Biomimetic enantioselective synthesis of \(\beta,\beta\)-difluoro-\(\alpha\)-amino acid derivatives

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Although utilization of fluorine compounds has a long history, synthesis of chiral fluorinated amino acid derivatives with structural diversity and high stereoselectivity is still very appealing and challenging. Here, we report a biomimetic study of enantioselective [1,3]-proton shift of \(\beta,\beta\)-difluoro-\(\alpha\)-imine amides catalyzed by chiral quinine derivatives. A wide range of corresponding \(\beta,\beta\)-difluoro-\(\alpha\)-amino amides were achieved in good yields with high enantioselectivities. The optically pure \(\beta,\beta\)-difluoro-\(\alpha\)-amino acid derivatives were further obtained, which have high application values in the synthesis of fluoro peptides, fluoro amino alcohols and other valuable fluorine-containing molecules.

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Organic fluorides are a class of unique fluorine compounds, which feature with strong electronegativity, similar size to hydrogen, great influence on pKa, and lipophilicity, and behavior of hydrogen-bond receptors. Although fluorine-containing compounds have special physicochemical and biochemical properties, naturally occurring organofluorinated compounds are extremely rare. Fluorine earns other extensive applications in modern chemistry. For example, at least 25% of drugs and 50% agrochemicals contain fluorine. Chiral fluorinated amino acids are a class of fluorine-containing building blocks, which are wildly utilized in biorthogonal chemistry, drug modification, and asymmetric catalysis. However, the development of chiral fluorine-containing amino acids still has numerous limitations as below. (i) The preparation of fluorine-containing peptides from natural amino acids is extremely rare, and the application of natural chemical connection is still in its infancy; (ii) compared with aromatic fluorination, the structural diversity of alkyl-fluorinated amino acids needs further study; (iii) the self-disproportionation of enantiomers is also an important factor to prevent the formation of optically pure fluorinated amino acids.

Difluoromethylene has the highest dipole moment in the fluoromethane series and also exerts as biosostere of ketone and ether, which may change the protein structure. Several fluorinated amino acids are a class of unique fluorine compounds, and fluorinated alanine isomers exhibit as bioisostere of ketone and ether, which may change the protein structure.

Fig. 1 Representative β,β-difluoro-α-amino acid derivatives. I: β,β-difluorophenylalanyl puromycin; II: 3,3-difluoro-3,4-dideoxy-KRN7000 analog; III: CCRF-CEM poly-γ-glutamate synthetase.

**Table 1** Optimization of asymmetric isomerization of 1a.

| Entry | NHC | Solvent | Yield [%] | ee [%] |
|-------|-----|---------|-----------|--------|
| 1     | A   | Toluene | 34        | 81     |
| 2     | B   | Toluene | 18        | 2      |
| 3     | C   | Toluene | 79        | 93     |
| 4     | D   | Toluene | 76        | 95     |
| 5     | E   | Toluene | 83        | 90     |
| 6     | F   | Toluene | 43        | 95     |
| 7     | G   | Toluene | 87        | 95     |
| 8     | H   | Toluene | 80        | 95     |
| 9     | G   | DCM     | 81        | 93     |
| 10    | G   | CHCl3   | 85        | 94     |
| 11    | G   | CH3CN   | 51        | 84     |
| 12    | G   | THF     | 49        | 77     |
| 13a   | G   | Toluene | 86        | 95     |
| 14a   | G   | Toluene | 27        | 96     |

Fig. 2 Strategies for asymmetric synthesis of β,β-difluoro-α-amino acid derivatives. Strategies, a enzymatic hydrolysis; b asymmetric hydrogenation; c chiral auxiliaries. Valuable molecules: difluoro peptides, difluoro amino alcohols, and difluoro alkoil.
rapid synthesis of chiral fluoro amino acids, we report here an approach to achieve dFAAS via biomimetic enantioselective [1,3]-proton-shift reactions. Notably, the yielded chiral dFAAs are useful synthons for producing various valuable molecules, such as difluoro peptides, difluoro amino alcohols, and difluoro alkaloids.

**Results and discussion**

**Reaction optimization.** We first studied the reaction of N-benzyl-2-((2-chlorobenzyl)imino)-3,3-difluoro-5-phenylpentanamide 1a as model substrate and toluene as solvent. The results are shown in Table 1. When cinchonan-6′, diol A was exploited, the expected 2a was acquired in 34% yield and 81% ee (entry 1). Almost racemic 2a was obtained by replacing the catalyst with quinine B (entry 2), while the yield and enantioselectivity were improved by using catalysts C and D (entries 3, 4). Further tests on catalysts E and F showed no significant change in yield or ee (entries 5, 6). To our delight, when G was tested, 2a was successfully formed with 87% yield and 95% ee and pseudoe-nantiomer H was also tested for delivering the enantiomer of 2a with 80% yield and 95% ee (entries 7 and 8). Solvent evaluation experiments reveal that toluene is the best choice (entries 9–12). Furthermore, reducing the loading of catalyst G to 5 mol% (entry 13) without decreasing the yield or ee implies that this highly enantioselective biomimetic [1,3]-proton shift can be achieved under suitable conditions. Even if the time is extended to 24 h and the loading is further reduced to 1 mol% (entry 14), the requirement of useless yield can not be met.

**Substrate scope.** Under the optimized catalytic conditions, we turned our attention to explore the generality of biomimetic [1,3]-proton-shift reaction. As illustrated in Fig. 3, R1 group was examined first. In the process of generating the [1,3]-proton-shift products 2a and 2b, it was found that the yield and enantioselectivity are independent of the adjacent CH2CH2 groups of the substituents (1a and 1b). Alkene (1c) is also compatible under this condition. Furthermore, the CH2 adjacent groups containing cyclohexyl, 1,3-dioxolan-2-yl, isopropyl, phenyl, methyl and alkene were well tolerated and provided good yields and high enantioselectivities (2d–2i).

β,β-difluoro-2-aminobutyric amide (2j) was obtained with good yield and excellent ee. In the case of 2k that is a hydrolyzed derivative and bearing ester group, 72% ee and 62% yield were observed. Then,
we turned to amide group R², where isobutyl can be installed smoothly (2m). It is gratifying that dipeptides (2n and 2o) bearing leukine have been successfully provided for peptide synthesis and the diastereoisomers indicated that the source of chirality is induced by the catalyst rather than substrate itself. Pleasingly, the imines with or without substituted phenyl group are well tolerated under these conditions (2p–2r). β,β-difluoro natural amino acid derivatives (e.g., β,β-difluoro glutamine, β,β-difluoro leucine, and β,β-difluoro phenylalanine) were all obtained in good yields and high ee’s.

Mechanistic studies and postulated mechanism. Next, we started to investigate the reaction mechanism. We want to understand whether the [1,3]-proton-shift process is intermolecular or intramolecular. Benzyl-deuterated 1a-d₂ was prepared and the reaction carried out under standard conditions. The results show that 2k has no erosion on deuterated ratio (Fig. 4a). As a contrast, when deuterium oxide was added to the reaction system, no deuterium product was found (Fig. 4b). When 1a-d₂ cross-reacted with 1t, no deuterium was found in product 2t (Fig. 4c). These experiments reveal that the [1,3]-proton-shift process is intramolecular. The parallel kinetic isotope effects (KIE) were measured with kH/kD of 4.0, indicating that the rate-determining step (RDS) is the process of hydrogen leaving from benzyl group (Fig. 5).

Based on the condition optimization, substrate scope, and the initial mechanistic experiments, a plausible mechanism for the biomimetic enantioselective [1,3]-proton-shift reaction was proposed (Fig. 6). First, the possible intramolecular hydrogen-bond interaction (blue) of the amide NH to imine of the substrate is essential, and without it, the ee decreased dramatically (e.g., 2l bearing an ester group resulted in 72% ee). Then, the catalyst with free OH of phenol is needful (e.g., cat. B with OMe only induced 2% ee) for assembling another hydrogen bond between it and the N of the amide (green). The bulk isopentyloxy of the catalyst enforced deprotonation of the inner H of benzyl (red) and this process was a RDS (TS-I). Finally, the asymmetric protonation of 2-azaallyl anion from Si face (TS-II) to deliver the target product with R-configuration.

Synthetic transformations and applications. In addition, our protocol is potentially suitable for large-scale preparation. As illustrated in Fig. 7, the use of 2 mol% catalyst G is sufficient to produce 2i (0.91 g) in 80% yield and 95% ee. Compound 3 can be achieved via hydrolysis and N-Phth protection. Next, the conversion of amide to ester group can generate the key intermediate 4. Finally, 3,3-difluoro-3,4-dideoxy-KRN7000 analog II can be made smoothly from 4a based on formal synthesis. Meanwhile, the corresponding Fmoc-protected amino acid 5 can be prepared from 4b for further medicinal
study. To further prove the practicability of dFAAs, difluoro leukemia was assembled into linear difluoro-oxytocin (28% yield for overall steps), which could be folded smoothly by using glutathione oxidation (63% yield) (Fig. 8). Additionally, we demonstrated that the stability of folded difluoro-oxytocin (3 mM GSH, 100 mM PBS, pH 7.0, rt) is higher than WT oxytocin \(^{64-67}\) (Fig. 9).

In summary, a biomimetic enantioselective [1,3]-proton shift of difluorimines has been developed. This new protocol allows the rapid assembly of enantiomerically enriched dFAAs from readily available starting materials under mild conditions. Preliminary mechanism studies show that the proton transfer is intramolecular and the deprotonation step is RDS. More details on enantiocontrol are being conducted in our laboratory and will be reported in due course.

**Methods**

**Synthesis of 2.** To a flame-dried Schlenk reaction tube equipped with a magnetic stir bar, was added the 1 (0.1 mmol) and cat. G (1.9 mg, 0.005 mmol). The Schlenk tube was closed with a septum, and toluene (1.0 mL) was added. The mixture was then stirred at 25 °C and monitored by TLC until 1 was consumed. The mixture was concentrated under reduced pressure and purified by column chromatography on silica gel (hexane/EtOAc = 20:1–10:1) to afford the desired product 2. Full experimental details can be found in the Supplementary Methods.

**Fig. 7 Gram-scale synthesis and transformations.** The gram-scale synthesis of 1l. The transformation of 2i to II and 2t to 5.

**Fig. 8 Solid-phase peptide synthesis of difluorinated oxytocin.**

- a About 20% piperidine/DMF, 10 min, double; b 1.5 equiv. fluorinated leucine, 1.5 equiv. HOBT, 1.5 equiv. DIC, minimum DMF, rt, 16 h; c 10% piperidine/DMF, 3 mins, double; d 3 equiv. amino acid, 2.9 equiv. HBTU, 6 equiv. DIPEA, minimum DMF, rt, 1.5 h; e 10% piperidine/DMF, 3 mins, double; f TFA cocktail, global deprotection, rt, 3 h; g GSH/GSSG folding, rt, over-night. Fmoc = fluorenylmethoxycarbonyl, HOBT = hydroxybenzotriazole, DIC = O-(1H-benzotriazol-1-yl)-uronium hexafluorophosphate, HBTU = N,N,N',N'-tetramethyl-O-[(1H-benzotriazol-1-yl)-uronium hexafluorophosphate, DIPEA = N,N-diisopropylethylamine, GSSG = glutathione disulfide.
Fig. 9 Folded difluoro-oxytocin stable than folded WT oxytocin. s4: fold WT-oxytocin, s5: linear WT oxytocin; s4’: fold difluoro-oxytocin, s5’: linear difluoro-oxytocin, s1=s3/s6=s9 scrambling, s1’=s3’/s6’=s9’ scrambling.

Data availability
For 1H NMR, 13C NMR, and 19F NMR spectra see Supplementary Figs. 1–75 and highperformance liquid-chromatography spectra see Supplementary Figs. 76–118. The X-ray crystallographic coordinates for Fmoc-2I (Supplementary Data 1) reported in this article have been deposited at the Cambridge Crystallographic Data Centre (CCDC), under deposition number CCDC 2109856. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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Author contributions

Q. P. conducted the main chemical part experiments; B. Y. conducted peptide part experiments; F. L., M. L., B. Z. and D. G. prepared several starting materials. D. B. and J. W. conceptualized and directed the project, and drafted the paper with assistance from coauthors. All authors contributed to discussions.

Competing interests

The authors declare no competing interests.

Additional information

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