H. Ligand Template Strategies for Catalyst Encapsulation. *Acc. Chem. Res.* **2018**, *51*, 2115–2128.

(7) Raynal, M.; Ballester, P.; Vidal-Ferran, A.; Van Leeuwen, P. W. N. M. Supramolecular Catalysis. Part 2: Artificial Enzyme Mimics. *Chem. Soc. Rev.* **2014**, *43*, 1734–1787.

(8) Kujpers, P. F.; Otte, M.; Durr, M.; Ivanovic-Burmazovic, I.; Reek, J. N. H.; de Bruin, B. A Self-Assembled Molecular Cage for Substrate-Selective Epoxidation Reactions in Aqueous Media. *ACS Catal.* **2016**, *6* (5), 3106–3112.

(9) Masar, M. S.; Gianneschi, N. C.; Oliveri, C. G.; Stern, C. L.; Nguyen, S. T.; Mirkin, C. A. Allosterically Regulated Supramolecular Catalysis of Acyl Transfer Reactions for Signal Amplification and Detection of Small Molecules. *J. Am. Chem. Soc.* **2007**, *129* (33), 10149–10158.

(10) Guo, D.; Liu, Y. Supramolecular Chemistry of p-Sulfonato-Calix[*n*]arenes and Its Biological Applications. *Acc. Chem. Res.* **2014**, *47* (7), 1925–1934.

(11) Fang, Y.; Powell, J. A.; Li, E.; Wang, Q.; Perry, Z.; Kirchon, A.; Yang, X.. Xiao, Z. Zhu, C.; Zhang, L.; Huang, F.; Zhou, H. Catalyti Reactions Within the Cavity of Coordination Cages. *Chem. Soc. Rev.* **2019**, *48*, 4707–4730.

(12) Zhang, Q.; Tiefenbacher, K. Terpene Cyclization Catalysed inside a Self-Assembled Cavity. *Nat. Chem.* **2015**, *7* (3), 197.

(13) Zhang, Q.; Catti, L.; Pleiss, J.; Tiefenbacher, K. Terpene Cyclizations inside a Supramolecular Catalyst: Leaving-Group-Controlled Product Selectivity and Mechanistic Studies. *J. Am. Chem. Soc.* **2017**, *139* (33), 11482–11492.

(14) Karunanithi, P. S.; Zerbe, P. Terpene Synthases as Metabolic Gatekeepers in the Evolution of Plant Terpenoid Chemical Diversity. *Front. Plant Sci.* **2019**, *10*, 1166.

(15) Croteau, R.; Alonso, W. R.; Koepp, A. E.; Johnson, M. A. Biosynthesis of Monoterpenes: Partial Purification, Characterization, and Mechanism of Action of 1,8-Cineole Synthase. *Arch. Biochem. Biophys.* **1994**, *309* (1), 184–192.

(16) Chapman, K.; Holmes, M.; Seckl, J. 11β-Hydroxysteroid Dehydrogenases: Intracellular Gate-Keepers of Tissue Glucocorticoid Action. *Physiol Rev* **2013**, *93* (3), 1139–1206.

(17) Takezawa, H.; Kanda, T.; Nanjo, H.; Fujita, M. Site-Selective Functionalization of Linear Diterpenoids through U-Shaped Folding in a Confined Artificial Cavity. *J. Am. Chem. Soc.* **2019**, *141* (13), 5112–5115.

(18) Bender, T. A.; Bergman, R. G.; Raymond, K. N.; Toste, F. D. A Supramolecular Strategy for Selective Catalytic Hydrogenation Independent of Remote Chain Length. *J. Am. Chem. Soc.* **2019**, *141* (30), 11806–11810.

(19) Kontaxi, C.; Piccardo, P.; Gill, A. C. Lysine-Directed Post-Translational Modifications of Tau Protein in Alzheimer's Disease and Related Tauopathies. *Front. Mol. Biosci.* **2017**, *4,* 56.

(20) Walsh, C. T.; Garneau-Tsodikova, S.; Gatto, G. J. Protein Posttranslational Modifications: The Chemistry of Proteome Diversifications. *Angew. Chem. Int. Ed.* **2005**, *44* (45), 7342–7372.

(21) Baalmann, M.; Ziegler, M. J.; Werther, P.; Wilhelm, J.; Wombacher, R. Enzymatic and Site-Specific Ligation of Minimal-Size Tetrazines and Triazines to Proteins for Bioconjugation and Live-Cell Imaging. *Bioconjugate Chem.* **2019**, *30* (5), 1405–1414.

(22) Lobba, M. J.; Fellmann, C.; Marmelstein, A. M.; Maza, J. C.; Kissman, E. N.; Robinson, S. A.; Staahl, B. T.; Urnes, C.; Lew, R. J.; Mogilevsky, C. S.; Doudna, J. A.; Francis, M. B. Site-Specific Bioconjugation through Enzyme-Catalyzed Tyrosine–Cysteine Bond Formation. *ACS Cent. Sci.* **2020**, *6* (9), 1564–1571.

(23) Matsumoto, T.; Tanaka, T.; Kondo, A. Enzyme-Mediated Methodologies for Protein Modification and Bioconjugate Synthesis. *Biotechnol. J.* **2012**, *7* (9), 1137–1146.

(24) Yang, C.; Mi, J.; Feng, Y.; Ngo, L.; Gao, T.; Yan, L.; Zheng, Y. G. Labeling Lysine Acetyltransferase Substrates with Engineered Enzymes and Functionalized Cofactor Surrogates. *J. Am. Chem. Soc.* **2013**, *135* (21), 7791–7794.

(25) Sunbul, M.; Yin, J. Site Specific Protein Labeling by Enzymatic Posttranslational Modification. *Org. Biomol. Chem.* **2009**, *7* (17), 3361–3371.

(26) Hughes, D. L. Biocatalysis in Drug Development—Highlights of the Recent Patent Literature. *Org. Process Res. Dev.* **2018**, *22* (9), 1063–1080.

(27) Savile, C. K.; Janey, J. M.; Mundorff, E. C.; Moore, J. C.; Tam, S.; Jarvis, W. R.; Colbeck, J. C.; Krebber, A.; Fleitz, F. J.; Brands, J.; Devine, P. N.; Huisman, G. W.; Hughes, G. J. Biocatalytic Asymmetric Synthesis of Chiral Amines from Ketones Applied to Sitagliptin Manufacture. *Science* **2010**, *329* (5989), 305–309.

(28) Noey, E. L.; Tibrewal, N.; Jiménez-Osés, G.; Osuna, S.; Park, J.; Bond, C. M.; Cascio, D.; Liang, J.; Zhang, X.; Huisman, G. W.; Tang, Y.; Houk, K. N. Origins of Stereoselectivity in Evolved Ketoreductases. *PNAS* **2015**, *112* (51), E7065–E7072.

(29) Hastings, C. J.; Pluth, M. D.; Bergman, R. G.; Raymond, K. N. Enzymelike Catalysis of the Nazarov Cyclization by Supramolecular Encapsulation. *J. Am. Chem. Soc.* **2010**, *132* (20), 6938–6940.

(30) Bierschenk, S. M.; Bergman, R. G.; Raymond, K. N.; Toste, F. D. A Nanovessel-Catalyzed Three-Component Aza-Darzens Reaction. *J. Am. Chem. Soc.* **2020**, *142* (2), 733–737.

(31) Bomann, M. D.; Guch, I. C.; DiMare, M. A Mild, Pyridine-Borane-Based Reductive Amination Protocol. *J. Org. Chem.* **1995**, *60* (18), 5995–5996.

(32) Kawase, M.; Kikugawa, Y. Chemistry of Amine–Boranes. Part 5. Reduction of Oximes, O-Acyloximes, and O-Alkyloximes with Pyridine–Borane in Acid. *J. Chem. Soc., Perkin Trans. 1* **1979**, No. 0, 643–645.

(33) Pelter, A.; Rosser, R. M.; Mills, S. Reductive Aminations of Ketones and Aldehydes Using Borane–Pyridine. *J. Chem. Soc., Perkin Trans. 1* **1984**, No. 0, 717–720.

(34) While not directly analogous, changes in the regioselectivity of hydride reductions of enones have also been demonstrated using an NHC-capped cyclodextrin host by Sollogoub and coworkers: Xu, G.; Leloux, S.; Zhang, P.; Suarez, J. M.; Zhang, Y.; Derat, E.; Menand, M.; Birstri-Aslanoff, O.; Roland, S.; Leyssens, T.; Riant, O.; Sollogoub, M. Capturing the Monomeric (L)CuH in NHC-Capped Cyclodextrin: Cavity-Controlled Chemoselective Hydrosilylation of α,β-Unsaturated Ketones. *Angew. Chem. Int. Ed.* **2020**, *59* (19), 7591–7597.

(35) Hoffman, C.; Tanke, R. S.; Miller, M. J. Synthesis of Alpha-Amino Acids by Reduction of Alpha-Oximino Esters with Titanium (III) Chloride and Sodium Borohydride. *J. Org. Chem.* **1989**, *54* (15), 3750–3751.

(36) Liu, S.; Yang, Y.; Zhen, X.; Li, J.; He, H.; Feng, J.; Whiting, A. Enhanced Reduction of C–N Multiple Bonds Using Sodium Borohydride and an Amorphous Nickel Catalyst. *Org. Biomol. Chem.* **2011**, *10* (3), 663–670.

(37) Periasamy, M.; Thirumalaikumar, M. Methods of Enhancement of Reactivity and Selectivity of Sodium Borohydride for Applications in Organic Synthesis. *J. Organomet. Chem.* **2000**, *609* (1), 137–151.

(38) Chen, D.; Disotuar, M. M.; Xiong, X.; Wang, Y.; Chou, D. H.-C. Selective N-Terminal Functionalization of Native Peptides and Proteins. *Chem. Sci.* **2017**, *8* (4), 2717–2722.

(39) Chan, A. O.-Y.; Ho, C.-M.; Chong, H.-C.; Leung, Y.-C.; Huang, J.-S.; Wong, M.-K.; Che, C.-M. Modification of N-Terminal α-Amino Groups of Peptides and Proteins Using Ketenes. *J. Am. Chem. Soc.* **2012**, *134* (5), 2589–2598.

(40) Chilamari, M.; Purushottam, L.; Rai, V. Site-Selective Labeling of Native Proteins by a Multicomponent Approach. *Chem. Eur. J.* **2017**, *23* (16), 3819–3823.

(41) Matos, M. J.; Oliveira, B. L.; Martínez-Sáez, N.; Guerreiro, A.; Cal, P. M. S. D.; Bertoldo, J.; Maneiro, M.; Perkins, E.; Howard, J.; Deery, M. J.; Chalker, J. M.; Corzana, F.; Jiménez-Osés, G.; Bernardes, G. J. L. Chemo- and Regioselective Lysine Modification on Native Proteins. *J. Am. Chem. Soc.* **2018**, *140* (11), 4004–4017.

(42) Spokoyny, A. M.; Zou, Y.; Ling, J. J.; Yu, H.; Lin, Y.-S.; Pentelute, B. L. A Perfluoroaryl-Cysteine SNAr Chemistry Approach to Unprotected Peptide Stapling. *J. Am. Chem. Soc.* **2013**, *135* (16), 5946–5949.

(43) Birchall, J. M.; Green, M.; Haszeldine, R. N.; Pitts, A. D. The Mechanism of the Nucleophilic Substitution Reactions of Polyfluoroarenes. *Chem. Commun.* **1967**, No. 7, 338–339.

(44) Becer, C. R.; Hoogenboom, R.; Schubert, U. S. Click Chemistry beyond Metal-Catalyzed Cycloaddition. *Angew. Chem. Int. Ed.* **2009**, *48* (27), 4900–4908.

(45) Østergaard, M.; Mishra, N. K.; Jensen, K. J. The ABC of Insulin: The Organic Chemistry of a Small Protein. *Chem. Eur. J.* **2020**, *26* (38), 8341–8357.

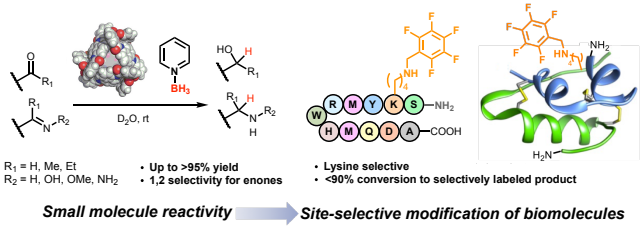
(46) Mei, H.; Yu, X. C.; Chan, K. K. NBl-C16-Insulin: Site-Specific Synthesis, Purification, and Biological Activity. *Pharm Res* **1999**, *16* (11), 1680–1686.

(47) Means, G. E.; Feeney, R. E. Reductive Alkylation of Amino Groups in Proteins. *Biochemistry* **1968**, *7* (6), 2192–2201.

(48) While PEt_4^+ has a higher affinity for host-**1** than NEt_4^+, both ammonium and phosphonium salts have large association constants ($K_{eq} > 10^4$ M^{-1} in D_2O) and can generally be used interchangeably for most control reactions. For the blocked-host control reaction with insulin, however, PEt_4^+ was required to adequately inhibit host reactivity due to the excess in host and micromolar concentrations of the protein substrate.

(49) Burns, J. A.; Butler, J. C.; Moran, J.; Whitesides, G. M. Selective Reduction of Disulfides by Tris(2-Carboxyethyl)Phosphine. *J. Org. Chem.* **1991**, *56* (8), 2648–2650.

TOC:


D2O, rt
BH3
R1 = H, Me, Et
R2 = H, OH, OMe, NH2
• Up to >95% yield
• 1,2 selectivity for enones
• Lysine selective
• <90% conversion to selectively labeled product
Small molecule reactivity
Site-selective modification of biomolecules