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Catalytic asymmetric aza-Michael addition of fumaric monoacids with multifunctional thiourea/boronic acids

Kenichi Michigami, Hiroki Murakami, Takeru Nakamura, Noboru Hayama, and Yoshiji Takemoto*

The first chemical enantioselective synthesis of N-hydroxyaspartic acid derivatives using chiral multifunctional thiourea/boronic acid organocatalysts was developed. A series of fumaric monoacids underwent intermolecular asymmetric aza-Michael addition of O-alkyl hydroxylamines in excellent regioselectivity. The addition of another carboxylic acid raised the enantiomeric enrichment up to 97% ee. O-Deprotection of the aza-Michael adduct provided an aspartate-derived hydroxylamine fragment applicable for KAHA (α-keto acid-hydroxylamine) ligation.

Chemoselective peptide conjugation by enzymes is a ubiquitous process in nature, producing only recyclable phosphates as by-products. Meanwhile, a general chemical approach for the amide C=N bond formation is based on stoichiometric activation of carboxylic acids by coupling reagents. Though this method is highly reliable, reagent-derived non-recyclable side products are inevitably generated. In addition, protection of nucleophilic functional groups is required due to incompatibility with condensation reagents. The development of environmentally benign alternatives is thus highly demanded. Among large numbers of strategies reported to circumvent these issues, elegant examples have been disclosed in recent years by Bode and co-workers. The protocol of simply mixing α-keto acids and hydroxylamines in aqueous solvent under mild conditions afforded amides concomitant with the release of carbon dioxide (CO2) and water. This clean amide synthesis, KAHA ligation, has manifested significant advantages in polypeptide synthesis because the connection of peptide segments proceeds without protection of side chains and the loss of enantiopurity. However, low accessibility of both α-keto acids and hydroxylamines underlies a major problem that affects the practical convenience. In marked contrast to α-amino acid-derived α-keto acids, synthetic research for hydroxylamine counterparts is still less exploited: only a few simple derivatives including cyclic analogues of homoserine, serine, and aspartic acid semialdehyde (Asa) have been synthesised and applied to KAHA peptide ligation (Scheme 1A). Since these nucleophiles require long-step routes for preparation, a straightforward and enantioselective synthesis of such hydroxylamines represents a major challenge for KAHA strategy in view of general peptide synthesis.

Scheme 1. Strategy for the Synthesis of α-Amino Acid-Derived Hydroxylamines for KAHA Peptide Ligation

We have recently focused on a direct catalytic asymmetric aza-Michael addition of BnONH2 to α,β-unsaturated carboxylic acids using organocatalysts consisting of aryloboronic acid and chiral trans-1,2-cyclohexanediamine-based aminothiourea (Scheme 1B). Notably, our multifunctional catalysts only promoted 1,4-addition: The 1,2-adducts, N-benzoylhydrimides, which are usually formed in organoboron-catalysed dehydrative amidation, were donors.
not observed. Encouraged by the efficient protocols free from “pre-activation” and “protection” of carboxylic acids,\textsuperscript{12} we targeted \(N\)-hydroxysuccinamic acid derivatives to demonstrate synthetic versatility of our catalytic systems. Although biocatalytic synthesis of enantipure \(N\)-hydroxy and \(N\)-alkoxyaspartates have been reported,\textsuperscript{13} chemical methods are still under developed, and to the best of our knowledge, no example has emerged for catalytic asymmetric variant.\textsuperscript{14} Herein, we report the asymmetric synthesis of \(N\)-hydroxysuccinamic acid derivatives catalysed by multifunctional thiourea/boronic acids, which would offer a new, facile access to aspartate-derived hydroxylamine fragments suitable for KAHA peptide ligation.

First, initial investigations focused on catalytic asymmetricaza-Michael addition using mono-tert-butyl fumarate (1a) and \(O\)-benzyldihydroxylamine (2a) as a nucleophile. In line with our previous studies, a chiral integrated thiourea/boronic acid catalyst A was employed in the presence of 4 Å molecular sieves (4 Å MS) at room temperature. Though no reaction took place in DMF and MeCN (Table 1, entries 1 and 2), the reaction in less polar solvents, Et\(_2\)O and CH\(_2\)Cl\(_2\), furnished the corresponding \(N\)-benzyloxysuccinamic acid diester 3aa after treatment with TMSCHN\(_2\). When the reaction was conducted in toluene, both the yield and ee drastically increased (entry 5). CCl\(_4\) was found to be the optimal solvent, providing 3aa in 80% yield and 88% ee (entry 6). Next, thiourea moiety of the catalyst was deviated. Loss of both yields and ees was observed with electron-withdrawing thioureas B and C (entries 7 and 8). The ortho-Me substituted catalyst D gave slightly higher ee, but the yield dropped to 59%. \(N\)-Methyl thiourea catalyst E also promoted the reaction, albeit in moderate yield and ee (entry 10). Therefore, we continued the following experiments with catalyst A.

Afterwards, a range of carboxylic acid was employed as additives, since our previous works suggested three molecules of carboxylic acid were involved in catalytic cycle: Two of them bind to the catalyst and the other one works as a proton shuttle (vide infra).\textsuperscript{8c} No reaction occurred with 1 equivalent of HCO\(_2\)H and AcOH or \(^7\)BuCO\(_2\)H did not exhibit beneficial effects. Meanwhile, both the yield and ee improved with PhCO\(_2\)H, affording 3aa in 88% yield and 93% ee (entry 14). In sharp contrast, a highly acidic \(p\)-TsOH inhibited the reaction completely, probably due to the protonation of the nucleophile and/or the catalyst (entry 15). Control experiments revealed that both the catalyst and 4 Å MS were essential for the reaction (entries 16 and 17). All reactions proceeded at \(\beta\) position of the carboxylic acid exclusively, which indicates that the carboxylic acid was predominantly activated in situ by boronic acid over the alkyl ester moiety.\textsuperscript{15}

Table 1. Optimisation of the Reaction Conditions.

<table>
<thead>
<tr>
<th>entry</th>
<th>catalyst</th>
<th>additive</th>
<th>solvent</th>
<th>3aa (%)</th>
<th>ee (%)</th>
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<td>-</td>
</tr>
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<td>MeCN</td>
<td>0</td>
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<td>A</td>
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<td>60</td>
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<td>A</td>
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<td>CCl(_4)</td>
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<td>7</td>
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<td>CCl(_4)</td>
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<td><strong>93</strong></td>
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<td>CCl(_4)</td>
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<tr>
<td>17(c)</td>
<td>A</td>
<td>-</td>
<td>CCl(_4)</td>
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</table>

Reaction conditions: 1a (0.10 mmol), 2a (0.11 mmol, 1.1 equiv), catalyst (0.010 mmol, 10 mol%), additive (0.10 mmol, 1.0 equiv), 4 Å MS (50 mg), solvent (1.0 mL, 0.1 M), rt, 24 h. Isolated yields are shown. Ees were estimated by chiral HPLC analysis. The reaction was performed without 4 Å MS.

With the optimal conditions in hand, we next explored the substrate scope of the aza-Michael addition of fumaric acid monoesters with 2a in the presence of catalyst A. (Figure 1). Benzyl ester 1b and ethyl ester 1c were converted into the corresponding aspartates 3ba and 3ca, respectively in moderate yields with high ees. Remarkably, in addition to fumaric acid monoesters, phenylalanine-derived fumaric acid monoamides 1d and 1e underwent the aza-Michael addition. By employing catalyst A with (\(R,R\)) configuration, \(d\)-Phe-\(d\)-Asp derivative 3ea was furnished in higher diastereoselectivity than that of \(L\)-Phe-\(d\)-Asp derivative 3da. The catalyst was switched to (\(S,S\))-A (ent-A) for the reaction of \(N\)-fumaryl-\(L\)-amino esters 1f and 1g, producing Gly-L-Asp and \(L\)-Ser-L-Asp derivatives 3fa and 3ga, respectively. Even though the stereoselectivity is moderate, these results suggest the feasibility of the synthesis of \(N\)-hydroxyaspartate-derived peptides through this protocol.
Isolated yields are shown. 

Figure 1. Substrate scope of fumaric monoacids (0.40 mmol). Isolated yields are shown. Ees were estimated by chiral HPLC analysis. aDr was determined by 1H NMR analysis. bent-A was used as a catalyst.

Figure 2. Screening of Nucleophiles (0.20–0.40 mmol). Isolated yields are shown. Ees were estimated by chiral HPLC analysis. Ee was determined after N-benzyolization.

Based on the experimental results, including perfect regioselectivity and mechanistic studies performed previously, a proposed transition state of the addition of hydroxylamine is depicted in Figure 3. Since no reaction occurred in the absence of 4 Å MS (Table 1, entry 17), removal of water is indispensable for this reaction. In a plausible intermediate containing diacyloxyboronate, the hydrogen-bond interaction between the thiourea NH protons and one of the carboxy group facilitates conjugate addition of hydroxylamine. The high enantioselectivity would be attributed to an additional interaction between the nucleophile NH proton and another carboxy ligand, accelerating Re-face attack of hydroxylamine. The nucleophilic addition is accompanied with intermolecular proton transfer, which is promoted by the third molecule of carboxylic acid.

Figure 3. Proposed Transition State of Nucleophilic Addition.

A quick access to various chiral N-alkoxyaspartate derivatives prompted us to examine O-deprotection of the Michael adducts for the application to KAHA ligation. Various deprotection conditions tested revealed that compound 3be, obtained from 1b and 2e, was the substrate of choice. First, treatment of 3be with TFA and subsequent careful quenching with water gave an OH-free hydroxymine 4b in 42% yield. The following KAHA ligation using α-keto acid 5 proceeded smoothly and the corresponding amide 6b was obtained without significant loss of enantiomeric excess. The same reaction with Fmoc-l-leucine-derived α-keto acid 7 was also afforded dipeptide 8b in moderate yield without epimerisation.

Scheme 3. O-De-protection and KAHA Ligation of Aspartate-Derived Hydroxylamine Derivatives

In summary, we have developed a direct aza-Michael addition of hydroxylamine derivatives to various fumaric monoacids catalysed by multifunctional thiourea/boronic acids. The process enables the first catalytic asymmetric chemical synthesis of N-hydroxyspartic acid derivatives with perfect regioselectivity and high enantioselectivity. Deprotection of the SEMONH₂-adduct provides a new aspartate-derived hydroxylamine fragment for KAHA peptide ligation. Further research into the synthetic application is actively ongoing, and the results will be reported in due course.

Conflicts of interest

There are no conflicts to declare.
Acknowledgement

This paper is dedicated to the memory of our talented colleague, Mr. Takeru Nakamura, who passed away on 25 October, 2017. This work was financially supported by JSPS KAKENHI, Grant No. JP16H06384.

Notes and references


2 Acknowledgement


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takemoto@pharm.kyoto-u.ac.jp

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(A-1) Optimisation Details: Tables S1-S8

Several reaction parameters of the enantioselective aza-Michael addition were investigated. In each tables are described isolated yields.

Table S1. Investigation of Solvent Effect

<table>
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<tr>
<td>2</td>
<td>MeCN</td>
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</tr>
<tr>
<td>3</td>
<td>EtOAc</td>
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<td>5</td>
<td>CH₂Cl₂</td>
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</tr>
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Table S2. Deviation of Thioureas of Multifunctional Organoboron Catalysts

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<td>E</td>
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<td>F</td>
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Table S3. Screening of Acid Additives

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<th>ee (%)</th>
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<td>88</td>
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Table S4. Investigation of Nucleophiles

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<td>3</td>
<td>2c</td>
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<td>4</td>
<td>2d</td>
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<td>5a</td>
<td>2e</td>
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<td>85</td>
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<tr>
<td>6</td>
<td>S2a</td>
<td>0</td>
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</table>

*a Ee was determined after N-benzoxylation.

Figure S1. Unsuccessful Substrates
Monomethyl fumarate (S1a) and fumaryl monoanilide (S1b) did not undergo the aza-Michael addition, probably due to low solubility in CCl₄.
(A-2) Determination of Stereochemistry

The aza-Michael adduct 3aa was converted into N-Fmoc-aspartic diester S3aa and the stereochemistry was determined as R configuration by comparison of HPLC charts and optical rotations with (S)-N-Fmoc-aspartic diester derived from the commercially available mono-tert-butyl-L-aspartate (Scheme S1).

Scheme S1

\[
\begin{align*}
\text{BnO} & \quad \text{NH} \\
\text{^{t}BuO_2C} & \quad \text{CO}_2\text{Me} \\
3aa & \quad \text{(91\% ee)}
\end{align*}
\]

1) 10\% Pd/C, MeOH, rt, H \text{2 atm}
2) FmocCl, \text{iPr}_2\text{NEt}, \text{CH}_2\text{Cl}_2, 0 \degree \text{C to rt}

\[
\begin{align*}
\text{NHFmoc} & \quad \text{NH} \\
\text{^{t}BuO_2C} & \quad \text{CO}_2\text{Me} \\
\text{S3aa} & \quad \text{(R)}
\end{align*}
\]

61\% (2 steps), 87\% ee

\[
\begin{align*}
\text{NHFmoc} \\
\text{^{t}BuO_2C} & \quad \text{CO}_2\text{H}
\end{align*}
\]

commercially available

\[
\begin{align*}
\text{toluene/MeOH, 0 \degree \text{C}} \\
\text{TMSCHN}_2
\end{align*}
\]

99\%

\[
\begin{align*}
\text{NHFmoc} & \quad \text{NH} \\
\text{^{t}BuO_2C} & \quad \text{CO}_2\text{Me} \\
\text{commercially available} & \quad \text{(S)}
\end{align*}
\]

\[
\begin{align*}
\text{toluene/MeOH, 0 \degree \text{C}} \\
\text{TMSCHN}_2
\end{align*}
\]

99\%

\[
\begin{align*}
\left[\alpha\right]_{D}^{23} &= -7.12 \ (c \ 0.73, \ \text{CHCl}_3) \\
\left[\alpha\right]_{D}^{23} &= +5.96 \ (c \ 7.51, \ \text{CHCl}_3)
\end{align*}
\]
(B) General

All manipulations were carried out under argon atmosphere unless otherwise noted. Infrared (IR) spectra were recorded on a JASCO FT/IR-4100 Fourier transform infrared spectrophotometer. NMR spectra were recorded on a JEOL ECP-400 spectrometer and JEOL ECA-500 spectrometer, operating at 400 MHz (1H) or 100 MHz (13C) and 500 MHz (1H) or 125 MHz (13C), respectively. Chemical shifts in CDCl3, DMSO-d6, and CD3OD were reported in the scale relative to CHCl3 (7.26 ppm), DMSO (2.50 ppm), and MeOH (3.31 ppm) for 1H NMR, and to CDCl3 (77.0 ppm) for 13C NMR as internal references, respectively. NMR data are reported as follows: chemical shifts, multiplicity (s: singlet, d: doublet, t: triplet, q: quartet, quin: quintet, m: multiplet, br: broad signal), coupling constant (Hz), and integration. ESI-HRMS spectra were measured on a Shimadzu LCMS-IT-TOF fitted with an ESI. Optical rotations were measured on a JASCO P-2200 digital polarimeter with a path length of 1 cm; concentrations are quoted in grams per 100 mL. [α]D values are measured in 10⁻¹ deg cm²/g. Chiral HPLC analyses were carried out using a SHIMADZU DGU-20A5. Column chromatography was performed with Cica silica gel 60N (40-100 μm, spherical, neutral). Dry solvents were purchased from Wako Pure Chemical Industries, Ltd. and used as received. Organocatalysts A-F were prepared according to our developed procedures.¹
(C) Materials and Methods

(C-1) Preparation of Substrates

Monoethyl fumarate (1c) was purchased from Tokyo Chemical Industry Co., Ltd. Monomethyl fumarate (S1a) was purchased from Sigma-Aldrich Co. LLC. mono-tert-butyl fumarate (1a) and (E)-4-oxo-4-(phenylamino)but-2-enoic acid (S1b) were prepared according to the reported procedures.

Mono-tert-butyl fumarate (1a): White solids.

![1a](image)

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 6.87 (d, $J = 15.6$ Hz, 1H), 6.76 (d, $J = 15.6$ Hz, 1H), 1.52 (s, 9H) ppm.

(E)-4-Oxo-4-(phenylamino)but-2-enoic acid (S1b): White solids.

![S1b](image)

IR (neat) $\tilde{\nu}$: 1696, 1654 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 12.99 (br s, 1H), 10.51 (s, 1H), 7.67 (d, $J = 8.0$ Hz, 2H), 7.34 (t, $J = 7.6$ Hz, 2H), 7.14 (d, $J = 15.2$ Hz, 1H), 7.09 (t, $J = 7.6$ Hz, 1H), 6.65 (d, $J = 15.2$ Hz, 1H) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 166.4, 161.6, 138.6, 137.2, 130.8, 128.9, 124.0, 119.4 ppm; HRMS (ESI) $m/z$ calcd. for [M-H]-: 190.0510, found: 190.0521.

Monobenzyl fumarate (1b): To a solution of benzyl tert-butyl fumarate (262.1 mg, 1.0 mmol, 1 equiv) in CH$_2$Cl$_2$ (4.0 mL) was added TFA (1.8 mL) and stirred at room temperature for 7 h. The mixture was concentrated, and the resulting solids were recrystallised from CH$_2$Cl$_2$ and hexane to afford 1b as white solids (138.4 mg, 0.67 mmol, 67%).

IR (neat) $\tilde{\nu}$: 2940, 1719, 1694 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 7.39-7.34 (m, 5H), 6.99 (d, $J = 16.0$ Hz, 1H), 6.88 (d, $J = 16.0$ Hz, 1H), 5.25 (s, 2H) ppm.

Ethyl (S,E)-4-((1-(tert-butoxy)-1-oxo-3-phenylpropan-2-yl)amino)-4-oxobut-2-enoate (S1d):

A mixture of L-phenylalanine tert-butyl ester hydrochloride (1.32 g, 6.0 mmol, 1 equiv), monoethyl fumarate (944.0 mg, 6.6 mmol, 1.1 equiv), HOBt (972.0 mg, 7.2 mmol, 1.2 equiv), Et$_3$N (1.82 g, 18.0 mmol, 3.0 equiv) and EDCI (1.38 g, 7.2 mmol, 1.2 equiv) in DMF (30 mL) was stirred at room temperature for 20 h. The solution was diluted with brine (20 mL) and extracted with Et$_2$O (20 mL, 2 times). The combined organic phase was dried over Na$_2$SO$_4$ followed by filtration and concentration under reduced pressure. The residue was then purified by silica-gel column chromatography (eluent: hexane/EtOAc, 3:1) to afford S1d as white solids (1.43 g, 4.12 mmol, 67%).

m.p. 139.3-140.0 °C; [a]$_D^{10}$ 106.9 (c 0.89, CHCl$_3$); IR (neat) $\tilde{\nu}$: 3312, 1734, 1716, 1637 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 7.29-7.22 (m, 3H), 7.14 (d, $J = 6.0$ Hz, 2H), 6.92 (d, $J = 15.5$ Hz, 1H), 6.80 (d, $J = 15.5$ Hz, 1H), 6.45 (d, $J = 7.5$ Hz, 1H), 4.84 (dt, $J = 7.0$, 6.0 Hz, 1H), 4.22 (q, $J = 7.5$ Hz, 2H), 3.14 (d, $J = 6.0$ Hz, 2H), 1.42 (s, 9H), 1.30 (t, $J = 7.5$ Hz, 3H), $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$: 170.2, 165.5, 163.0, 135.9, 130.9, 129.6, 128.5, 127.2, 82.9, 61.3, 53.9, 37.9, 28.0, 14.2 ppm; HRMS (ESI) $m/z$ calcd. for C$_{19}$H$_{25}$NO$_5$ [M+Na]$^+$: 370.1625, found: 370.1588.

(S,E)-4-((1-(tert-Butoxy)-1-oxo-3-phenylpropan-2-yl)amino)-4-oxobut-2-enoic acid (1d): To a solution
of S1d (1.43 g, 4.12 mmol, 1.0 equiv) in THF (20 mL) and water (8.0 mL) was added LiOH (172.8 mg, 4.12 mmol, 1.0 equiv) and stirred at room temperature for 12 h. The mixture was washed with Et₂O, and the aqueous phase was acidified with 1 M HCl aq. The solution was extracted with EtOAc (20 mL, 2 times) and the combined organic layer was washed with brine. After drying over Na₂SO₄ followed by filtration, the solvent was removed under reduced pressure to afford 1d as white solids (565.3 mg, 1.77 mmol, 43%).

m.p. 118.7-119.1 °C; [α]₂⁰D 105.8 (c 0.68, CHCl₃); IR (neat) ν: 3350, 1726, 1659, 1642 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ: 7.29-7.24 (m, 3 H), 7.15-7.14 (m, 2 H), 7.00 (d, J = 15.5 Hz, 1H), 6.83 (d, J = 7.5 Hz, 1H), 6.82 (d, J = 15.0 Hz, 1H), 4.89 (dt, J = 8.0, 7.0 Hz, 1H), 3.13 (d, J = 6.0 Hz, 2H), 1.42 (s, 9H) ppm; ¹³C NMR (125 MHz, CDCl₃) δ: 170.9, 169.4, 163.1, 137.6, 135.7, 130.3, 129.6, 128.6, 127.3, 83.4, 54.0, 38.1, 28.0 ppm; HRMS (ESI) m/z calcd. for C₁₇H₂₁NO₅ [M+Na]⁺: 342.1312, found: 342.1295.

Ethyl (R,E)-4-((1-(tert-butoxy)-1-oxo-3-phenylpropan-2-yl)amino)-4-oxobut-2-enoate (S1e): A mixture of D-phenylalanine t-butyl ester hydrochloride 6 (1.32 g, 6.0 mmol, 1.0 equiv), monoethyl fumarate (944.0 mg, 6.6 mmol, 1.1 equiv), HOBt (972.0 mg, 7.2 mmol, 1.2 equiv), Et₃N (1.82 g, 18.0 mmol, 3.0 equiv) and EDCI (1.38 g, 7.2 mmol, 1.2 equiv) in DMF (30 mL) was stirred at room temperature for 20 h. The solution was diluted with brine (20 mL) and extracted with Et₂O (20 mL, 2 times). The combined organic phase was dried over Na₂SO₄ followed by filtration and concentration under reduced pressure. The residue was then purified by silica-gel column chromatography (eluent: hexane/EtOAc, 3:1) to afford S1d as white solids (1.46 g, 4.23 mmol, 70%).

m.p. 135.5-137.8 °C; [α]₁⁸D -91.3 (c 0.94, CHCl₃); IR (neat) ν: 3310, 1734, 1716, 1636 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ: 7.27-7.22 (m, 3H), 7.15-7.13 (m, 2H), 6.94 (d, J = 15.5 Hz, 1H), 6.81 (d, J = 14.5 Hz, 1H), 6.54 (d, J = 7.5 Hz, 1H), 4.85 (dt, J = 7.5, 6.0 Hz, 1H), 4.21 (q, J = 7.0 Hz, 2H), 3.14 (d, J = 6.0 Hz, 2H), 1.41 (s, 9H), 1.29 (t, J = 7.5 Hz, 3H), ¹³C NMR (125 MHz, CDCl₃) δ: 170.3, 165.5, 163.0, 135.95, 135.91, 130.9, 129.5, 128.5, 127.1, 82.8, 61.3, 53.9, 37.9, 28.0, 14.2 ppm; HRMS (ESI) m/z calcd. for C₁₉H₂₅NO₅ [M+Na]⁺: 370.1625, found: 370.1576.

(R,E)-4-((1-(tert-Butoxy)-1-oxo-3-phenylpropan-2-yl)amino)-4-oxobut-2-enoic acid (1e): To a solution of S1e (1.46 g, 4.23 mmol, 1.0 equiv) in THF (20 mL) and water (8.0 mL) was added LiOH. (177.4 mg, 4.23 mmol, 1.0 equiv) and stirred at room temperature for 12 h. The mixture was washed with Et₂O, and the aqueous phase was acidified with 1 M HCl aq. The solution was extracted with EtOAc (20 mL, 2 times) and the combined organic layer was washed with brine. After drying over Na₂SO₄ followed by filtration, the solvent was removed under reduced pressure to afford 1e as white solids (855.0 mg, 2.67 mmol, 65%).

m.p. 118.6-119.2 °C; [α]₂¹D -95.0 (c 0.48, CHCl₃); IR (neat) ν: 3351, 1726, 1660, 1642 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ: 7.29-7.24 (m, 3H), 7.14 (d, J = 7.0 Hz, 2H), 6.99 (d, J = 15.5 Hz, 1H), 6.81 (d, J = 15.0 Hz, 1H), 6.74 (d, J = 8.0 Hz, 1H), 4.88 (dt, J = 8.0, 6.0 Hz, 1H), 3.14 (d, J = 6.5 Hz, 2H), 1.42 (s, 9H) ppm; ¹³C NMR (125 MHz, CDCl₃) δ: 170.9, 169.4, 163.0, 137.6, 130.2, 129.6, 128.6, 127.3, 83.4, 54.0, 38.0,
28.0 ppm; HRMS (ESI) m/z calcld. for C_{17}H_{21}NO_{5} [M+Na]^+ : 342.1312, found: 342.1291.

Ethyl (E)-4-(((tert-butoxycarbonylmethyl)amino)-4-oxobut-2-enolate (S1f): A mixture of glycine tert-butyl ester hydrochloride (835.0 mg, 4.98 mmol, 1.0 equiv), monoethyl fumarate (788.4 mg, 5.47 mmol, 1.1 equiv), HOBt (1.01 g, 7.47 mmol, 1.5 equiv), and EDCI (1.43 g, 7.47 mmol, 1.5 equiv) in DMF (13.5 mL) was stirred at room temperature for 20 h. The solution was diluted with brine (30 mL) and extracted with Et₂O (50 mL, 3 times). The combined organic phase was dried over Na₂SO₄ followed by filtration and concentration under reduced pressure. The residue was then purified by silica-gel column chromatography (eluent: hexane/EtOAc, 1:1) to afford S1f as yellow oil (716.7 mg, 2.78 mmol, 56%).

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\text{IR (neat)} \nu: 3301, 2980, 1724, 1668 \text{ cm}^{-1}; \quad \text{1H NMR (500 MHz, CDCl}_3\text{)} \delta: 6.94 (d, J = 15.0 \text{ Hz, 1H}), 6.82 (d, J = 15.0 \text{ Hz, 1H}), 4.24 (q, J = 7.0 \text{ Hz, 2H}), 4.03 (d, J = 4.5 \text{ Hz, 2H}), 1.47 (s, 9H), 1.31 (t, J = 6.5 \text{ Hz, 3H}) \text{ppm; 13C NMR (125 MHz, CDCl}_3\text{)} \delta: 168.6, 165.5, 163.6, 135.5, 131.1, 82.9, 61.3, 42.4, 28.1, 14.2 \text{ ppm; HRMS (ESI) m/z calcld. for [M+Na]^+: 280.1155, found: 280.1157.}
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(E)-4-(((tert-Butoxycarbonylmethyl)amino)-4-oxobut-2-enic acid (1f): A solution of S1f (716.0 mg, 2.78 mmol, 1 equiv) in THF (14 mL) was treated with 1 M LiOH aq. (2.78 mL, 2.78 mmol, 1.0 equiv) and stirred at ambient temperature for 4 h. The mixture was acidified with 1 M HCl aq. and extracted with CHCl₃ (30 mL, 3 times). After drying over Na₂SO₄ followed by filtration, the solvent was removed under reduced pressure to afford 1f as white solids (366.7 mg, 1.60 mmol, 60%).

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\text{m.p. 229.4 °C (decomp.); IR (neat)} \nu: 3339, 2868, 1732, 1689, 1658, 1637 \text{ cm}^{-1}; \quad \text{1H NMR (500 MHz, CDCl}_3\text{)} \delta: 7.02 (d, J = 15.5 \text{ Hz, 1H}), 6.84 (d, J = 15.5 \text{ Hz, 1H}), 6.54 (br s, 1H), 4.06 (d, J = 4.5 \text{ Hz, 2H}), 1.48 (s, 9H) \text{ppm; 13C NMR (125 MHz, CDCl}_3\text{)} \delta: 168.6, 166.9, 165.3, 135.6, 130.5, 81.7, 41.6, 26.9 \text{ ppm; HRMS (ESI) m/z calcld. for [M-H]^+: 228.0877, found: 228.0868.}
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Ethyl (S,E)-4-((1,3-bis(tert-butoxy)-1-oxopropan-2yl)amino)-4-oxobut-2-enolate (S1g): A mixture of O-tert-butyl-L-serine tert-butyl ester hydrochloride (2.53 g, 10.0 mmol, 1.0 equiv), monoethyl fumarate (1.72 g, 12.0 mmol, 1.2 equiv), HOBt (1.62 g, 12.0 mmol, 1.2 equiv), and EDCI (2.87 g, 15.0 mmol, 1.5 equiv) in DMF (27 mL) was stirred at room temperature for 12 h. The solution was diluted with brine (50 mL) and extracted with Et₂O (50 mL, 3 times). The combined organic phase was dried over Na₂SO₄ followed by filtration and concentration under reduced pressure. The residue was then purified by silica-gel column chromatography (eluent: hexane/EtOAc, 1:1) to afford S1g as white solids (3.06 g, 8.91 mmol, 89%).

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\text{m.p. 79.4- 80.5 °C; [}\alpha{]}_D^{25} 43.2 (c 0.45, CHCl}_3\text{); IR (neat)} \nu: 3309, 2981, 1741, 1715, 1656 \text{ cm}^{-1}; \quad \text{1H NMR (500 MHz, CDCl}_3\text{)} \delta: 6.99 (d, J = 15.5 \text{ Hz, 1H}), 6.96 (d, J = 8.5 \text{ Hz, 1H}), 4.60 (dt, J = 8.0, 3.0 \text{ Hz, 1H}), 4.13 (q, J = 7.0 \text{ Hz, 2H}), 3.69 (dd, J = 8.0, 3.0 \text{ Hz, 1H}), 3.46 (dd, J = 8.0, 3.0 \text{ Hz, 1H}), 1.35 (s, 9H), 1.18 (t, J = 7.0 \text{ Hz, 3H}), 1.01 (s, 9H) \text{ppm; 13C NMR (125 MHz, CDCl}_3\text{)} \delta: 169.0, 165.5, 163.2, 136.3, 130.5, 81.9, 73.0, 62.1, 61.1, 53.5, 27.9, 27.2, 14.1 \text{ ppm; HRMS (ESI) m/z calcld. for C}_{17}\text{H}_{29}\text{NO}_{6}[M+Na]^+: 366.1887,}
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(S,E)-4-((1,3-Bis(tert-butoxy)-1-oxopropan-2-yl)amino)-4-oxobut-2-enoic acid (1g): A solution of S1g (1.03 g, 3.0 mmol, 1.0 equiv) in THF (15 mL) was treated with 1 M LiOH aq. (3.0 mL, 3.0 mmol, 1.0 equiv) and stirred at ambient temperature for 4 h. The mixture was acidified with 1 M HCl aq. and extracted with CHCl3 (20 mL, 3 times). After drying over Na2SO4 followed by filtration, the solvent was removed to afford 1g as white solids (836.9 mg, 2.65 mmol, 88%).

m.p. 165.5-167.9˚C; [α]D23 25.6 (c 0.42, CHCl3); IR (neat) ν: 3331, 3074, 1720, 1706, 1631 cm\(^{-1}\); 1H NMR (500 MHz, CDCl3) δ: 7.19 (d, \(J = 8.5\) Hz, 1H), 7.07 (d, \(J = 15.5\) Hz, 1H), 6.88 (d, \(J = 15.5\) Hz, 1H), 4.73 (dt, \(J = 9.0, 3.0\) Hz, 1H), 3.80 (dd, \(J = 9.0, 3.0\) Hz, 1H), 3.56 (dd, \(J = 9.0, 3.0\) Hz, 1H), 1.45 (s, 9H), 1.12 (s, 9H) ppm; 13C NMR (125 MHz, CDCl3) δ: 169.7, 168.8, 137.3, 130.5, 82.8, 73.5, 62.2, 53.6, 28.0, 27.3 ppm; HRMS (ESI) m/z calcd. for C15H25NO6[M+Na]⁺: 338.1574, found: 338.1554.

(C-2) Preparation of Nucleophiles
O-benzylhydroxylamine (2a) was prepared by neutralization of BnONH2·HCl by 4 M NaOH aq. After extraction with CHCl3, general work up and dried under vacuum to afford 2a. O-Benzoylhydroxylamine (S2a) was prepared according to the reported procedure.

O-(4-Methoxybenzyl)hydroxylamine (2b): A mixture of N-hydroxyphthalimide (1.80 g, 11.0 mmol, 1.1 equiv), 4-methoxybenzyl chloride (1.86 g, 10.0 mmol, 1.0 equiv), and Et3N (1.22 g, 12.1 mmol, 1.1 equiv) in CH2Cl2 (25 mL) was stirred at room temperature for 4 h. The suspension was washed with brine (20 mL, 3 times) and the combined organic phase was dried over Na2SO4. After the solvent was removed under reduced pressure, the residue was dissolved in CHCl3/MeOH = 3:1 (50 mL). To the solution was added to N2H4·H2O (750.9 mg, 15.0 mmol, 1.5 equiv) and stirred at room temperature for 2 h. The mixture was filtered through a pad of Celite® and the filtrate was concentrated under reduced pressure. The residue was purified by silica-gel column chromatography (eluent: hexane/EtOAc, 1:1) to afford 2b as colorless oil (1.10 g, 6.2 mmol, 62%).

IR (neat) ν: 2977, 2917 cm\(^{-1}\); 1H NMR (500 MHz, CDCl3) δ: 7.29 (d, \(J = 9.0\) Hz, 2H), 6.89 (d, \(J = 9.0\) Hz, 2H), 5.33 (br s, 2H), 4.61 (s, 2H), 3.80 (s, 3H) ppm; 13C NMR (125 MHz, CDCl3) δ: 159.5, 130.1, 129.4, 113.9, 113.7, 77.7, 53.3 ppm; HRMS (ESI) m/z calcd. for C8H9NO, [M]+: 176.0682, found: 176.0556.

O-(4-Trifluoromethylbenzyl)hydroxylamine (2c): 2c was prepared through the procedure analogous to that of 2b. Alkylation was performed using N-hydroxyphthalimide (1.35 g, 7.0 mmol, 1.4 equiv), 4-(trifluoromethyl)benzyl chloride (815.0 mg, 5.0 mmol, 1.0 equiv), and Et3N (708.3 mg, 7.0 mmol, 1.4 equiv) in CH2Cl2 (20 mL). Deprotection of phthalimide was conducted with N2H4·H2O (525.6 mg, 10.5 mmol, 1.5 equiv) in CH3Cl/MeOH = 3:1 (15 mL), which afforded 2c as colorless oil (810.0 mg, 4.2 mmol, 84%).

IR (neat) ν: 2940, 1323 cm\(^{-1}\); 1H NMR (500 MHz, CDCl3) δ: 7.63 (d, \(J = 8.0\) Hz, 2H), 7.48 (d, \(J = 8.0\) Hz, 2H), 5.48 (br s, 2H), 4.75 (s, 2H) ppm; 13C NMR (125 MHz, CDCl3) δ: 141.7, 130.1, 129.4, 113.9, 113.7, 77.7, 53.3 ppm; HRMS (ESI) m/z calcd. for C8H9NO, [M]+: 176.0682, found: 176.0556.
125.4 (q, J = 3.9 Hz), 76.9 ppm; HRMS (ESI) m/z calcd. for C8H8FNO, [M+H]+: 192.0631, found: 192.0579.

**O-(Benzzyloxymethyl)hydroxylamine (2d):** 2d was prepared through the procedure analogous to that of 2b. Alkylation was performed using N-hydroxyphthalimide (322.6 mg, 2.0 mmol, 1.0 equiv), chloromethyl benzyl ether (439.1 mg, 2.8 mmol, 1.4 equiv), and Et3N (286.0 mg, 2.8 mmol, 1.4 equiv) in CH2Cl2 (4.5 mL). Deprotection of phthalimide was conducted with N2H4·H2O (150.2 mg, 3.0 mmol, 1.5 equiv) in CH3Cl/MeOH = 3:1 (5 mL), which afforded 2d as colorless oil (213.4 mg, 1.4 mmol, 70%).

IR (neat) ν: 2871 cm⁻¹; 1H NMR (500 MHz, CDCl3) δ: 7.38-7.34 (m, 4H), 7.32-7.29 (m, 1H), 5.51 (br s, 2H), 4.85 (s, 2H), 4.67 (s, 2H) ppm; 13C NMR (125 MHz, CDCl3) δ: 137.1, 128.4, 127.7, 98.5, 69.9 ppm; HRMS (ESI) m/z calcd. for C8H12NO2, [M]+: 154.0865, found: 154.0805.

**O-((2-Trimethylsilylethoxy)methyl)hydroxylamine (2e):** 2e was prepared through the procedure analogous to that of 2b. Alkylation was performed using N-hydroxyphthalimide (815.5 mg, 5.0 mmol, 1.0 equiv), chloromethyl 2-(trimethylsilyl)ethyl ether (1.16 g, 7.0 mmol, 1.4 equiv), and Et3N (708.3 mg, 7.0 mmol, 1.4 equiv) in CH2Cl2 (11.4 mL). Deprotection of phthalimide was conducted with N2H4·H2O (375.5 mg, 7.5 mmol, 1.5 equiv) in CH3Cl/MeOH = 3:1 (10 mL), which afforded 2e as colorless oil (550.5 mg, 3.1 mmol, 62%).

IR (neat) ν: 2953 cm⁻¹; 1H NMR (500 MHz, CDCl3) δ: 5.47 (br s, 2H), 4.73 (s, 2H), 3.64 (t, J = 8.0 Hz, 2H), 0.96 (t, J = 8.0 Hz, 2H), 0.01 (s, 9H) ppm; 13C NMR (125 MHz, CDCl3) δ: 98.8, 65.7, 18.3 ppm; HRMS (ESI) m/z calcd. for C6H17NO2Si, [M+H]+: 164.1107, found: 164.1018.

**O-Benzoylhydroxylamine (S2a):** Colorless oil.

1H NMR (400 MHz, CDCl3) δ: 8.00 (d, J = 2.0 Hz, 2H), 7.58 (t, J = 7.6 Hz, 1H), 7.45 (t, J = 7.6 Hz, 2H), 6.60 (br s, 2H) ppm.
(C-3) General Procedure for Catalytic Aza-Michael Addition

Prior to the reaction, 4 Å MS was dried by heat-gun (>300 ºC, 15 min) under vacuum (ca. 2 Torr). To an oven-dried 10 mL screw tube were placed an organocatalyst (10 mol%), substrate 1 (1.0 equiv), and benzoic acid (1.0 equiv), which were suspended in CCl₄ (0.2 M) and sealed with a Teflon-coated screw cap. After stirring at room temperature for 10 min, pre-heated 4 Å MS (500 mg/mmol) was added and the tube was capped and further stirred for 5 min. Hydroxylamine 2 (1.1 equiv) was then added and the system was closed again followed by stirring at ambient temperature for the indicated time. After the reaction progress was monitored by ¹H NMR analysis (a small amount of the mixture was transferred into an NMR tube). The reaction mixture was diluted in toluene/MeOH (3:1, 1 mL) and treated with TMSCHN₂ (10% in hexane, 1 mL) and stirred for 30 min. The excess TMSCHN₂ was quenched with AcOH, then the mixture was filtered through Celite® and the cake was washed with MeOH. After the solvent was removed under reduced pressure, the residue was purified by silica-gel column chromatography (eluent: hexane/EtOAc, 1:1) to afford the product 3. The ee of 3 was estimated by chiral HPLC analysis.

1-tert-Butyl 4-methyl N-benzyloxy-D-aspartate (3aa): The reaction was carried out using 1a (34.4 mg, 200 µmol, 1.0 equiv) and 2a (27.0 mg, 220 µmol, 1.1 equiv) in the presence of catalyst A (7.9 mg, 20 µmol, 10 mol%), benzoic acid (24.4 mg, 200 µmol, 1.0 equiv) and 4 Å MS (100.0 mg) for 24 h. The crude product was purified by silica-gel column chromatography (eluent: hexane/EtOAc, 3:1) afforded 3aa as colorless oil (40.5 mg, 164 µmol, 80%, 93% ee).

[α]D²⁴⁺5.4 (c 1.00, CHCl₃); IR (neat) ν: 3275, 1731 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ: 7.31 (m, 5H), 6.18 (br s, 1H), 4.69 (s, 2H), 3.92 (br s, 1H), 3.68 (s, 3H), 2.76 (dd, J = 16.0, 6.4 Hz, 1H), 2.63 (dd, J = 16.0, 6.4 Hz, 1H), 1.47 (s, 9H) ppm; ¹³C NMR (125 MHz, CDCl₃) δ: 171.3, 170.6, 137.5, 128.3, 128.2 127.7, 76.3, 64.2, 60.1, 51.8, 34.3, 27.9 ppm; HRMS (ESI) m/z calcd. for C₁₆H₂₃NO₅, [M+Na⁺]: 332.1468, found: 332.1470. The enantiomeric excess was estimated by chiral HPLC analysis (DAICEL CHIRALPAK IB, eluent: hexane/2-propanol = 99/1, flow rate: 1.0 mL/min. detector: UV at 220 nm), tR = 12.0 min (minor), 10.5 min (major).

1-Benzyl 4-methyl N-benzyloxy-D-aspartate (3ba): The reaction was carried out using 1b (82.4 mg, 400 µmol, 1.0 equiv) and 2a (54.2 mg, 440 µmol, 1.1 equiv) in the presence of catalyst A (15.9 mg, 40 µmol, 10 mol%), benzoic acid (48.8 mg, 400 µmol, 1.0 equiv) and 4 Å MS (200.0 mg) for 24 h. The crude product was purified by silica-gel column chromatography (eluent: hexane/EtOAc, 2:1) afforded 3ba as colorless oil (69.6 mg, 201 µmol, 50%, 91% ee).

[α]D²⁶⁺4.2 (c 0.94, CHCl₃); IR (neat) ν: 1738 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ: 7.34 (m, 10H), 6.26 (br s, 1H), 5.23 (d, J = 12.0 Hz, 1H), 5.19 (d, J = 12.0 Hz, 1H), 4.71 (d, J = 12.0 Hz, 1H), 4.68 (d, J = 12.0 Hz, 1H), 3.58 (s, 3H) ppm; ¹³C NMR (125 MHz, CDCl₃) δ: 170.6, 137.5, 128.3, 128.2 127.7, 76.3, 64.2, 60.1, 51.8, 34.3, 27.9 ppm; HRMS (ESI) m/z calcd. for C₁₆H₂₃NO₅, [M+Na⁺]: 332.1468, found: 332.1470. The enantiomeric excess was estimated by chiral HPLC analysis (DAICEL CHIRALPAK IB, eluent: hexane/2-propanol = 99/1, flow rate: 1.0 mL/min. detector: UV at 220 nm), tR = 12.0 min (minor), 10.5 min (major).
Hz, 1H), 4.09 (t, J = 7.0 Hz, 1H), 3.64 (s, 3H), 2.85 (dd, J = 16.0, 6.0 Hz, 1H), 2.70 (dd, J = 16.0, 6.0 Hz, 1H) ppm; ¹³C NMR (125 MHz, CDCl₃) δ: 171.5, 171.2, 137.4, 135.4, 128.68, 128.60, 128.4, 128.3, 128.0, 76.6, 67.2, 60.2, 52.0, 34.2 ppm; HRMS (ESI) m/z calcd. for C₁₉H₂₁NO₅, [M+Na⁺]: 366.1312, found: 366.1290. The enantiomeric excess was estimated by chiral HPLC analysis (DAICEL CHIRALPAK IB, eluent: hexane/2-propanol = 98/2, flow rate: 1.0 mL/min. detector: UV at 254 nm), tᵣ = 16.1 min (major), 17.4 min (minor).

1-Ethyl 4-methyl N-benzyloxy-D-aspartate (3ca): The reaction was carried out using 1c (57.6 mg, 400 µmol, 1.0 equiv) and 2a (54.2 mg, 440 µmol, 1.1 equiv) in the presence of catalyst A (15.9 mg, 40 µmol, 10 mol%), benzoic acid (48.8 mg, 400 µmol, 1.0 equiv) and 4 Å MS (200.0 mg) for 24 h. The crude product was purified by silica-gel column chromatography (eluent: hexane/EtOAc, 2:1) afforded 3ca as colorless oil (55.2 mg, 197.6 µmol, 49%, 94% ee).

[α]D²⁶ 6.1 (c 1.04, CHCl₃); IR (neat) ν: 3265, 1732 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ: 7.31 (m, 5H), 6.22 (br s, 1H), 4.69 (s, 2H), 4.23 (q, J = 6.8 Hz, 2H), 4.01 (t, J = 6.4 Hz, 1H), 3.68 (s, 3H), 2.81 (dd, J = 16.4, 6.4 Hz, 1H), 1.28 (t, J = 6.8 Hz, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ: 171.5, 171.2, 137.4, 128.4, 128.3, 127.8, 76.5, 61.4, 60.0, 51.9, 34.1 ppm; HRMS (ESI) m/z calcd. for C₁₄H₁₉NO₅, [M+Na⁺]: 304.1155, found: 304.1140. The enantiomeric excess was estimated by chiral HPLC analysis (DAICEL CHIRALPAK @, eluent: hexane/2-propanol = 99/1, flow rate: 1.0 mL/min. detector: UV at 254 nm), tᵣ = 16.9 min (major), 20.1 min (minor).

Methyl (R)-3-((benzyloxy)amino)-4-((S)-1-((tert-butoxy)-1-oxo-3-phenylpropan-2-yl)amino)-4-oxobutanoate (3da): The reaction was carried out using 1d (133.2 mg, 400 µmol, 1.0 equiv) and 2a (54.2 mg, 440 µmol, 1.1 equiv) in the presence of catalyst A (15.9 mg, 40 µmol, 10 mol%), benzoic acid (48.8 mg, 400 µmol, 1.0 equiv) and 4 Å MS (200.0 mg) for 24 h. The crude product was purified by silica-gel column chromatography (eluent: hexane/EtOAc, 2:1) afforded 3da as colorless oil (120.0 mg, 263 µmol, 66%, 67:33 dr).

For major diastereomer: [α]D²¹ 39.8 (c 0.51, CHCl₃); IR (neat) ν: 3381, 1730, 1675 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ: 7.34-7.14 (m, 10H), 6.19 (d, J = 5.5 Hz, 1H), 4.73 (dt, J = 7.5, 6.0 Hz, 1H), 4.68 (d, J = 12.0 Hz, 1H), 4.61 (d, J = 12.0 Hz, 1H), 3.81 (m, 1H), 3.66 (s, 3H), 3.11 (dd, J = 15.0, 6.0 Hz, 1H), 3.07 (dd, J = 15.0, 6.0 Hz, 1H), 2.87 (dd, J = 17.5, 9.0 Hz, 1H), 2.77 (dd, J = 17.0, 9.0 Hz, 1H), 1.40 (s, 9H) ppm; ¹³C NMR (125 MHz, CDCl₃) δ: 172.5, 170.4, 170.2, 137.2, 136.3, 129.6, 128.57, 128.52, 128.4, 128.1, 127.0, 82.4, 76.3, 60.5, 53.6, 52.0, 38.2, 32.2, 28.0 ppm; HRMS (ESI) m/z calcd. for C₂₅H₃₂N₂O₆, [M+H⁺]: 457.2333, found: 457.2353.

For minor diastereomer: [α]D²¹ 19.7 (c 0.60, CHCl₃); IR (neat) ν: 3388, 1732, 1674 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ: 7.33-7.17 (m, 10H), 6.18 (d, J = 6.0 Hz, 1H), 4.73 (dt, J = 7.5, 6.0 Hz, 1H), 4.62 (d, J = 11.5 Hz, 1H), 4.59 (d, J = 11.5 Hz, 1H), 3.82 (ddd, J = 8.0, 7.0, 4.5 Hz, 1H), 3.65 (s, 3H), 3.11 (dd, J = 14.0, 6.5 Hz, 1H), 3.08 (dd, J = 14.0, 6.0 Hz, 1H), 2.83 (dd, J = 12.0, 4.5 Hz, 1H), 2.71 (dd, J = 12.0, 8.0 Hz, 1H), 1.44 (s, 9H) ppm; ¹³C NMR (125 MHz, CDCl₃) δ: 172.3, 170.47, 170.42, 170.42, 136.3, 129.6, 128.57, 128.52, 128.4, 128.1, 127.0, 82.4, 76.2, 60.6, 53.6, 52.0, 38.1, 32.5, 28.0 ppm; HRMS (ESI) m/z calcd. for C₂₅H₃₂N₂O₆,
Methyl (R)-3-((benzyloxy)amino)-4-(((R)-1-(tert-butoxy)-1-oxo-3-phenylpropan-2-yl)amino)-4-oxobutanoate (3ea): The reaction was carried out using 1e (133.2 mg, 400 µmol, 1.0 equiv) and 2a (54.2 mg, 440 µmol, 1.1 equiv) in the presence of catalyst A (15.9 mg, 40 µmol, 10 mol%), benzoic acid (48.8 mg, 400 µmol, 1.0 equiv) and 4 Å MS (200.0 mg) for 24 h. The crude product was purified by silica-gel column chromatography (eluent: hexane/EtOAc, 2:1) afforded 3ea as colorless oil (128.2 mg, 280 µmol, 70%, 75:25 dr).

For major diastereomer: $\left[\alpha\right]_{D}^{21} = -15.0$ (c 0.66, CHCl3); IR (neat) $\tilde{\nu}$: 3381, 1731, 1674 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl3) $\delta$: 7.34-7.16 (m, 10H), 6.19 (br s, 1H), 4.73 (dt, $J = 7.5, 6.0$ Hz, 1H), 4.61 (d, $J = 11.5$ Hz, 1H), 4.59 (d, $J = 11.5$ Hz, 1H), 3.82 (m, 1H), 3.66 (s, 3H), 3.11 (dd, $J = 14.0, 6.5$ Hz, 1H), 3.08 (dd, $J = 14.0, 6.5$ Hz, 1H), 2.83 (dd, $J = 12.0, 4.5$ Hz, 1H), 2.71 (dd, $J = 17.0, 8.0$ Hz, 1H), 1.41 (s, 9H) ppm; $^{13}$C NMR (125 MHz, CDCl3) $\delta$: 172.3, 170.47, 170.42, 137.1, 136.2, 129.7, 128.5, 128.4, 128.1, 127.0, 82.4, 76.2, 60.6, 53.6, 52.0, 38.1, 32.5, 28.0 ppm; HRMS (ESI) $m/z$ calcd. for C$_{25}$H$_{32}$N$_2$O$_6$, [M+H]$^+$: 457.2333, found: 457.2328.

For minor diastereomer: $\left[\alpha\right]_{D}^{22} = -43.9$ (c 0.57, CHCl3); IR (neat) $\tilde{\nu}$: 3383, 1729, 1674 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl3) $\delta$: 7.32-7.14 (m, 10H), 6.19 (d, $J = 6.0$ Hz, 1H), 4.74 (dt, $J = 7.5, 6.5$ Hz, 1H), 4.65 (d, $J = 11.5$ Hz, 1H), 4.59 (d, $J = 11.5$ Hz, 1H), 3.81 (m, 1H), 3.66 (s, 3H), 3.12 (dd, $J = 14.0, 6.0$ Hz, 1H), 3.07 (dd, $J = 12.0, 4.5$ Hz, 1H), 2.87 (dd, $J = 17.0, 5.0$ Hz, 1H), 2.77 (dd, $J = 17.5, 8.0$ Hz, 1H), 1.40 (s, 9H) ppm; $^{13}$C NMR (125 MHz, CDCl3) $\delta$: 172.5, 170.4, 170.2, 137.1, 136.3, 129.5, 128.49, 128.46, 128.0, 127.0, 82.4, 76.2, 60.4, 53.6, 51.9, 38.1, 32.2, 28.0 ppm; HRMS (ESI) $m/z$ calcd. for C$_{25}$H$_{32}$N$_2$O$_6$, [M+H]$^+$: 457.2333, found: 457.2355.

Methyl (S)-3-((benzyloxy)amino)-4-(((tert-butoxycarbonylmethyl)amino)-4-oxobutanoate (3fa): The reaction was carried out using 1f (91.6 mg, 400 µmol, 1.0 equiv) and 2a (54.2 mg, 440 µmol, 1.1 equiv) in the presence of catalyst ent-A (15.9 mg, 40 µmol, 10 mol%), benzoic acid (48.8 mg, 400 µmol, 1.0 equiv) and 4 Å MS (200.0 mg) for 24 h. The crude product was purified by silica-gel column chromatography (eluent: hexane/EtOAc, 2:1) afforded 3fa as colorless oil (241.4 mg, 266 µmol, 66%, 63% ee).

$\left[\alpha\right]_{D}^{24} = -4.6$ (c 1.46, CHCl3); IR (neat) $\tilde{\nu}$: 1733, 1669 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl3) $\delta$: 7.34-7.28 (m, 4H), 7.19 (m, 1H), 6.46 (br s, 1H), 4.73 (s, 2H), 4.03 (d, $J = 5.5$ Hz, 1H), 3.88 (dd, $J = 5.0, 2.0$ Hz, 1H), 3.85 (dd, $J = 5.0, 2.0$ Hz, 1H), 3.66 (s, 3H), 2.89 (dd, $J = 17.0, 8.0$ Hz), 2.78 (dd, $J = 17.0, 8.0$ Hz), 1.46 (s, 9H) ppm; $^{13}$C NMR (125 MHz, CDCl3) $\delta$: 172.5, 170.8, 168.7, 130.6, 128.7, 128.5, 128.1, 82.3, 76.2, 60.4, 52.0, 42.0, 32.3, 28.1 ppm; HRMS (ESI) $m/z$ calcd. for C$_{18}$H$_{26}$N$_2$O$_6$, [M+H]$^+$: 367.1864, found: 367.1789. The enantiomeric excess was estimated by chiral HPLC analysis (DAICEL CHIRALPAK IB, eluent: hexane/2-propanol = 96/4, flow rate: 1.0 mL/min. detector: UV at 254 nm), $t_R = 29.6$ min (major), 27.7 min (minor).
Methyl (R)-3-((benzyloxy)amino)-4-((S)-1,3-di-tert-butoxy-1-oxopropan-2-yl)amino)-4-oxobutanoate (3ga): The reaction was carried out using 1g (126 mg, 400 µmol, 1.0 equiv) and 2a (54.2 mg, 440 µmol, 1.1 equiv) in the presence of catalyst ent-A (15.9 mg, 40 µmol, 10 mol%), benzoic acid (48.8 mg, 400 µmol, 1.0 equiv) and 4 Å MS (200.0 mg) for 24 h. The crude product was purified by silica-gel column chromatography (eluent: hexane/EtOAc, 2:1) afforded 3da as colorless oil (366.6 mg, 322 µmol, 81%, 73:27 dr).

For major diastereomer: \([\alpha]_D^{21} 33.2 (c 1.02, \text{CHCl}_3); \text{IR (neat)} \ \nu: 3403, 1735, 1679 \text{ cm}^{-1}; \ \text{1H NMR (500 MHz, CDCl}_3) \ \delta: 7.66 (d, J = 8.0 \text{ Hz, 1H}), 7.37-7.31 (m, 5H), 6.26 (d, J = 10.0 \text{ Hz, 1H}), 4.80 (dd, J = 15.0, 11.5 \text{ Hz, 1H}), 4.60 (dt, J = 9.0, 2.5 \text{ Hz, 1H}), 3.90 (dt, J = 7.5, 4.5 \text{ Hz, 1H}), 3.81 (dd, J = 17.0, 4.5 \text{ Hz, 1H}), 3.67 (s, 3H), 3.54 (dd, J = 8.5, 3.0 \text{ Hz, 1H}), 2.91 (dd, J = 17.0, 4.5 \text{ Hz, 1H}), 2.76 (dd, J = 17.0, 9.0 \text{ Hz, 1H}), 1.47 (s, 9H), 1.13 (s, 9H) \text{ ppm}; \ \text{13C NMR (125 MHz, CDCl}_3) \ \delta: 172.4, 170.5, 169.2, 137.3, 128.6, 128.5, 128.0, 81.8, 76.4, 73.1, 60.7, 53.2, 52.0, 32.6, 28.1, 27.4 \text{ ppm}; \ \text{HRMS (ESI)} \ m/z \ \text{calcd. for C}_{25}\text{H}_{32}\text{N}_2\text{O}_6, [M+H]^+: 453.2595, \text{ found: 453.2570.}

For minor diastereomer: \([\alpha]_D^{21} 1.0 (c 0.96, \text{CHCl}_3); \text{IR (neat)} \ \nu: 3405, 1737, 1679 \text{ cm}^{-1}; \ \text{1H NMR (500 MHz, CDCl}_3) \ \delta: 7.61 (d, J = 8.5 \text{ Hz, 1H}), 7.34-7.26 (m, 5H), 6.26 (d, J = 6.5 \text{ Hz, 1H}), 4.78 (dd, J = 17.0, 11.0 \text{ Hz, 2H}), 4.61 (dt, J = 8.5, 2.5 \text{ Hz, 1H}), 3.91 (ddd, J = 9.0, 6.5, 3.0 \text{ Hz, 1H}), 3.81 (dd, J = 9.0, 3.0 \text{ Hz, 1H}), 3.67 (s, 3H), 3.54 (dd, J = 9.0, 3.0 \text{ Hz, 1H}), 2.93 (dd, J = 17.5, 5.0 \text{ Hz, 1H}), 2.80 (dd, J = 17.5, 9.0 \text{ Hz, 1H}), 1.47 (s, 9H), 1.12 (s, 9H) \text{ ppm}; \ \text{13C NMR (125 MHz, CDCl}_3) \ \delta: 172.4, 170.5, 169.3, 137.3, 128.5, 128.4, 128.0, 81.9, 76.4, 73.1, 60.7, 53.2, 51.9, 32.8, 28.1, 27.4 \text{ ppm}; \ \text{HRMS (ESI)} \ m/z \ \text{calcd. for C}_{25}\text{H}_{32}\text{N}_2\text{O}_6, [M+H]^+: 453.2595, \text{ found: 453.2524.}

1-tert-Butyl 4-methyl N-(4-methoxybenzyl)oxy-D-aspartate (3ab): The reaction was carried out using 1a (68.8 mg, 400 µmol, 1.0 equiv) and 2b (67.4 mg, 440 µmol, 1.1 equiv) in the presence of catalyst A (15.9 mg, 40 µmol, 10 mol%), benzoic acid (48.8 mg, 400 µmol, 1.0 equiv) and 4 Å MS (200.0 mg) for 24 h. The crude product was purified by silica-gel column chromatography (eluent: hexane/EtOAc, 2:1) afforded 3ab as colorless oil (101.8 mg, 300 µmol, 75%, 89% ee).

\([\alpha]_D^{23} 8.2 (c 0.68, \text{CHCl}_3); \text{IR (neat)} \ \nu: 1732 \text{ cm}^{-1}; \ \text{1H NMR (500 MHz, CDCl}_3) \ \delta: 7.26 (t, J = 7.0 \text{ Hz, 2H}), 6.86 (d, J = 7.0 \text{ Hz, 2H}), 6.12 (br s, 1H), 4.61 (s, 2H), 3.80 (s, 3H), 3.68 (s, 3H), 2.75 (dd, J = 16.0, 7.5 \text{ Hz, 1H}), 2.62 (dd, J = 16.0, 7.5 \text{ Hz, 1H}), 1.46 (s, 9H) \text{ ppm}; \ \text{13C NMR (125 MHz, CDCl}_3) \ \delta: 171.4, 170.7, 159.4, 130.1, 129.7, 113.7, 82.1, 76.1, 60.7, 55.3, 51.9, 34.4, 28.0 \text{ ppm}; \ \text{HRMS (ESI)} \ m/z \ \text{calcd. for C}_{17}\text{H}_{25}\text{NO}_6, [M+H]^+: 340.1755, \text{ found: 340.1744.}\) The enantiomeric excess was estimated by chiral HPLC analysis (DAICEL CHIRALPAK IB, eluent: hexane/2-propanol = 98/2, flow rate: 1.0 mL/min. detector: UV at 254 nm), \(t_R = 13.3 \text{ min (major), 14.1 min (minor).}\)
1-tert-Butyl 4-methyl N-(4-trifluoromethyl)benzoyloxy-D-aspartate (3ac): The reaction was carried out using 1a (34.4 mg, 200 µmol, 1.0 equiv) and 2c (42.0 mg, 220 µmol, 1.1 equiv) in the presence of catalyst A (7.9 mg, 20 µmol, 10 mol%) and benzoic acid (48.8 mg, 400 µmol, 1.0 equiv) and 4 Å MS (200.0 mg) for 24 h. The crude product was purified by silica-gel column chromatography (eluent: hexane/EtOAc, 3:1) afforded 3ac as colorless oil (48.2 mg, 128 µmol, 64%, 96% ee).

\[\alpha\] D 6.6 (c 1.90, CHCl3); IR (neat) \(\nu\): 1732, 1324 cm\(^{-1}\); 1H NMR (400 MHz, CDCl3) \(\delta\): 7.60 (d, \(J = 8.0\) Hz, 1H), 7.44 (d, \(J = 6.0\) Hz, 1H), 6.25 (d, \(J = 6.0\) Hz, 1H), 4.75 (s, 2H), 4.75 (s, 2H), 3.75 (s, 3H), 2.75 (dd, \(J = 16.0, 6.5\) Hz, 1H), 2.61 (dd, \(J = 16.0, 6.5\) Hz, 1H), 1.48 (s, 9H) ppm; 13C NMR (125 MHz, CDCl3) \(\delta\): 171.1, 170.5, 141.7, 129.9 (q, \(J = 32.6\) Hz), 128.2, 125.2 (q, \(J = 3.7\) Hz), 124.1 (q, \(J = 270\) Hz), 82.2, 75.4, 60.7, 51.8, 51.7, 34.2, 27.9 ppm; HRMS (ESI) m/z calcd. for C\(_{17}\)H\(_{22}\)NO\(_5\)F\(_3\), [M+Na]+: 400.1342, found: 400.1333. The enantiomeric excess was estimated by chiral HPLC analysis (DAICEL CHIRALPAK IB, eluent: hexane/2-propanol = 99/1, flow rate: 1.0 mL/min. detector: UV at 254 nm), \(t_R = 15.8\) min (minor), 20.2 min (major).

1-tert-Butyl 4-methyl N-(benzloyloxy)methoxy-D-aspartate (3ad): The reaction was carried out using 1a (34.4 mg, 200 µmol, 1.0 equiv) and 2d (36.7 mg, 220 µmol, 1.1 equiv) in the presence of catalyst A (7.9 mg, 20 µmol, 10 mol%) and benzoic acid (48.8 mg, 400 µmol, 1.0 equiv) and 4 Å MS (200.0 mg) for 24 h. The crude product was purified by silica-gel column chromatography (eluent: hexane/EtOAc, 3:1) afforded 3ad as colorless oil (40.0 mg, 122 µmol, 60%, 87% ee).

\[\alpha\] D 4.4 (c 1.06, CHCl3); IR (neat) \(\nu\): 1731 cm\(^{-1}\); 1H NMR (400 MHz, CDCl3) \(\delta\): 7.31 (m, 5H), 6.43 (d, \(J = 6.4\) Hz, 1H), 4.86 (s, 2H), 4.86 (s, 2H), 3.98 (dt, \(J = 10.0, 6.4\) Hz, 1H), 3.69 (s, 3H), 2.77 (dd, \(J = 16.4, 6.4\) Hz, 1H), 2.67 (dd, \(J = 16.4, 6.4\) Hz, 1H), 1.48 (s, 9H) ppm; 13C NMR (100 MHz, CDCl3) \(\delta\): 171.2, 170.6, 137.7, 128.4, 127.79, 127.67, 82.1, 69.9, 60.8, 51.8, 34.4, 27.9 ppm; HRMS (ESI) m/z calcd. for C\(_{17}\)H\(_{25}\)NO\(_6\), [M+Na]+: 362.1574, found: 362.1588. The enantiomeric excess was estimated by chiral HPLC analysis (DAICEL CHIRALPAK IB, eluent: hexane/2-propanol = 98/2, flow rate: 1.0 mL/min. detector: UV at 220 nm), \(t_R = 16.1\) min (major), 17.4 min (minor).

1-tert-Butyl 4-methyl N-(2-trimethylsilyloxy)methoxy-D-aspartate (3ae): The reaction was carried out using 1a (34.4 mg, 200 µmol, 1.0 equiv) and 2e (33.6 mg, 220 µmol, 1.1 equiv) in the presence of catalyst A (7.9 mg, 20 µmol, 10 mol%), benzoic acid (48.8 mg, 400 µmol, 1.0 equiv) and 4 Å MS (200.0 mg) for 24 h. The crude product was purified by silica-gel column chromatography (eluent: hexane/EtOAc, 3:1) afforded 3ae as colorless oil (48.9 mg, 142 µmol, 70%). Since 3ae was not detectable on HPLC, N-benzylation was conducted to estimate the ee of 3ae.

\[\alpha\] D +4.3 (c 1.00, CHCl3); IR (neat) \(\nu\): 1738 cm\(^{-1}\); 1H NMR (400 MHz, CDCl3) \(\delta\): 6.36 (d, \(J = 6.4\) Hz, 1H), 4.75 (s, 2H), 3.94 (dt, \(J = 12.8\) Hz, 6.4 Hz), 3.68 (s, 3H), 3.62 (t, \(J = 8.4\) Hz, 2H), 1.45 (s, 9H), 0.94 (t, \(J = 8.4\) Hz, 2H) ppm; 13C NMR (125 MHz, CDCl3) \(\delta\): 171.2, 170.6, 97.7, 82.0, 65.7, 60.8, 51.8, 34.4, 27.9, 18.1 ppm; HRMS (ESI) m/z calcd. for C\(_{15}\)H\(_{31}\)NO\(_6\)Si, [M+Na]+: 372.1813, found: 372.1820.
1-tert-Butyl 4-methyl N-benzoyl-N-(2-trimethylsilylethoxy)methoxy-D-aspartate (3ae): To a solution of 3ae (34.9 mg, 100 μmol, 1.0 equiv) in EtOAc (1mL) were added BzCl (21.1 mg, 150 µmol, 1.5 equiv) and sat. NaHCO₃ aq. (1 mL) at 0 ºC. The mixture was allowed to warm to room temperature and stirred for 3 h. Added brine (2 mL) and extracted with EtOAc (5 mL, 2 times). The crude product was purified by silica-gel column chromatography (eluent: hexane/EtOAc, 5:1) afforded 3ae’ as colorless oil (38.8 mg, 85 μmol, 85%).

\[ \alpha \] D 25 0.71 (c 5.84, CHCl3); IR (neat) ν: 1736, 1648 cm⁻¹; 1H NMR (500 MHz, CDCl3) δ: 7.67 (d, J = 7.0 Hz, 2H), 7.46 (t, J = 7.0 Hz, 1H), 7.41 (t, J = 7.0 Hz, 2H) 5.06 (br, 1H), 4.85 (br, 2H), 3.73 (s, 3H), 3.49 (s, 2H), 3.21 (dd, J = 17.0, 7.0 Hz, 1H), 2.97 (dd, J = 17.0, 7.0 Hz, 1H), 1.48 (s, 9H), 0.85 (t, J = 8.0 Hz, 2H), 0.018 (s, 9H) ppm; 13C NMR (125 MHz, CDCl 3) δ: 198.7, 171.3, 167.6, 134.2, 130.9, 128.3, 128.2, 99.6, 82.9, 67.7, 61.1, 52.0, 34.0, 27.9, 17.9 ppm; HRMS (ESI) m/z calcd. for C22H35NO7Si, [M+H]+: 453.2111, found: 453.2183. The enantiomeric excess was estimated by chiral HPLC analysis (DAICEL CHIRALPAK IB, eluent: hexane/2-propanol = 1, flow rate: 1.0 mL/min. detector: UV at 254 nm), tR = 18.9 min (major), 23.7 min (minor).

1-Benzyl 4-methyl N-(2-trimethylsilylethoxy)methoxy-L-aspartate (3be): The reaction was carried out using 1b (82.5 mg, 400 μmol, 1.0 equiv) and 2e (67.3 mg, 440 µmol, 1.1 equiv) in the presence of catalyst ent-A (15.9 mg, 40 μmol, 10 mol%), benzoic acid (48.8 mg, 400 µmol, 1.0 equiv) and 4 Å MS (200.0 mg) for 24 h. The crude product was purified by silica -gel column chromatography (eluent: hexane/EtOAc, 3:1) afforded 3be as colorless oil (268.4 mg, 284 μmol, 70%, 86% ee).

\[ \alpha \] D 26 +4.7 (c 1.00, CHCl3); IR (neat) ν: 1739 cm⁻¹; 1H NMR (500 MHz, CDCl3) δ: 7.35 (m, 5H), 6.42 (d, J = 7.0 Hz, 1H), 5.21 (s, 2H), 4.76 (s, 2H), 4.12 (dt, J = 7.0, 6.0Hz, 1H), 3.65 (s, 3H), 3.62 (dt, J = 8.5, 1.0 Hz, 1H), 2.85 (dd, J = 16.0, 6.0Hz, 1H) 2.74 (dd, J = 16.0, 6.0 Hz, 1H), 0.94 (dt, J = 8.5, 1.2 Hz, 2H), 0.01 (s, 9H) ppm; 13C NMR (125 MHz, CDCl3) δ: 171.4, 170.9, 135.2, 128.5, 128.3, 128.2, 97.8, 67.1, 65.8, 60.2, 51.9, 34.1, 18.0 ppm; HRMS (ESI) m/z calcd. for C18H29NO6Si, [M+Na]+: 406.1656, found: 406.1643. The enantiomeric excess was estimated by chiral HPLC analysis (DAICEL CHIRALPAK IB, eluent: hexane/2-propanol = 99/1, flow rate: 1.0 mL/min. detector: UV at 254 nm), tR = 16.4 min (major), 18.2 min (minor).

1-tert-Butyl 4-methyl 9H-fluoren-9-ylmethoxycarbonyl-D-aspartate (S3aa): 3aa (25.3 mg 100 µmol, 1.0 equiv) was dissolved in MeOH (30 mL) and added to 10% Pd/C (80 mg) under an atmosphere of argon. The reaction was carefully flushed with hydrogen gas and stirred at room temperature for 5 h. The atmosphere was replaced with argon before filtration through a pad of Celite®. The filtrate was concentrated under reduced pressure and the residue was dissolved in CH₂Cl₂ (1.0 mL). To the solution were added Pr₂NEt (64.6 mg, 500 µmol, 5.0 equiv) and FmocCl (25.8 mg, 120 µmol, 1.2 equiv). The resulting mixture was stirred at ambient temperature for 17 h, then the solvent was evaporated. The residue was purified by silica-gel column chromatography (eluent:
hexane/EtOAc, 1:1) to afford S3aa as colorless oil (26.0 mg, 61 μmol, 61%).

\[ \alpha \] D 23 -7.1 (c 0.73, CHCl₃); IR (neat) \( \tilde{\nu} \): 3368, 1721 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) \( \delta \): 7.75 (d, \( J = 7.5 \) Hz, 2H), 7.60 (d, \( J = 7.5 \) Hz, 2H), 7.40 (t, \( J = 7.5 \) Hz, 2H), 5.80 (d, \( J = 8.5 \) Hz, 1H), 4.54 (dt, \( J = 8.5, 4.5 \) Hz, 1H), 4.38 (dt, \( J = 17.0, 7.0 \) Hz, 2H), 4.23 (t, \( J = 7.0 \) Hz, 1H), 3.71 (s, 3H), 3.00 (dd, \( J = 17.0, 4.0 \) Hz, 1H), 1.48 (s, 9H) ppm; ¹³C NMR (125 MHz, CDCl₃) \( \delta \): 171.3, 169.6, 156.0, 143.9, 143.8, 141.3, 127.8, 127.1, 125.2, 120.0, 82.7, 67.2, 52.0, 51.0, 47.2, 36.8, 27.9 ppm; HRMS (ESI) m/z calcld. for 448.1731, [M+Na]+ found: 448.1757. The enantiomeric excess was estimated by chiral HPLC analysis (DAICEL CHIRALPAK IA, eluent: hexane/2-propanol = 96/4, flow rate: 1.0 mL/min. detector: UV at 254 nm), \( t_R \) = 20.3 min (major), 26.6 min (minor).

(D) O-Deprotection and KAHA Ligation

1-Benzyl 4-methyl N-hydroxy-L-aspartate (4b): To a solution of 3be (192.7 mg, 500 μmol, 1.0 equiv) in CH₂Cl₂ (5 mL) was added TFA (172.5 mg, 1.5 mmol, 3.0 equiv) at room temperature and stirred for 1 h. H₂O (5 mL) was added and further stirred at ambient temperature for 1 h, and the mixture was dried over Na₂SO₄. The solids were filtered off and the solvent was removed under reduced pressure. The residue was then purified by silica-gel column chromatography (eluent: hexane/EtOAc, 1:2) to afford 4b as colorless oil (53.1 mg, 210 μmol, 42%). Since 4b was gradually degraded on standing, 4b was used for KAHA ligation soon after purification.

\(^1\)H NMR (500 MHz, CDCl₃) \( \delta \): 7.32 (m, 5H), 5.22 (s, 2H), 4.06 (dt, \( J = 8.0, 5.0 \) Hz, 1H), 3.66 (s, 3H), 2.87 (dd, \( J = 16.0, 5.0 \) Hz, 1H), 2.80 (dd, \( J = 16.0, 5.0 \) Hz, 1H) ppm.

1-Benzyl 4-methyl (3-phenylpropanoyl)-L-aspartate (6b): A mixture of 4b (23.3 mg, 92 µmol, 1.0 equiv) and 2-oxo-4-phenylbutanoic acid (5) (16.3 mg, 92 µmol, 1.0 equiv) in DMSO/H₂O (9:1, 200 µL) was stirred at 40 ºC for 24 h. The crude mixture was extracted with EtOAc (2 mL, 3 times) and then purified by silica-gel column chromatography (eluent: hexane/EtOAc, 1:2) to afford 6b as yellow oil (26.1 mg, 70 μmol, 60%, 82% ee).

\[ \alpha \] D 24 -13.2 (c 1.75, CHCl₃); IR (neat) \( \tilde{\nu} \): 3311, 1732, 1652 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) \( \delta \): 7.38-7.30...
(m, 6H), 7.27-7.24 (m, 1H), 7.19-7.17 (m, 3H), 6.42 (d, J = 8.0 Hz, 1H), 5.17 (dd, J = 28.0, 12.5 Hz, 2H), 4.88 (dt, J = 8.0, 4.0 Hz, 1H), 3.00 (dd, J = 17.5, 4.0 Hz, 1H), 2.95 (t, J = 8.0 Hz, 2H), 2.75 (dd, J = 17.5, 4.0 Hz, 1H), 2.59 (ddd, J = 18.0, 15.0, 8.0 Hz, 1H), 2.49 (ddd, J = 18.0 Hz, 15.0, 8.0 Hz/ppm; \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\): 171.8, 171.4, 170.5, 140.5, 135.1, 128.55, 128.45, 128.26, 126.1, 67.5, 51.9, 48.3, 38.0, 36.0, 31.4 ppm; HRMS (ESI) \(m/z\) calcd. For C\(_{21}\)H\(_{23}\)NO\(_5\), [M+H\]^+\): 370.1649, found: 370.1676. The enantiomeric excess was estimated by chiral HPLC analysis (DAICEL CHIRALPAK IB, eluent: hexane/2-propanol = 99/1, flow rate: 1 mL/min. detector: UV at 254 nm), \(t_R = 18.2\) min (minor), 16.4 min (major).

**1-Benzyl 4-methyl 9\(H\)-fluoren-9-ylmethoxycarbonyl-L-leucyl-L-aspartate (8b):** A mixture of 4b (60.1 mg, 156 \(\mu\)mol, 1.0 equiv) and (S)-3-(9\(H\)-fluoren-9-ylmethoxycarbonyl)amino)-5-methyl-2-oxohexanoic acid (7) (57.1 mg, 150 \(\mu\)mol, 1.0 equiv) in DMSO/H\(_2\)O (9:1, 15 mL) was stirred at 40 °C for 15 h. The crude mixture was extracted with EtOAc (2 mL, 3 times) and then purified by silica-gel column chromatography (eluent: hexane/EtOAc, 1:2) to afford 8b as yellow oil (41.2 mg, 72 \(\mu\)mol, 50%, 93:7 dr). \([\alpha]_D^{23}\) -3.20 (c 1.20, CHCl\(_3\)); IR (neat) \(\tilde{\nu}\): 3314, 1738, 1661 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\): 7.75 (d, \(J = 7.0\) Hz, 2H), 7.57 (d, \(J = 5.5\) Hz, 2H), 7.39-7.29 (m, 9H), 6.95 (d, \(J = 8.0\) Hz, 1H), 5.34 (d, \(J = 8.0\) Hz, 1H), 5.15 (dd, \(J = 21.0, 12.0\) Hz, 2H), 4.91 (t, \(J = 4.0\) Hz, 1H), 4.36 (m, 2H) 4.21 (m, 2H), 3.58 (s, 3H), 3.05 (dd, \(J = 17.0, 3.5\) Hz, 1H), 2.81 (dd, \(J = 17.0, 3.5\) Hz, 1H), 1.63 (m, 2H), 1.50 (m, 1H), 0.89 (s, 6H) ppm; \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\): 172.2, 171.5, 170.3, 156.1, 143.9, 143.8, 141.3, 135.1, 128.69, 128.60, 128.4, 127.8, 127.1, 125.1, 120.0, 67.7, 67.1, 53.4, 52.1, 48.5, 41.9, 36.0, 24.6, 22.9, 22.0 ppm; HRMS (ESI) \(m/z\) calcd. For C\(_{33}\)H\(_{36}\)N\(_2\)O\(_7\), [M+Na\]^+\): 595.2415, found: 595.2396.
(E) References

(F) $^1$H NMR and $^{13}$C NMR Spectra
$\text{BnO}_2\text{C} \rightleftharpoons \text{CO}_2\text{H}$

1b

$X$: parts per Million: Proton
BnO₂C

\[ \text{CO}_2\text{H} \]

1b

X: parts per Million: Carbon13

S23
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https://repository.kulib.kyoto-u.ac.jp

X : parts per Million : 13C
A Self-archived copy in
Kyoto University Research Information Repository
https://repository.kulib.kyoto-u.ac.jp

\[
\text{S1e}
\]

\[
\begin{array}{c}
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\text{N} \\
\text{Ph} \\
\text{CO}_2\text{Et}
\end{array}
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\[X \text{: parts per Million : } ^{13}\text{C}\]
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A Self-archived copy in
Kyoto University Research Information Repository
https://repository.kulib.kyoto-u.ac.jp

X: parts per Million: 13C
A Self-archived copy in
Kyoto University Research Information Repository
https://repository.kulib.kyoto-u.ac.jp

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BnO<sub>2</sub>NH

EtO<sub>2</sub>C<sup>−</sup>CO<sub>2</sub>Me

3ca

X: parts per Million: Proton
$\text{BnO}^+\text{NH}$

$\text{EtO}_2\text{C} - \text{CO}_2\text{Me}$

$3ca$
CO₂Me
H
O
N
₄Bu₂C
H
NH
3fa

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X: parts per Million: III

S62
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Kyoto University Research Information Repository
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X : parts per Million : 13C
A Self-archived copy in Kyoto University Research Information Repository
https://repository.kulib.kyoto-u.ac.jp

![Chemical structure](image)

**3ga-minor**

![NMR spectrum](image)

X: parts per Million: 13C
MeO
\[\text{O} \quad \text{NH} \quad \text{CO}_2\text{Me}\]
\[\text{BuO}_2\text{C} \quad \text{CO}_2\text{Me}\]

3ab

X: parts per Million: 13C
$\text{BnO} - \text{O} - \text{NH}$

$\text{tBuO}_2\text{C} - \text{CO}_2\text{Me}$

$3\text{ad}$
BnO $\overset{\text{NH}}{\rightleftharpoons}$ tBuO$_2$C $\overset{\text{CO$_2$Me}}{\rightleftharpoons}$

3ad

$\text{X: parts per Million : Carbon13}$
X : parts per Million : 111
A Self-archived copy in Kyoto University Research Information Repository
https://repository.kulib.kyoto-u.ac.jp
(G) HPLC Traces

![HPLC Traces Diagram](https://repository.kulib.kyoto-u.ac.jp)

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