JACS Au
AN OPEN ACCESS JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

pubs.acs.org/jacsau
Article

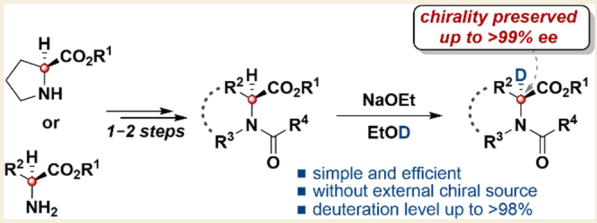
Simple and Efficient Enantioselective α-Deuteration Method of α-Amino Acids without External Chiral Sources

Soojun Park, Jae Hyun Kim, Dongjun Kim, Yeonjoon Kim, Seonah Kim, and Sanghee Kim*

Cite This: JACS Au 2024, 4, 2246−2251
Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Deuterium-labeled α-amino acids are useful in research related to drug discovery and biomedical science. However, a high degree of site selectivity and stereoselectivity in the deuterium incorporation process is still difficult to achieve. Herein, we report a new enantioselective deuteration method at the α-position of several amino acids without external chiral sources. The proposed deuteration methods (NaOEt and EtOD) are highly selective and simple. Additionally, we provide a mechanistic study for this enantioretentive deuteration.



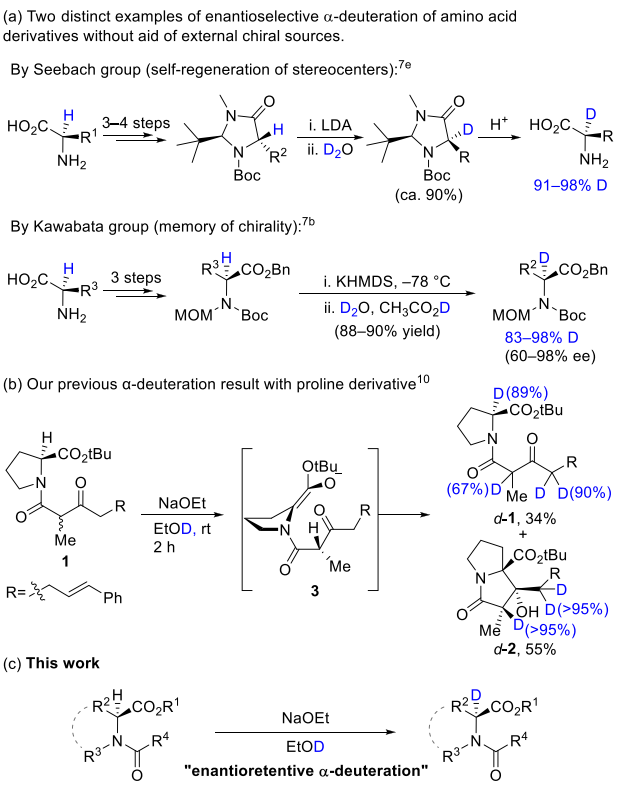
KEYWORDS: α-deuteration, α-amino acids, enantioretentive, memory of chirality, α-deuterated amino acids

INTRODUCTION

Owing to the kinetic isotope effect, deuterium-labeled α-amino acids have found numerous important applications in research, especially related to drug discovery and biomedical science.[1−4] For example, they are used in elucidating secondary and tertiary structures of proteins.[2] They are also widely used to investigate biochemical processes such as the metabolism of peptides and the mechanism of enzymes.[3] Further, compounds with an α-deuterated amino acid showed improved metabolic stability compared to those with an α-amino acid moiety.[4] Therefore, various methods have been developed to obtain chiral deuterated amino acids.[5,6] Notable recent methods include the use of chiral metal complexes and organocatalysts.[5] However, these methods are associated with poor site- and stereoselectivity depending on the amino acid type.[5a,c,e,f] Other notable methods are based on the use of enzymes.[6] They are often limited in substrate scope or provide deuterated amino acids with moderate enantioselectivity and deuterium incorporation.

While most developed methods for asymmetrical deuterium attachment require external chiral sources, a few methods can access deuterated amino acids without using external chiral intervention.[7] The principles of Seebach's "self-regeneration of stereocenters (SRS)"[8] have been mainly employed.[7c,e] Although the SRS-based methods could effectively and asymmetrically incorporate deuterium, they required a strong base (e.g., LDA) under anhydrous conditions and additional steps to transfer carbon-centered chirality to adjacent carbons and remove accessory groups (Scheme 1a). Kawabata group reported a different method[7b] that used the concept of "memory of chirality (MOC)[9]", a phenomenon in which the sp^3 chirality of the starting material is preserved in a reactive intermediate as an axial chirality.[9c] Unlike SRS-based methods,

Scheme 1. Enantioretentive α-Deuteration of Amino Acids



Received: February 29, 2024
Revised: May 9, 2024
Accepted: May 9, 2024
Published: May 21, 2024

it does not need preinstallation of carbon-centered chirality. However, this method still required a strong base under anhydrous conditions at a low temperature (−78 °C). Therefore, the development of a convenient and practical production approach for α-deuterated amino acids is still in great demand.

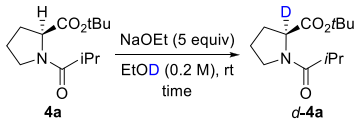
In the course of our previous study on asymmetric total synthesis,[10] we obtained a valuable clue for asymmetrically incorporating a deuterium atom into the α-position of amino acids without external chiral intervention under conventional reaction conditions. When the proline derivative **1** was treated with NaOEt in EtOD at room temperature, the aldol product *d*-**2** was formed with retention of the C-α configuration (Scheme 1b). Interestingly, the analysis of the incomplete reaction mixtures showed that the hydrogen at the α-position of the remaining aldol substrate was replaced with deuterium to give *d*-**1** while preserving its original chirality. Unlike Seebach's SRS-based and Kawabata's MOC-based methods, this stereoselective deuteration did not require the use of an anhydrous strong base. We envisioned that this phenomenon could be utilized to rapidly and efficiently access α-deuterated chiral prolines and other amino acids from readily available amino acids under conventional reaction conditions.

Herein, we report a new deuteration method at the α-position of amino acids, including proline, serine, cysteine, phenylalanine, and lysine (Scheme 1c). The developed deuteration method was simple and did not require expensive equipment, strong bases, or extra chiral sources. A mechanistic study for this enantio-retentive deuteration is also provided.

RESULT AND DISCUSSION

Initially, *N*-isobutyryl L-proline ester **4a** was designed as a model substrate (Scheme 2). Unlike proline derivative **1**,

Scheme 2. Enantioretentive α-Deuteration of Proline Derivatives 4a[a,b,c,d]



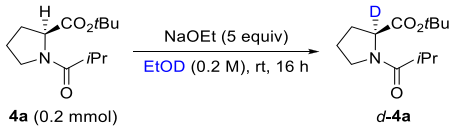
time	yield[b]	%D[c]	ee%[d]
4 h	95%	72%	98%
8 h	94%	98%	97%
16 h	94%	98%	97%

[a]Reagents and conditions: **4a** (0.2 mmol), NaOEt (5.0 equiv), EtOD (0.2 M), room temperature. [b]The indicated yields are isolated. [c]The DL is estimated based on ^{1}H NMR analysis. [d]The enantiomeric excess (*ee*) value is estimated by using chiral HPLC.

substrate **4a** does not possess a carbonyl group in the amide side chain and therefore cannot undergo intramolecular aldol reaction. When **4a** was treated with NaOEt (5.0 equiv) in EtOD (0.2 M) at room temperature, deuteration proceeded gradually to afford *d*-**4a** in 94% yield after 8 h. The deuteration level (DL) of thus obtained *d*-**4a** was excellent (98%) and its enantiomeric purity was greater than 97%. The prolonged reaction time (16 h) did not affect the reaction outcomes. Interestingly, negligible deuterium incorporation was observed at the α-position of the amide carbonyl group.

We modified the reaction conditions to understand the reaction. The reaction concentration did not considerably affect the reaction efficiency (Table 1, entries 2 and 3). The change in the cation of the ethoxide base did not alter the reaction outcome (entries 4 and 5). The influence of the solvent composition on the reaction outcome was noteworthy. The use of THF as a major cosolvent resulted in a lower yield

Table 1. Modification of the Reaction Conditions[a]



entry	deviation	yield[b]	% D[c]	*ee*[d]
1	none	94%	98%	97%
2	EtOD (0.1 M)	96%	98%	98%
3	EtOD (1.0 M)	98%	96%	98%
4	LiOEt (5.0 equiv)	95%	95%	97%
5	KOEt (5.0 equiv)	97%	98%	98%
6	THF/EtOD = 10:1	75%	89%	75%
7	THF/EtOD = 10:1, − 10 °C, 48 h	91%	92%	94%
8	DMF/EtOD = 10:1	42%	84%	56%
9	toluene/EtOD = 10:1	46%	96%	4%
10	EtOH/EtOD = 1:1	95%	43%	97%
11	EtOH/EtOD = 1:1, 60 h	94%	60%	97%
12[e]	EtOD (1.0 M), NaOEt (1.0 equiv)	87%	94%	96%

[a]Reaction conditions: **4a** (0.2 mmol), NaOEt (5.0 equiv), EtOD (0.2 M), room temperature, 16 h. [b]Isolated yields. [c]Determined based on ^{1}H NMR analysis. [d]Determined using chiral HPLC. [e]40 mmol scale.

and the enantiomeric purity of *d*-**4a** compared to the reaction in EtOD only (entry 1 vs 6). However, at −10 °C, the chemical yield and enantiomeric purity were similar, although the reaction time was prolonged (48 h; entry 7). The use of polar DMF substantially decreased the reaction efficiency and selectivity (entry 8). Further, the addition of a nonpolar solvent, i.e., toluene, led to a considerable decrease in the yield and enantioselectivity (entry 9). When EtOH was used as a cosolvent, the yield and *ee* values were the same as those obtained from the reaction in EtOD only; however, the deuterium level decreased at the given reaction time (entry 1 vs 10). The deuterium level continuously increased with the reaction time while maintaining the yield and enantioselectivity (entries 10 and 11). The deuteration reaction could be enlarged without significantly losing the reaction efficiency and selectivity. For instance, at 1.0 M and 1.0 equiv of NaOEt, a 40 mmol (9.6 g) scale reaction of **4a** afforded *d*-**4a** in 87% yield, with a DL and enantiomeric purity of 94 and 96%, respectively (entry 12).

Based on the aforementioned results, the enantioretentive deuteration mechanism was investigated. Inter- or intramolecular metal coordination mechanisms were not considered because no substantial differences were observed in the reaction outcomes between countercations (Table 1, entries 1, 4, and 5). Deuteration transfer from the amide moiety to the proline moiety was not considered because no deuterium incorporation was observed at the α-position of the amide carbonyl group in **4a**. Thus, a conceivable deuteration pathway is associated with the deuteration of chiral enolates directly from the EtOD solvent.

We conducted a density functional theory (DFT)-based computational investigation using the M06-2X functional with the 6-311++G(d,p) basis set to understand the deuteration mechanism. The calculated free energy profile of EtOD at room temperature is shown in Figure 1. Based on the computational conformation analysis of **4a**, 10 distinct conformers were found (see SI for details). The most stable conformers were **4a-I**, which was used as a starting point for our computational studies. Deprotonation at the proline α-

https://doi.org/10.1021/jacsau.4c00185
JACS Au 2024, 4, 2246−2251

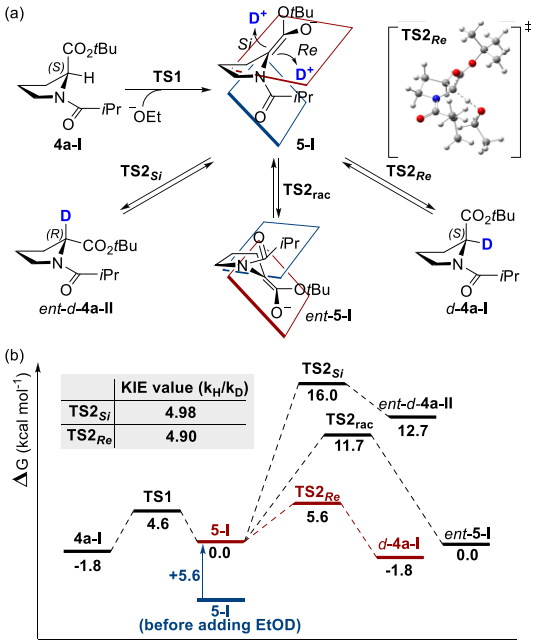


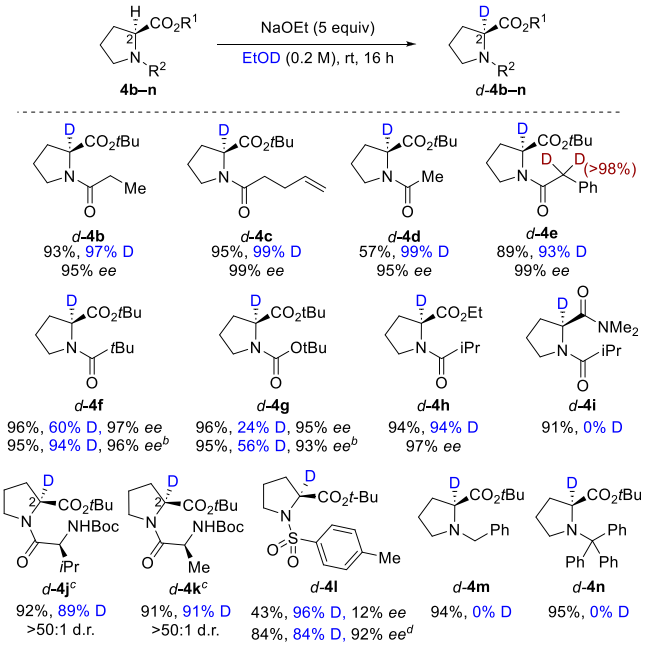
Figure 1. Plausible reaction pathways and free energy profiles of enantioretentive α-deuteration of **4a**.

position on conformers **4a-I** generated an axially chiral enolate **5-I** with a free energy barrier ($\Delta G^{\ddagger}$) of 6.4 kcal/mol (**TS1**). The transient enolate could undergo several reaction pathways, including the epimerization of axial chirality, reprotonation, and deuteration by EtOD. The $\Delta G^{\ddagger}$ of deuteration from the *Re* face ($\mathbf{TS2}_{Re}$), which lead to *d*-**4a-I**, is only 5.6 kcal/mol.[11] On the other hand, deuteration from the Si face resulted in less stable conformer *ent*-*d*-**4a-II** with the free energy barrier ($\Delta G^{\ddagger}$) of 16.0 kcal/mol ($\mathbf{TS2}_{Si}$). These results suggested the preferential deuteration of the *Re* face, providing *d*-**4a** as a deuteration product.[12] The $\Delta G^{\ddagger}$ value of epimerization from enolate **5-I** to *ent*-**5-I** was 11.7 kcal/mol ($\mathbf{TS}_{rac}$).[13] This implies that deuteration from the *Re* face can occur faster than epimerization in the deuterated solvent.

We also considered the entropic penalties stemming from coordinating the substrate and EtOD in **5-I** by calculating their free energies separately. The alignment of substrate and EtOD for deuteration results in the free energy penalty of 5.6 kcal/mol, leading to the overall $\Delta G^{\ddagger}$ of 11.2, 17.3, 21.6 kcal/mol for $\mathbf{TS2}_{Re}$, $\mathbf{TS2}_{rac}$, and $\mathbf{TS2}_{Si}$, respectively (Figure 1b). Accounting for translational entropies of substrates and EtOD did not change the trends of the free energy barriers of these three TSs. The kinetic isotope effect (KIE) was also investigated for $\mathbf{TS2}_{Re}$ and $\mathbf{TS2}_{Si}$; the calculated reaction rate of deuterium transfer was 4.9−5.0 times slower than that of proton transfer for both cases.[14] Overall, the computational calculation results agreed well with the experimental outcomes of enantioretentive deuteration.

The scope of the enantioretentive deuteration process was examined by varying the amide group (Table 2). The employed conditions were 5 equiv of NaOEt in 0.2 M EtOD at room temperature. For practical reasons, the reaction time was fixed at 16 h, although a longer reaction time might lead to higher levels of deuterium exchange. The simple unbranched aliphatic-acid-conjugated proline ester underwent a high degree of enantioretentive deuteration. The reaction efficiency was almost the same as that of substrate **4a** with an isobutyryl

Table 2. Substrate Scope[a]



[a]Standard reaction conditions: **4** (0.2 mmol), NaOEt (5.0 equiv), EtOD (0.2 M), room temperature, 16 h. The indicated yields are isolated. The DL and diastereomeric ratio (d.r.) were estimated by ^{1}H NMR analysis. The *ee* value was estimated using chiral HPLC. [b]The reaction was processed for 48 h. [c]The reaction was processed at 0 °C for 24 h. [d]The reaction was processed at −10 °C for 48 h.

group. For instance, the substrates **4b** and **4c** with propionyl or pentenoyl group obtained the high yields and *ee* values of the corresponding deuterated compounds. Further, substrate **4d** with the acetyl group successfully provided *d*-**4d** in high DL and high *ee*, albeit in a lower yield (57%) due to the instability of the acetylamide group under the aforementioned conditions.[15] Moreover, when the phenylacetyl group was employed, the obtained *ee* value and DL of *d*-**4e** were high. In this case, almost complete deuterium incorporation was observed at the α-position of the amide carbonyl group. When substrate **4f** with a bulky pivaloyl group was subjected to the above reaction conditions, the DL was only 60% at the given reaction time. After 48 h, the deuterium level reached 94%, and the enantiomeric purity of the obtained *d*-**4f** was 96%. We attributed the slow deuteration rate of **4f** to the bulky pivaloyl group because it might cause a low kinetic acidity for the C-2 hydrogen atom. Further, substrate **4g** with an *N*-Boc group produced the high yield and *ee* values of the corresponding deuterated compounds but with a low DL (24%). The prolonged reaction time (48 h) led to an increased DL (56%) without compromising the yield and enantioselectivity.

When the *t*-butyl ester moiety of **4a** was changed to ethyl ester (**4h**), deuteration proceeded effectively while maintaining almost the same *ee* value and DL. On the other hand, When the *t*-butyl ester of **4a** was changed to the dimethylamide group (**4i**), deuterium incorporation was not realized due to the lower acidity for the C-2 hydrogen atom of **4i**. Based on this result, we envisioned that site-selective deuteration at the C-terminal of polypeptides would be possible. As a feasibility study, dipeptides **4j** (Boc-L-Val-L-Pro-O^{t}Bu) and **4k** (Boc-L-Ala-L-Pro-O^{t}Bu) were subjected to NaOEt/EtOD conditions. Both dipeptides showed site-selective deuteration at the C-2

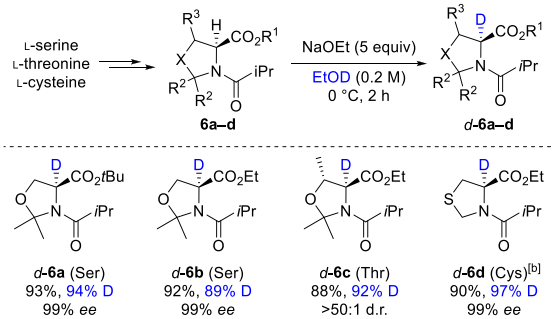
https://doi.org/10.1021/jacsau.4c00185
JACS Au 2024, 4, 2246−2251

position with high DL and stereoselectivity. This result suggests our method could be applied to longer peptides.

To further examine the substrate scope and limitations, we tested several substrates **4l**–**n** with a noncarbonyl *N*-protecting group. When *N*-tosyl substrate **4l** was subjected to the above reaction conditions, *ee* was only 12% at the given reaction time and temperature. However, when the reaction was performed at −10 °C for 48 h, the enantiomeric purity of *d*-**4l** was increased to 92% *ee* with 84% DL. When a benzyl (**4m**) or trityl group (**4n**) was employed as an *N*-protecting group, no deuteration products were observed. These results suggested the importance of the carbonyl group of the *N*-protecting group in the enantio-retentive deuteration.

The scope of the deuteration process was expanded to other amino acid derivatives. The pseudoprolines of serine, threonine, and cysteine were first examined (Table 3). They

Table 3. α-Deuteration of Pseudo-proline Derivatives[a]

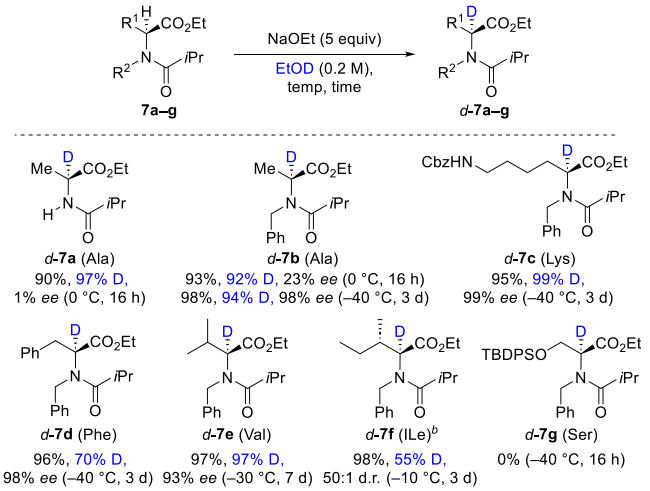


[a]Standard reaction conditions: **6** (0.2 mmol), NaOEt (5.0 equiv), EtOD (0.2 M), 0 °C, 2 h. The indicated yields are isolated. The DL and diastereomeric ratio (d.r.) were estimated by ^{1}H NMR analysis. The enantiomeric excess (*ee*) value was estimated using chiral HPLC. [b]The reaction was processed at −50 °C for 12 h.

underwent deuteration more rapidly than proline derivatives under the given reaction conditions at room temperature; however, the chemical yields were lower due to the instability. Thus, the reactions were tested at 0 °C for 2 h. The oxazolidine-protected serine (**6a** and **6b**) gave results similar to those obtained with the corresponding proline derivatives (**4a** and **4h**). Moreover, the oxazolidine-protected threonine also afforded the deuterated product *d*-**6c** in >50:1 d.r. and 88% yield. In contrast, the cysteine-derived thiazolidine derivate **6d** gave the corresponding deuterated compound in high yield but with almost complete racemization. This racemization could be overcome by performing the deuteration at lower reaction temperatures. At −50 °C, **6d** produced *d*-**6d** (97% DL) in 90% yield with perfect enantioselectivity (For computational details, see SI and ref 16).

While the enantioretentive deuteration of proline was readily extended to the aforementioned pseudoprolines, the reaction protocols could not be directly applied to the acyclic amino acid derivatives. For instance, the alanine derivative **7a** with an isobutyryl group as the only amine substituent produced a high yield of the α-deuterated compound at 0 °C (Table 4). However, almost complete racemization was observed. Meanwhile, the alanine derivative **7b** possessing both isobutyryl and benzyl groups at the nitrogen atom exhibited a low degree of chirality preservation (23% *ee*) at 0 °C. The enantiomeric purity of *d*-**7b** was increased to 98% *ee* with 94% DL when the reaction was performed at −40 °C for 3 d. Under these

Table 4. α-Deuteration of Acyclic Amino Acid Derivatives[a]



[a]Standard reaction conditions: **7** (0.2 mmol), NaOEt (5.0 equiv), EtOD (0.2 M). The indicated yields are isolated. The DL was estimated based on ^{1}H NMR analysis. The enantiomeric excess (*ee*) value was estimated using chiral HPLC. [b]Reaction runs by using EtOD/THF = 10:1 (0.2 M) as the solvent.

conditions, N^{ε}-Cbz-protected lysine derivative **7c** showed enantioretentive (99% *ee*) α-deuteration in excellent yield (95%) and DL (99%). Moreover, phenylalanine derivative **7d** produced the corresponding deuterated compound *d*-**7d** in high yield and *ee* values with 70% DL. Similarly, the valine derivative **7e** yielded good results in terms of both yield and DL, achieving 93% *ee* when the reaction was conducted at −30 °C for 7 d. The isoleucine-derived substrate **7f** also showed good results in yield and selectivity at −10 °C for 3 d. However, its deuteration was rather slow compared to less sterically bulky derivatives **7b** and **7d**. The *O*-protected serine derivative **7g** failed to produce the corresponding deuterated compounds because of the rapid β-elimination under the conditions.

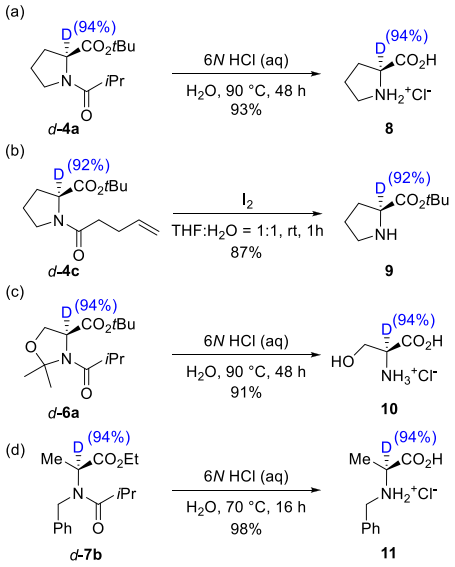
To demonstrate the synthetic utility of the current methodology in preparing deuterated amino acids, the transformations of deuterated products were explored. The proline ester *d*-**4a** with an isobutyryl group was converted to the deuterated proline **8** in high yield by acid-catalyzed hydrolysis without loss of the DL (Scheme 3). The pentenoyl group in *d*-**4c** was selectively removed upon treatment with iodine in aqueous THF to give a high yield of the deuterated proline *t*-butyl ester **9**. The deuterated serine **10** was obtained in good yield from the oxazolidine-protected serine *d*-**6a** by acid-catalyzed hydrolysis. Further, acyclic amino ester derivative *d*-**7b** was successfully hydrolyzed in acidic conditions to afford *N*-benzyl amino acid **11** without lowering DL.

CONCLUSIONS

In summary, we established an efficient method for the site-selective and stereoselective synthesis of α-deuterated chiral amino acids from readily available amino acids themselves. The substrate proline and pseudoproline derivatives, possessing the *N*-isobutyryl group, were readily prepared. The reaction conditions were simple (NaOEt in EtOD solvent), and the developed method did not require any expensive equipment, strong bases, or extra chiral sources. The deuterated product was afforded with a high level of enantiopurity and deuteration.

https://doi.org/10.1021/jacsau.4c00185
JACS Au 2024, 4, 2246−2251

Scheme 3. Transformation of α-Deuterated α-Amino Acid Derivatives[a]



The EtOD amount can be reduced while maintaining enantioselectivity by using THF as a major cosolvent. Our mechanistic study suggested that the enantioretentive H/D exchange involves the enantioselective generation of axially chiral enolates and the facial-selective deuteration of enolates. This study demonstrated the first case of the retention of chiral information on optically active α-amino acid derivatives during α-deuteration in a protic solvent. Moreover, the scope of the enantioretentive deuteration process was extended to acyclic amino acids. The several acyclic amino ester derivatives possessing isobutyryl and benzyl groups at the nitrogen atom exhibited an excellent degree of chirality preservation at a low temperature of −40 °C. Further studies are being conducted to extend this base-promoted enantioretentive hydrogen isotope exchange reaction for asymmetrically assessing various isotope compounds.

METHOD

General Information

All chemicals were of reagent grade and were used as purchased. All reactions were performed under an inert atmosphere of dry nitrogen by using distilled dry solvents. The reactions were monitored with TLC analysis using silica gel 60 F-254 thin-layer chromatography plates. Compounds on the TLC plates were visualized under UV light and by spraying with either potassium permanganate or anisaldehyde solutions. Flash column chromatography was conducted on silica gel 60 (230−400 mesh). Melting points were measured using a Buchi B-540 melting point apparatus without correction. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-ECZ400S/L1 (400 MHz) and JEOL JNM-ECA-600 (600 MHz) at 298 K if not noted otherwise. The IR spectra were measured by an Agilent Technologies 5500 Series FT-IR spectrometer. High-resolution mass spectra (HRMS) were recorded using fast atom bombardment (FAB), or electrospray ionization (ESI) mass spectrometry.

General Procedure for α-Deuteration of Amino Acids

To a solution of amino acid derivatives (0.2 mmol, 1.0 equiv) in EtOD (1.0 mL) was added NaOEt (1.0 mmol, 5.0 equiv) at a given temperature. After stirring for the corresponding reaction time, the reaction mixture was quenched with saturated NH_4Cl aqueous solution (3.0 mL). Then, EtOD was removed under reduced pressure. The resulting mixture was poured into water (5.0 mL) and extracted with EtOAc three times (3 × 10 mL). The combined organic fraction was dried in $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel to give α-deuterated amino acid derivatives.

Computational Mechanistic Studies

We describe in detail in the Supporting Information.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacsau.4c00185.

Experimental procedures of all new compounds; spectroscopic data analysis; copies of NMR spectra; computational studies (PDF)

AUTHOR INFORMATION

Corresponding Author

Sanghee Kim − *College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea;* orcid.org/0000-0001-9125-9541; Phone: +82-2-880-2487; Email: pennkim@snu.ac.kr

Authors

Soojun Park − *College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea*

Jae Hyun Kim − *College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea; College of Pharmacy, Chung-Ang University, Seoul 06974, Republic of Korea;* orcid.org/0000-0001-8600-5950

Dongjun Kim − *College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea*

Yeonjoon Kim − *Chemistry Department, Colorado State University, Fort Collins, Colorado 80523, United States; Department of Chemistry, Pukyong National University, Busan 48513, Republic of Korea;* orcid.org/0000-0002-4784-7925

Seonah Kim − *Chemistry Department, Colorado State University, Fort Collins, Colorado 80523, United States;* orcid.org/0000-0001-9846-7140

Complete contact information is available at: https://pubs.acs.org/10.1021/jacsau.4c00185

Author Contributions

CRediT: **Soojun Park** data curation, formal analysis, investigation, methodology, resources, visualization, writing-original draft; **Jae Hyun Kim** conceptualization, data curation, formal analysis, methodology, resources, validation, visualization; **Dongjun Kim** data curation, methodology, validation, visualization; **Sanghee Kim** conceptualization, formal analysis, funding acquisition, investigation, project administration, supervision, writing-review & editing.

Notes

The authors declare no competing financial interest.

https://doi.org/10.1021/jacsau.4c00185
JACS Au 2024, 4, 2246−2251

■ ACKNOWLEDGMENTS

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (RS-2023-00209322 and NRF-2018R1A5A2024425).

■ REFERENCES

(1) (a) Yu, Y.; Lv, X. X.; Li, J. S.; Zhou, Q.; Cui, C.; Hosseinzadeh, P.; Mukherjee, A.; Nilges, M. J.; Wang, J. Y.; Lu, Y. Defining the Role of Tyrosine and Rational Tuning of Oxidase Activity by Genetic Incorporation of Unnatural Tyrosine Analogs. *J. Am. Chem. Soc.* **2015**, *137*, 4594−4597. (b) Dayal, P. V.; Singh, H.; Busenlehner, L. S.; Ellis, H. R. Exposing the Alkanesulfonate Monooxygenase Protein Protein Interaction Sites. *Biochemistry* **2015**, *54*, 7531−7538. (c) Furuta, T.; Takahashi, H.; Kasuya, Y. Evidence for a Carbanion Intermediate in the Elimination of Ammonia from L-Histidine Catalyzed by Histidine Ammonia-Lyase. *J. Am. Chem. Soc.* **1990**, *112*, 3633−3636. For review, see; (d) Kopf, S.; Bourriquen, F.; Li, W.; Neumann, H.; Junge, K.; Beller, M. Recent Developments for the Deuterium and Tritium Labeling of Organic Molecules. *Chem. Rev.* **2022**, *122*, 6634−6718.

(2) (a) Lian, L. Y.; Middleton, D. A. Labelling approaches for protein structural studies by solution-state and solid-state NMR. *Prog. Nucl. Magn. Reson. Spectrosc.* **2001**, *39*, 171−190. (b) Sack, I.; Balazs, Y. S.; Rahimipour, S.; Vega, S. Solid-State NMR Determination of Peptide Torsion Angles: Applications of ^{2}H-Dephased REDOR. *J. Am. Chem. Soc.* **2000**, *122*, 12263−12269. (c) Gardner, K. H.; Kay, L. E. Production and Incorporation of ^{15}N, ^{13}C, ^{2}H (^{1}H-δ1 Methyl) Isoleucine into Proteins for Multidimensional NMR Studies. *J. Am. Chem. Soc.* **1997**, *119*, 7599−7600.

(3) (a) Borno, A.; van Hall, G. Quantitative amino acid profiling and stable isotopically labeled amino acid tracer enrichment used for *in vivo* human systemic and tissue kinetics measurements. *J. Chromatogr. B: Anal. Technol. Biomed Life Sci.* **2014**, *951*−*952*, 69−77. (b) Nelson, S. D.; Trager, W. F. The use of deuterium isotope effects to probe the active site properties, mechanism of cytochrome P450-catalyzed reactions, and mechanisms of metabolically dependent toxicity. *Drug Metab. Dispos.* **2003**, *31*, 1481−1497. (c) Rose, J. E.; Leeson, P. D.; Gani, D. Mechanisms and Stereochemistry of the Activation of (2s)-Serine and (2r)-Serine O-Sulfate as Suicide Inhibitors for Escherichia-Coli Glutamic-Acid Decarboxylase. *J. Chem. Soc., Chem. Commun.* **1992**, 1784−1786.

(4) (a) DeWitt, S.; Czarnik, A. W.; Jacques, V. Deuterium-Enabled Chiral Switching (DECS) Yields Chirally Pure Drugs from Chemically Interconverting Racemates. *ACS Med. Chem. Lett.* **2020**, *11*, 1789−1792. (b) Maltais, F.; Jung, Y. C.; Chen, M.; Tanoury, J.; Perni, R. B.; Mani, N.; Laitinen, L.; Huang, H.; Liao, S.; Gao, H.; Tsao, H.; Block, E.; Ma, C.; Shawgo, R. S.; Town, C.; Brummel, C. L.; Howe, D.; Pazhanisamy, S.; Raybuck, S.; Namchuk, M.; Bennani, Y. L. In Vitro and In Vivo Isotope Effects with Hepatitis C Protease Inhibitors: Enhanced Plasma Exposure of Deuterated Telaprevir versus Telaprevir in Rats. *J. Med. Chem.* **2009**, *52*, 7993−8001.

(5) (a) Li, A. B.; Song, X. J.; Ren, Q.; Bao, P. W.; Long, X. Y.; Huang, F. L.; Yuan, L.; Zhou, J. S.; Qin, X. R. Cobalt-Catalyzed Asymmetric Deuteration of α-Amidoacrylates for Stereoselective Synthesis of α,β-Dideuterated α-Amino Acids. *Angew. Chem., Int. Ed.* **2023**, *62*, No. e202301091. (b) Fu, C.; Chang, X.; Xiao, L.; Wang, C. J.; Fu, C.; Chang, X.; Xiao, L.; Wang, C. J. Stereodivergent Synthesis of Enantioenriched α-Deuterated α-Amino Acids via Cascade Cu(I)-Catalyzed H−D Exchange and Dual Cu- and Ir-Catalyzed Allylation. *Org. Lett.* **2022**, *24*, 5562−5567. (c) Zhong, H. Y.; Shevlin, M.; Chirik, P. J. Cobalt-Catalyzed Asymmetric Hydrogenation of α,β-Unsaturated Carboxylic Acids by Homolytic H_2 Cleavage. *J. Am. Chem. Soc.* **2020**, *142*, 5272−5281. (d) Galan, S. R. G.; Wickens, J. R.; Dadova, J.; Ng, W.-L.; Zhang, X.; Simion, R. A.; Quinlan, R.; Pires, E.; Paton, R. S.; Caddick, S.; Chudasama, V.; Davis, B. G. Post-translational site-selective proteinback bone α-dueteration. *Nat. Chem. Biol.* **2018**, *14*, 955−963. (e) Bhatia, S.; Spahlinger, G.; Boukhumseen, N.; Boll, Q.; Li, Z. L.; Jackson, J. E. Stereoretentive H/D Exchange via an Electroactivated Heterogeneous Catalyst at sp^3 C−H Sites Bearing Amines or Alcohols. *Eur. J. Org. Chem.* **2016**, *2016*, 4230−4235. Angew. Chem. 2015, 127, 10620−10623. (f) Taglang, C.; Martinez-Prieto, L. M.; del Rosal, I.; Maron, L.; Poteau, R.; Philippot, K.; Chaudret, B.; Perato, S.; Lone, A. S.; Puente, C.; Dugave, C.; Rousseau, B.; Pieters, G. Enantiospecific C−H Activation Using Ruthenium Nanocatalysts. *Angew. Chem., Int. Ed.* **2015**, *54*, 10474−10477. (g) Moozeh, K.; So, S. M.; Chin, J. Catalytic Stereoinversion of L-Alanine to Deuterated D-Alanine. *Angew. Chem., Int. Ed.* **2015**, *54*, 9381−9385.

(6) (a) Doyon, T. J.; Buller, A. R. Site-Selective Deuteration of Amino Acids through Dual-Protein Catalysis. *J. Am. Chem. Soc.* **2022**, *144*, 7327−7336. (b) Rowbotham, J. S.; Ramirez, M. A.; Lenz, O.; Reeve, H. A.; Vincent, K. A. Bringing biocatalytic deuteration into the toolbox of asymmetric isotopic labelling techniques. *Nat. Commun.* **2020**, *11*, 1454. (c) Chun, S. W.; Narayan, A. R. H. Biocatalytic, Stereoselective Deuteration of α-Amino Acids and Methyl Esters. *ACS Catal.* **2020**, *10*, 7413−7418.

(7) (a) Navo, C. D.; Oroz, P.; Mazo, N.; Blanco, M.; Peregrina, J. M.; Jimenez-Oses, G. Stereoselective α-Deuteration of Serine, Cysteine, Selenocysteine, and 2,3-Diaminopropanoic Acid Derivatives. *Org. Lett.* **2022**, *24*, 6810−6815. (b) Ohtsuki, H.; Takashima, M.; Furuta, T.; Kawabata, T. Direct asymmetric synthesis of α-deuterated α-amino acid derivatives from the parent α-amino acids via memory of chirality. *Tetrahedron Lett.* **2018**, *59*, 1188−1191. (c) Brunner, M.; Saarenketo, P.; Straub, T.; Rissanen, K.; Koskinen, A. M. P. Stereocontrolled α-Alkylation of Fully Protected L-Serine. *Eur. J. Org. Chem.* **2004**, *2004*, 3879−3883. (d) Elemes, Y.; Ragnarsson, U. Synthesis of enantiopure α-deuteriated Boc-L-amino acids. *J. Chem. Soc., Perkin Trans.* **1996**, *1*, 537−540. (e) Seebach, D.; Dziadulewicz, E.; Behrendt, L.; Cantoreggi, S.; Fitzi, R. Synthesis of Nonproteinogenic (R) or (S)-Amino Acids Analogs of Phenylalanine, Isotopically Labeled and Cyclic Amino-Acids from *tert*-Butyl 2-(*tert*-Butyl)-3-Methyl-4-Oxo-1-Imidazolidinecarboxylate (Boc-Bmi). *Liebigs Ann. Chem.* **1989**, *12*, 1215−1232.

(8) (a) Seebach, D.; Sting, A. R.; Hoffmann, M. Self-regeneration of stereocenters (SRS)−Applications, limitations, and abandonment of a synthetic principle. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2708−2748.

(9) (a) For reviews, see: Alezra, V.; Kawabata, T. Recent Progress in Memory of Chirality (MOC): An Advanced Chiral Pool. *Synthesis* **2016**, *48*, 2997−3016. (b) Campolo, D.; Gastaldi, S.; Roussel, C.; Bertrand, M. P.; Nechab, M. Axial-to-central chirality transfer in cyclization processes. *Chem. Soc. Rev.* **2013**, *42*, 8434−8466. (c) Zhao, H. W.; Hsu, D. C.; Carlier, P. R. Memory of Chirality: An Emerging Strategy for Asymmetric Synthesis. *Synthesis* **2005**, *2005*, 1−16.

(10) Kim, J. H.; Lee, S.; Kim, S. Biomimetic Total Synthesis of (−)-Penibruguieramine A Using Memory of Chirality and Dynamic Kinetic Resolution. *Angew. Chem., Int. Ed.* **2015**, *54*, 10875−10878.

(11) The relative free energies of **4a-I** and *d*-**4a-I** are slightly different (below 0.1 kcal/mol) because the latter does have deuterium (see SI for details).

(12) The calculated energy profile of the protonation of **5-I** with EtOH is very similar to that of the deuteration of **5-I** (see SI for details).

(13) The calculated energy profile of the deuteration of *ent*-**5-I** is the same as that of **5-I**.

(14) The KIE values deviated from 1.0 indicate that the deuterium isotope was properly considered in DFT calculations.

(15) The acetylamide compound (**4e**) is deacetylated under the standard condition listed in Table 2.

(16) Our DFT computation revealed that the $\Delta G^{\ddagger}$ difference between the deuteration and epimerization associated with **6d** at 0 °C was only 1.2 kcal/mol. This racemization is thermodynamically driven and dominant at 0 °C, whereas the dominant reaction path is altered into the kinetically-driven deuteration at−50 °C (see SI for details).

https://doi.org/10.1021/jacsau.4c00185
JACS Au 2024, 4, 2246−2251