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Site-Selective Deuteration of α-Amino Esters with 2-Hydroxynicotinaldehyde as a Catalyst

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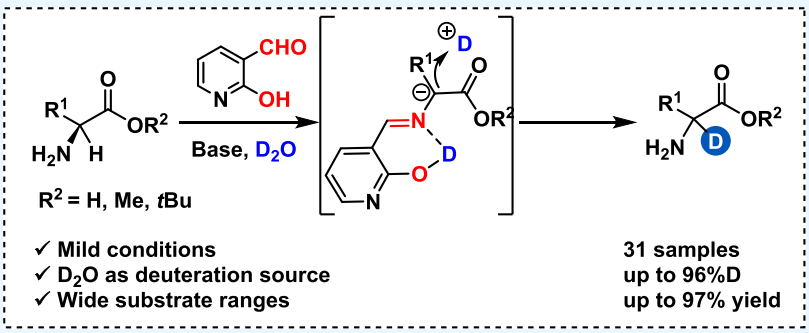

ABSTRACT: An efficient method has been developed for the synthesis of α-deuterated α-amino esters via hydrogen isotope exchange of α-amino esters in D_2O with 2-hydroxynicotinaldehyde as a catalyst under mild conditions. This methodology exhibits a wide range of substrate scopes, remarkable functional group tolerance, and affording the desired products in good yields with excellent deuterium incorporation. Notably, the ortho-hydroxyl group and the pyridine ring of the catalyst play a crucial role in the catalytic activity, which not only stabilizes the carbon-anion intermediates but also enhances the acidity of the amino esters' α−C−H bond.

INTRODUCTION

Deuterium compounds have recently played an important role in fundamental research and commercial endeavors,[1,2] including drug discovery and development (Scheme 1a).[3−7] Among various deuterated molecules, the α-deuterated amino acids represent a specific class of isotopically labeled compounds.[8] They have been extensively used in bio-organic chemistry research and play an essential role in the emerging field of clinical functional metabolomics,[9−11] which can provide important data on amino acid metabolism and protein turnover in vivo. In addition, precise and selective incorporation of deuterium sites into the α-stereocenter of α-amino acids can effectively suppress the epimerization to improve the bioavailability and decrease the potential toxicity.[10,12,13] Those applications have led to a high demand for methods that can selectively generate deuterated α-amino acids.[14,15]

While various methods have been reported for the synthesis of α-deuterated α-amino acids, the hydrogen isotope exchange (HIE) of amino acids and their derivates with different deuterium sources is considered the most feasible choice (Scheme 1b).[16] Lygo et al. developed a phase transfer asymmetric alkylation of glycine-derived ketimine using KOD/D_2O to access deuterium-labeled L-amino acids in good yield and high deuterium degree.[17] Song et al. developed the organocatalytic dynamic kinetic resolution (DKR) reaction of racemic azlactones with EtOD as a nucleophile as well as a deuterium source for the synthesis of α-deuterium-labeled amino acids.[18] However, these methods often require prefunctionalization of substrates,[19−21] harsh conditions,[22,23] and poor tolerance of functional groups.[24−27] Takeda developed an approach for the preparation of α-deuterated amino acids by the DKR of racemates with the formation of Ni(II) complexes derived from amino acids. The reaction can yield up to 96% α-deuteration but requires CD_3OD as a deuterium source and an equimolar amount of $NiCl_2$.[28] The heterogeneous catalysts, including Ru/C, RuNP@PVP, Ru/Pt NPs, and Pd/Ni NPs, have been developed in H/D exchange reactions of amino acids; nevertheless, those methods suffer from several disadvantages, such as poor regioselectivity, limited substrate scope, and the use of D_2 as a deuterium source.[29] Lämmerhofer developed a deuteration reaction for amino acids using salicylaldehyde as the catalyst, but this

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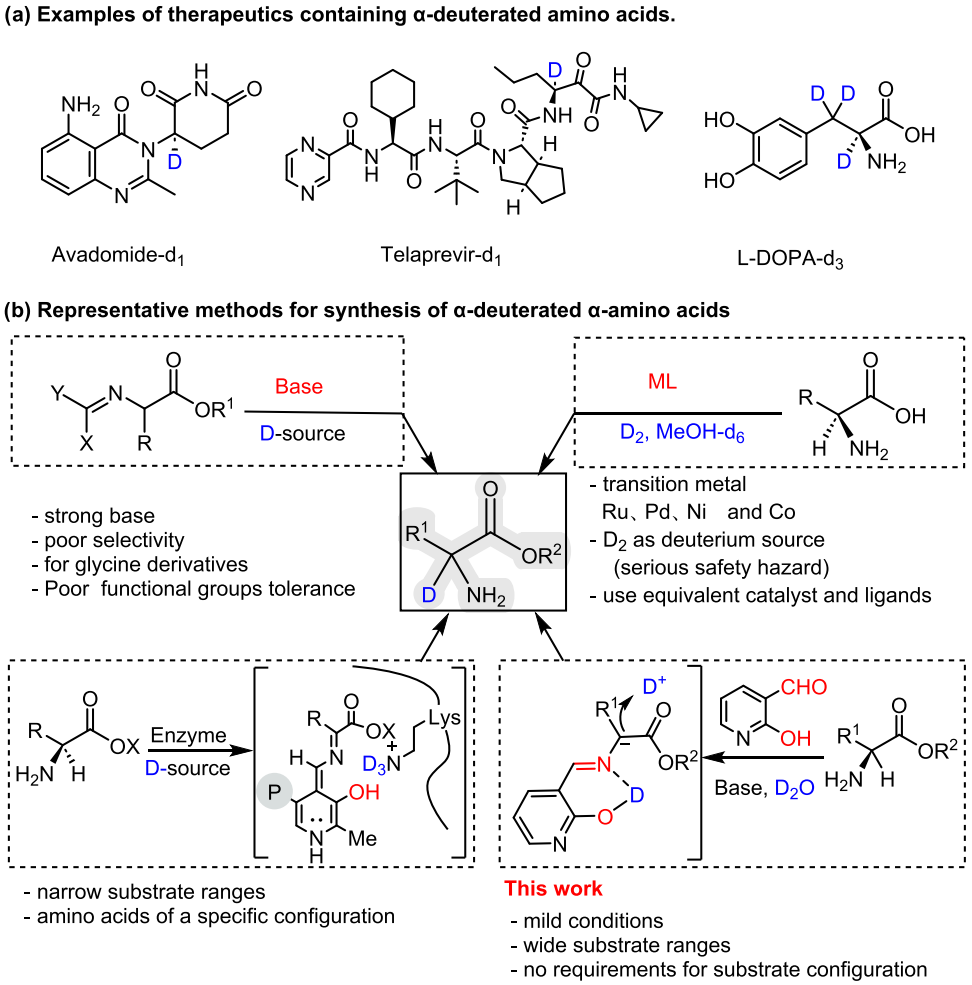
Scheme 1. Development of α-Deuterated Amino Acids



method required the use of CH_3COOD as the deuterium source and high reaction temperatures.[30] Chin developed a deuteration reaction for amino acids using 3,5-dichlorosalicylaldehyde as a catalyst with the D_2O as the deuterium source.[31] They found that the rate of deuteration of alanine decreased about 50-fold when the 3,5-dichlorosalicylaldehyde catalyst was replaced with salicylaldehyde, suggesting that the electron-withdrawing chlorides on salicylaldehyde benefit the reaction. Narayan and Buller developed enzyme-catalyzed methods for α-deuterated α-amino acids using D_2O as the deuterium source, including α-hydroxylamine synthase (SxtA AONS) and aminotransferase (DsaD).[32,33] Although biocatalysis has shown superior regioselectivity and chemoselectivity compared to other methods, the specificity of the enzymes limits the applicability of the substrates and, therefore, prevents their widespread use. Despite the great advancements made in the past, there is still a need for an efficient method to synthesize α-deuterated α-amino acids from readily available materials with a high level of deuterium incorporation and a wide array of substrates.

Due to the unique structure of α-amino acids, their α-H atoms have a certain degree of acidity, requiring a strong base to remove the α-hydrogen. In the biocatalytic approaches to transforming amino acids into α-deuterated analogs, typically the formation of imines from amino acids can activate the α–C–H bond by increasing its polarity. We assumed that the aromatic aldehydes with an auxiliary group as catalysts could activate the C–H bond of α-amino acids, while the imine formed between the aldehydes and free amino groups and the auxiliary group could facilitate Schiff base formation. The acidity of the α–C–H bond of amino esters can be controlled through intramolecular resonance by tuning the electronic properties of the aromatic ring.[31] Herein, we report a simple approach for the selective deuteration of various α-amino esters in high yields and deuterium incorporation using 2-hydroxynicotinaldehyde as a catalyst. This protocol allows us to convert amino esters to the deuterated counterparts directly without prefunctionalization and with the convenience of D_2O as the deuterium source.

RESULTS AND DISCUSSION

We initially investigated the HIE reaction of *tert*-butyl phenylalanine (L-Phe-O*t*Bu) using salicylaldehyde as the catalyst in D_2O at room temperature. The deuterated Phe-O*t*Bu with 20% deuteration at the α position was obtained (Scheme 2A). To clarify the role of the 2-hydroxyl group of the aldehyde, benzaldehyde, 2-methylbenzaldehyde, and 2-methoxybenzaldehyde were used as catalysts for the deuteration reaction; no deuterated ester was observed, indicating that the hydroxyl group adjacent to the aldehyde group is an indispensable condition in the catalytic system (Scheme 2A). Then, the different structures of aldehydes with adjacent hydroxyl groups were further investigated. Possibly due to the influence of the hydroxyl group at the 5-position on the

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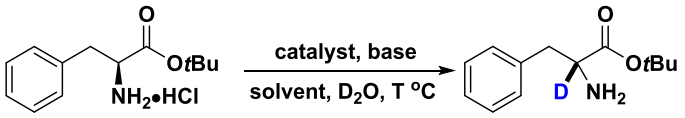
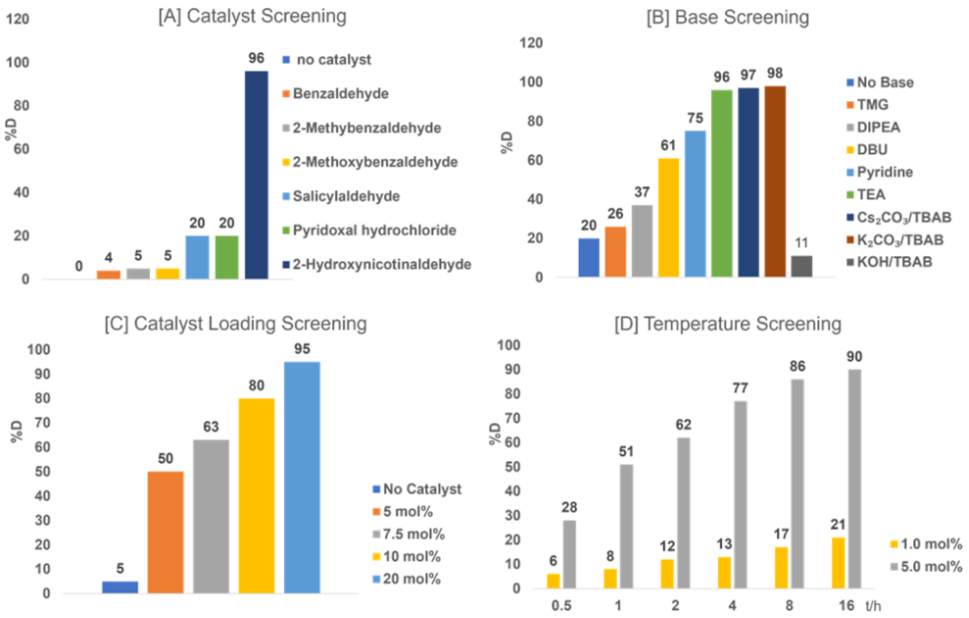
Scheme 2. Reaction Optimization[aa]





[a]Reaction condition (unless noted otherwise): [A] L-Phe-O*t*Bu (1.0 mmol), catalyst (20 mol %), TEA (4.0 equiv), DCM/D_2O, 25 °C, 24 h; [B] L-Phe-O*t*Bu (1.0 mmol), 2-hydroxynicotinal (20 mol %), base (4.0 equiv), DCM/D_2O; [C] L-Phe-O*t*Bu (1.0 mmol), 2-hydroxynicotinal (*x* mol %), K_2CO_3 (4.0 equiv), TBAB (10 mol %), DCM/D_2O, 25 °C, 24 h; [D] L-Phe-O*t*Bu (1.0 mmol), 2-hydroxynicotinal (*x* mol %), K_2CO_3 (4.0 equiv), TBAB (10 mol %), DCM/D_2O, 50 °C.

formation of Schiff bases, pyridoxal hydrochloride gave 20% D. To our delight, 2-hydroxynicotinaldehyde as a catalyst was able to obtain 96% D, while other aldehydes failed to reach 50% D. The pyridine ring of 2-hydroxynicotinaldehyde, which is deficient in electrons, can enhance the stabilization of the carbon-anion intermediate by means of intramolecular resonance and thus promote the activation of the α-H in the amino ester.[31,34,35] In addition, amino esters could not be deuterated without the catalyst.

Next, a variety of organic and inorganic bases, such as triethylamine, pyridine, potassium carbonate, and cesium carbonate, have been investigated (Scheme 2B). Among them, triethylamine and potassium carbonate achieved 96% and 98% D, respectively. Potassium carbonate was selected for further optimization, as the use of an inorganic base facilitates post-treatment and avoids the possibility that the presence of trace amounts of water during post-treatment can lead to a decrease in the deuteration degree of the reaction when the catalyst and organic base are in the same phase. In addition, we found that the deuteration of amino esters could be partially achieved by 2-hydroxynicotinaldehyde without a base. Next, the loading of the catalyst was further examined (Scheme 2C). It is observed that as the amount of catalyst is reduced, the reaction rate slows down, and without a catalyst, the reaction does not proceed to deuteration. In particular, the reaction rate showed a significant increase at 50 °C with 5 mol % catalyst as compared to the room temperature (Scheme 2D). Finally, the optimized reaction condition was the use of 5 mol % 2-hydroxynicotinaldehyde as a catalyst, 4.0 eq. K_2CO_3 as the base, 10 mol % TBAB as an additive, DCM as the solvent, and 1 mL of D_2O as the deuterium source at 50 °C for 24 h under a nitrogen atmosphere (for more information on reaction optimization, see the Supporting Information).

Once the optimal conditions had been determined, the performance of the deuterated systems was tested with different structures of the L-amino esters (Table 1). The products were separated as Boc-protected amino esters to facilitate the separation and analysis. L-Phe-OMe and L-Phg-OMe derivatives with different substituents (Me, NO_2, OH, Br, Cl) on the aryl ring (**1-*d*-15-*d***) were tested first, and the deuterated esters (racemate) were obtained in good to excellent yields (80−97%) and deuterium incorporation (43−96% D). Deuterium incorporation was not affected by the electronic properties of the substituents on the aryl ring, even with nitro and hydroxy groups (**6-*d*-8-*d***). However, the incorporation of deuterium was influenced by the position of the substituents. o-Chlorophenylalanine methyl ester (**12-*d***) and *o*-chlorophenylglycine methyl ester (**15-*d***) have only 72, and 43% deuteration. We hypothesize that the orthochlorine atoms create a steric hindrance, thus impeding the proximity of endogenous bases to the target position. This limited deuterium incorporation may stem from the exogenous bases' inability to capture hydrogen at the α-position of the amino acids.

Encouraged by the studies presented above, we also performed deuterated reactions of other commonly used natural L-amino esters (**16-*d*-23-*d***). Under the optimized conditions, various α-deuterated amino esters (racemate), such as Trp-OMe (**16-*d***), Asp-O*t*Bu (**18-*d***), Glu-OMe (**19-*d***), Leu-OMe (**20-*d***), Ala-OMe (**21-*d***), and Lys-OMe (**23-*d***), were

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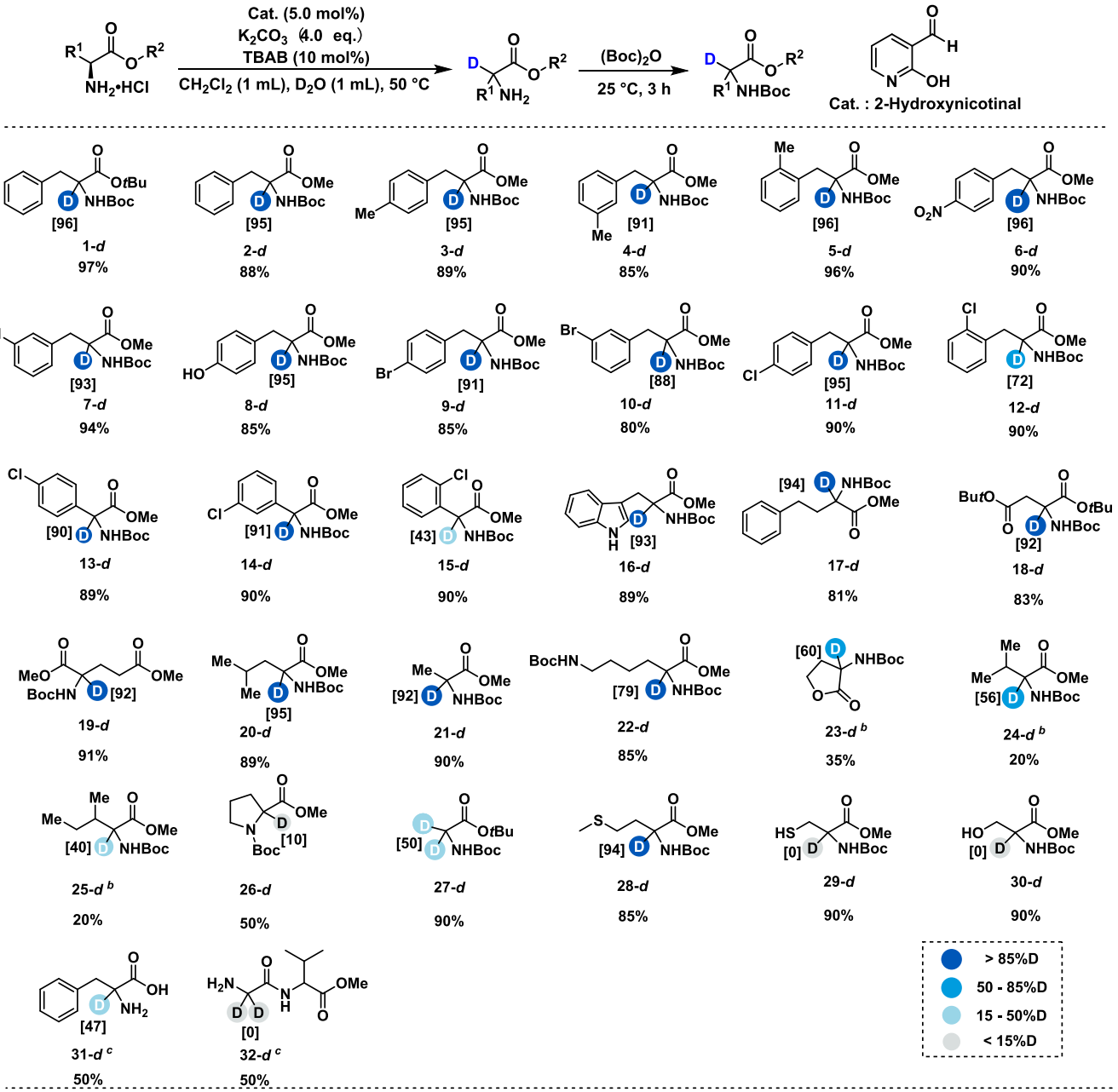
Table 1. Substrate Scope[a]



[a]Reaction condition (unless noted otherwise): L-amino acid methyl ester (1 mmol), 2-hydroxynicotinal (5.0 mol %), K_2CO_3 (4.0 equiv), TBAB (10 mol %), DCM (1.0 mL), and D_2O (1.0 mL, 55 equiv), 50 °C, 24 h; $(Boc)_2O$ (1.5 equiv), 25 °C, 3 h. Isolated yield. Deuterium incorporation was calculated by the peak integrity of ^1H NMR for symmetric positions, with only 1% D shown. [b]2-Hydroxynicotinal (20.0 mol %), 80 °C, 24 h. [c]The yield and the degree of deuterium incorporation were determined after the protection.

obtained in good yields (81−92%) and deuterium incorporation (79−95% D). Heterocyclic, distal ester, and amine groups in amino esters have no significant effect on catalytic efficiency or reaction results. However, the deuterations of Ile-OMe and Val-OMe were not successful due to a steric hindrance.[23] Although the yield and degree of deuteration are not ideal, deuteration of these amino esters can be achieved by increasing the catalyst loading and reaction temperature (**24-*d*-26-*d***). The tolerance of this method to functional groups containing active hydroxyl and sulfhydryl groups was also investigated, such as L-Cys-OMe (**29-*d***) and L-Ser-OMe (**30-*d***), but neither of them was deuterated. When L-Met-OMe is used as a substrate, the racemate product (**28-*d***) is obtained in 85% yield and 94% D. We speculated that the active hydroxyl or thiol and amine groups in the amino esters would react with the catalyst to form acetals, leading to catalyst inactivation. To further illustrate the potential of the method, we studied free amino acid deuteration under optimal conditions where phenylalanine can be obtained in 47% D (**31-*d***). Deuteration of dipeptides such as glycyl-L-valine (**32-*d***) has also been attempted. However, no obvious trace of deuteration was found, probably due to the reduced acidity of their α−C−H bonds.

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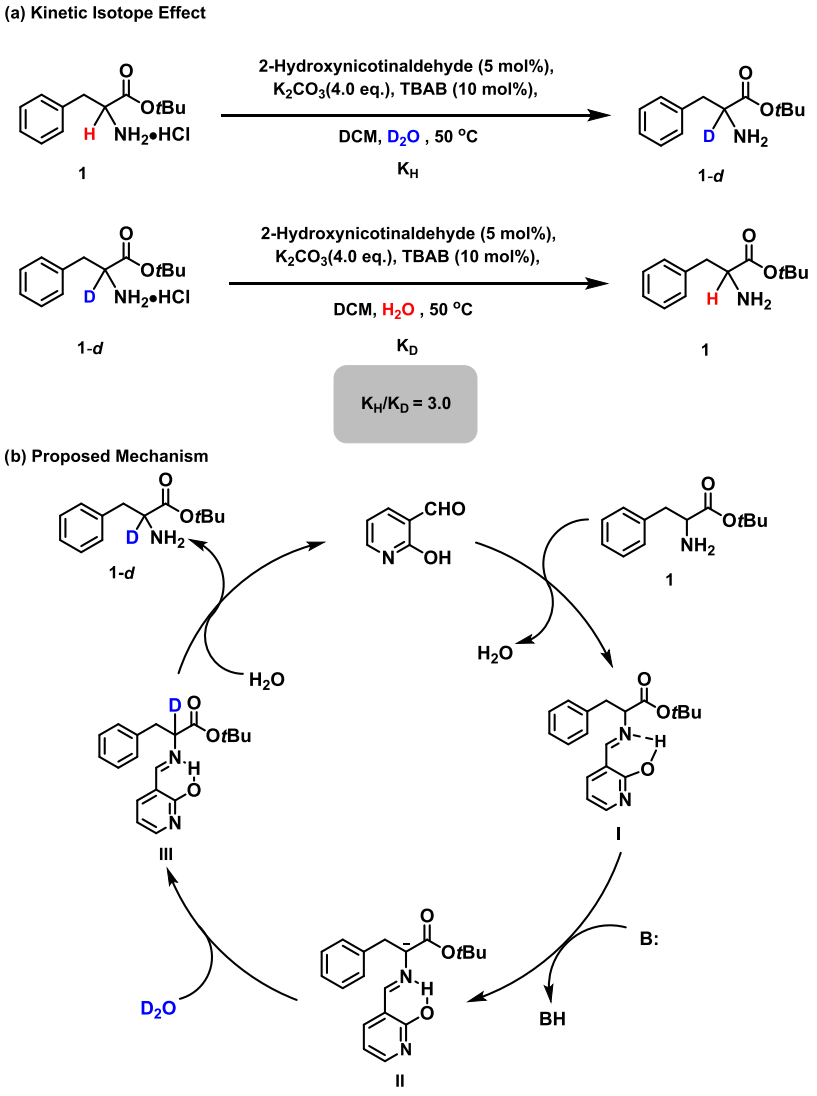
Scheme 3. Mechanism Studies



To gain deep insights into the reaction mechanism, a kinetic isotope experiment was performed using Phe-O*t*Bu (**1**) and deuterated Phe-O*t*Bu (**1**-*d*) as substrates (Scheme 3a). By integration of ^{1}H NMR, the value of K_H/K_D is 3.0, which suggests that α−C−H bond cleavage of amino ester is involved in the rate-determining step. On the basis of the results and literature reports,[34,35] we proposed the following mechanism (Scheme 3b). First, the Schiff base (**I**) was generated in situ from 2-hydroxynicotinaldehyde and amino ester, which stabilized by intramolecular resonance assisted of ortho-hydrogen bonds. Subsequently, the imine intermediates are deprotonated by the base to form the carbanion intermediate (**II**). The electron-deficient pyridine ring can enhance the stabilization of the carbon-anion intermediate and facilitate the activation of α-H in the amino ester. The carbanion is deuterated under heavy water to generate a deuterated imino ester (**III**), which is hydrolyzed to give deuterated products and regenerate the catalyst.

With the successful development of this aldehyde-catalyzed HIE of amino esters, a gram-scale experiment was initiated to further explore the application of this reaction. As shown in Scheme 4a, under optimized reaction conditions, 1.16 g of deuterated Boc-Phe-O*t*Bu (racemate) was obtained in 90% yield with the degree of deuteration essentially unchanged (96% D). Furthermore, the asymmetric deuteration reaction of L-amino esters has been preliminarily studied (Scheme 4b). It was disappointing that chiral aldehydes or phase transfer catalysts could not be used to achieve stereoselective control of the reaction. In addition, the derivatization of deuterated amino acid methyl esters was carried out. As shown in Scheme 4c, deuterated amino acids and amino amides can be obtained in good yield from deuterated amino esters, with no effect on the degree of deuteration.

CONCLUSIONS

In summary, we have developed a practical protocol for the selective deuteration of L-amino esters at the α-positions using aldehydes as catalysts. The method provides α-deuterated α-amino esters (racemates) in good yields with uniformly high levels of deuterium incorporation. The process displays several features, including mild reaction conditions, low catalyst loading, D_2O as a deuterium reagent, as well as compatibility with a wide range of α-amino acid derivatives. Experiments suggested that the ortho-hydroxyl group and the pyridine ring of the catalyst are essential for the catalytic system, not only for the stabilization of reaction intermediates but also for the

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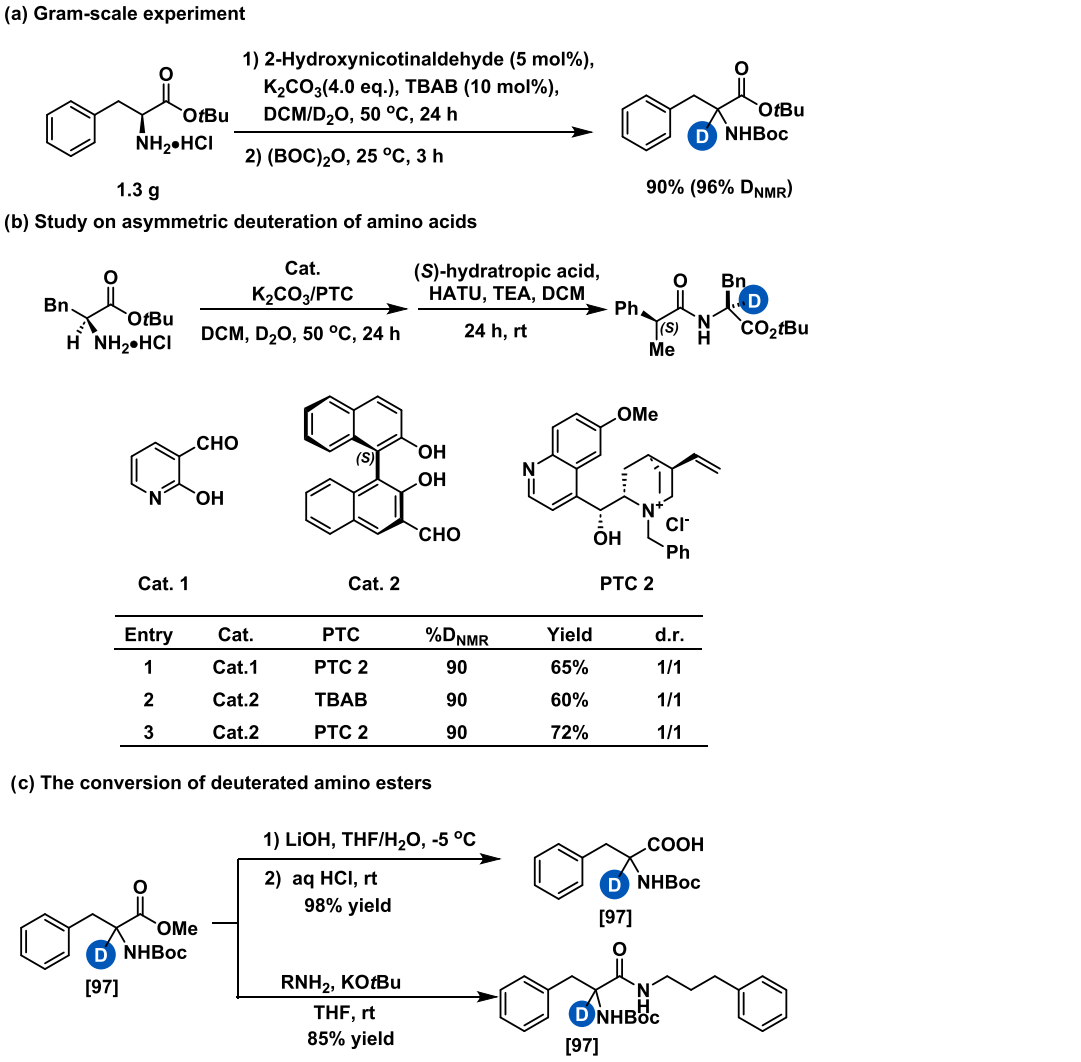
Scheme 4. Application and Design



Entry	Cat.	PTC	%D_{NMR}	Yield	d.r.
1	Cat.1	PTC 2	90	65%	1/1
2	Cat.2	TBAB	90	60%	1/1
3	Cat.2	PTC 2	90	72%	1/1

promotion of α-H activation of amino esters. Further investigations for the development of a new and efficient chiral aldehyde catalytic system for the preparation of chiral deuterated amino acids are currently underway in our laboratory. Meanwhile, the derivatization studies of amino acids have improved the complementary method for the further application of deuterated amino acids.

EXPERIMENTAL SECTION

General Information. Commercially available reagents were purchased from Aladdin, Bidepharm, and Leyan Chemicals, which were used directly without further purification unless stated otherwise. The purchased raw materials of amino esters are all in the L-configuration. The deuterated solvents were supplied by Ningbo Cuiying Chemicals. The D_2O for the reaction was fetched and transferred to the reaction in a glove box with a nitrogen atmosphere. Analytic thin-layer chromatography (Leyan chemicals) was used to check the formation of unexpected side reactions. Visualization was achieved by ultraviolet light (254 and 365 nm) and iodine staining. Flash chromatography was performed on silica gel (200−300 mesh) with the indicated solvent systems. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded on a Bruker Ascend 400 spectrometer, and chemical shifts are reported in ppm downfield from TMS and are referenced to the residual proton in $CDCl_3$ or DMSO-d_6. The spectra for deuterated substrates are reported as observed, while integration differences of less than 5% are ignored. The NMR data are reported as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet with J = coupling constant in Hz, and the deuterated position is marked as "Labeled". The high-resolution mass spectra (ESI-TOF-HRMS) were performed on an Agilent 6530 using a time-of-flight mass spectrometer equipped with an ESI-TOF resource. The degree of deuterium incorporation was calculated based on ^{1}H NMR methods, which had been described in our previous work.[36]

General Procedure for Standard Condition. To an oven-dried heavy wall pressure vessel, substrate (1.0 mmol), 2-hydroxynicotinaldehyde (6.2 mg, 5 mol %), K_2CO_3 (552.3 mg, 4.0 mmol), TBAB (32.3 mg, 0.1 mmol), and DCM (1.0 mL) were added in D_2O (1.0 mL) under the N_2 atmosphere. The vessel was purged with a nitrogen stream and sealed by Teflon bushing with Viton O-ring and then placed into the inside circle of a preheated aluminum block on a magnetic stirrer and stirred at 50 °C for 24 h. After the reaction finished, the vessel was allowed to cool to room temperature in water. Then, the di-*tert*-butyl dicarbonate (0.345 mL) was added to the

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solution. The mixture was then heated and stirred for 3 h at 25 °C. The organic layer was washed with distilled water and brine and dried over Na_2SO_4. The solvent was removed under vacuum. After purification by column chromatography, the α-deuterated amino esters were obtained in the racemate.

*tert-Butyl (tert-butoxycarbonyl)phenylalaninate-d (**1-d**).* White powder (312.5 mg, 97%), R_f = 0.66 (Petroleum ether/EtOAc = 4:1), 96% D-incorporation at α-positions; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.33−7.11 (m, 5H), 4.99 (s, 1H), 4.45 (q,*J*= 6.7 Hz, 0.04H, **Labeled**), 3.04 (s, 2H), 1.40 (d, *J* = 9.8 Hz, 18H).

*Methyl (tert-butoxycarbonyl)phenylalaninate-d (**2-d**).* Colorless oil (246.5 mg, 88%), R_f = 0.66 (Petroleum ether/EtOAc = 4:1), 95% D-incorporation at α-positions; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.36−7.23 (m, 3H), 7.18−7.12 (m, 2H), 5.00 (s, 1H), 4.62 (d, *J* = 7.4 Hz, 0.05H, **Labeled**), 3.74 (s, 3H), 3.10 (q, *J* = 13.8 Hz, 2H), 1.44 (s, 9H).

*Methyl 2-((tert-butoxycarbonyl)amino)-3-(p-tolyl)-propanoate-d (**3-d**).* Colorless oil (261.8 mg, 89%), R_f = 0.5 (Petroleum ether/EtOAc = 5:1), 95% D-incorporation at α-positions; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.10 (d, *J* = 7.9 Hz, 2H), 7.00 (d, *J* = 8.0 Hz, 2H), 4.95 (s, 1H), 4.56 (d, *J* = 7.5 Hz, 0.05H, **Labeled**), 3.71 (s, 3H), 3.10−2.96 (m, 2H), 2.31 (s, 3H), 1.42 (s, 9H).

*Methyl 2-((tert-butoxycarbonyl)amino)-3-(m-tolyl)-propanoate-d (**4-d**).* Colorless oil (261.8 mg, 85%), R_f = 0.5 (Petroleum ether/EtOAc = 5:1), 91% D-incorporation at α-positions; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.18 (t, *J* = 7.5 Hz, 1H), 7.05 (d, *J* = 7.6 Hz, 1H), 6.92 (d, *J* = 10.5 Hz, 2H), 4.95 (s, 1H), 4.56 (d, *J* = 7.5 Hz, 0.09H, **Labeled**), 3.71 (s, 3H), 3.03 (q, *J* = 13.9 Hz, 2H), 2.32 (s, 3H), 1.42 (s, 9H).

*Methyl 2-((tert-butoxycarbonyl)amino)-3-(o-tolyl)-propanoate-d (**5-d**).* Colorless oil (282.4 mg, 96%), R_f = 0.5 (Petroleum ether/EtOAc = 5:1), 96% D-incorporation at α-positions; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.19−7.01 (m, 4H), 4.99 (s, 1H), 4.56 (d, *J* = 7.7 Hz, 0.04H, **Labeled**), 3.69 (s, 3H), 3.13 (d, *J* = 14.0 Hz, 1H), 2.97 (d, *J* = 14.1 Hz, 1H), 2.34 (s, 3H), 1.39 (s, 9H).

*Methyl 2-((tert-butoxycarbonyl)amino)-3-(4-nitrophenyl)-propanoate-d (**6-d**).* Colorless oil (292.8 mg, 90%), R_f = 0.2 (Petroleum ether/EtOAc = 5:1) with 96% D-incorporation at α-positions; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.19−8.13 (m, 2H), 7.35−7.28 (m, 2H), 5.04 (s, 1H), 4.63 (s, 0.04H, **Labeled**), 3.73 (s, 3H), 3.26 (d, *J* = 13.7 Hz, 1H), 3.11 (d, *J* = 13.7 Hz, 1H), 1.41 (s, 9H).

*Methyl 2-((tert-butoxycarbonyl)amino)-3-(3-nitrophenyl)-propanoate-d (**7-d**).* Colorless oil (305.8 mg, 94%), R_f = 0.2 (Petroleum ether/EtOAc = 5:1) with 93% D-incorporation at α-positions; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.11 (dt, *J* = 6.4, 2.5 Hz, 1H), 8.00 (d, *J* = 2.5 Hz, 1H), 7.48 (dd, *J* = 4.9, 2.3 Hz, 2H), 5.08 (s, 1H), 4.61 (d, *J* = 7.1 Hz, 0.07H, **Labeled**), 3.75 (s, 3H), 3.28 (d, *J* = 13.8 Hz, 1H), 3.10 (d, *J* = 13.9 Hz, 1H), 1.40 (s, 9H).

*Methyl (tert-butoxycarbonyl)tyrosinate-d (**8-d**).* Colorless oil (251.9 mg, 85%), R_f = 0.2 (Petroleum ether/EtOAc = 5:1) with 95% D-incorporation at α-positions; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.15−7.05 (m, 4H), 4.98 (s, 1H), 4.58 (dd, *J* = 16.3, 8.5 Hz, 0.05H, **Labeled**), 3.69 (s, 3H), 3.09 (d, *J* = 13.9 Hz, 1H), 3.02 (d, *J* = 14.2 Hz, 1H), 1.40 (s, 9H).

*Methyl 3-(4-bromophenyl)-2-((tert-butoxycarbonyl)-amino)propanoate-d (**9-d**).* Colorless oil (305.4 mg, 85%), R_f = 0.2 (Petroleum ether/EtOAc = 5:1) with 91% D-incorporation at α-positions; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.37−7.31 (m, 2H), 6.96−6.90 (m, 2H), 4.92 (s, 1H), 4.49 (q, *J* = 6.7 Hz, 0.09H, **Labeled**), 3.64 (s, 3H), 3.01 (d, *J* = 13.8 Hz, 1H), 2.91 (d, *J* = 13.9 Hz, 1H), 1.34 (s, 9H).

*Methyl 3-(3-bromophenyl)-2-((tert-butoxycarbonyl)-amino)propanoate-d (**10-d**).* Colorless oil (287.4 mg, 80%), R_f = 0.5 (Petroleum ether/EtOAc = 5:1) with 88% D-incorporation at α-positions; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.37 (dt, *J* = 8.0, 1.4 Hz, 1H), 7.27 (d, *J* = 6.3 Hz, 1H), 7.16 (t, *J* = 7.8 Hz, 1H), 7.06 (d, *J* = 7.7 Hz, 1H), 5.00 (s, 1H), 4.56 (q, *J* = 6.7 Hz, 0.12H, **Labeled**), 3.72 (s, 3H), 3.10 (d, *J* = 13.8 Hz, 1H), 2.98 (d, *J* = 13.9 Hz, 1H), 1.42 (s, 9H).

*Methyl 2-((tert-butoxycarbonyl)amino)-3-(4-chlorophenyl)propanoate-d (**11-d**).* Colorless oil (283.3 mg, 90%), R_f = 0.66 (Petroleum ether/EtOAc = 3:1) with 95% D-incorporation at α-positions; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.22−7.15 (m, 2H), 6.99 (d, *J* = 8.4 Hz, 2H), 4.96 (s, 1H), 4.50 (dd, *J* = 9.9, 4.4 Hz, 0.05H, **Labeled**), 3.64 (s, 3H), 3.02 (d, *J* = 13.8 Hz, 1H), 2.92 (d, *J* = 13.9 Hz, 1H), 1.34 (s, 9H).

*Methyl 2-((tert-butoxycarbonyl)amino)-3-(2-chlorophenyl)propanoate-d (**12-d**).* Colorless oil (283.3 mg, 90%), R_f = 0.66 (Petroleum ether/EtOAc = 3:1) with 72% D-incorporation at α-positions; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.29 (dq, *J* = 5.9, 2.6 Hz, 1H), 7.12 (d, *J* = 2.5 Hz, 3H), 4.98 (s, 1H), 4.57 (q, *J* = 7.6 Hz, 0.28H, **Labeled**), 3.65 (s, 3H), 3.22 (dd, *J* = 13.8, 6.6 Hz, 1H), 3.04 (dd, *J* = 13.8, 6.5 Hz, 1H), 1.31 (s, 9H).

*Methyl 2-((tert-butoxycarbonyl)amino)-2-(4-chlorophenyl)acetate-d (**13-d**).* Colorless oil (267.7 mg, 89%), R_f = 0.66 (Petroleum ether/EtOAc = 3:1) with 90% D-incorporation at α-positions; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.36−7.26 (m, 4H), 5.59 (s, 1H), 5.29 (d, *J* = 6.4 Hz, 0.10H, **Labeled**), 3.72 (s, 3H), 1.42 (s, 9H).

*Methyl 2-((tert-butoxycarbonyl)amino)-2-(3-chlorophenyl)acetate-d (**14-d**).* Colorless oil (270.7 mg, 90%), R_f = 0.66 (Petroleum ether/EtOAc = 3:1) with 91% D-incorporation at α-positions; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.36 (q, *J* = 1.4 Hz, 1H), 7.31−7.19 (m, 3H), 5.60 (s, 1H), 5.30 (d, *J* = 7.2 Hz, 0.09H, **Labeled**), 3.73 (d, *J* = 2.3 Hz, 3H), 1.43 (s, 9H).

*Methyl 2-((tert-butoxycarbonyl)amino)-2-(2-chlorophenyl)acetate-d (**15-d**).* Colorless oil (270.7 mg, 90%), R_f = 0.66 (Petroleum ether/EtOAc = 3:1) with 43% D-incorporation at α-positions; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94−7.82 (m, 1H), 7.53−7.44 (m, 1H), 7.44−7.30 (m, 3H), 5.65 (d, *J* = 8.5 Hz, 0.57H, **Labeled**), 3.64 (s, 3H), 1.39 (s, 9H)

*Methyl (tert-butoxycarbonyl)tryptophanate-d (**16-d**).* Colorless oil (284.2 mg, 89%), R_f = 0.5 (petroleum ether/EtOAc = 3:1) with 93% D-incorporation at α-positions. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.17 (s, 1H), 7.55 (d, *J* = 7.8 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.26−7.08 (m, 2H), 7.00 (d, *J* = 2.3 Hz, 1H), 5.08 (s, 1H), 4.65 (s, 0.07H, **Labeled**), 3.67 (s, 3H), 3.30−3.26 (m, 2H), 1.43 (s, 9H).

*Methyl 2-((tert-butoxycarbonyl)amino)-4-phenylbutanoate -d (**17-d**).* Colorless oil (238.4 mg, 81%), R_f = 0.66 (Petroleum ether/EtOAc = 5:1) with 94% D-incorporation at the α-positions. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.24−7.16 (m, 2H), 7.11 (ddd, *J* = 8.5, 7.0, 1.7 Hz, 3H), 5.02 (s, 1H), 4.28 (d, *J* = 8.7 Hz, 0.06H, **Labeled**), 3.63 (s, 3H), 2.60

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(ddd, J = 9.3, 6.7, 2.2 Hz, 2H), 2.12−2.00 (m, 1H), 1.86 (ddd, J = 13.8, 9.1, 6.7 Hz, 1H), 1.38 (s, 9H).

Di-tert-butyl (tert-butoxycarbonyl)aspartate-d (**18-d**). Colorless oil (287.5 mg, 83%), R_f = 0.66 (Petroleum ether/EtOAc = 5:1) with 92% D-incorporation at α-positions. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.46 (s, 1H), 4.37 (dt, J = 9.0, 4.6 Hz, 0.08H, **Labeled**), 2.82 (d, J = 16.8 Hz, 1H), 2.65 (d, J = 16.7 Hz, 1H), 1.43 (s, 18H).

Dimethyl (tert-butoxycarbonyl)glutamate-d (**19-d**). White powder (251.4 mg, 91%), R_f = 0.66 (Petroleum ether/EtOAc = 5:1) with 92% D-incorporation at α-positions.^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.93 (s, 1H), 4.16 (d, J = 8.1 Hz, 0.08H, **Labeled**), 3.57 (s, 3H), 3.50 (s, 3H), 2.32−2.12 (m, 2H), 2.00 (dt, J = 14.8, 7.6 Hz, 1H), 1.82−1.71 (m, 1H), 1.26 (s, 9H).

Methyl (tert-butoxycarbonyl)leucinate-d (**20-d**). White powder (219.2 mg, 89%), R_f = 0.66 (Petroleum ether/EtOAc = 5:1) with 95% D-incorporation at the α-positions. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.92 (s, 1H), 4.28 (d, J = 6.4 Hz, 0.05H, **Labeled**), 3.69 (s, 3H), 1.74−1.57 (m, 5H), 1.57−1.45 (m, 1H), 1.40 (s, 9H), 0.91 (dd, J = 6.6, 2.8 Hz, 6H).

Methyl (tert-butoxycarbonyl)-L-alaninate-d (**21-d**). Colorless oil (183.8 mg, 90%), R_f = 0.66 (Petroleum ether/EtOAc = 5:1) with 92% D-incorporation at α-positions.^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.01 (s, 1H), 4.32−4.21 (m, 0.08H, **Labeled**), 3.73 (s, 3H), 1.44 (s, 10H), 0.92 (t, J = 7.5 Hz, 3H).

Methyl N^2,N^6-bis(tert-butoxycarbonyl)lysinate-d (**22-d**). White powder (307.2 mg, 85%), R_f = 0.66 (Petroleum ether/EtOAc = 5:1) with 79% D-incorporation at α-positions.^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.13 (s, 1H), 4.66 (s, 1H), 4.23 (q, J = 7.8 Hz, 0.21H, **Labeled**), 3.69 (s, 3H), 3.05 (s, 2H), 1.82−1.69 (m, 1H), 1.59 (ddd, J = 13.4, 10.1, 5.6 Hz, 1H), 1.39 (s, 22H).

tert-Butyl (2-oxotetrahydrofuran-3-yl)carbamate-d (**23-d**). Colorless oil (70.7 mg, 35%), R_f = 0.66 (Petroleum ether/EtOAc = 5:1) with 60% D-incorporation at α-positions.^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.05 (s, 1H), 4.44 (td, J = 9.0, 1.2 Hz, 1H), 4.35 (s, 0.40H, **Labeled**), 4.25 (ddd, J = 11.4, 9.3, 5.8 Hz, 1H), 2.83−2.68 (m, 1H), 2.27−2.11 (m, 1H), 1.46 (s, 9H).

tert-Butyl (tert-butoxycarbonyl)valinate-d (**24-d**). Colorless oil (54.9 mg, 20%), R_f = 0.66 (Petroleum ether/EtOAc = 5:1) with 56% D-incorporation at α-positions.^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.00 (s, 1H), 4.22 (dd, J = 9.2, 4.8 Hz, 0.44H, **Labeled**), 3.73 (d, J = 1.9 Hz, 3H), 2.11 (dt, J = 13.5, 6.4 Hz, 1H), 1.44 (s, 9H), 1.02−0.77 (m, 6H).

tert-Butyl 2-((tert-butoxycarbonyl)amino)-3-methylpentanoate-d (**25-d**). Colorless oil (57.7 mg, 20%), R_f = 0.66 (petroleum ether/EtOAc = 5:1) with 40% D-incorporation at α-positions. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.98 (d, J = 8.3 Hz, 1H), 4.32 (dd, J = 8.4, 4.5 Hz, 0.60H, **Labeled**), 1.82 (tdd, J = 9.3, 7.0, 4.8 Hz, 1H), 1.45 (d, J = 3.4 Hz, 18H), 1.28−1.09 (m, 1H), 0.91 (td, J = 7.1, 3.9 Hz, 6H).

1-(tert-Butyl) 2-methyl pyrrolidine-1,2-dicarboxylate-d (**26-d**). Colorless oil (115.1 mg, 50%), R_f = 0.66 (Petroleum ether/EtOAc = 5:1) with 10% D-incorporation at α-positions.^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.22 (ddd, J = 40.3, 8.6, 3.8 Hz, 0.90H, **Labeled**), 3.68 (d, J = 1.6 Hz, 3H), 3.56−3.27 (m, 2H), 2.16 (tdd, J = 16.1, 7.7, 4.1 Hz, 1H), 1.99−1.76 (m, 3H), 1.39 (d, J = 20.7 Hz, 9H).

tert-Butyl (tert-butoxycarbonyl)glycinate-d (**27-d**). Colorless oil (209.8 mg, 90%), R_f = 0.66 (petroleum ether/EtOAc = 5:1) with 50% D-incorporation at the α-positions. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.09 (s, 1H), 3.69 (d, J = 4.4 Hz, 1H, **Labeled**), 1.38 (d, J = 8.1 Hz, 18H).

Methyl (tert-butoxycarbonyl)methioninate-d (**28-d**). Colorless oil (224.5 mg, 85%), R_f = 0.66 (petroleum ether/EtOAc = 5:1) with 94% D-incorporation at the α-positions. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.11 (s, 1H), 4.42 (s, 0.06H, **Labeled**), 3.75 (s, 3H), 2.53 (dd, J = 8.2, 6.9 Hz, 2H), 2.10 (s, 4H), 1.92 (dt, J = 14.3, 7.4 Hz, 1H), 1.44 (s, 9H).

2-((tert-Butoxycarbonyl)amino)-3-phenylpropanoic pivalic anhydride-d (**31-d**). White powder (175.1 mg, 50%), R_f = 0.66 (petroleum ether/EtOAc = 5:1) with 47% D-incorporation at the α-positions. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.40−7.09 (m, 5H), 5.00 (d, J = 8.1 Hz, 1H), 4.45 (q, J = 6.8 Hz, 0.53H, **Labeled**), 3.05 (d, J = 6.3 Hz, 2H), 1.41 (d, J = 9.5 Hz, 18H).

■ ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its Supporting Information.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c09974.

Optimization of the reaction conditions; analytical date for the labeled and unlabeled compounds; and copies of ^{1}H NMR spectrum (PDF)

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Notes

The authors declare no competing financial interest.

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