Formaldehyde-mediated Hydride Liberation of Alkylamines for Intermolecular Reactions in HFIP

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Abstract: The ability of alkylamines to spontaneously liberate hydride ions is typically restrained, except under specific intramolecular reaction settings. Herein, we demonstrate that this reactivity can be unlocked through the simple treatment with formaldehyde in hexafluoroisopropanol (HFIP) solvent, thereby enabling various intermolecular hydride transfer reactions of alkylamines under mild conditions. Besides transformations of small molecules, these reactions enable unique late-stage modification of complex peptides. Mechanistic investigations uncover that the key to these intermolecular hydride transfer processes lies in the accommodating conformation of solvent-mediated macrocyclic transition states, where the aggregates of HFIP molecules act as dexterous proton shuttles. Importantly, negative hyperconjugation between the lone electron pair of nitrogen and the anti-bonding orbital of amine's α C-H bond plays a critical role in the C-H activation, promoting its hydride-liberation.

One-sentence Summary: Unlocking the redox reactivity of alkylamines through mild, metal-free hydride liberation.

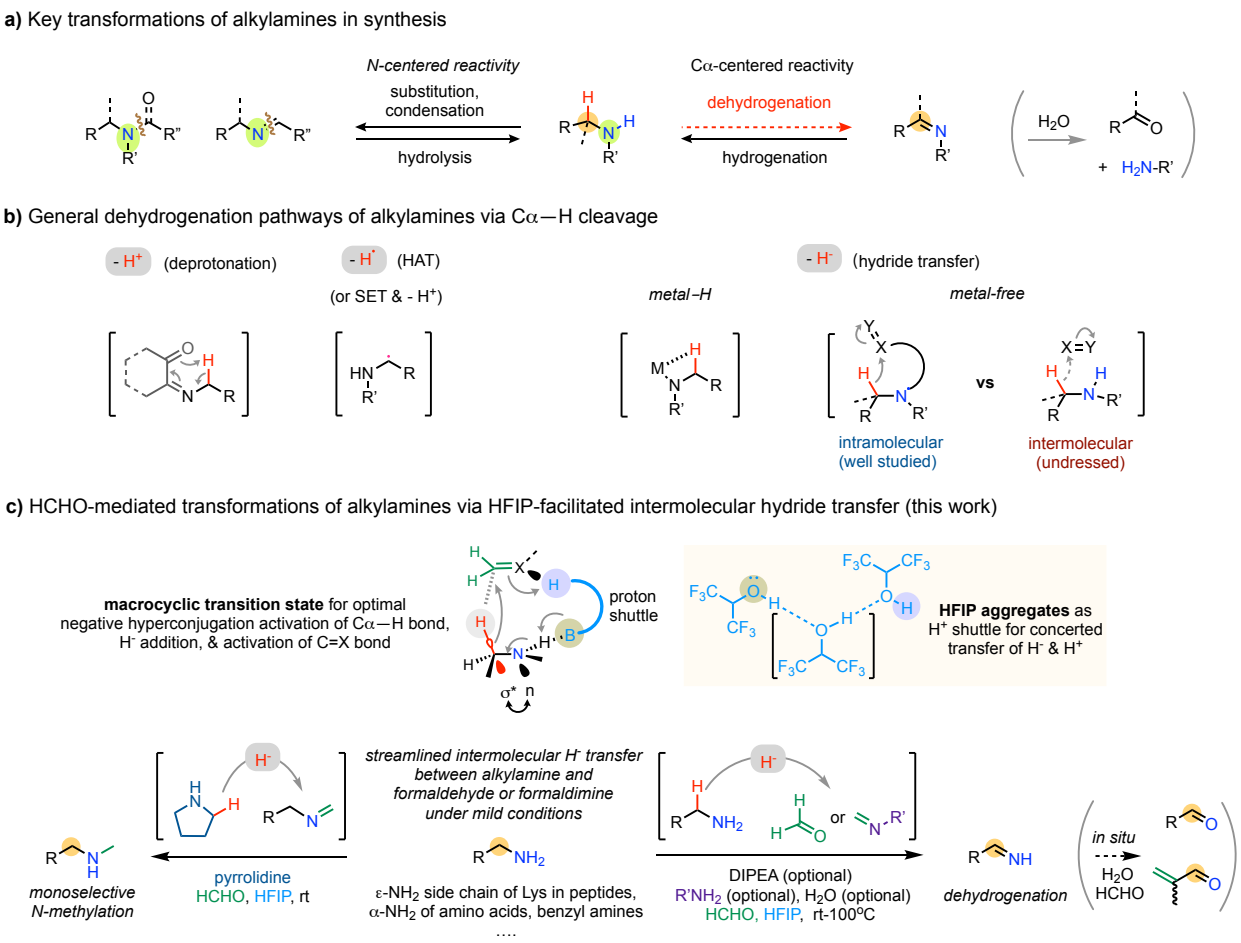


Figure 1. Dehydrogenative transformations of alkylamines via intermolecular hydride transfer.

Alkylamines constitute a fundamental class of organic compounds with diverse roles in both natural processes and the synthesis of human-made materials.(*1,2*) The reactivity of alkylamines is primarily dictated by the nucleophilicity of the nitrogen atom, as exemplified in various *N*-centered substitution or condensation reactions where N-H bonds of amines are transformed into N-C bonds (**Fig. 1a**). Despite their predominant non-redox reactivity, alkylamines also possess distinctive redox capabilities.(*3*) Of particular significance is the dehydrogenation of alkylamines into imines or iminiums, with the potential to yield aldehydes or ketones upon hydrolysis.(*4,5*) This process could open up exciting opportunities for altering alkylamines at their otherwise inert α carbon positions and enable a powerful "crossover" between amine and carbonyl compounds. While non-redox N-H substitution reactions are well-established, harnessing the redox reactivity of alkylamines for the generation of imines has presented substantial challenges for chemists. Mechanistically, the crux of the alkylamine dehydrogenation revolves around the cleavage of the unreactive α C-H bond.(*6-8*) As illustrated in **Fig. 1b**, various strategies have been explored to effect this Cα-H bond cleavage, including deprotonation (H^+ transfer) with vicinal dicarbonyl reagents,(*9*) hydrogen atom transfer (HAT) or single electron transfer/deprotonation,(*10-12*) and metal-catalyzed hydride (H^-) transfer reactions.(*13,14*) In addition to metal-catalyzed pathways, hydride transfer within alkylamines can also occur without metal assistance, although primarily in intramolecular settings.(*15-21*) Despite the significant advances in intramolecular reactions, our understanding of amine

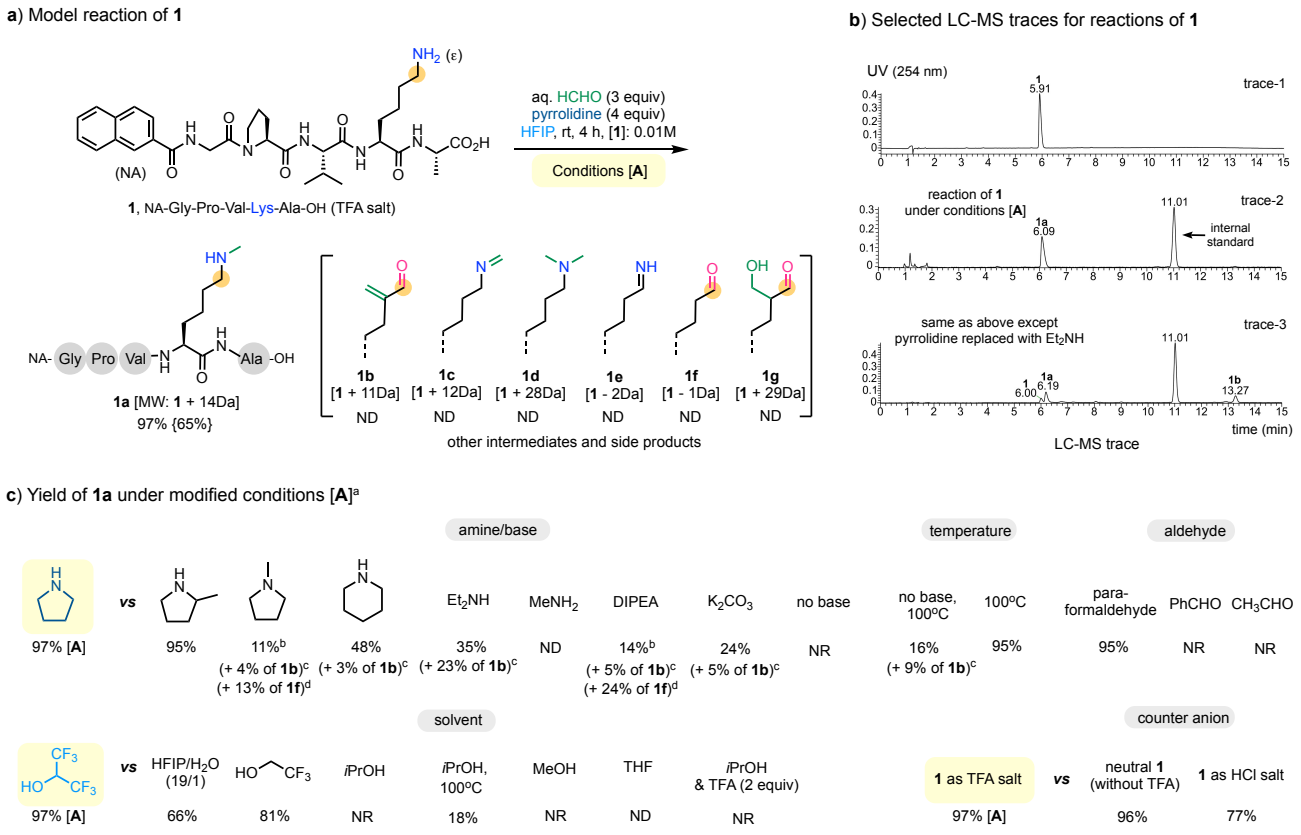
hydride liberation remains limited. Our ability to control this process is restricted, and practical methods for metal-free dehydrogenation of alkylamines via intermolecular hydride transfer have remained elusive.

In this work, we report a straightforward approach employing formaldehyde (HCHO) reagent in hexafluoroisopropanol (HFIP) solvent to harness the hydride-liberation potential of alkylamines, thereby enabling a range of intermolecular hydride transfer reactions with formaldehyde or formaldimines under mild conditions. By leveraging pyrrolidine as an external hydride donor, primary alkylamines can undergo selective *N*-methylation via the formaldimine intermediates at room temperature (rt). By tuning the reaction conditions to neutral or mildly basic settings, alkylamines can undergo selective dehydrogenation via direct hydride transfer to HCHO, yielding imines that can be further converted to other carbonyl products. In addition to its applicability across small molecule substrates, these reactions offer unique methods for mono-selective *N*-methylation of amino side chains within peptides, or the conversion of lysine's nucleophilic amino side chain within peptides into an electrophilic acrolein moiety. Our mechanistic investigations uncover that the key to these intermolecular hydride transfer processes lies in the engagement of solvent-mediated macrocyclic transition states. These transition states can accommodate multiple delicate yet vital orbital interactions, facilitating the concerted transfer of both hydride and proton. In particular, the adoption of suitable conformations that foster strong negative hyperconjugation between the lone electron pair of nitrogen (n) and the anti-bonding orbital (σ*) of the α C-H bond of the amine constitutes a potent activator of the Cα-H bond, promoting hydride-liberation. The aggregates of HFIP molecules also play a pivotal role by acting as the critical proton shuttles within the macrocyclic transition state.

Mono-selective *N*-methylation of alkylamines: Lysine (Lys), an essential proteinogenic amino acid (AA), assumes an indispensable role in governing the functions and physicochemical properties of peptides and proteins.(*22-25*) Nature has crafted a large array of enzyme-catalyzed pathways for post-translational modification (PTM) on Lys's ε-NH_2 side chains.(*26*) Among these, the *N*ε-methylation of Lys stands out as one of the most important PTMs in the domain of epigenetic control over cellular functions.(*27,28*) Besides the side chain *N*-methylation, nature also extensively uses backbone *N*α-methylation of all kinds of αAA units to modulate the physicochemical properties, particularly membrane permeability, of peptide natural products.(*29*) Despite the seemingly simple structures, the preparation of secondary methyl alkylamines remains problematic due to the competing *N, N*-dimethylation side reactions and the difficulty in separating those homologues.(*30-33*) Besides PTMs, the Lys side chain within peptides and proteins serves as a coveted handle for various bioconjugation applications.(*22-25*) Despite remarkable strides made over the past half-century, the quest for novel approaches to selectively modify Lys continues unabated.

HCHO is a ubiquitous metabolite in living systems and is involved in a variety of nonspecific *in vivo* modifications of biomolecules such as proteins and nucleic acids.(*34-37*) Recently, we reported a highly efficient and selective method for peptide stapling through a simple treatment with aq. HCHO (37 wt. % in H_2O) in HFIP solvent at rt.(*38*) HCHO can effectively crosslink the side chains of Lys and tyrosine (Tyr) in nearby positions, forging a methylene linkage. This crosslinking proceeded through a Mannich-type reaction involving the Lys-derived formaldimine group and the phenol side chain of Tyr. In addition, HCHO can also crosslink the Lys and the guanidine side chain of arginine (Arg) via a tetrahydrotriazine linkage.(*39*) Importantly, HFIP solvent plays a key role in enhancing the reactivity of Lys-Tyr crosslinking at rt, although the mechanism behind this solvent promotion effect remained unclear.(*40-*

43) During the course of these studies, we made an intriguing observation that the treatment of Lys-containing peptide substrates lacking Tyr or Arg residues, such as pentapeptide **1**, with HCHO in HFIP at rt could generate a small amount of *N*-methylamine **1a** and acrolein **1b**, along with other side products (**Fig. 1a**). We postulated that the *N*-methylation product was formed through a homologous intermolecular hydride transfer between substrate **1** and formaldimine intermediate **1c**. In this process, **1** acted as the hydride donor, while **1c** served as the hydride acceptor. The resultant imine intermediate **1e** could subsequently undergo hydrolysis to yield aldehyde **1f**, which gave **1b** upon Aldol condensation with another HCHO. Intrigued by the unique hydricity of the Lys amino side chain, we questioned whether the introduction of external amine reagents as hydride donors could facilitate a hetero-selective hydride transfer, ultimately leading to the selective *N*-methylation of Lys.(*44, 45*) Gratifyingly, the use of cyclic secondary alkylamine pyrrolidine can indeed significantly enhance the *N*-methylation. Treatment of **1** with 3 equiv of aq. HCHO and 4 equiv of pyrroline in HFIP at rt for 4 hours gave product **1a** in near quantitative yield as determined by LC-MS analysis (conditions [**A**], see LC trace-2 in **Fig. 2b**).



c) Yield of **1a** under modified conditions [**A**][a]

		amine/base								temperature		aldehyde		
	vs				Et_2NH	$MeNH_2$	DIPEA	K_2CO_3	no base	no base, 100°C	100°C	para-formaldehyde	PhCHO	CH_3CHO
97% [**A**]		95%	11%[b] (+ 4% of **1b**)[c] (+ 13% of **1f**)[d]	48% (+ 3% of **1b**)[c]	35% (+ 23% of **1b**)[c]	ND	14%[b] (+ 5% of **1b**)[c] (+ 24% of **1f**)[d]	24% (+ 5% of **1b**)[c]	NR	16% (+ 9% of **1b**)[c]	95%	95%	NR	NR

		solvent						counter anion		
	vs	$HFIP/H_2O$ (19/1)	HOCF_3	*i*PrOH	*i*PrOH, 100°C	MeOH	THF	*i*PrOH & TFA (2 equiv)	**1** as TFA salt	*vs*	neutral **1** (without TFA)	**1** as HCl salt
97% [**A**]		66%	81%	NR	18%	NR	ND	NR	97% [**A**]		96%	77%

Figure 2. Optimization of the *N*-methylation of lysine side chain within peptide **1**. Reactions were performed on a 0.01 mmol scale. Yields were based on the UV absorption of LC traces using *trans*-cinnamic acid as an internal standard. HPLC-isolated yields were shown in braces. ND: not detected. NR: no reaction. [a]The reaction parameters of conditions [**A**] were modified as per the specifications. [b]A complex mixture of products was formed. [c]A varied amount of **1b** was observed. [d]A varied amount of **1f** was observed.

The *N*-terminal 1-naphthoyl group (NA) was incorporated to enhance the UV detection of peptides. Starting material **1**, a salt with trifluoroacetic acid (TFA), was prepared by standard solid phase peptide synthesis protocols. The choice of amine reagent had a significant impact on the reaction (**Fig. 2c**): 2-

methylpyrrolidine gave very similar results to pyrrolidine; tertiary amines such as *N*-methyl pyrrolidine and *N,N*-diisopropylethylamine (DIPEA) gave **1a** in less than 20% yield, accompanied by a complex mixture of **1b**, **1f** and other side products; 6-membered piperidine was considerably less effective than pyrrolidine (48% of **1a**); the use of K_2CO_3 as a base gave a low yield of **1a**, along with a small amount of **1b**; replacing pyrrolidine with its acyclic analog, Et_2NH, formed **1a** in 35% yield, along with a 23% of acrolein **1b** (see LC trace-3). Polyfluorinated alcohol solvents such as HFIP proved critical in achieving a high yield of **1a**. Replacing it with trifluoroethanol (TEF) gave a moderately diminished yield of **1a** (81%). Adding a small amount of water cosolvent (HFIP/H_2O: 19/1) reduced the yield of **1a** to 66%. Regular alcohol solvents such as MeOH and *i*PrOH, as well as non-alcoholic solvents like THF, gave a negligible amount of **1a** at rt. However, when the reaction in *i*PrOH was conducted at an elevated temperature (100°C), a small amount of **1a** (18%) was obtained, along with other side products. The residual TFA in **1** exerted minimal influence on the reaction, as evidenced by the similarity of results when **1** in free amine form was used. Using paraformaldehyde instead of aq. HCHO produced the same results. Notably, substituting HCHO with other alkyl or aryl aldehydes such as CH_3CHO and PhCHO did not give any corresponding alkylation products, underscoring the unique ability of HCHO in promoting this intermolecular hydride transfer reaction. Finally, no *N*, *N*-dimethylation product **1d** was detected under these pyrrolidine-mediated conditions.

Current methods for mono-selective *N*-methylation of primary alkylamines mostly require the use of a protecting group to block one of the N-H bonds.(*30*) This necessity considerably inflates the cost of synthesizing *N*-methylated αAA building blocks and limits applications in late-stage modification of complex molecules like peptides. As shown in **Fig. 3a**, our HCHO/pyrrolidine/HFIP protocol offers a convenient and efficient late-stage approach for the mono-selective introduction of a methyl group onto the amino side chain of peptides with diverse composition and length under standard conditions [**A**]. The method exhibits excellent tolerance towards functional groups such as CO_2H and OH groups. Unprotected αAA residues, including histidine (His, **6a**), serine (Ser, **5a**), threonine (Thr, **5a**), tyrosine (Tyr, **4a**), and glutamic acid (Glu, **7a**), were compatible, whereas unprotected cysteine (Cys), tryptophan (Trp), and Arg resulted in undesired side products through their reaction with HCHO. Product **7a**, a 15-mer peptide containing multiple nucleophilic AA residues, was formed in 90% LC yield. The *N*-methylation of ornithine (Orn), a non-proteinogenic αAA unit with a side chain one methylene unit shorter than that of Lys, also worked well (**3a**). Natural product Gramicidin S with potent antibiotic activity underwent *N*-methylation on both of its Orn side chains, giving **8a** in 95% LC yield. However, as exemplified by substrate **9**, *N*-methylation of the *N*-terminal NH_2 group of peptides often resulted in a mixture of products since HCHO can also react with the terminal N and the neighboring amide NH to form a cyclic addition product (see **9x**). In comparison, the terminal *N*-methylation of peptides bearing a proline next to the *N*-terminal αAA can proceed effectively under modified conditions [**A**] at elevated temperatures (see **10a**).

As shown in **Fig. 3b**, the *N*-methylation of *N*-naphthyl (Na) 6-aminohexanamide **11** with D_2-deuterated formaldehyde DCOD and pyrrolidine under standard conditions [**A**] gave D_2-labeled **11a-2D** in high yield. Compared to the reaction with normal pyrrolidine, the reaction of **11** with 2,2,5,5-D_4-deuterated pyrrolidine was slower but still proceeded cleanly to give the corresponding D_1-labeled **11a-D** in excellent yield in 4 hours, affirming the pyrrolidine as the hydride donor. A kinetic isotope effect (KIE) of 3.1 indicated that the C-H cleavage of pyrrolidine is the rate-limiting step of the intermolecular hydride transfer reaction. As shown in **Fig. 3c**, our *N*-methylation conditions can work well for a variety of

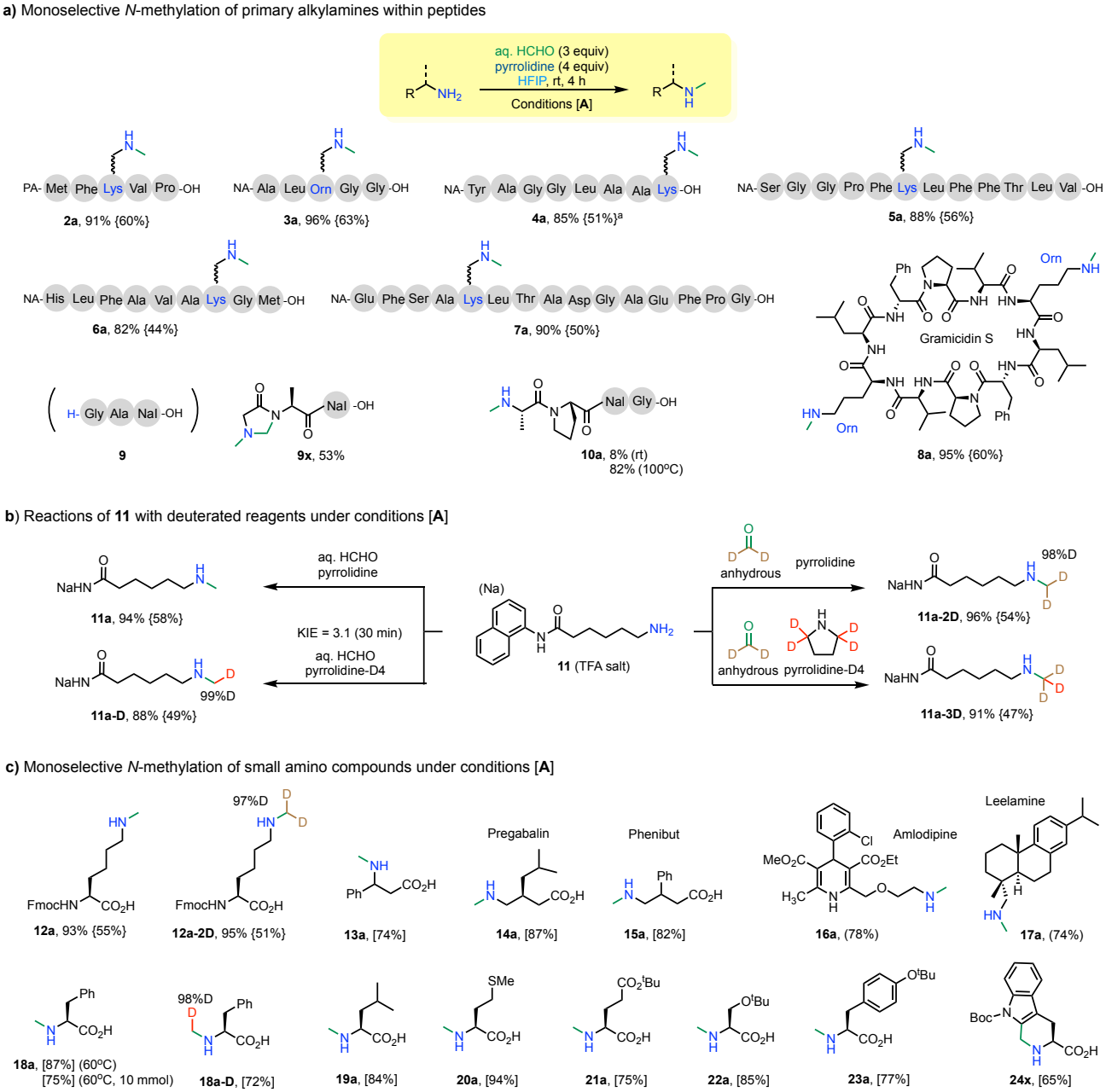


Figure 3. The substrate scope of mono-selective *N*-methylation of primary alkylamines. Reactions were performed on a 0.03-0.05 mmol scale for peptides and a 0.2 mmol scale for small molecules. Yields were based on the UV absorption of LC traces unless otherwise specified. HPLC-isolated yields were shown in braces, column chromatography-isolated yields were shown in parenthesis, and trituration-isolated yields were shown in square brackets. PA: 2-picolinamide cap. Nal: 3-(2-naphthyl)-L-alanine. [a]2 equiv of HCHO and pyrrolidine were used respectively.

normal primary alkylamines. For example, the reactions of drug molecules Amlodipine and Leelamine gave the corresponding *N*-methylated products **16a** and **17a** in good yield. The *N*ε-methylation of *N*α-Fmoc-Lys-OH **12** gave the high-priced *N*α-Fmoc-*N*ε-Me-Lys-OH, in both regular and deuterated forms (**12a** and **12a-2D**), in excellent yield. Notably, a simple optimization of the reaction workup provided a highly efficient and practical protocol to prepare *N*-methyl AAs bearing a free CO_2H group without the

need for any column chromatography purification: simply removing HFIP solvent from the reaction mixture under reduced pressure followed by trituration with diethyl ether provided the desired products with excellent purity (>95%) and mono-selectivity. For example, the reactions of β-AA **13**, γ-AAs Pregabelin and Phenibut gave products **13a**-**15a** in high yield. As shown by **18a**-**23a**, a series of *N*α-Me αAAs can be prepared from the easily accessible αAA precursors in good to excellent yield. *N*α-Me-Phe-OH **18a** was prepared in 75% yield on a gram scale synthesis. These types of *N*-Me αAAs are valuable building blocks for the synthesis of peptides with *N*-methylated backbones and usually require a three-step synthesis. It is worth mentioning that the preparation of *N*α-Me-Trp(Boc)-OH was unsuccessful due to the precursor H-Trp(Boc)-OH **24** undergoing a Pictet-Spengler reaction with HCHO (see **24x**).

Dehydrogenative reactions of alkylamines: As discussed above, the choice of base or amine additive had a significant impact on the HCHO-mediated intermolecular hydride transfer reaction of **1** in HFIP. Adding tertiary amines or inorganic bases such as K_2CO_3 typically led to an increased formation of dehydrogenative Aldol condensation product **1b**. Regrettably, none of the common primary and secondary amine additives tested enabled a highly efficient and selective dehydrogenative transformation of Lys in **1** by generating a suitable formaldimine or iminium intermediate as a hydride acceptor. Interestingly, it was HCHO itself that emerged as the most effective hydride acceptor for this purpose. The yield of **1b** was significantly improved, reaching 76%, when the reaction of **1** was conducted at 100°C for 4 h in the mixed solvents of HFIP and H_2O (19/1) in the presence of DIPEA base (conditions [**B**], **Fig. 4a**, LC trace-4). A small amount of *N, N*-dimethylamine **1d** (11%) was formed as the only appreciable side product. In comparison, the reaction conducted without any base at 100°C produced a mixture of *N*-methylamine **1a**, **1b**, and other identified side products (**Fig. 4c**, LC trace-6). It is worth noting that DIPEA was not directly involved in the hydride transfer, as other bases such as NEt_3, DABCO, and K_2CO_3 gave comparable results. The noninvolvement of the external amine base indicated that the amino side chain of Lys served as the sole hydride donor, while HCHO served as the main hydride acceptor during the dehydrogenation of Lys. Additionally, the formaldimine intermediate of Lys served as a minor hydride acceptor, leading to the formation of **1a**, which can be further methylated to produce **1c** at high temperatures. The dehydrogenative Aldol condensation of **1** can occur at rt, yielding **1b** with a significantly reduced yield (21%) along with the formation of **1a** and other unidentified side products (see LC trace-5). At temperatures higher than 80°C, the formation of those side products was considerably suppressed, although higher amounts of *N*, *N*-dimethyl amine **1d** were generated. Consistent with the mono-selective *N*-methylation protocol, the use of polyfluorinated alcohol solvent was critical for achieving a high yield. The addition of a small amount of H_2O cosolvent to HFIP (HFIP/H_2O: 19/1) slightly improved the yield of **1b**. No conversion of **1** occurred when the reaction was conducted in the mixed solvent of *i*PrOH/H_2O (19/1) at rt; however, raising the temperature to 100°C gave small amounts of **1a** and **1b**. The reaction of **1** in its free amine form gave similar results, indicating that the residual TFA had a negligible impact. Little dehydrogenative products of **1** were formed when HCHO was replaced with other aldehydes, such as acetaldehyde or benzaldehyde. Interestingly, this HCHO-mediated dehydrogenative condensation reaction offered an unprecedented method for converting the nucleophilic amino side chain of Lys into an electrophilic acrolein group bearing two potential reaction sites. As shown in **Fig. 4d**, treatment of **1b** with hydrazone **25** in HFIP at rt selectively gave conjugation product **26**, while treatment with both 1-aminopiperidine **27** and *p*-toluenethiol **28** gave the double conjugation product **29** in excellent yield.

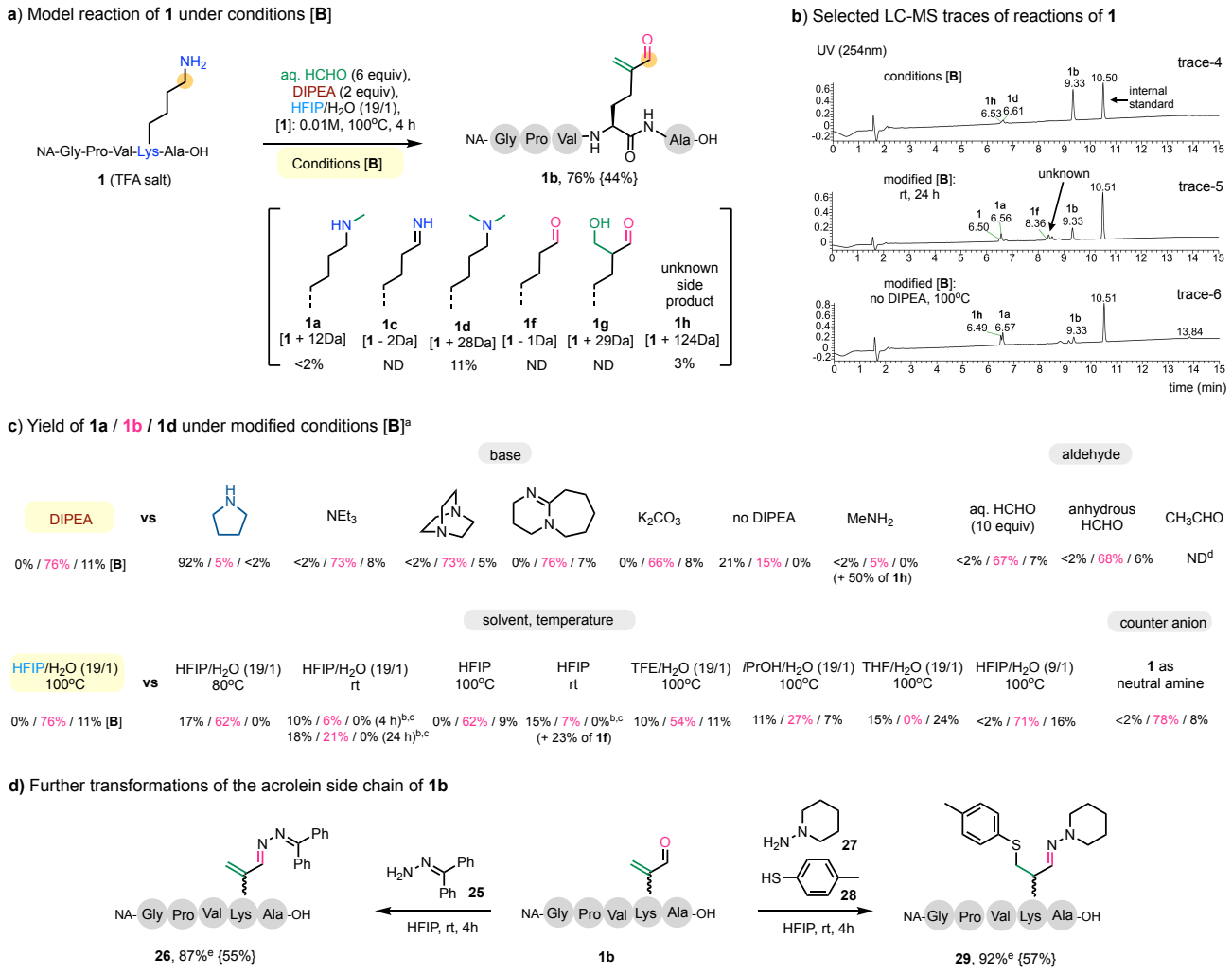


Figure 4. Reaction optimization of the dehydrogenative Aldol condensation of peptide **1**. Reactions were performed on a 0.01 mmol scale. Yields were based on the UV absorption of LC traces using 2-naphthoic acid as an internal standard unless otherwise specified. HPLC-isolated yields were shown in braces. [a]The reaction parameters of the standard conditions [**B**] were modified as per the specifications. [b]A complex mixture of products was obtained. [c]A varied amount of **1f** was observed. [d]Negligible amount of dehydrogenative products was observed. [e]Yields were based on ^{1}H-NMR analysis of the crude reaction mixture on a 0.05 mmol scale after workup.

As depicted in **Fig. 5a**, the dehydrogenative Aldol condensation of Lys proved to be effective for a variety of peptide substrates under standard conditions [**B**]. In most of these reactions, *N, N*-dimethylamines were formed as the major side product in small amounts, typically around 10%. Functional groups such as CO_2H of glutamic acid (Glu in **31b**), $CONH_2$ (**30b**), alkyl OH of Ser and Thr (**30b**, **32b**), imidazole of His (**32b**), and methyl sulfide of methionine (Met, **32b**) were compatible. A 15-mer peptide product **7b** was obtained in moderate yield. Unlike the *N*-methylation reactions, the reaction of Orn did not give the corresponding acrolein product in appreciable yield (<5%) (see **3b**). Primary amines bearing a long alkyl chain, such as the 7-amino-heptanoyl group on the *N*-terminus of peptide **33**, showed similar reactivities to Lys's side chain. However, as the alkyl chain shortened, the presence of nearby nucleophilic groups might interfere with the post-dehydrogenation transformation, leading to the formation of more side products. For example, product **34b** was obtained with a lower yield than its counterpart **11b**, which

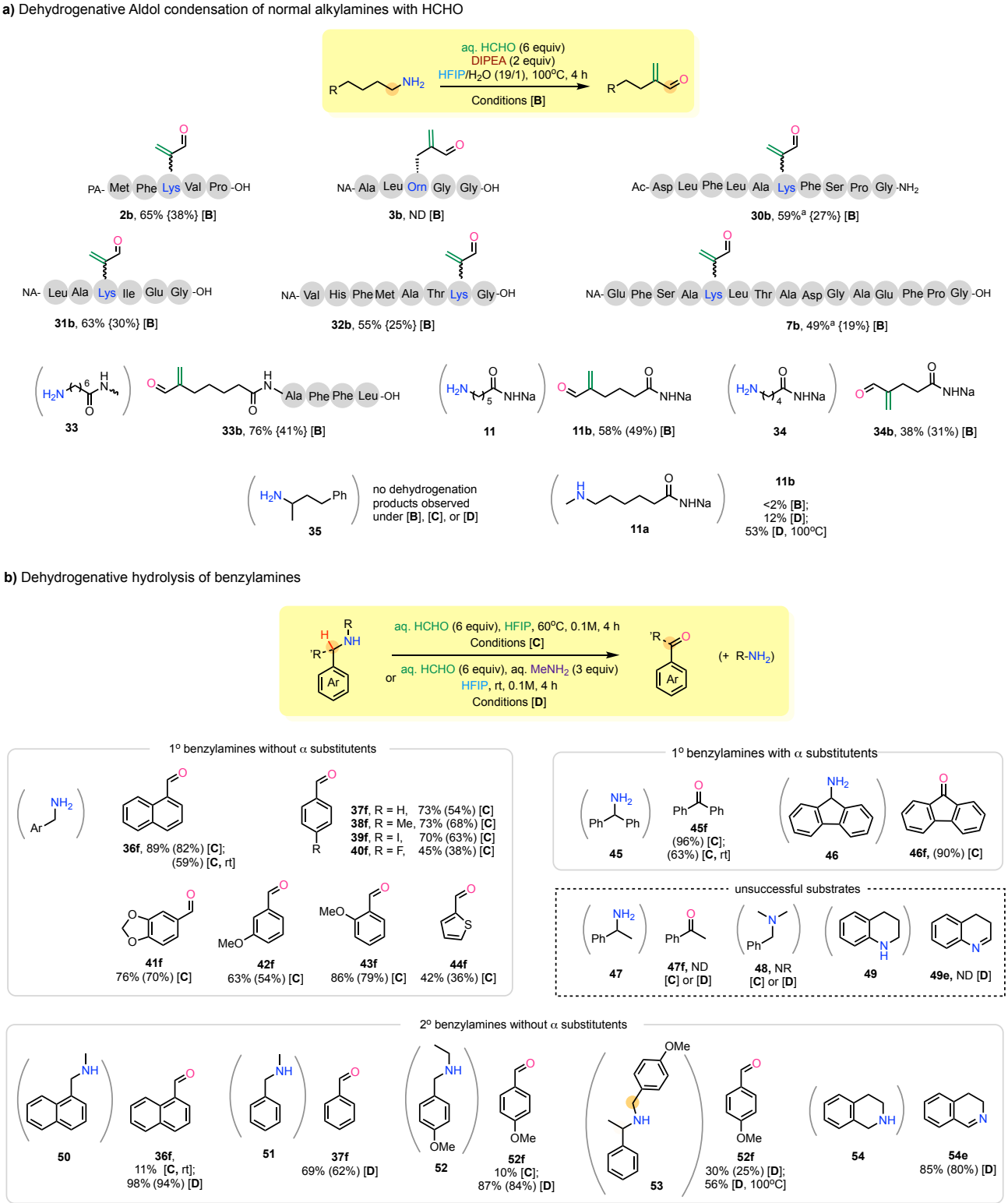


Figure 5. HCHO-mediated dehydrogenative reactions of alkylamines. Yields are based on ^{1}H-NMR analysis of the crude reaction mixture on a 0.03-0.05 mmol scale for peptides and a 0.2 mmol scale for small molecules. HPLC-isolated yields were shown in braces, and column chromatography-isolated yields were shown in parenthesis. Amine substrates were shown in gray parenthesis. [a]Yields were based on the UV absorption of LC traces.

bears one more methylene group. Notably, the performance of the dehydrogenation reactions was found to be sensitive toward the steric influence around the amino group. For example, the reaction of alkylamine **35**, which bears a Cα-methyl substituent, did not give any dehydrogenation products under various conditions tested.

Because alkyl aldehydes can readily undergo condensation at α position with HCHO, the conditions for the above HCHO-mediated dehydrogenative transformation of Lys were tailored to produce more stable acrolein products via a tandem sequence at high reaction temperatures. In principle, the dehydrogenation reaction of alkylamines with a blocked Cβ reaction site could be stopped at the imine or aldehyde stage. As shown in **Fig. 5b**, we were pleased to find benzylamines can react with HCHO to give benzaldehydes in good to excellent yield. For example, the reaction of 1-naphthylmethylamine **36** with 6 equiv of aq. HCHO in HFIP at rt for 4 h gave 1-naphthaldehyde **36f** in 59% yield along with a 35% unreacted starting material. The reaction of **36** at a gently elevated temperature (60°C) proceeded cleanly to generate **36f** in 82% isolated yield (conditions [**C**]). As free amine starting materials were used, no external base was needed. The dehydrogenative hydrolysis reaction worked well for a variety of primary benzylamines without any α-substituents (**37f-39f**，**41f-43f**). Benzylamines featuring an electron-deficient arene motif exhibited lower reactivity (see **40f**). As seen in the dehydrogenative Aldol condensation reactions, the steric hindrance around the Cα atom of benzylamines could significantly diminish the dehydrogenation reactivity. For instance, the reaction of α-methylbenzylamine **47** did not give any imine or hydrolyzed ketone product **47f**. In comparison, the α-aryl benzylamines such as compounds **45** and **46** worked well under conditions [**C**] to form the corresponding ketones in excellent yield, probably due to the significantly weakened benzylic C-H bonds.

As exemplified by **50**, secondary benzyl alkyl amines typically did not work well under conditions [**C**]. Notably, the addition of alkyl amine additives can enhance the reactivity. Among the alkylamines examined, methylamine worked the best, and the addition of 3 equiv of aq. $MeNH_2$ (40% wt. % in H_2O) to conditions [**C**] can significantly improve the dehydrogenative hydrolysis of secondary benzylamines at rt (conditions [**D**]). For example, the reaction of **50** gave product **36f** in near quantitative yield under conditions [**D**]. The reaction of **52** gave *para*-methoxy benzaldehyde **52f** in 84% isolated yield. The dehydrogenation of dibenzylamine **53** selectively took place at the more accessible benzylic position to give **52f** in moderate yield at an elevated temperature. The steric hindrance of the secondary alkyl group on N might have caused the lower reactivity of the methylene benzylic C-H bond of **53**. Notably, the dehydrogenation reaction of tetrahydroisoquinoline **54** selectively took place at the benzylic position, giving the corresponding 3,4-dihydroisoquinoline **54e** in an excellent yield. In contrast, tetrahydroquinoline **49** showed little reactivity under either conditions [**C**] or [**D**], highlighting the impact of C-H bond strength and conformation toward the dehydrogenation reactivity. Normal secondary alkylamines such as **11a** exhibited minimal dehydrogenation activity under conditions [**B**] or [**C**]. Their reactivity can be enhanced by the addition of $MeNH_2$ at elevated reaction temperature. For example, the reaction of **11a** under modified conditions [**D**] at 100°C gave acrolein product **11b** in moderate yield (53%). Finally, tertiary benzylamines such as *N,N*-dimethylbenzylamine **48** show little reactivity under conditions [**C**] or [**D**].

Mechanistic investigations: The ability of alkyl amines to spontaneously liberate hydride ions is typically restrained, except under specific intramolecular reaction conditions.(*15-20*) Remarkably, this potential can be unlocked through the simple treatment with HCHO in HFIP solvent. Nevertheless, the

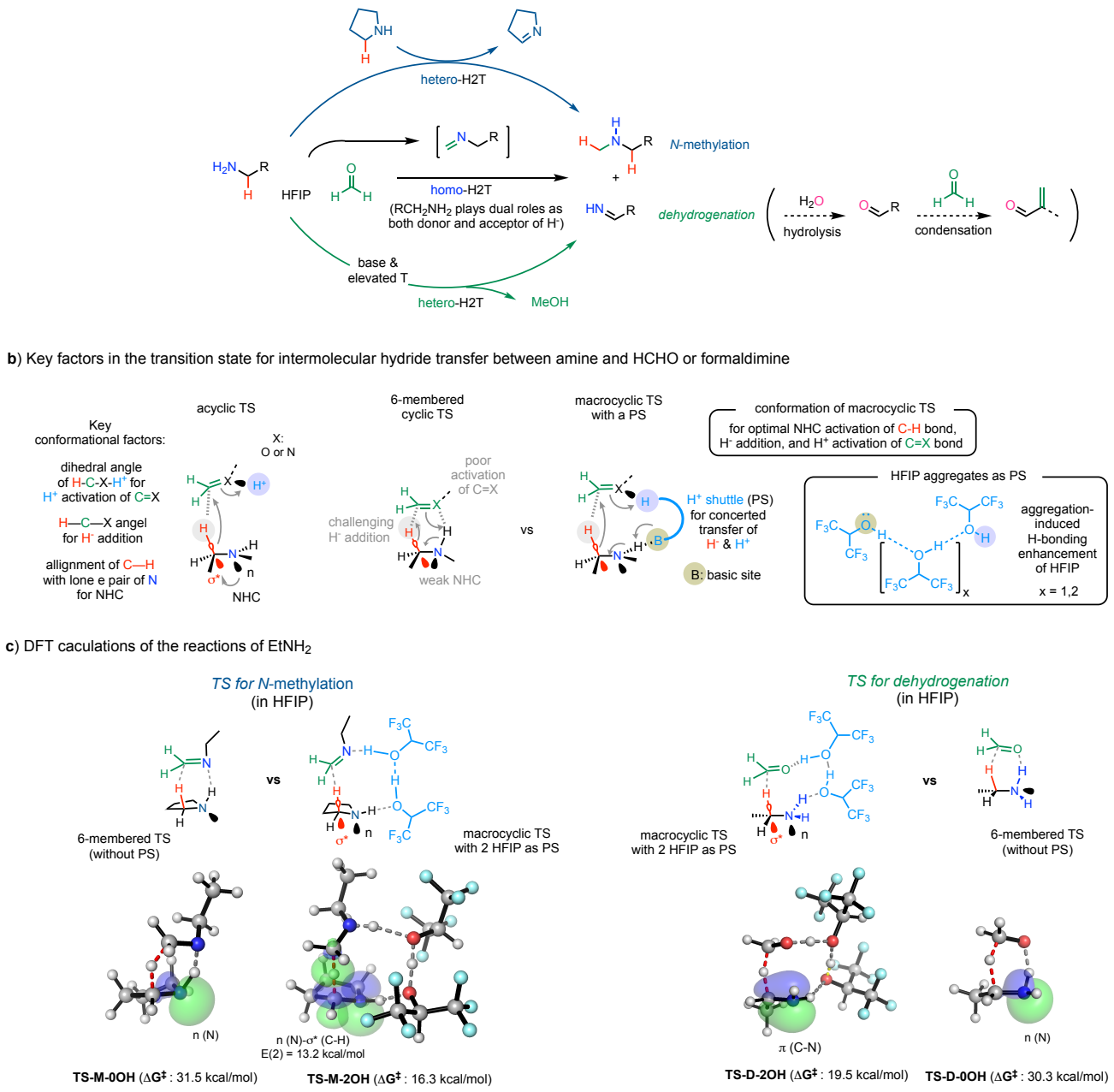


Figure 6. Mechanistic analyses. See SI (**fig. S28** and **fig. S29**) for the details of DFT calculations and other macrocyclic transition states involving different HFIP aggregates as proton shuttles.

reactivity of amines must be carefully controlled by pairing them with suitable reaction partners to achieve selective and efficient transformations. As outlined in **Fig. 6a**, the mixture of primary alkyl amines and HCHO in HFIP can undergo homologous hydride transfer between the amine and the resultant formaldimine intermediate, yielding a mixture of *N*-methylation and dehydrogenation products. Depending on the amine's structure, the resultant imine can be hydrolyzed into an aldehyde, which can further react with HCHO to generate additional products such as acrolein. We refer to this metal-free hydride transfer process, which involves both hydride and proton transfer (2 Hs), as H2T for clarity. Notably, in the homo-H2T reaction, the amine serves a dual role, acting as both a hydride acceptor via formaldimine and a

hydride donor. To tailor the reactivity for synthetic applications, it is imperative to prioritize one of the amine's dual roles in a hetero-selective H2T manner. In our *N*-methylation reaction, the addition of pyrrolidine with strong hydride-releasing capacities can effectively steer the alkyl amine's reaction toward a hetero-selective H2T pathway, where the amine exclusively serves as the hydride acceptor. Conversely, in our dehydrogenative Aldol condensation reaction, the utilization of a base and elevated reaction temperature can direct the alkyl amine's reaction along an alternative hetero-H2T pathway, with the amine acting as the hydride donor and HCHO as the hydride acceptor. It's worth noting that, unlike the *N*-methylation protocol, the homo-H2T reactivity of the amine is not entirely suppressed in the dehydrogenative condensation system. The addition of the base can only partially inhibit the formation of the formaldimine intermediate by sequestrating the residual TFA, thus resulting in the formation of *N*-methylation side products. In our dehydrogenative hydrolysis of secondary benzylamines with HCHO and $MeNH_2$, benzylamines serve as the hydride donor, and methylene imine serves as the hydride acceptor.

Previous research has shown that intramolecular hydride transfer between alkylamines and polar π-bonds (C=X) can efficiently occur under mild conditions, indicating the thermodynamic favorability of this process. Most intramolecular reactions of this nature involve a kinetically favored medium-sized cyclic transition state (TS), where the appropriate conformation for hydride attack to the C=X group and acid-enhanced electrophilicity of C=X are crucial for achieving high reactivity. In contrast, intermolecular hydride transfer, due to kinetic barriers, presents significantly greater difficulties. The conventional 6-membered cyclic TS model falls short in accommodating optimal conformation for hydride attack and enabling the activation of the C=X bond. Notably, the observed solvent dependence in our reactions suggests that HFIP plays a pivotal role in facilitating HCHO-mediated intermolecular hydride transfer. Aside from its high acidity ($pK_a = 9.3$), HFIP is known for forming relatively stable aggregates of varying lengths (usually up to 4 units) and arrangements through H-bonding. (*42*) Such aggregation can further enhance HFIP's H-bonding capabilities. As depicted in **Fig. 6b**, we propose that HFIP aggregates act as a dexterous proton shuttle (PS) to promote intermolecular hydride transfer between alkyl amines and HCHO or formaldimine through macrocyclic transition states.(*46-50*) This PS-mediated macrocyclic TS enables a concerted transfer of both a hydride and a proton. The O atom of HFIP captures the proton from the amine's NH group and relays it through the H-bonded network of PS to the C=X group. The inability of the methylene iminium intermediates of *N*-methyl alkyl amine to form an H-bond with the H atom of HFIP might account for the low reactivity of the *N*, *N*-dimethylation.

Our density functional theory (DFT) calculations for the *N*-methylation and dehydrogenation of a model substrate, $EtNH_2$, confirmed that such macrocyclic TSs are indeed significantly more favorable than the conventional 6-membered TSs without PS (see **TS-M-0OH** and **TS-D-0OH** in **Fig. 6c**). In the *N*-methylation of $EtNH_2$ with HCHO and pyrrolidine, TSs featuring 2 to 5 linearly aggregated HFIP molecules exhibit considerably lower free energy barriers (< 20 kcal/mol, see (**fig. S28**) detailed structures in Supporting Information) compared to $\Delta G^{\ddagger}$ value of 31.5 kcal/mol for **TS-M-0OH** in HFIP solvent. For the dehydrogenation reaction of $EtNH_2$ with HCHO, transition states featuring linearly aggregated HFIP as a proton shuttle have an energy barrier of around 20 kcal/mol (see (**fig. S29**) detailed structures in SI), as opposed to 30.3 kcal/mol for **TS-D-0OH** without a PS. Interestingly, TSs featuring branched aggregates of HFIPs as PS, such as **TS-M-3OHb** and **TS-D-3OHb** (see (**fig. S28** and **fig. S29**) detailed structures in SI), exhibit an even smaller energy barrier than the ones containing linearly aggregated HFIPs. In contrast to HFIP, the aggregates of *i*PrOH cannot reduce the energy barrier for the corresponding TSs

($\Delta G^{\ddagger}$>40 kcal/mol). This phenomenon is likely attributed to the electrophilicity enhancement of the C=X group (where X = O or N) through strong H-bonding with HFIP aggregates. Notably, the local conformations of macrocyclic TSs, particularly around the Cα of the amine, appear to be critical to determining their stabilities. As evidenced by the representative transition states **TS-M-2OH** and **TS-D-2OH**, both involving 2 HFIPs, the alignment of α C-H bond with the lone pair electrons of N in both $EtNH_2$ and pyrrolidine set up favorable conformations for negative hyperconjugation (NHC) between the C-H σ* orbital and the long pair n orbital of N.(*51-53*) Such NHC effect can activate the α C-H bond and enhance the hydricity of amines. Natural bond orbital (NBO) analysis of **TS-M-2OH** shows that such NHC significantly contributes to its stability (n (N)→σ* (C-H) = 13.2 kcal/mol). The strong NHC results in the development of a characteristic C=N π-bond in **TS-M-2OH** for the dehydrogenation of $EtNH_2$. In comparison, the C-H σ* orbital and the n orbital of N were misaligned in the 6-membered transition states, preventing activation of the α C-H bond. The structures of both aldehyde and amine reactants could strongly influence their ability to adopt optimal conformations within macrocyclic transition states for the desired hydride transfer, likely accounting for the reactivity variations observed with different substrates and reactants. Given the mixed forms of HFIP aggregates in solution, we believe that the assembly of various HFIP-assisted macrocyclic TSs enables the facile intermolecular hydride transfer step in these HCHO-mediated H2T reactions at rt. In the case of dehydrogenative Aldol condensation, the elevated reaction temperature is likely necessary for the subsequent steps to achieve a rapid and clean overall transformation. Our calculations also indicate that TFA can act as a PS to facilitate the H2T step through a similar macrocyclic TS (see SI (**fig. S29**) for details). However, our control experiments show that TFA is not a prerequisite for these reactions, and combining TFA with other solvents, such as *i*PrOH, does not significantly promote these reactions at rt (see **Fig. 2c**).

In summary, we have developed an unprecedented metal-free reaction strategy for liberating hydrides from alkylamines via a simple treatment with HCHO in HFIP solvent under mild conditions. This strategy enables efficient and selective transformations of both small molecules and complex peptides, heralding new possibilities in harnessing the redox reactivity of alkylamines. Mechanistic studies have unveiled that solvent-mediated macrocyclic transition states possess a unique ability to adopt optimal conformations for the activation of amine C-H bonds through negative hyperconjugation. The involvement of these macrocyclic transition states, facilitated by HFIP aggregates, is likely to have broader implications in various other reactions conducted in HFIP.

Materials and Methods:

A typical procedure for mono-selective *N*-methylation of lysine within peptides under conditions [A]: To a solution of linear peptides (TFA salt, 0.01 mmol, 1.0 equiv) in 1.0 mL of HFIP were added aq. HCHO (2.4 μL, 0.03 mmol, 3.0 equiv, 37 wt.% in H_2O) and pyrrolidine (2.8 mg, 0.04 mmol, 4.0 equiv). The reaction mixture was stirred under an air atmosphere for 4 hours at rt before being concentrated *in vacuo*. The resulting residues were dissolved in a small amount of H_2O/CH_3CN and purified by semipreparative HPLC with a reverse phase C18 column (H_2O and MeCN with 0.1% HCO_2H as eluents) to give corresponding products as HCO_2H salts after lyophilization.

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Supplementary Information: Detailed synthetic procedures, additional control experiments, compound characterization, LC-MS trace, NMR spectra, and computational experiments.

Data and materials availability: All data are available in the main text or the supplementary materials.

Acknowledgments: This work was partially supported by the National Key R&D Program of China (2022YFA1504303), National Natural Science Foundation of China (92256302), and Frontiers Science Center for New Organic Matter (63181206).

Author Contributions: S.C., H.T., and B.L. made equal contributions to this work. B.L. was responsible for the initial discovery of the mono-selective *N*-methylation reaction, H.T. discovered the dehydrogenative Aldol condensation reaction, and undertook preliminary reaction optimizations and substrate scope investigations. S.C. concluded the project with improved reaction optimizations and additional scope investigations. C.X. helped with expanding the substrate scope. Y.S., D.Z., and H.Z. performed the DFT calculations. G.H. supervised part of the experimental studies. X.X. supervised the calculation studies and edited part of the manuscript. G.C. oversaw the entire project and prepared the manuscript.

Competing financial interests

The authors declare no competing financial interests.