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Access to *N*-α-deuterated amino acids and DNA conjugates via Ca(II)-HFIP-mediated reductive deutero-amination of α-oxo-carbonyl compounds

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The development of practical and selective strategies for deuterium incorporation to construct deuterated molecules, particularly deuterium-labeled amino acids, has become as a growing focus of basic research, yet it remains a formidable challenge. Herein, we present a bioinspired calcium-HFIP-mediated site-selective reductive deutero-amination of α-oxo-carbonyl compounds with amines. Utilizing d_2-Hantzsch ester as the deuterium source, this reaction attains remarkable deuteration efficiency (> 99% deuteration). It enables the synthesis of *N*-α-deuterated amino acid motifs with a wide range of functionality, as evidenced by over 130 examples. The method exhibits compatibility with diverse substrates, such as amino acids, peptides, drug molecules, and natural products bearing different substituents. Moreover, the application of this strategy in the synthesis of DNA-tagged *N*-α-deuterated amino acids/peptides has been demonstrated. This work offers an efficient and innovative solution for deuterated amino acid chemistry and holds substantial application potential in organic synthesis, medicinal chemistry, and chemical biology.

Deuterium has emerged as a protagonist of medicinal chemistry research over the past two decades. Recently, there has been a surge of interest in deuterated drugs to decrease the potential toxicity, improve metabolic stability, and other pharmacokinetic properties[1–3]. The earliest efforts to incorporate deuterium into bioactive molecules trace back to the 1960s, revealing reduced metabolism in d_2-tyramine and decreased toxicity in d_3-morphine compared to their non-deuterated counterparts[4,5]. Indeed, pharmaceutical drugs commonly undergo oxidative metabolism mediated by cytochrome P450 (CYP450) enzymes, and the substitution of C-H bonds with more stable C-D bonds at the metabolic sites of drugs, particularly at the α-position to heteroatoms, serves as a bioisostere having the robust potential to enhance the pharmacokinetic profile of drugs[6–13]. These endeavors resulted in the successful development of the pioneering deuterated drug, deutetrabenazine, which received approval from the American Food and Drug Administration (FDA) in 2017 for the treatment of Huntington's disease[12]. In recent years, several novel deuterated drugs, such as deucravacitinib, donafenib, VV116, and deuruxolitinib, have been developed for diverse medicinal applications based on the deuterium labeling strategy[6,7]. Except for this, deuterium labeling has also

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found attractive utility in various other scientific applications, such as the investigation of metabolites, the use of NMR spectroscopy as internal standards, and tags to elucidate mechanisms in synthetic organic chemistry[13]. Therefore, exploring the chemical space of deuterium-labeled molecules can offer promising avenues for the innovation and the discovery of functional molecules across multiple scientific disciplines (Fig. 1a)[14–17].

Amino acids, as we know, are fundamental chemical building blocks of life, widely found in small-molecule pharmaceuticals and serving as key components of peptide-based therapeutics and

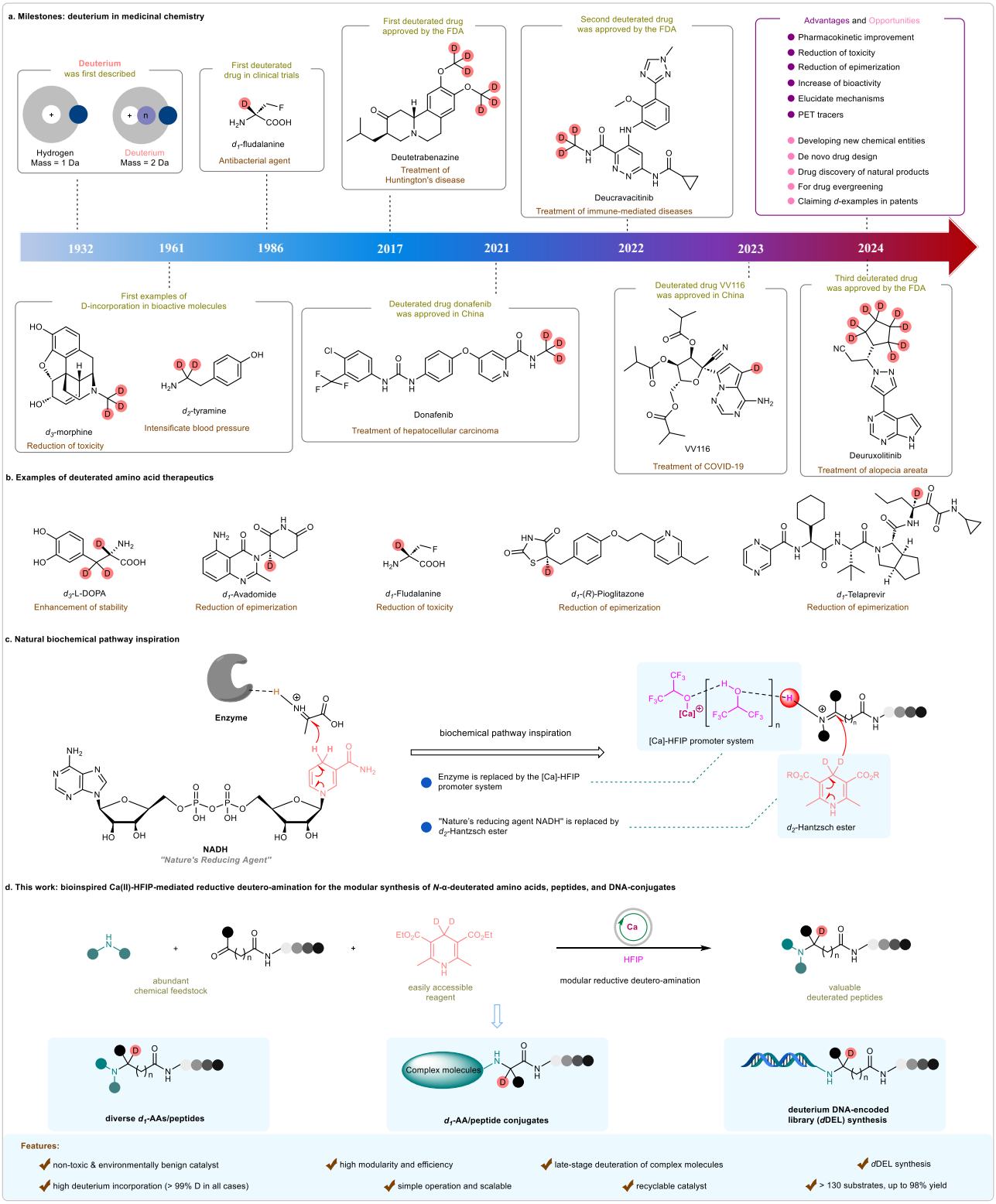

Fig. 1 | Strategies to access deuterated amino acids/peptides. a Milestones: deuterium in medicinal chemistry. **b** Examples of deuterated amino acid therapeutics. **c** Natural biochemical pathway inspiration. **d** This work: bioinspired Ca(II)-HFIP-mediated reductive deutero-amination for the modular synthesis of *N*-α-deuterated amino acids.

biologics[18–20]. Deuterium-labeled amino acids and their derivatives have shown widespread utility for elucidating biosynthetic pathways[21,22], enzyme mechanisms[23], structures of peptides/proteins[24], improving absorption distribution metabolism and excretion (ADME) profiling, and enhancing the efficacy[25]. Morever, the pharmacokinetics and predicted metabolic sites of amino acid drug candidates, deuterated at the α-position to nitrogen, have been widely investigated (Fig. 1b), which underpins their demand and motivates researchers to design improved strategies to access these compounds[6]. As a result, several versatile strategies, involving transition-metal-catalyzed or biocatalyzed H/D exchange of α-amino acids[26,27] and glycine-derived imines[28], organophoto-catalyzed deuteration of the Beckwith–Karady alkene[29] as well as transition-metal-catalyzed reductive deuteration of α-amidoacrylates[30], have been dislosed to date toward their synthesis[31–33]. Despite these remarkable advancements, existing protocols generally encounter limitations, including the requirement for pre-synthesized sophisticated substrates, severe conditions, the employment of noble and/or toxic catalysts, and only moderate levels of deuterium incorporation. This falls short of market demands for deuterated active pharmaceutical ingredients (APIs) that require high isotopic purity, ideally exceeding 98%[13]. Moreover, the incorporation of multiple deuterium atoms can sometimes result in unforeseen alterations in the physical properties of the compounds, including solubility and lipophilicity, which could result in notable variations in plasma protein binding (as seen with d_9-caffeine), as well as differences in the extent and/or rate of absorption, while also increasing the overall cost[6]. In particular, within the domain of deuterated amino acid drug discovery, achieving site-selective incorporation, especially at the α-position relative to heteroatoms, with a minimal number of deuterium atoms and a high level of deuterium incorporation is critically important for enhancing pharmacokinetic properties. However, this remains an insurmountable challenge. In contrast, nature's biosynthesis creates essential amino acids via the enzymatic reduction of H-bond activated simple pyruvate-derived ketimines[34,35]. Inspired by the concept of biological reductive amination, we developed a modular three-component reductive deutero-amination protocol, which is mediated by alkaline-earth calcium(II) in combination with hexafluoroisopropanol (HFIP)[36–40]. In this approach, the natural H-bond donor enzyme and the reducing agent NADH are replaced by the Ca(II)-HFIP promoter system and the d_2-Hantzsch ester (d_2-HE)[41,42], respectively. Specifically, we envisioned that the exposure of amine and ketoester or ketoacid to the active $Ca(NTf_2)_2$-HFIP system would lead to the intermediate generation of an iminium intermediate that undergoes reductive deutero-amination in the presence of a suitable d_2-Hantzsch ester, thereby allowing the modular assembly of diverse deuterated amino acids even peptides in high yields and excellent levels of deuterium incorporation at the α-position to *N*-heteroatom (Fig. 1c). To the best of our knowledge, alkaline-earth metal-mediated strategies for direct deuterium incorporation into chemical scaffolds have been not been reported previously. Additionally, the bioinspired Ca(II)-HFIP-mediated approach for the site-selective synthesis of deuterated amino acids has remained unexplored. Consequently, we were motivated to validate this hypothesis and we present herein our findings. The salient aspects of this work are as follows: (1) In terms of the methodology, Ca(II)-HFIP-mediated modular and efficient reductive deutero-amination protocol that combines three readily accessible feedstocks—amines, ketoesters, and d_2-HEs is developed for the one-pot and modular synthesis of diverse deuterated amino acids under mild reaction conditions (room temperature) and in high yields with the exclusive incorporation of deuterium into the α-position to *N*-heteroatom, (2) in terms of the scope, diverse amino acids, peptides, drug molecules as well as natural products with various substituted forms is found to be well-compatible, (3) in terms of the catalyst recycling run, the catalyst's high water solubility facilitates its recovery, thereby enabling multiple subsequent runs (up to eight runs) with the maintenance of the catalytic efficiency, and (4) in terms of the utility, the application in the on-DNA synthesis of DNA-tagged *N*-α-deuterated amino acids/peptides is also found to be feasible (Fig. 1d).

Results

Optimization of reaction conditions

Very recently, calcium(II)-catalyzed transformations, leveraging alkaline-earth metal chemistry, have emerged as a compelling alternative to traditional transition-metal catalysis, driven by considerations of cost, inherent low toxicity, and easy handling properties[42–49]. Continuing our research endeavors, we aim to establish robust and sustainable methodologies employing calcium catalysis to access scaffolds that hold significant value for medicinal chemists[50–56]. Thus, to investigate the feasibility of this proposal, in the initial attempt, we probed a reaction of aniline (1 equiv.) and ethyl 2-oxoacetate (1 equiv.) with and **d_2-HE1** (1.5 equiv.) in the presence of $Ca(NTf_2)_2$ at 25 °C for 12 h in HFIP (Supplementary Table 1, entry 1). To our delight, the desired d_1-glycine derivative (**1**) was obtained in 72% yield and >99% deuteration. Indeed, recent studies have shown that combining $Ca(NTf_2)_2$ with HFIP[36–40] creates acidity-enhanced Ca(II)/HFIP clusters, which can initiate reactions that are unlikely to occur in conventional organic solvents[50–53,57,58]. Further improvement could be obtained by modifying the ratio of analine, ethyl 2-oxoacetate, and **d_2-HE1** to 1:1.2:1.5, which provided the desired product **1** in 94% yield (see Supplementary Table 1 for details). When the deuterium source was replaced by **d_2-HE2 to d_2-HE5**, the desired product was obtained in diminished yields (Fig. 2a). Based on our preliminary results as well as

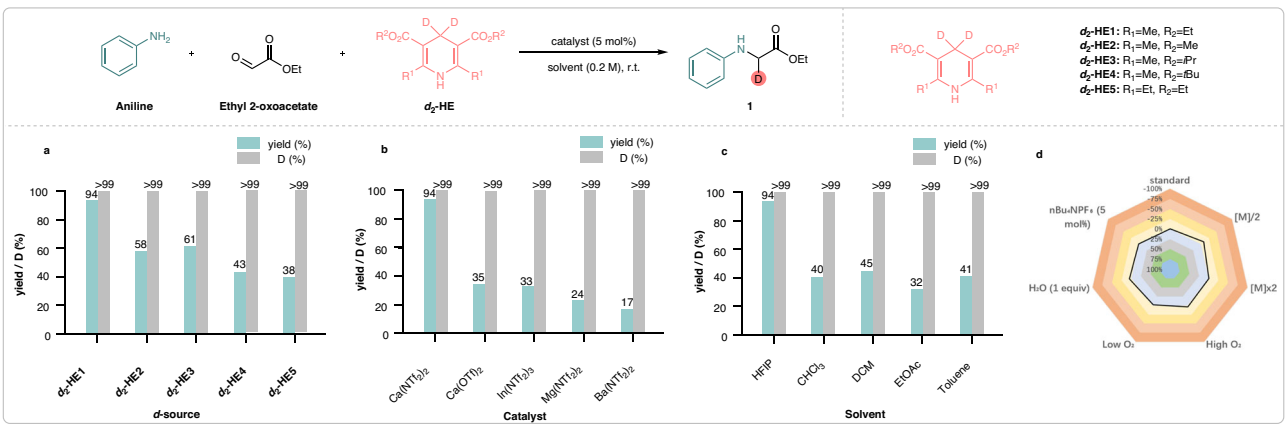

Fig. 2 | Optimization of reaction conditions. Standard reaction conditions: aniline (0.2 mmol, 1 equiv.), ethyl glyoxylate (1.2 equiv.), d_2-HE1 (1.5 equiv.), $Ca(NTf_2)_2$ (5 mol%) in HFIP (0.2 M) at r.t. for 12 h. **a** Optimization of deuterium sources. **b** Optimization of catalysts. **c** Optimization of solvents. **d** Glorius' sensitivity assessment.

the literature survey, the effect of the Lewis acid proved to be very important for the selective reductive amination process. Hence, a variety of Lewis acids such as $Ca(OTf)_2$, $In(NTf_2)_3$, $Mg(NTf_2)_2$, and $Ba(NTf_2)_2$, were tested in the reductive deutero-amination, which all give a negligible result, thereby indicating that $Ca(NTf_2)_2$ has obvious superiority (Fig. 2b). When the solvent was switched from HFIP to $CHCl_3$, DCM, EtOAc, or Toluene, the yield of the deuterated product was significantly decreased (Fig. 2c), which further confirmed the key role of HFIP in the reaction development, particularly with sensitive substrates such as peptide[59,60]. Finally, the reaction failed to proceed in the absence of $Ca(NTf_2)_2$ (see Supplementary Table 1 for details), revealing an indispensable role of the Ca(II) catalyst. Finally, we were pleased to find that the reaction could proceed in air, eliminating the necessity for anaerobic conditions. Next, we investigated the effects of the reaction conditions on this transformation following Glorius' sensitivity assessment (Fig. 2d)[61]. We found that this protocol was not significantly affected when a significant amount of the additive (nBu_4NPF_6) was used for the activation of the catalyst. Furthermore, this reaction exhibited a high degree of tolerance towards substrate concentration, water, and oxygen. This observation underscores the generality and practicality of the established protocol.

Scope for the synthesis of deuterated amino acids

With the optimized conditions for the synthesis of ***d*$_1$-Gly** (**1**) in hand, we next tried to explore the compatibility of this system. Initially, we focused our efforts on the synthesis of deuterated glycine derivatives with an array of diverse amines (Fig. 3). Methyl, isopropyl, and *tert*-butyl groups were tolerated and provided the target deuterated amino acids (2–4) in excellent yields. Halogen-substituted anilines also were all compatible to deliver the corresponding products **5**–**8** in 78% to 98% yields, which thus provided additional opportunities for further derivatization via metal-catalyzed cross-couplings. Moreover, the reaction could be applied to a wide range of electronically diverse substrates. In general, the electron-donating group worked well, furnishing the desired products **9** and **10** in good yields. Electron-withdrawing groups at para-position, including $-CO_2Me$, $-SF_5$, $-OCF_3$, $-CONH_2$, -CONHMe, $-CONMe_2$, -CN, $-NO_2$ groups, could be regarded as viable substrates, affording the corresponding products **11**-**18** in good to excellent yields. It should be emphasized that, with the introduction of the reducible functional groups such as cyano, nitro, amides, or ester group to the aniline, the high chemoselectivity was observed, as these functional groups remained unreduced by d_2-HE1. The structure of deuterated amino acid **16** was further confirmed by X-ray crystallography. The position of the substituent on the phenyl ring had no obvious effect on the outcome of the reaction, as *ortho*- or *meta*-substituted anilines were well-compatible (**19** and **20**). Additionally, ketoacids could serve as versatile substrates to yield the corresponding compounds **21**–**24**, each featuring free carboxylic acids at the C-terminal. Further examination revealed that the disubstituted and trisubstituted anilines were also suitable substrates, delivering the corresponding products **25**–**29** effectively. The protocol was not limited to anilines, and 2-naphthylamine could be applied to the reaction, generating the corresponding **30** and **31** in good yields. *S*-, *O*-, and *N*-heterocycles remained intact throughout this transformation (**32**–**34**). Notably, primary alkyl amines were also well tolerated under the reaction conditions, enabling the desired product to be obtained in an excellent yield (**35, 36**). To our delight, it was discovered that secondary aryl amines exhibited good compatibility with the developed calcium(II)-mediated system. Symmetrical (**37**–**41**) and unsymmetrical diaryl amines (**42**–**45**), aryl-alkyl amines (**46, 47**), as well as cyclic amines (**48**–**50**) were all well tolerated, yielding the corresponding ***d*$_1$-Gly** in good to excellent yields. Importantly, our protocol was also compatible with secondary alkyl amines, thus giving the corresponding products with good yields (**51, 52**). Encouraged by these results, the versatility of this protocol was further examined by testing various ketoesters or ketoacids for the assembly of diverse natural/unnatural deuterated amino acid derivatives. Employing methyl pyruvate as the raw material and reacting it with different amines, a series of deuterated alanine derivatives (***d*$_1$-Ala**) have been obtained smoothly (**53**–**64**). Diverse alkyl-substituted ketoesters/acids are suitable for the synthesis of deuterated natural amino acid derivatives. Thus, the corresponding ***d*$_1$-Phe** (**65**), ***d*$_1$-Val** (**66**), ***d*$_1$-Tyr** (**67**), ***d*$_1$-Trp** (**68**) were obtained in moderate to good yields. Unnatural amino acids, which facilitate the design of proteins with enhanced stability and functionality, have attracted considerable interest in drug discovery. We were pleased to find that ethyl-substituted ketoesters, cyclic ketoesters, as well as longer carbon chain ketoesters could be regarded as feasible substrates, yielding the corresponding deuterated unnatural amino acid derivatives (***d*$_1$-UAAs 69-73**) in 74% to 89% yields.

Scope for the synthesis of deuterated peptides

The extensive functional group tolerance and remarkable site-selectivity observed in our study piqued our interest in investigating the synthesis of deuterated peptides (Fig. 4). Firstly, diverse amines, including primary amines and secondary amines have been evaluated in the optimized conditions. As a result, a series of deuterated dipeptide derivatives (***d*$_1$-Gly-Phe**) have been successfully synthesized (**74**-**84**). The structure of deuterated dipeptide **83** was confirmed by X-Ray crystallography. Importantly, the functional molecule can perfectly maintain the chirality of the original skeleton in this strategy (see Supplementary Fig. 3 for details). A clean reductive deutero-amination was observed to furnish deuterated dipeptides **85**-**89** containing non-polar side chains such as alanine, isoleucine, tyrosine, valine, phenylalanine, and tryptophan in excellent yields with moderate to good diastereoselectivity. To further enhance diastereoselectivity, we explored modifications to the reaction conditions, including solvent, temperature, and reaction time, as these factors might influence the Ca(II)-HFIP-mediated reactions (see Supplementary Table 2 for details). However, attempts to increase the diastereoselectivity under such conditions were unfruitful. It is worth noting that minor disparities in steric hindrance could potentially account for the enhanced diastereoselectivity witnessed in dipeptide **88**. We found that the diastereoselectivity of dipeptide **89** is relatively better than others. Deuterated dipeptides **90** and **91** bearing basic side chains as derived from lysine were also isolated in 67% and 55% yields. Deuterated dipeptide **92** bearing polar uncharged side chains incorporated by using serine, and protected carboxylate side chains introduced by employing glutamic acid (**93**) were effectively prepared in good yields. Under optimized conditions, deuterated dipeptide **94** containing the special amino acid methionine was also successfully generated. Inspired by the successful synthesis of deuterated dipeptides, our curiosity was piqued to investigate the influence of increasing chain length on the assembly of deuterated peptides. It is noteworthy that deuterated tripeptides (**95**–**98**) containing alanine, phenylalanine, proline, glycine, and leucine can be synthesized with high yields. Significantly, the nucleophilic residues derived from amino acids such as serine, tyrosine, glutamic acid, proline, methionine, and lysine can form covalent bonds with the Michael acceptor-type electrophilic warheads found in targeted covalent inhibitors (TCIs), thus, this synthesis potential holds significant value for the discovery of targeted covalent drugs[62].

Late-stage functionalization of bioactive molecules

Late-stage functionalization of pharmaceutically relevant molecules holds immense potential for rapidly assembling a library of medicinal scaffolds[63]. Encouraged by the above investigations on the synthesis of deuterated amino acids/peptides via Ca(II)-HFIP-mediated reductive deutero-amination process, we questioned whether such protocol could be applied for the bioactive pharmaceutical molecules-deuterated amino acids ligation. As shown in Fig. 5, Amlodipine is a

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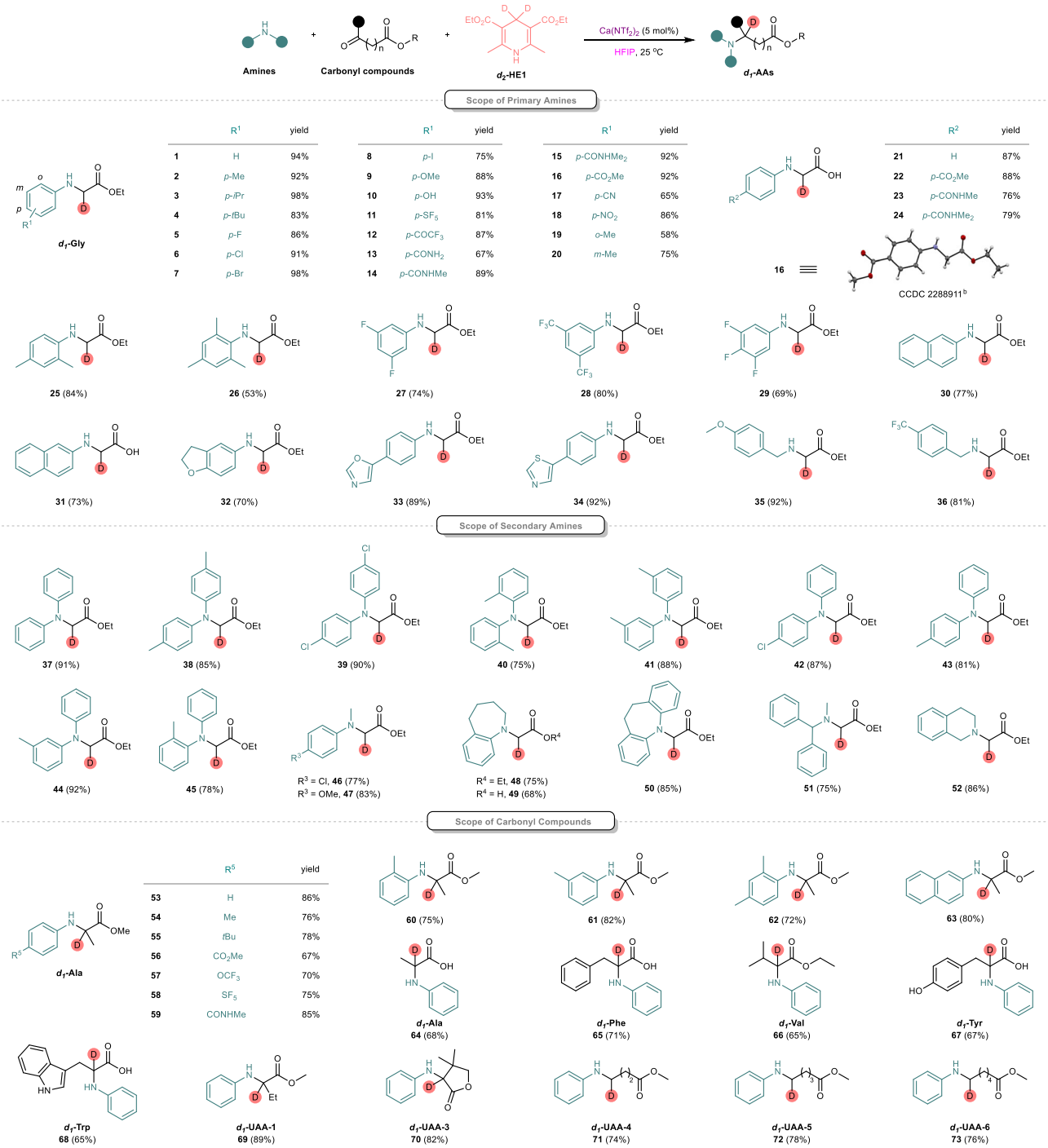

	R^1	yield
1	H	94%
2	*p*-Me	92%
3	*p*-*i*Pr	98%
4	*p*-*t*Bu	83%
5	*p*-F	86%
6	*p*-Cl	91%
7	*p*-Br	98%

	R^1	yield
8	*p*-I	75%
9	*p*-OMe	88%
10	*p*-OH	93%
11	*p*-SF_5	81%
12	*p*-$COCF_3$	87%
13	*p*-$CONH_2$	67%
14	*p*-CONHMe	89%

	R^1	yield
15	*p*-$CONHMe_2$	92%
16	*p*-CO_2Me	92%
17	*p*-CN	65%
18	*p*-NO_2	86%
19	*o*-Me	58%
20	*m*-Me	75%

	R^2	yield
21	H	87%
22	*p*-CO_2Me	88%
23	*p*-CONHMe	76%
24	*p*-$CONHMe_2$	79%

25 (84%) 26 (53%) 27 (74%) 28 (80%) 29 (69%) 30 (77%)
31 (73%) 32 (70%) 33 (89%) 34 (92%) 35 (92%) 36 (81%)
37 (91%) 38 (85%) 39 (90%) 40 (75%) 41 (88%) 42 (87%) 43 (81%)
44 (92%) 45 (78%) 50 (85%) 51 (75%) 52 (86%)

	R^5	yield
53	H	86%
54	Me	76%
55	*t*Bu	78%
56	CO_2Me	67%
57	OCF_3	70%
58	SF_5	75%
59	CONHMe	85%

60 (75%) 61 (82%) 62 (72%) 63 (80%)
64 (68%) 65 (71%) 66 (65%) 67 (67%)
68 (65%) 69 (89%) 70 (82%) 71 (74%) 72 (78%) 73 (76%)

Fig. 3 | Substrate scope for the synthesis of deuterated amino acids and their derivatives. Standard reaction conditions: amine (0.2 mmol, 1 equiv.), carbonyl compound (1.2 equiv.), d_2-HE1 (1.5 equiv.), Ca(NTf$_2$)$_2$ (5 mol%) in HFIP (0.2 M) at r.t. for 12 h. The yields are isolated yields. > 99% deuteration in all cases. [b]ORTEP drawing of compound **16**. Thermal ellipsoids are shown at 30% probability level.

calcium channel blocker medication widely used clinically to treat hypertension and coronary artery disease. Using it as a substrate to react with ethyl glyoxylate, the corresponding d_1-Amlodipine-Gly **99** was obtained in 83% yield. Later, a panel of drug molecules including Sulphamethoxazole (**100**), Benzocaine (**101**), Oseltamivir (**102**), Dapsone (**103**), Crizotinib (**104**), Indomethacin (**105**), Flurbiprofen (**106**), Menbutone (**107**), Probenecid (**108**), Lsoxepac (**109**), Ibuprofen (**110**), Gemfibrozil (**111**), and Febuxostat (**112**) proceeded the deuterated reductive amination with 68-89% yields. Besides, natural products such as Monomethyl Succinate (**113**), Lauric Acid (**114**), and Pyrenebutyric Acid (**115**) could also tolerate the reaction conditions to give the corresponding conjugates in good yields. Encouraged by the successful ligation of deuterated amino acids with drugs/natural products, we advanced in stitching dipeptides to drug scaffolds. Gratifyingly, dipeptides were efficiently conjugated with drug scaffolds, leading to the conjugate d_1-Menbutone-Gly-Phe **116** and d_1-Menbutone-Ala-Val **117** in a site-selective manner. Notably, these results validated that amide, halogens, sulfone, ester, ketone, and heterocycles, are all unperturbed to the reaction system. Undoubtedly, the Ca(II)-HFIP-mediated late-stage modification provides a highly efficient and appealing strategy for the synthesis of new chemical entities, which can be readily screened and explored for potential drug discovery.

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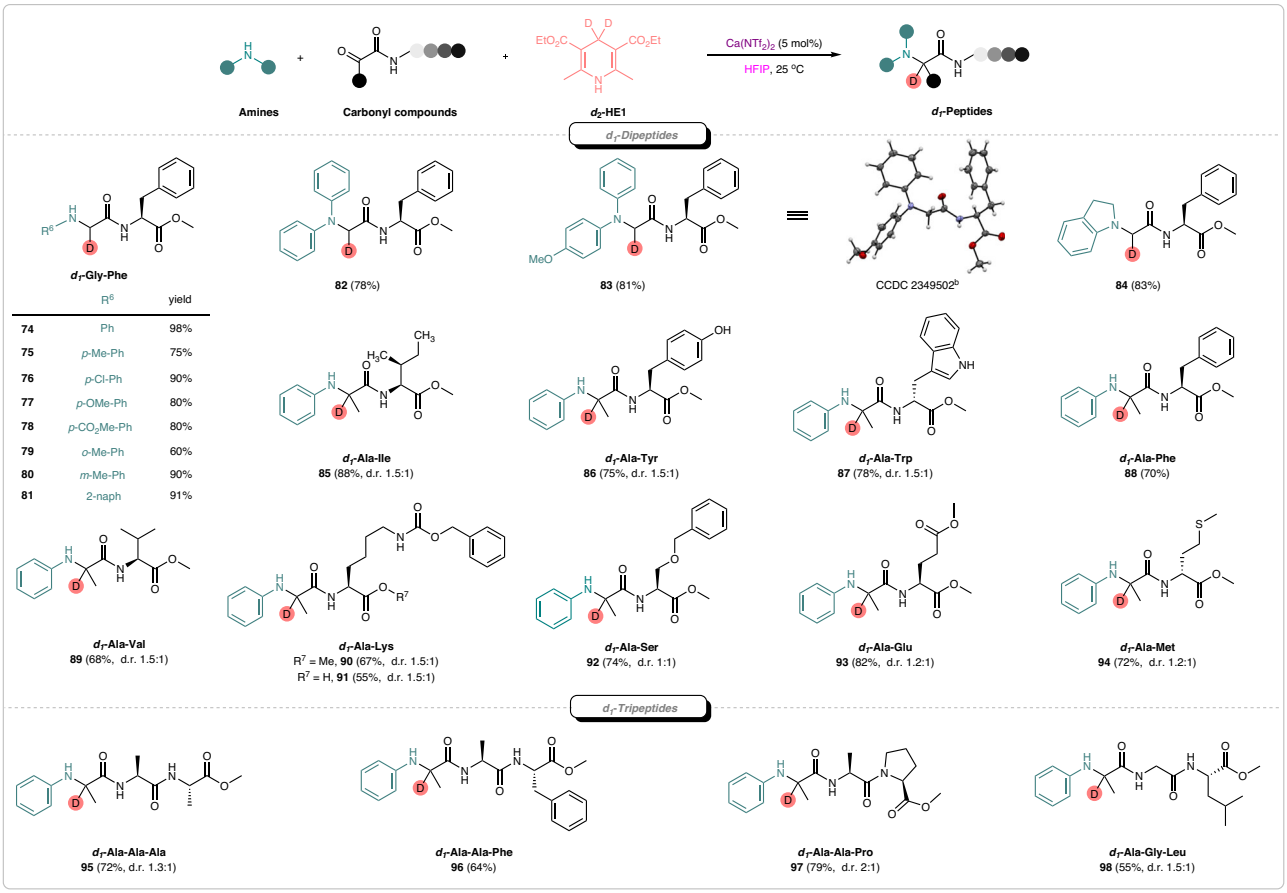

Fig. 4 | Substrate scope for the synthesis of deuterated peptides. Standard reaction conditions: amine (0.2 mmol, 1 equiv.), carbonyl compound (1.2 equiv.), d_2-HE1 (1.5 equiv.), $Ca(NTf_2)_2$ (5 mol%) in HFIP (0.2 M) at r.t. for 12 h. The yields are isolated yields. The d.r. was determined by ^{1}H NMR analysis. >99% deuteration in all cases. [b]ORTEP drawing of compound **83**. Thermal ellipsoids are shown at 30% probability level.

Human microsomal metabolic assay

To further demonstrate the functionality of introducing the deuterium into the chemical entity for improving metabolic stability. we preliminarily performed a human microsomal metabolic assay to evaluate the metabolic stability using **68** as the model antioxidant compound. After 180 min of incubation in human microsomes, the amount of remaining **68** was higher than that of **68-H**. The calculated half-life of **68** was also prolonged compared with that of **68-H** (**68-H,** $t_{1/2}$ = 4.46 h; **68,** $t_{1/2}$ = 5.91 h), suggesting that metabolic tolerance is obviously improved by deuterium incorporation at the α-position of the amino acid (Fig. 6a). The results further exemplify the remarkable value of the current protocol in facilitating the site-selective synthesis of deuterated amino acids/peptides.

Gram-scale synthesis and reuse of $Ca(NTf_2)_2$

The practicability of this protocol was further successfully explored as evidenced by a gram-scale synthesis of deuterated amino acid **1** and d_1-amino acid conjugate **102** in 90% and 76% yields without any setback (Fig. 6b). The approach described in this paper allows the catalyst to be recovered fairly simply as the catalyst could be extracted from the reaction by water. The catalysts can then be reused in a consecutive run. The excellent catalytic activity remained for up to eight runs. The robustness of the process was shown when different amines, as well as drug molecules, were used in the recycling experiment (Fig. 6c).

Mechanistic investigations

To better gain insight into the mechanism, a series of control experiments have been conducted (Fig. 7a). When the radical scavenger (2,2,6,6-tetramethylpiperidin-1-yl) oxidanyl (TEMPO) was added to the model reaction, the reactions proceeded well without loss of yields. AIBN was a common radical initiator used in the radical processed reaction. However, the yield of the reaction could not be improved with AIBN under standard conditions. On the basis of these experiments, we excluded the possibility of a radical mechanism. The subsequent deuterium experiment showed that no deuterium incorporation was observed when d_1-HE1 or d_2-HFIP was utilized, which revealed that the deuterium in the products came from the 4-D of the Hantzsch ester. Thus, we suggest that the *d*-transfer reaction may follow a concerted mechanism, akin to a related transfer-hydrogenation process. The kinetic isotope effect (k_H/k_D = 4.8) was observed in the competitive reaction between H- and D-Hantzsch ester, which clearly implied that cleavage of the C-H bond is the rate-limiting step.

Based on the above results and literature precedents[43,64,65], a possible reaction pathway involving a concerted mechanism for the Ca(II)-HFIP-mediated reductive deutero-amination is proposed in Fig. 7b. In our previous work, it was found that $[Ca(NTf_2)(HFIP)_2]^+$, with two HFIP molecules coordinated to calcium, represents the most active cluster[57,58]. For substrates possessing O and N atoms as anchor points[57], chelation is formed through a Ca–O coordination bond and an N–H hydrogen bond with one HFIP ligand. The second HFIP ligand stabilizes the complex via F–Ca and O–H bonding. In the current situation, this association would give rise to species **A**. Subsequently, ***d*$_2$-HE1** deuterates the imine to produce the target product. Finally, the catalyst is regenerated by proton transfer of **C**, accompanied by the release of **D** (confirmed by NMR analysis) as a by-product. To confirm this hypothesis, we initially carried out acidity studies using Child's method. The combination of HFIP (2 equiv) with $Ca(NTf_2)_2$ led

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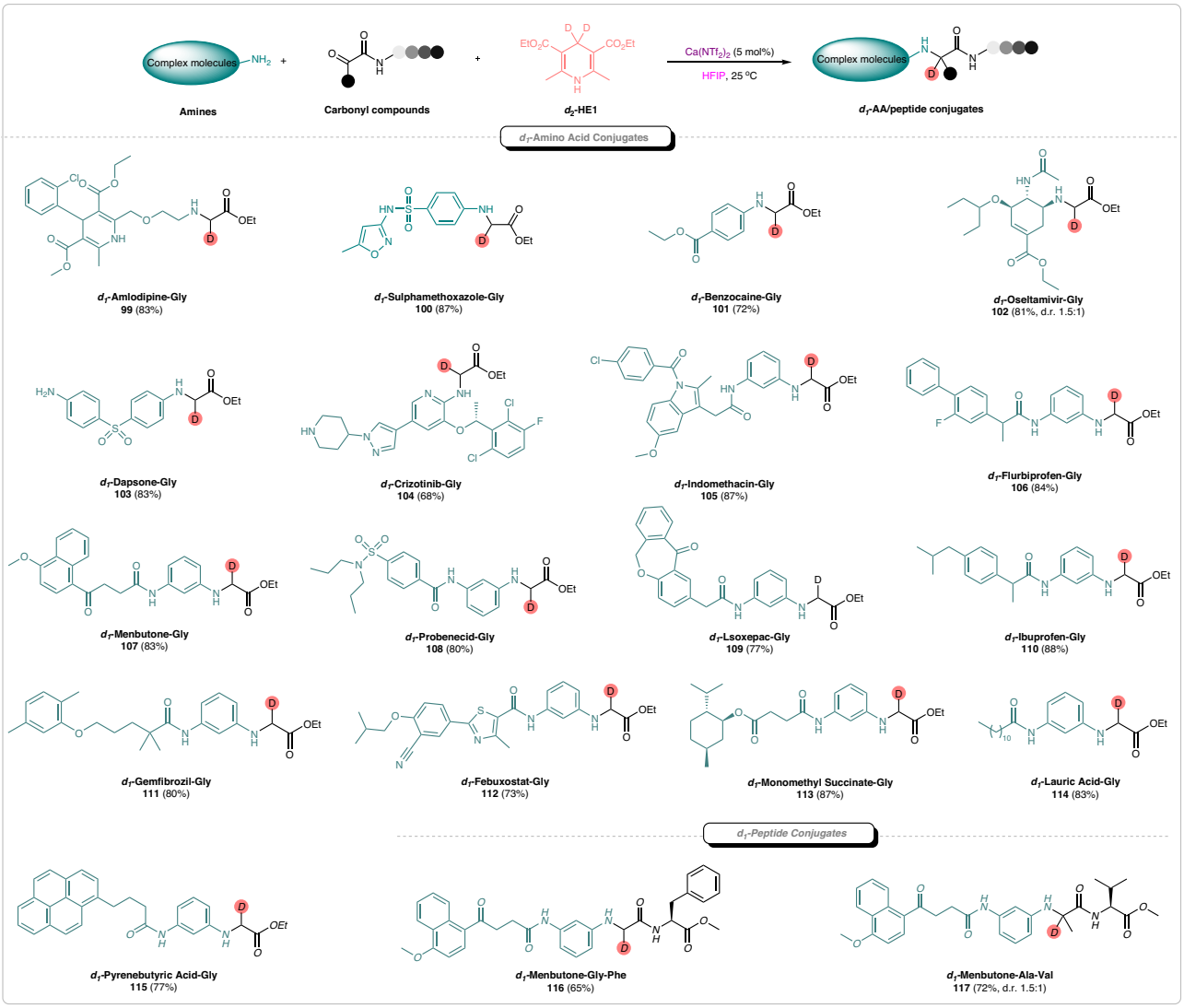

Fig. 5 | Late-stage functionalizations. Standard reaction conditions: amine (0.2 mmol, 1 equiv.), carbonyl compound (1.2 equiv.), d_2-HE1 (1.5 equiv.), $Ca(NTf_2)_2$ (5 mol%) in HFIP (0.2 M) at r.t. for 12 h. The yields are isolated yields. The d.r. was determined by ^1H NMR analysis. >99% deuteration in all cases.

to the largest chemical shift variation for H^β at 7.26 ppm (Fig. 7c). These results corroborate the hypothesis that the Ca(II)-HFIP-mediated system demonstrates enhanced acidity. Then, we executed M06-2X/6-31 + G(d,p) (SMD) computations with methyl (*E*)-2-(phenylimine) acetate, 1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylic acid dimethyl ester, and the aforementioned complex $[Ca(NTf_2)(HFIP)_2]^+$ (Fig. 7d). The activation of the imine acetate as described above does indeed enable the simultaneous transfer of a hydride from the Hantzsch ester and a proton from HFIP at a reasonable free energy cost of 16.7 kcal/mol. This step is significantly exergonic by 17.2 kcal/mol.

Synthesis of DNA conjugates and diversified transformations

In 1992, Lerner and Brenner introduced the DNA-encoded library (DEL), a revolutionary technology that seamlessly integrates chemistry and biology for drug discovery[66,67]. It is a collection of small molecules, each distinctly labeled with a DNA barcode, facilitating the highly efficient pooled screening of millions or even billions of compounds against a specific target protein. A DEL is typically built through multiple cycles of enzymatic DNA barcoding and DNA-compatible chemical reactions. As a result, advancing DNA-compatible reactions constitutes one of the crucial aspects in the DEL research domain, although it still poses a substantial challenge[68,69]. The complexity stems from the necessity to strike a balance between the water solubility and sensitivity of highly functionalized DNA barcodes while safeguarding their integrity during the reaction process. Despite these obstacles, the mild reaction conditions, high yields, and the suitability for late-stage functionalization of complex molecules have motivated us to adapt this developed reductive deutero-amination into on-DNA synthesis (DNA-compatible reductive deutero-amination).

As predicted, the on-DNA reaction proceeded well at room temperature, affording the target DNA-conjugated d_1-amino acid **118** in 94% conversion, by using 20 equivalents of $Ca(NTf_2)_2$, 1000 equivalents of the carbonyl compound, 1000 equivalents of ***d*$_2$-HE1**, and HFIP as solvent. Notably, this represents the pioneering example of on-DNA synthesis via calcium catalysis. And, the on-DNA reactions were carried out on a small scale (~2 nmol), and even with 1000 equivalents of reagents, there are no significant atom economy issues. Furthermore, the product, being attached to water-soluble DNA, allows for easy removal of excess reagents through ethanol precipitation. With the optimized reaction conditions established, we then sought to evaluate the reaction scope of this Ca(II)-HFIP-mediated on-DNA reductive deutero-amination. By rational design, we have successfully synthesized a variety of DNA-conjugated d_1-amino acids/peptides. As depicted in Fig. 8a, DNA-conjugated amine **HP-1** reacted with different carbonyl compounds could successfully generate the target DNA-d_1-

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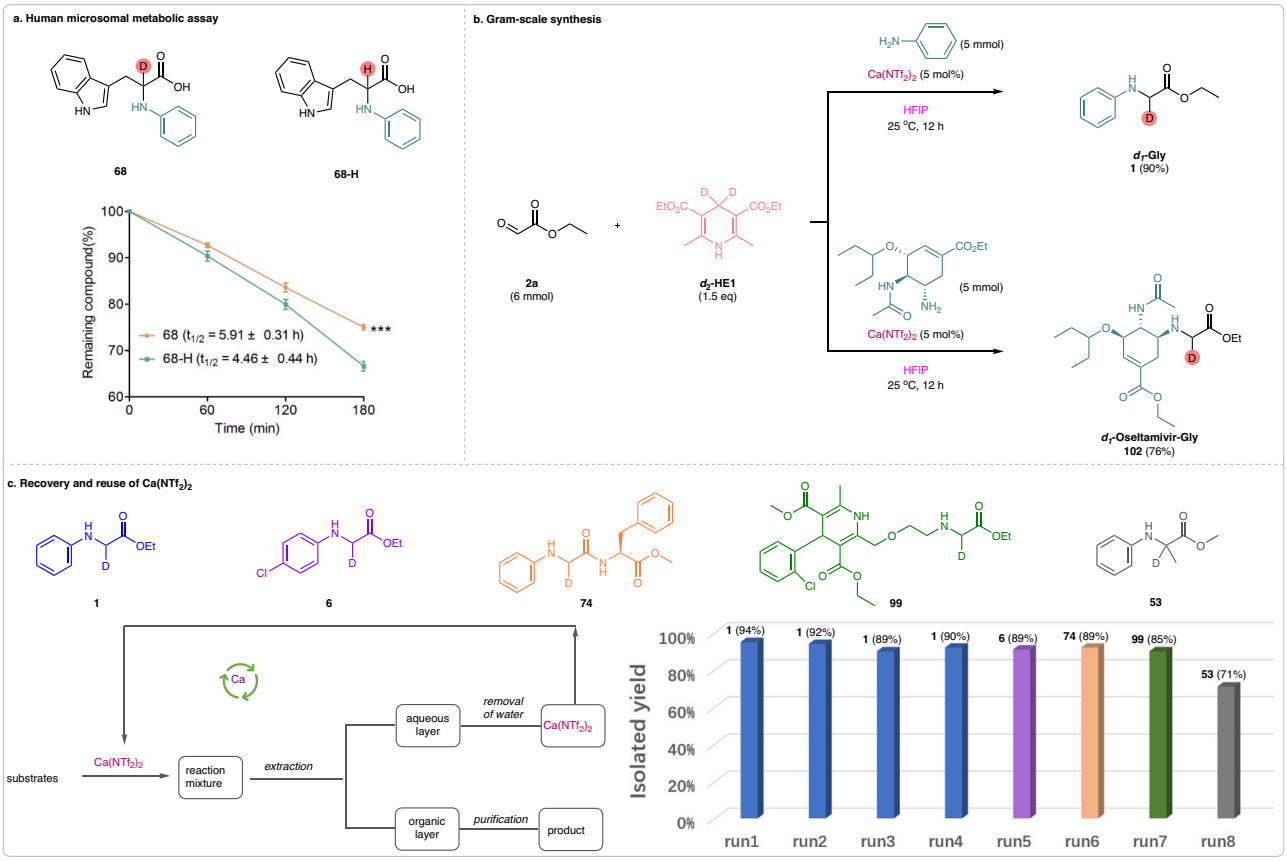

Fig. 6 | Biological analysis and synthetic applications. a Human microsomal metabolic assay. Values represent the mean accompanied by the standard error of the mean (SEM) ($n = 3$). Measurements were taken from distinct samples. The estimated half-life ($t_{1/2}$) of **68** was extended in comparison to that of **68-H**, suggesting that the metabolic stability of **68** in human microsomes was augmented through deuteration. ***$p < 0.001$ compared with **68-H**. Source data are provided as a Source Data file. **b** Gram-scale synthesis. **c** Recovery and reuse of $Ca(NTf_2)_2$.

AA conjugates (**118**), DNA-d_1-UAA conjugates (**119**–**121**), and DNA-d_1-peptide conjugates (**122**) in moderate to excellent conversions. Of note, the position of the substituent on the phenyl ring of the amino group had no significant effect on the outcome of the reaction, as *meta*-substituted amines were well-compatible and afforded the desired DNA conjugates **123**–**127** in 44–95% conversions. To further probe the generality of this on-DNA reductive deutero-amination, the long chain linked DNA-conjugated amine **HP-2** and **HP-3** were synthesized and proved as good reactants to react with diverse carbonyl compounds, yielding the desired DNA-conjugated d_1-AAs/UAAs/peptide in moderate to good conversions (**128**–**137**).

As demonstrated, the Ca(II)-HFIP-mediated on-DNA reductive deutero-amination protocol produces the desired target products, DNA-conjugated d_1-amino acids with an additional anchor–carboxy group. This feature will facilitate their use in diversity-oriented synthesis. Thus, to comprehensively evaluate the potential utility of this methodology in the development of chemodivergent synthesis, we investigated the potential of on-DNA synthesis of DNA-d_1-complex molecular conjugates in a DEL rehearsal. As shown in Fig. 8b, the Ca(II)-HFIP-mediated on-DNA reductive deutero-amination of DNA-conjugated amine (**HP-1**) with carbonyl substrate proceeded smoothly to give DNA-conjugated d_1-amino acid (**118**), which, upon further reaction with various amines through the classical amidation under preliminarily defined conditions, resulted in the formation of the desired DNA-conjugated d_1-complex molecules **138** and **139** in synthetically useful yields. These results demonstrate the compatibility of the developed protocol and highlight its significant potential for DEL construction.

In summary, we herein reported the direct access to the deuterated amino acids/peptides through the bioinspired reductive deutero-amination of simple ketoesters with amines. This versatile protocol was triggered by an earth-abundant and environmentally benign calcium-based catalyst using bioinspired d_2-hantzsch ester as a deuterium source in HFIP. The protocol features mild conditions, high yields, good substrate scope, excellent deuterium levels, and site-selective deuterium incorporation at the α-position to *N*-heteroatom. The catalyst's high water solubility facilitates its recovery, enabling multiple subsequent runs and underscoring the efficiency and sustainability of this protocol. The practicability and robustness of this protocol have been demonstrated through its application in gram-scale synthesis and late-stage deuteration of drug molecules and natural products. Additionally, the functionality of the developed deuterium-labeled strategy for exhibiting increased metabolic stability has been illustrated. Finally, the calcium catalysis has successfully been applied in DEL synthesis, and the on-DNA synthesis of DNA-tagged deuterated amino acids/peptides/drugs has been obtained smoothly, which manifested great potential for the synthesis of deuterium DEL (*d*DEL), thereby efficiently exploring the ultra-large deuterium chemical space.

Methods

General procedure for the synthesis of d_1-amino acids/peptides

Amine (0.20 mmol, 1.0 equiv.), carbonyl compound (0.24 mmol, 1.2 equiv.), d_2-HE1 (0.30 mmol, 1.5 equiv.), $Ca(NTf_2)_2$ (5 mol%.) and HFIP (0.2 M) and solvent were added to a 4 mL Schlenk tube equipped with a Teflon screw cap. The reaction was stirred at room temperature for 12 h. Then, the reaction mixture was quenched with saturated NH_4Cl aqueous solution and extracted with ethyl acetate (10 mL × 3). The combined organic layers were washed with brine, dried over $MgSO_4$,

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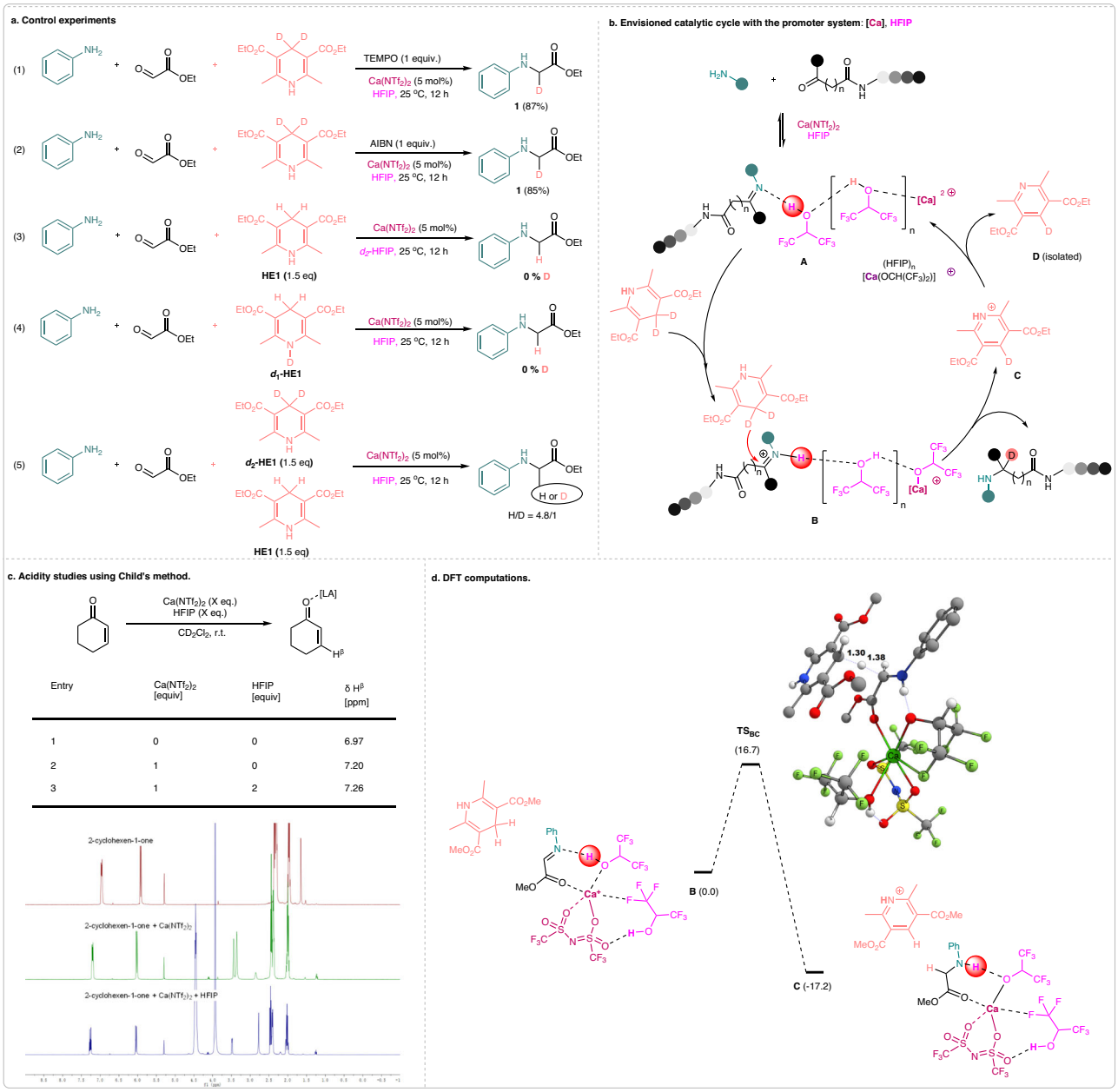

Fig. 7 | Mechanistic investigations. a Control experiment. **b** Envisioned catalytic cycle with the promoter system: [Ca], HFIP. **c** Acidity studies using Child's method. Conditions: mixture of 2-cyclohexen-1-one (1 equiv), $Ca(NTf_2)_2$ (0 or 1 equiv) and HFIP (0 or 2 equiv) stirred at r.t. for 1 hour. **d** DFT computations. Computed free energy of the calcium-mediated reduction of methyl (*E*)−2-(phenylimine)acetate (ΔG_{298}, kcal/mol; selected distances in Å; some hydrogen atoms have been omitted for clarity).

and filtered. The solvent was removed under vacuum. The crude product was purified by preparative TLC (crude product is deposited in a horizontal thin line at the bottom of the plate, and the plate is run in the appropriate solvent system as usual) to afford the desired products.

General Procedure for the synthesis of DNA conjugates

To the headpiece (4 μL, 0.5 mM in ddH_2O) was added 1000 equiv of carbonyl compounds (4 μL, 500 mM in HFIP), 1000 equiv of d_2-HE1 (4 μL, 500 mM in HFIP) and 20 equiv of $Ca(NTf_2)_2$ (4 μL, 10 mM in HFIP) in 20 μL HFIP. The mixture was vortexed and stood at 25 °C for 12 h. After the reaction was completed, added sodium carbonate (4 μL, 4 μL, 10 mM in ddH_2O) to the mixture, and heated the reaction mixture at 60 °C for at least 30 min. Then the mixture was centrifuged at 4 °C for 15 min at 4000 × *g*, and the resultant supernatant was collected. Add aqueous 5 N NaCl solution (10% by volume) and cold ethanol (2.5 times by volume, ethanol stored at −20 °C) to the resultant supernatant. The mixture was vortexed and stored at a −80 °C freezer for more than 1 h. The sample was centrifuged for around 30 min at 4 °C in a micro-centrifuge at 5300 × *g*. The above supernatant was removed, and the pellet (precipitate) was dissolved in deionized water for LC-MS detection.

General procedure of Ca(II)-HFIP-mediated on-DNA synthesis of d_1-amino acids/peptides in a DEL rehearsal

To DNA-d_1-Gly 111 (4 μL, 2 nmol, 0.5 mM in ddH_2O) was added 13.5 μL of MOPS buffer (pH = 7, 50 mM in ddH_2O) and 3 μL of condensation reagent (500 mM in MOPS buffer). The resulting mixture was vortexed and stirred at 45 °C for 30 minutes. Then 7.5 μL of desired amine (100 mM in DMSO) was added to the mixture and stirred at 45 °C for

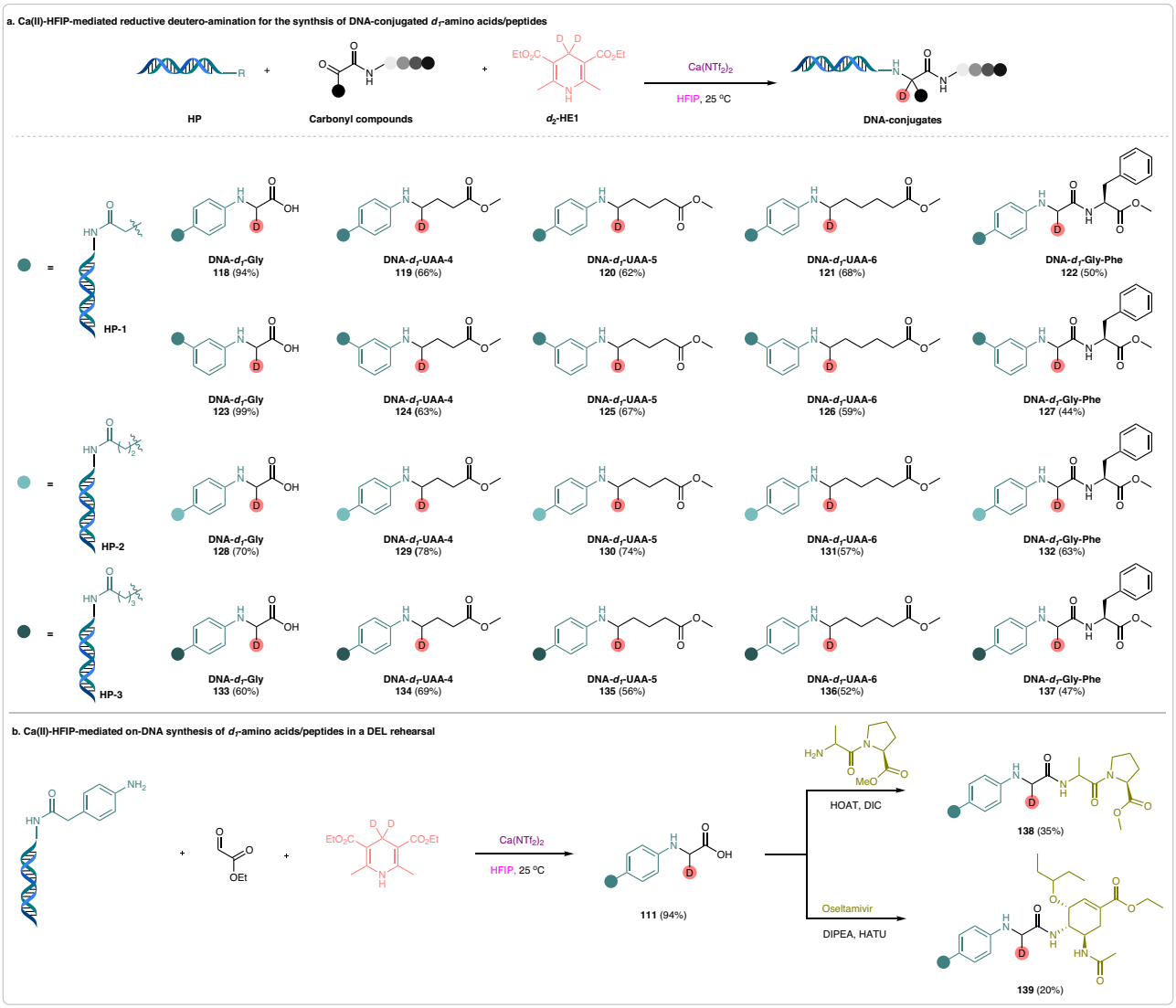

Fig. 8 | Synthesis of DNA-conjugated d_1-amino acids/peptides and diversified transformations. a Ca(II)-HFIP-mediated reductive deutero-amination for the synthsis of DNA-conjugated d_1-amino acids/peptides. **b** Ca(II)-HFIP-mediated on-DNA synthesis of d_1-amino acids/peptides in a DEL rehearsal.

12 hours. After that 5 M NaCl solution (10% by volume) and cold ethanol (2.5 times by volume, ethanol stored at −20 °C) were added, vortexed, and incubated at −80 °C for at least 30 minutes. The sample was centrifuged for 30 minutes at 4 °C in a microcentrifuge at 5300 × *g* to remove the supernatant. The resulting pellet (precipitate) was re-dissolved in ddH_2O (10 µL) for LC-MS detection.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Detailed experimental procedures and characterization data are provided in the Supplementary Information. All data can be requested from the corresponding author. Crystallographic data for the structures reported in this article have been deposited at the Cambridge Crystallographic Data Center, under deposition numbers CCDC 2288911 (16), and 2349502 (83). Copies of the data can be obtained free of charge via https://www.ccdc.cam.ac.uk/structures/. The source data underlying Fig. 6a and DFT computations is provided as a Source Data file. Source data are provided with this paper.

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Author contributions

S.W., W.Y., and H.X. conceived the project, designed the experiments, and wrote the manuscript with input from all of the authors. H.L. and Y.L. performed the experiments and interpreted the data equally. S.Z. assisted with the DNA-encoded substrate synthesis and library construction. L.M., Z. Zeng, and Z. Zhou participated in the biological experiments. V.G. performed DFT calculations.

Competing interests

The authors declare no competing interests.

Additional information

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