Biocatalytic asymmetric synthesis of unnatural amino acids using C-N lyases
Fu, Haigen

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Rapid Chemoenzymatic Route to Glutamate Transporter Inhibitor L-TFB-TBOA and Related Amino Acids

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Abstract

The complex amino acid (L-\textit{threo})-3-{3-[4-(trifluoromethyl)benzoylamino]benzyloxy} aspartate (L-TFB-TBOA) and its derivatives are privileged compounds for studying the roles of excitatory amino acid transporters (EAATs) in regulation of glutamatergic neurotransmission, animal behavior, and in the pathogenesis of neurological diseases. The widespread use of L-TFB-TBOA stems from its high potency of EAAT inhibition and the lack of off-target binding to glutamate receptors. However, one of the main challenges in the evaluation of L-TFB-TBOA and its derivatives is the laborious synthesis of these compounds in stereoisomerically pure form. Here, we report an efficient and step-economic chemoenzymatic route that gives access to enantio- and diastereopure L-TFB-TBOA and its derivatives at multigram scale.

Keywords

Asymmetric synthesis, biocatalysis, amino acids, inhibitor, glutamate transport.
L-Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (CNS) and mediates numerous neuronal communications in the brain. However, accumulation of high levels of extracellular glutamate may lead to over-activation of glutamate-gated ion channels and, consequently, neuronal injury. Synaptic glutamate concentrations are strictly kept below levels of neurotoxicity by the family of excitatory amino acid transporters (EAATs) expressed on neurons and surrounding glial cells. Dysfunction of EAATs has been implicated in many neurological disorders, such as Alzheimer’s disease, epilepsy, amyotrophic lateral sclerosis, and Huntington’s disease.

L-Aspartate derivatives with aryloxy substituents at the C3 position, exemplified by (L-threo)-3-benzyloxyaspartate (1, L-TBOA, Scheme 1), were identified as the first class of non-transportable EAATs inhibitors. The importance of these L-aspartate derivatives as tools in neurobiological research was further highlighted by the identification of the most potent and widely used blocker, (L-threo)-3-[3-[4-(trifluoromethyl)benzoylamino]benzyloxy]aspartate (2a, L-TFB-TBOA, Scheme 1), which has nanomolar affinity to EAAT1 and EAAT2 and lacks affinity towards other glutamate receptors and transporters. However, the asymmetric synthesis of enantiopure L-TFB-TBOA and related compounds proved to be extremely challenging. Shimamoto and coworkers reported the asymmetric synthesis of enantiopure 2a through an elaborate 20-step synthetic procedure. Although a concise synthesis of 2a based on Sharpless aminohydroxylation with chiral ligand (DHQD)2PHAL was recently reported by Leuenberger et al., this procedure provided 2a with only 40% enantiomeric excess (ee). Efficient asymmetric synthesis of enantiopure L-TFB-TBOA and its derivatives would facilitate the further discovery of EAAT subtype-selective inhibitors, which are highly useful tools for the elucidation of the exact physiological roles of distinct EAATs in glutamate accumulation, synaptic transmission and animal behavior. Hence, alternative procedures that provide efficient and more step-economic access to 2a and related compounds are in great demand. Herein, we report a rapid chemoenzymatic route that gives convenient access to enantiopure L-TFB-TBOA and its derivatives at multigram scale. This method uses the late functionalization of a common precursor to provide a convenient way of divergent preparation of L-aspartate derivatives with large aryloxy substituents at the C3 position (Scheme 1).
Results and discussion

Retrosynthetic analysis suggests that L-TFB-TBOA (2a) could be derived from dimethyl (L-threo)-N-Boc-3-hydroxyaspartate (4) and substituted benzyl bromide 5a via O-alkylation and subsequent deprotection (Scheme 1). The key challenge was the formation of chiral building block 4 due to the possible difficulties in constructing the required L-threo configuration at vicinal chiral centers with a 1,2-aminoalcohol motif.17-21 We envisioned that this key precursor 4 could be readily generated from 1, which has the desired L-threo configuration, via protection and hydrogenolysis steps. While the chemical synthesis of 1 is a highly challenging 11-step procedure,10 a straightforward three-step chemoenzymatic methodology for the asymmetric synthesis of 1 (de >98%, ee >99%), starting from commercially available dimethyl acetylenedicarboxylate 3, has recently been reported (Scheme 2A).22,23

Scheme 1. Chemoenzymatic retrosynthesis of L-TFB-TBOA (2a).

To enable efficient multigram-scale synthesis of 1 (see SI), we first optimized the previously used procedure for the small-scale (150 mg) synthesis of 1.23 The addition of benzyl alcohol to 3 yields a mixture of cis and trans product isomers (Scheme 2A, step a). After ester hydrolysis of this isomeric mixture (step b), we purified the trans-2-benzyl oxyfumaric acid (6) by recrystallization (37% yield over two steps). This provided a higher yield of 6 when compared to the previously reported column chromatography method for isomer
separation. In addition, the efficiency of the methylaspartate ammonia lyase (MAL-L384A)-catalyzed amination of 6 (step c) was enhanced by using NH\textsubscript{3} (instead of NH\textsubscript{4}Cl) and pH 9.5 (instead of pH 9.0), leading to >98% conversion and affording product 1 in 80% isolated yield.

Starting from compound 1, the synthesis of key intermediate 4 was achieved in three successive reactions (steps d-f in Scheme 2B). Diesterification of 1 with SOCl\textsubscript{2} in dry methanol, followed by Boc-protection, delivered compound 8 without the need for purification. Hydrogenolysis of 8 using HCOONH\textsubscript{4}/Pd gave 4 in excellent yield (71% over three steps). Notably, exhaustive esterification of both carboxyl groups of compound 1 needs high SOCl\textsubscript{2} concentration (10 equivalents) under reflux, while mono-esterification of the C4-carboxyl group of 1 could be easily accomplished under ambient conditions with one equivalent of SOCl\textsubscript{2}. Another route (steps g-i in Scheme 2B) for preparing the chiral building block 4 was also designed by rearranging the three reactions described above. We initiated the synthetic procedure with debenzylation of 1, which provided 3-hydroxyaspartic acid (9), and which was followed by esterification and Boc-protection to afford 4 in three steps with 73% overall yield.

Scheme 2. Chemoenzymatic synthesis of L-TBOA (1) and chiral building block 4. Reagents and conditions: a) BnOH, DABCO, DCM, rt, 4 h. b) NaOH (2 M), reflux, 2h, then HCl (1 M), two-step yield after recrystallization was 37%. c) MAL-L384A (0.01 mol%), 5 M NH\textsubscript{3}/NH\textsubscript{4}Cl, 20 mM MgCl\textsubscript{2}, pH = 9.5, 24 h, conversion >98%, isolated yield 80%. d) SOCl\textsubscript{2}, MeOH, reflux, 6 h. e) di-tert-butyl dicarbonate, DIAEA, DCM, rt, 24 h. f) Pd/C, HCOONH\textsubscript{4}, MeOH, reflux, 45 min, 71% for 3 steps. g) Pd/C, HCOONH\textsubscript{4}, MeOH, reflux, 45 min. h) SOCl\textsubscript{2}, MeOH, reflux, 6 h. i) di-tert-butyl dicarbonate, DIAEA, DCM, rt, 24 h, 73% for 3 steps.
With chiral building block 4 in hand, the synthesis of target molecule L-TFB-TBOA (2a) could be accomplished through O-alkylation, followed by global deprotection (steps a-c in Scheme 3). This strategy provides a general synthesis route towards derivatives of L-threo-3-hydroxyaspartic acid, including valuable analogs of L-TBOA and L-TFB-TBOA. To facilitate efficient O-alkylation, which is effected by a nucleophilic substitution reaction of 4 and 5 (for synthesis details, see SI), the strong base NaH was used to deprotonate the hydroxyl group of 4. A low temperature (-20 °C) was needed to avoid epimerization, and the desired compound 11 (i.e., globally protected 2a) was obtained with an isolated yield of 45%. Subsequently, global deprotection of 11 was conducted via treatment with TFA and followed by hydrolysis with LiOH, providing the desired final product 2a (L-TFB-TBOA hydrochloride) in a yield of 59% over two deprotection steps.

As anticipated, product 2a was identified as the desired threo isomer (de >98%) by comparison of its 1H-NMR signals and J-coupling values to those of an authentic standard (commercially available L-TFB-TBOA) and chemically synthesized DL-threo and DL-erythro stereoisomers (Table S1). To determine the absolute configuration of product 2a, chiral HPLC analysis was conducted by using the authentic standard L-TFB-TBOA and chemically synthesized DL-TFB-TBOA as reference molecules. This analysis revealed that the chemoenzymatically produced 2a is present as a single enantiomer with exclusively the L-threo configuration (ee >99%, Table 1, Figure S2). The usefulness of our synthesis strategy was further demonstrated by the preparation of optically pure 2a at multigram-scale (2.1 g; see SI). In addition, two novel optically pure L-TFB-TBOA analogs, 2b (o-CF₃, de >98%, ee >99%) and 2c (m-CF₃, de >98%, ee >99%), were prepared using this newly developed methodology (Table 1).

Medicinal chemists are highly interested in building a large collection of meta-substituted analogs of L-TBOA to screen for potent and selective inhibitors of EAAT subtypes.12,25 In order to rapidly construct a library of L-TBOA analogs with various groups at the meta-position, we envisioned that intermediate 13, with a free m-NH₂ group, would be a convenient precursor for fast structural diversification by applying combinatorial chemistry methodologies. The synthesis of compound 13 was accomplished through a nucleophilic substitution reaction between 4 and m-nitrobenzylbromide, yielding 12, followed by conversion of the nitro group to an amino group with Pd/C/H₂, in a yield of 59% over two steps. To demonstrate the utility of intermediate 13, we synthesized two L-TFB-TBOA analogs (16a-b) with a longer alkyl spacer between the two phenyl rings (Scheme 3). Compound 15 was formed via amidation of the amino group of 13 with acyl chloride 14. Target products 16a and 16b were obtained in optically pure form after two steps of deprotection (Table 1).
Scheme 3. Synthesis of L-TFB-TBOA and its derivatives. Reagents and conditions: a) ArCH₂Br (5, see SI), NaH, DMF, -20 °C, 4 h, 42% - 50%. b) TFA/DCM (2:5, v/v), 0 °C, 1.5 h. c) THF/H₂O (1:1, v/v), LiOH, rt, 2 h, then HCl (1 M), 38% - 59%. d) 3-nitrobenzylbromide, NaH, DMF, -20 °C, 4 h, 61%. e) Pd/C, H₂, MeOH, 25 min, 92%. f) acyl chlorides (14, see SI), TEA, DCM, rt, 2 h, 51% - 65%.

Table 1. Absolute configuration of chemoenzymatically obtained L-TFB-TBOA and its derivatives.

<table>
<thead>
<tr>
<th>Entry</th>
<th>No.</th>
<th>R</th>
<th>n</th>
<th>de[^a] [%]</th>
<th>ee[^b] [%]</th>
<th>Abs. config.</th>
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<tr>
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<td>2a</td>
<td>p-CF₃</td>
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<td>(2S, 3S)^c</td>
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<td>2b</td>
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<td>&gt;98</td>
<td>&gt;99</td>
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</tr>
<tr>
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<td>2c</td>
<td>m-CF₃</td>
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<td>&gt;98</td>
<td>&gt;99</td>
<td>(2S, 3S)^c</td>
</tr>
<tr>
<td>4</td>
<td>16a</td>
<td>p-CF₃</td>
<td>1</td>
<td>&gt;98</td>
<td>&gt;99</td>
<td>(2S, 3S)^c</td>
</tr>
<tr>
<td>5</td>
<td>16b</td>
<td>p-CF₃</td>
<td>2</td>
<td>&gt;98</td>
<td>&gt;99</td>
<td>(2S, 3S)^c</td>
</tr>
</tbody>
</table>

[^a]: Diastereomeric excess (de) was determined by ¹H NMR.  
[^b]: Enantiomeric excess (ee) was determined by chiral HPLC (Figures S2-6).  
[^c]: Absolute configuration of 2a was determined unambiguously by comparison of ¹H NMR, chiral HPLC and optical rotation data to those of an authentic sample of L-TFB-TBOA (2S,3S).  
[^d]: Products 2a-b and 16a-b were assigned as (2S,3S)-configuration on the basis of analogy.
Conclusion

In conclusion, we have managed to construct the complex amino acid L-TFB-TBOA using only 9 steps with 6% overall yield, starting from commercially available dimethyl acetylenedicarboxylate. Compared with the previously reported 20-step synthesis of L-TFB-TBOA, this is a dramatic reduction in step count with fewer than half the steps. This chemoenzymatic synthesis methodology can be easily up-scaled to multigram-scale and gives convenient access to enantiopure derivatives of L-TBOA and L-TFB-TBOA.

Acknowledgements

This research was financially supported by the Netherlands Organisation for Scientific Research (KIEM grants 731.013.110 and 731.015.108). Haigen Fu and Jielin Zhang acknowledge funding from the China Scholarship Council. The authors thank Dr. Marianne de Villiers for insightful discussions.

Abbreviations

Boc, t-butoxy carbonyl; DABCO, 1,4-diazabicyclo[2.2.2]octane; DCM, dichloromethane; DIEA, N,N-diisopropylethylamine; (DHQD)$_2$PHAL, hydroquinidine 1,4-phthalazinediyl diether; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

Experimental section

For detailed experimental procedures and characterization of compounds, see Supplementary Information.

Notes

The authors declare no competing financial interest.
References


Supplementary Information

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I) General information

All chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) or Thermo Fisher Scientific Co. unless stated otherwise. Solvents were purchased from Biosolve (Valkenswaard, The Netherlands) or Sigma-Aldrich Chemical Co. The boiling point of the petroleum ether used for chemical purification was 40–60 °C. Authentic sample of L-TFB-TBOA was purchased from Tocris Bioscience. Ingredients for buffers and media were obtained from Duchefa Biochemie (Haarlem, The Netherlands) or Merck (Darmstadt, Germany). A previously engineered variant of methylyaspartate ammonia lyase (MAL-L384A) was overproduced in E. coli and purified as described previously.\(^1\) Ni-Sepharose 6 fast flow resin and prepacked PD-10 Sephadex G-25 columns for protein purification were purchased from GE Healthcare Bio-Sciences (Little Chalfont, UK). Proteins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing conditions on gels containing 10% polyacrylamide. The gels were stained with Coomassie brilliant blue. High performance liquid chromatography (HPLC) was performed with a Shimadzu LC-10AT HPLC with a Shimadzu SP-M10A ELSD detector. NMR analyses were performed on a Varian Inova 400 MHz machine at the NMR Center of the University of Groningen, or on a Brucker 500 MHz machine at the Drug Design laboratory of the University of Groningen. Chemical shifts (\(\delta\)) are reported in parts per million (ppm). Optical rotations were measured on a Schmidt+Haensch Polartronic MH8 polarimeter with a 10 cm cell (c given in g/100 mL). Electrospray ionization orbitrap high resolution mass spectrometry (HRMS) was performed by the Mass Spectrometry core facility of the University of Groningen.
II) Detailed experimental procedures

1. Chemoenzymatic synthesis of L-TBOA (1) at multigram scale

trans-2-Benzylxyfumaric acid (6)
To a stirred solution of dimethyl acetylenedicarboxylate (3, 2.84 g, 20.0 mmol) in DCM (150 mL) was added DABCO (0.22 g, 2.0 mmol) and benzyl alcohol (2.16 g, 20.0 mmol) at room temperature. After completion of the reaction (TLC monitoring), the solvent was removed under vacuum to provide crude products as a dark oil, which contained trans-S1 and cis-S1 isomers (trans/cis = 6/4). The crude product was dissolved in ethanol (50 mL) and subjected to basic hydrolysis using 2 M NaOH (50 mL) at reflux for 2 h. After complete hydrolysis, the reaction mixture was cooled to room temperature and extracted with EtOAc (2 x 50 mL). The aqueous layer was acidified with HCl (con.) until pH = 1 (in an ice-bath) and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (2 x 100 mL), dried over anhydrous Na₂SO₄ and evaporated to provide a dark yellow solid. Recrystallization was performed using hexane/Et₂O (v/v=1/3) to provide pure trans-2-(benzylxy)fumaric acid 6 (1.64 g, two steps yield 37%). 1H NMR (500 MHz, DMSO-d₆): δ 7.43 – 7.32 (m, 5H), 6.08 (s, 1H), 5.10 (s, 2H). The NMR data are in agreement with published data. Following the same procedures, we prepared 6 at multigram-scale (35.0 g) from dimethyl acetylenedicarboxylate (3, 60.0 g) and benzyl alcohol (44.3 g).

(L-threo)-3-benzylxyaspartate (1, L-TBOA)
To a slowly stirred solution of trans-2-(benzylxy)fumaric acid 6 (2.44 g, 11 mmol) in 220 mL of buffer (5 M NH₃/NH₄Cl, 20 mM MgCl₂, pH = 9.5) was added MAL L384A (0.01 mol%, 5 mL, 12.3 mg/mL), and the reaction mixture was incubated for 24 hours at room temperature. After completion of the enzymatic reaction (1H NMR monitoring, >98% conversion),
the reaction mixture was warmed up to 70 °C for 10 min until the enzyme precipitated, followed by filtration through cotton to remove the white precipitates. Most of the water in the reaction mixture was evaporated under vacuum, then the resulting concentrated mixture was acidified with HCl (conc.) to pH = 1 (in an ice-bath). The acidified solution was loaded onto a column packed with cation-exchange resin (1000 g of Dowex 50W X8, 50-100 mesh), which was pre-treated with 2 M aqueous ammonia (4 column volumes), 1 M HCl (2 column volumes) and distilled water (4 column volumes). The column was washed with water (2 column volumes) and the product was eluted with 2 M aqueous ammonia (2 column volumes). The ninhydrin-positive fractions were collected and lyophilized to yield the product L-TBOA (1) ammonium salt (white powder, 2.26 g, 80%). 1H NMR (500 MHz, D2O): δ 7.43 – 7.34 (m, 5H), 4.71 (d, \(J = 11.6\) Hz, 1H), 4.47 (d, \(J = 11.6\) Hz, 1H), 4.32 (d, \(J = 2.3\) Hz, 1H), 3.99 (d, \(J = 2.3\) Hz, 1H). The NMR data are in agreement with published data.1 Following the same procedures, we prepared L-TBOA at multigram-scale (1, 24.8 g) from 6 (25.0 g) in several batches.

2. Synthesis of chiral building block (4)

(L-threo)-dimethyl 2-amino-3-(benzyloxy)succinate hydrochloride (7)
To a stirred suspension of L-TBOA (1, 512 mg, 2 mmol) in dry MeOH (15 mL) at was added SOCl₂ (1.45 mL, 20 mmol) dropwise (in an ice-bath). After 20 min, the cooling system was removed and the reaction mixture was heated to reflux for 6 h. After completion of the reaction (TLC monitoring, MeOH/DCM 4:1, \(R_f = 0.8\), ninhydrin), the reaction mixture was cooled to room temperature, and solvent was removed to provide crude product 7 as a white solid (580 mg, 96%). No purification was needed, the crude product 7 was directly used for the next step. 1H NMR (500 MHz, DMSO-\(d_6\)): δ 8.78 (s, 3H), 7.39 – 7.32 (m, 5H), 4.77 (d, \(J = 11.8\) Hz, 1H), 4.64 (d, \(J = 3.6\) Hz, 1H), 4.55 (d, \(J = 11.8\) Hz, 1H), 4.46 (d, \(J = 3.6\) Hz, 1H), 3.75 (s, 3H), 3.64 (s, 3H); 13C NMR (126 MHz, DMSO-\(d_6\)): δ 168.4, 167.1, 136.6, 128.3 (2), 128.0 (2), 128.0, 75.2, 72.6, 54.1, 53.2, 52.8. HRMS (ESI⁺): calcd. for C13H16NO5 [M+H]⁺: 268.1180, found 268.1174.

(L-threo)-dimethyl 2-(benzyloxy)-3-[(tert-butoxycarbonyl)amino]succinate (8)
To a stirred solution of 7 (580 mg, 1.92 mmol) in dry DCM (20 mL) was added DIEA (495 µL, 3 mmol) and Boc₂O (436 mg, 2 mmol) under cooling in an ice-bath. After 10 min, the cooling system was removed and the reaction mixture was stirred at room temperature
for further 24 h. After completion of the reaction, the reaction mixture was diluted with DCM (20 mL), and washed with 0.5 M HCl (50 mL), saturated NaHCO₃ solution (50 mL) and brine (50 mL). The organic layer was dried over Na₂SO₄ and concentrated under vacuum to give crude product 8 as a clear oil (640 mg, 91%). No purification was needed, analytically pure product 8 was directly used for the next step. ³¹H NMR (500 MHz, DMSO-d₆): δ 7.36 – 7.28 (m, 5H), 7.05 (d, J = 9.5 Hz, 1H), 4.67 (d, J = 11.9 Hz, 1H), 4.62 (dd, J = 9.5, 4.3 Hz, 1H), 4.47 (d, J = 4.3 Hz, 1H), 4.42 (d, J = 11.9 Hz, 1H), 3.67 (s, 3H), 3.60 (s, 3H), 1.36 (s, 9H); ¹³C NMR (126 MHz, CDCl₃): δ 169.7 (2C), 155.4, 136.6, 128.4, 128.3 (2C), 128.2 (2C), 80.2, 76.8, 72.8, 56.0, 52.6, 52.4, 28.2 (3C). HRMS (ESI⁺): calcd. for C₁₈H₂₅NO₇Na [M+Na⁺]: 390.1523, found 390.1523.

(L-threo)-dimethyl 2-[(tert-butoxycarbonyl)amino]-3-hydroxysuccinate (4)

To a stirred solution of 8 (640 mg, 1.75 mmol) in dry MeOH (15 mL) was added Pd/C (0.6 g, 10 wt.% loading) and HCOONH₄ (0.7 g). The mixture was heated to reflux for 45 min. After completion of the reaction, the reaction mixture was filtered through Celite and evaporated under vacuum to provide crude product 4. Purification was conducted via flash chromatography (EtOAc/Petroleum ether, 15%, v/v) to provide compound 4 as clear oil (392 mg, 81%). ¹³H NMR (500 MHz, CDCl₃): δ 5.29 (d, J = 9.5 Hz, 1H), 4.78 (dd, J = 9.3, 2.0 Hz, 1H), 4.69 (dd, J = 5.8, 2.0 Hz, 1H), 3.82 (s, 3H), 3.80 (s, 3H), 3.22 (d, J = 5.7 Hz, 1H), 1.42 (s, 9H); ¹³C NMR (126 MHz, CDCl₃): δ 172.4, 169.8, 155.3, 80.4, 71.1, 56.1, 53.2, 52.9, 28.2 (3C). HRMS (ESI⁺): calcd. for C₁₁H₁₉NO₇Na [M+Na⁺]: 300.1054, found 300.1053. We prepared precursor 4 at multigram-scale (6.9 g) from 7 (9.8 g) following the same procedures.

(L-threo)-dimethyl 2-amino-3-hydroxysuccinate (10)

To a stirred solution of 1 (256 mg, 1 mmol) in dry MeOH (10 mL) was added Pd/C (0.25 g, 10 wt.% loading) and HCOONH₄ (0.35 g). The mixture was heated to reflux for 45 min. Subsequently, the reaction mixture was filtered through Celite and washed with MeOH (10 mL). The filtrate was collected and evaporated under vacuum to provide product (L-threo)-3-hydroxyaspartate (9). ¹³H NMR (500 MHz, D₂O): δ 4.54 (d, J = 2.1 Hz, 1H), 4.06 (d, J = 2.1 Hz, 1H); ¹³C NMR (126 MHz, D₂O): δ 176.6, 172.4, 70.7 (d, J = 12.6 Hz), 56.9 (t, J = 32.8 Hz). HRMS (ESI⁺): calcd. for C₄H₈NO₅ [M+H⁺]: 150.0397, found 150.0397.

In the subsequent step, compound 9 was dissolved in dry MeOH (10 mL), followed by dropwise addition of SOCl₂ (0.73 mL, 10 mmol) in an ice-bath. After 20 minutes, the cooling
system was removed and the reaction mixture was heated to reflux for 6 h. After completion of the reaction, the reaction mixture was cooled to room temperature and solvent was removed under vacuum to provide product 10 as white solid (192 mg, two-step yield 90%). No purification was needed, analytically pure product 10 was directly used for the next step.

\[ \text{HRMS (ESI\textsuperscript{+}): calcd. for C}_{6}\text{H}_{12}\text{NO}_{5} [M+H]\textsuperscript{+}: 178.0710, found 178.0712.} \]

(L-threo)-dimethyl 2-[(tert-butoxycarbonyl)amino]-3-hydroxysuccinate (4)

To a stirred solution of 10 (192 mg, 0.9 mmol) in dry DCM (10 mL) was added DIEA (330 µL, 2 mmol) and Boc\textsubscript{2}O (218 mg, 1 mmol) under cooling using an ice-bath. After 10 minutes, the cooling system was removed and the reaction mixture was stirred at room temperature for further 24 h. After completion of the reaction, the reaction mixture was diluted with DCM (10 mL), and washed with 0.5 M HCl (20 mL), saturated NaHCO\textsubscript{3} solution (20 mL) and brine (20 mL). The organic layer was dried over Na\textsubscript{2}SO\textsubscript{4} and evaporated under vacuum to give crude product 4, which was purified via flash chromatography (EtOAc/Petroleum ether, 15%, v/v) to provide pure compound 4 as a clear oil (202 mg, 81%).

3. Synthesis of substituted benzyl bromides (5)

N-[3-(hydroxymethyl)phenyl]-4-(trifluoromethyl)benzamide (S4a)

To a stirred solution of 3-aminobenzyl alcohol (S3, 861 mg, 7.0 mmol) and 4-trifluoromethylbenzoyl chloride (S2a, 1.04 g, 5.0 mmol) in THF (30 mL) was added triethylamine (707 mg, 7.0 mmol) under cooling using an ice-bath. After 10 minutes, the cooling bath was removed and reaction was run at room temperature for further 2 h. After completion of the reaction, the reaction mixture was diluted with ethyl acetate (50 mL) and washed with 1 M aqueous HCl (3 x 50 mL), saturated aqueous NaHCO\textsubscript{3} (2 x 50 mL) and brine (100 mL). The organic layer was dried over Na\textsubscript{2}SO\textsubscript{4} and evaporated under vacuum to give the crude product S4a. The product was precipitated from ethyl acetate/pentane to give pure S4a as a white powder (1.30 g, 88%). 1H NMR (400 MHz, Methanol-d\textsubscript{4}): d 8.09 (d, J = 8.0 Hz, 2H), 7.82 (d, J = 8.0 Hz, 2H), 7.71 (s, 1H), 7.61 (d, J = 8.0 Hz, 1H), 7.35 (t, J = 7.6 Hz, 1H), 7.17 (d,
N-[3-(hydroxymethyl)phenyl]-2-(trifluoromethyl)benzamide (S4b)

Compound S4b was prepared from 3-aminobenzyl alcohol (S3, 861 mg, 7.0 mmol), 2-trifluoromethylbenzoyl chloride (S2b, 1.04 g, 5.0 mmol) and triethylamine (707 mg, 7.0 mmol) following a procedure similar to that used for S4a. The compound S4b was obtained as a white solid (1.32 g, 89%). 1H NMR (400 MHz, DMSO-d6): \( \delta 10.46 (s, 1H) \), 8.32 (s, 1H), 8.28 (d, \( J = 8.0 \) Hz, 1H), 7.96 (d, \( J = 7.8 \) Hz, 1H), 7.80 – 7.76 (m, 2H), 7.69 (d, \( J = 8.1 \) Hz, 1H), 7.32 (t, \( J = 7.8 \) Hz, 1H), 7.08 (d, \( J = 7.5 \) Hz, 1H), 5.26 (t, \( J = 5.7 \) Hz, 1H), 4.53 (d, \( J = 5.6 \) Hz, 2H); 13C NMR (126 MHz, DMSO-d6): \( \delta 163.9, 143.2, 138.7, 135.8, 131.9, 129.7, 129.2 \) (q, \( J = 32.8 \) Hz), 128.4, 128.1 (q, \( J = 3.8 \) Hz), 124.0 (q, \( J = 273.4 \) Hz), 124.3 (q, \( J = 3.8 \) Hz), 122.1, 118.9, 118.6, 62.9; 19F NMR (376 MHz, DMSO-d6): \( \delta -61.1 \) (s). HRMS (ESI+): calcd. for C15H13F3NO2 [M+H]+: 296.0893, found 296.0885.

N-[3-(hydroxymethyl)phenyl]-3-(trifluoromethyl)benzamide (S4c)

Compound S4c was prepared from 3-aminobenzyl alcohol (S3, 861 mg, 7.0 mmol), 3-trifluoromethylbenzoyl chloride (S2c, 1.04 g, 5.0 mmol) and triethylamine (707 mg, 7.0 mmol) by following a procedure similar to that used for S4a. The title compound was obtained as a white solid (1.35 g, 91%). 1H NMR (500 MHz, DMSO-d6): \( \delta 10.54 (s, 1H) \), 7.85 (d, \( J = 7.8 \) Hz, 1H), 7.79 (t, \( J = 7.5 \) Hz, 1H), 7.72 – 7.68 (m, 3H), 7.53 (d, \( J = 8.3 \) Hz, 1H), 7.29 (t, \( J = 7.8 \) Hz, 1H), 7.06 (d, \( J = 7.6 \) Hz, 1H), 5.24 (t, \( J = 5.7 \) Hz, 1H), 4.50 (d, \( J = 5.7 \) Hz, 2H); 13C NMR (126 MHz, DMSO-d6): \( \delta 165.6, 143.3, 138.8, 136.3, 132.6, 130.0, 128.5, 128.4, 126.3 \) (q, \( J = 6.3 \) Hz), 125.8 (q, \( J = 31.5 \) Hz), 123.8 (q, \( J = 273.4 \) Hz), 122.0, 118.0, 117.7, 62.9; 19F NMR (376 MHz, Methanol-d4): \( \delta -60.6 \) (s). HRMS (ESI+): calcd. for C15H13F3NO2 [M+H]+: 296.0893, found 296.0886.

N-[3-(hydroxymethyl)phenyl]-2-[4-(trifluoromethyl)phenyl]acetamide (S4d).

To a stirred solution of 2-[4-(trifluoromethyl)phenyl]acetic acid (S5a, 0.50 g, 2.45 mmol) in dry DCM (20 mL) at room temperature was added oxalyl chloride solution (10 mL, 2 M in DCM) and one drop of dry DMF as catalyst. The reaction was stirred at same temperature for further 2 h. Subsequently, solvents and access oxalyl chloride were removed under reduced pressure, and the resulting acid chloride 14a was directly used for the next step.
The freshly prepared acid chloride 14a was diluted with dry THF (30 mL) under ice-bath condition, after which 3-aminobenzyl alcohol (361 mg, 2.94 mmol) and triethylamine (297 mg, 2.94 mmol) were added. After 10 minutes, the cooling system was removed, and the reaction mixture was stirred for another 2 hours at room temperature. After completion of the reaction, the reaction mixture was diluted with ethyl acetate (100 mL) and washed with 1 M HCl (3 x 100 mL), saturated aqueous NaHCO₃ (100 mL) and brine (100 mL). The organic phase was dried over Na₂SO₄ and the solvent was partially evaporated. The product S4d was precipitated from EtOAc/Petroleum ether to give white powder (0.61 g, 80%).

1H NMR (500 MHz, DMSO-d₆): δ 10.22 (s, 1H), 7.70 (d, J = 8.0 Hz, 2H), 7.58 – 7.54 (m, 3H), 7.48 (d, J = 8.4 Hz, 1H), 7.23 (t, J = 7.8 Hz, 1H), 6.98 (d, J = 7.6 Hz, 1H), 5.19 (t, J = 5.7 Hz, 1H), 4.46 (d, J = 5.7 Hz, 2H), 3.76 (s, 2H); 13C NMR (126 MHz, DMSO-d₆): δ 168.3, 143.2, 140.9, 138.9, 130.0 (2C), 128.4, 127.3 (q, J = 32.8 Hz), 125.1 (2C, q, J = 3.8 Hz), 124.4 (q, J = 272.2 Hz), 121.3, 117.4, 117.2, 62.8, 43.0; 19F NMR (376 MHz, DMSO-d₆): δ -60.9 (s). HRMS (ESI+): calcd. for C₁₆H₁₅F₃NO₂ [M+H]+: 310.1049, found 310.1049.

N-[3-(hydroxymethyl)phenyl]-3-[4-(trifluoromethyl)phenyl]propanamide (S4e)

Compound S4e was prepared from 3-[4-(trifluoromethyl)phenyl]propanoic acid (S5b, 0.50 g, 2.29 mmol) following a procedure similar to that used for S4d. The title compound was obtained as a white solid (0.62 g, 73%). 1H NMR (500 MHz, DMSO-d₆): δ 9.91 (s, 1H), 7.65 (d, J = 8.1 Hz, 2H), 7.54 (s, 1H), 7.48 (d, J = 8.0 Hz, 2H), 7.45 (d, J = 8.7 Hz, 1H), 7.22 (t, J = 7.8 Hz, 1H), 6.96 (d, J = 7.6 Hz, 1H), 5.18 (t, J = 5.7 Hz, 1H), 4.45 (d, J = 5.6 Hz, 2H), 3.00 (t, J = 7.6 Hz, 2H), 2.66 (t, J = 7.6 Hz, 2H); 13C NMR (126 MHz, DMSO-d₆): δ 170.0, 146.2, 143.2, 139.0, 129.1 (2C), 128.3, 126.7 (q, J = 31.5 Hz), 125.1 (2C, q, J = 3.8 Hz), 124.4 (q, J = 272.2 Hz), 121.1, 117.4, 117.1, 62.8, 37.3, 30.5; 19F NMR (376 MHz, DMSO-d₆): δ -60.7 (s). HRMS (ESI+): calcd. for C₁₆H₁₅F₃NO₂ [M+H]+: 324.1206, found 324.1199.

N-[3-(bromomethyl)phenyl]-4-(trifluoromethyl)benzamide (5a)

To a stirred solution of compound S4a (295 mg, 1.0 mmol) in DCM (20 mL), in an ice-bath, was added N-bromosuccinimide (213 mg, 1.2 mmol) and triphenylphosphine (314 mg, 1.2 mmol). The reaction was allowed to proceed at the same temperature for 2 h. After completion of the reaction, the solvent was evaporated and product 5a was purified by flash column chromatography (EtOAc/Petroleum ether, 10%, v/v) to give a white powder (299 mg, 84%). 1H NMR (400 MHz, DMSO-d₆): δ 10.51 (s, 1H), 8.13 (d, J = 8.0 Hz, 2H), 7.90 (d, J = 8.0 Hz, 2H), 7.89 (s, 1H), 7.68 (d, J = 7.6 Hz, 1H), 7.34 (t, J = 7.6 Hz, 1H), 7.19 (d,
N-[3-(bromomethyl)phenyl]-2-(trifluoromethyl)benzamide (5b)
Compound 5b was prepared from S4b (1.20 g, 4.07 mmol), NBS (866 mg, 4.88 mmol) and Ph$_3$P (1.28 g, 4.88 mmol) following a procedure similar to that used for 5a. The title compound was obtained as a white solid (1.10 g, 76%). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 10.63 (s, 1H), 7.89 – 7.83 (m, 2H), 7.80 (t, $J$ = 7.2 Hz, 1H), 7.72 (t, $J$ = 7.4 Hz, 2H), 7.57 – 7.55 (m, 1H), 7.34 (t, $J$ = 7.9 Hz, 1H), 7.20 (d, $J$ = 7.7 Hz, 1H), 4.71 (s, 2H); $^{13}$C NMR (126 MHz, Methanol-$d_4$): $\delta$ 168.7, 140.5, 140.0, 137.3, 133.4, 131.2, 130.3, 129.5, 128.2 (q, $J$ = 32.8 Hz), 127.5 (q, $J$ = 5.0 Hz), 126.4, 125.2 (q, $J$ = 273.4 Hz), 122.0, 121.3, 33.7; $^{19}$F NMR (376 MHz, Methanol-$d_4$): $\delta$ -60.54 (s). HRMS (ESI$^+$): calcd. for C$_{15}$H$_{12}$BrF$_3$NO [M+H]$^+$: 358.0049, found: 358.0049.

N-[3-(bromomethyl)phenyl]-2-(4-(trifluoromethyl)phenyl)acetamide (5d)
Compound 5d was prepared from S4d (0.61 g, 1.97 mmol), NBS (419 mg, 2.36 mmol) and Ph$_3$P (618 mg, 2.36 mmol) following a procedure similar to that used for 5a. The title compound was obtained as a white solid (0.47 g, 64%). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.65 (d, $J$ = 8.0 Hz, 2H), 7.55 (t, $J$ = 2.0 Hz, 1H), 7.46 (d, $J$ = 8.0 Hz, 2H), 7.35 (d, $J$ = 8.2 Hz, 1H), 7.27 (t, $J$ = 7.8 Hz, 1H), 7.19 (s, 1H), 7.14 (d, $J$ = 7.6 Hz, 1H), 4.43 (s, 2H), 3.78 (s, 2H); $^{13}$C NMR (126 MHz, DMSO-$d_6$): $\delta$ 168.3, 143.2, 140.9, 138.9, 130.0 (2C), 128.4, 127.3 (q, $J$ = 32.8 Hz), 125.1 (2C, q, $J$ = 3.8 Hz), 124.4 (q, $J$ = 272.2 Hz), 121.3, 117.4, 117.2, 62.8, 42.9; $^{19}$F NMR (376 MHz, Methanol-$d_4$): $\delta$ -63.9 (s). HRMS (ESI$^+$): calcd. for C$_{16}$H$_{14}$BrF$_3$NO [M+H]$^+$: 372.0205, found 372.0205.
N-[3-(bromomethyl)phenyl]-3-[4-(trifluoromethyl)phenyl]propanamide (5e)

Compound 5e was prepared from S4e (0.60 g, 1.86 mmol), NBS (396 mg, 2.23 mmol) and Ph3P (584 mg, 2.23 mmol) following a procedure similar to that used for 5a. The title compound was obtained as a white solid (0.42 g, 58%). 1H NMR (500 MHz, Methanol-d4): δ 7.61 (t, J = 1.7 Hz, 1H), 7.56 (d, J = 8.1 Hz, 2H), 7.45 – 7.39 (m, 3H), 7.25 (t, J = 7.9 Hz, 1H), 7.12 (d, J = 7.7 Hz, 1H), 4.50 (s, 2H), 3.07 (t, J = 7.7 Hz, 2H), 2.69 (t, J = 7.7 Hz, 2H); 13C NMR (126 MHz, DMSO-d6): δ 170.3, 146.3, 139.5, 138.6, 129.3 (2C), 129.2, 126.9 (q, J = 31.5 Hz), 125.3 (2C, q, J = 3.8 Hz), 124.6 (q, J = 272.2 Hz), 124.0, 119.9, 119.2, 37.4, 34.8, 30.6; 19F NMR (376 MHz, Methanol-d4): δ -63.9 (s). HRMS (ESI+): calcd. for C17H16BrF3NO [M+H]+: 386.0362, found 386.0362.

4. Synthesis of L-TFB-TBOA (2a) and its derivatives (2b-c)

(L-threo)-dimethyl 2-{[(tert-butoxycarbonyl)amino]-3-[3-(4(trifluoromethyl)benzoylamino)benzyloxy)succinate (11a)

To a stirred solution of compound 4 (50 mg, 0.18 mmol) in dry DMF (3 mL) was added 5a (129 mg, 0.36 mmol) at -20 °C. After 10 min, NaH (60% in mineral oil, 7.2 mg, 0.18 mmol) was added to the reaction mixture and the mixture was stirred at -20 °C for further 4 h. After completion of the reaction, the reaction mixture was quenched with cold water, extracted with EtOAc (3 x 20 mL), washed with brine (3 x 50 mL), and dried over Na2SO4. The solvent was evaporated to provide crude product 11a, which was purified via flash chromatography (EtOAc/Petroleum ether, 15%, v/v) to give 11a as a clear oil (45 mg, 45%). 1H NMR (500 MHz, CDCl3): δ 8.25 (s, 1H), 7.96 (d, J = 8.1 Hz, 2H), 7.70 (d, J = 8.1 Hz, 2H), 7.64 (d, J = 8.0 Hz, 1H), 7.53 (s, 1H), 7.31 (t, J = 7.8 Hz, 1H), 7.04 (d, J = 7.6 Hz, 1H), 5.36 (d, J = 9.9 Hz, 1H), 4.82 – 4.77 (m, 2H), 4.50 (d, J = 2.4 Hz, 1H), 4.36 (d, J = 11.9 Hz, 1H), 3.76 (s, 3H), 3.63 (s, 3H), 1.40 (s, 9H); 13C NMR (126 MHz, CDCl3): δ 169.9, 169.8, 164.7, 155.6, 138.2, 137.9, 137.9, 133.6 (q, J = 32.8 Hz), 129.3, 127.8 (2C), 125.9 (2C, q, J = 3.8 Hz), 123.7 (q, J = 272.2 Hz), 124.6, 120.3, 120.1, 80.5, 77.3, 72.7, 56.1, 52.8, 52.6, 28.3 (3C). HRMS (ESI+): calcd. for C26H30BrF5N2O8 [M+H]+: 555.1949, found 555.1954.
(L-threo)-dimethyl 2-[(tert-butoxycarbonyl)amino]-3-{3-[2-(trifluoromethyl)benzoyl-amino]benzyloxy}succinate (11b)

Compound 11b was prepared from 4 (50 mg, 0.18 mmol), 5b (129 mg, 0.36 mmol) and NaH (60% in mineral oil, 7.2 mg, 0.18 mmol) following a procedure similar to that used for 11a. The title compound was obtained as clear oil (50 mg, 50%). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.75 (d, $J$ = 7.8 Hz, 1H), 7.65 – 7.58 (m, 5H), 7.48 (s, 1H), 7.34 (t, $J$ = 7.8 Hz, 1H), 7.07 (d, $J$ = 7.6 Hz, 1H), 5.35 (d, $J$ = 10.0 Hz, 1H), 4.83 – 4.80 (m, 2H), 4.51 (d, $J$ = 2.4 Hz, 1H), 4.38 (d, $J$ = 12.0 Hz, 1H), 3.77 (s, 3H), 3.65 (s, 3H), 1.41 (s, 9H); $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 169.8, 169.8, 165.8, 155.6, 138.0, 137.8, 135.8, 132.4, 130.4, 129.4, 128.7, 127.4 (q, $J$ = 31.5 Hz), 126.7 (q, $J$ = 5.0 Hz), 124.7, 123.7 (q, $J$ = 273.4 Hz), 120.0, 119.8, 80.4, 77.3, 72.6, 56.1, 52.8, 52.6, 28.3 (3C). HRMS (ESI$^+$): calcd. for C$_{26}$H$_{30}$F$_3$N$_2$O$_8$ [M+H]$^+$: 555.1949, found 555.1942.

(L-threo)-dimethyl 2-[(tert-butoxycarbonyl)amino]-3-{3-[3-(trifluoromethyl)benzoyl-amino]benzyloxy}succinate (11c)

Compound 11c was prepared from 4 (50 mg, 0.18 mmol), 5c (129 mg, 0.36 mmol) and NaH (60% in mineral oil, 7.2 mg, 0.18 mmol) following a procedure similar to that used for 11a. The title compound was obtained as clear oil (42 mg, 42%). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.13 (s, 1H), 8.06 (d, $J$ = 7.8 Hz, 1H), 7.99 (s, 1H), 7.83 – 7.80 (m, 1H), 7.65 (dt, $J$ = 15.6, 7.9 Hz, 2H), 7.52 (s, 1H), 7.35 (t, $J$ = 7.8 Hz, 1H), 7.07 (d, $J$ = 7.6 Hz, 1H), 5.36 (d, $J$ = 10.0 Hz, 1H), 4.85 – 4.80 (m, 2H), 4.51 (d, $J$ = 2.3 Hz, 1H), 4.39 (d, $J$ = 11.9 Hz, 1H), 3.78 (s, 3H), 3.66 (s, 3H), 1.41 (s, 9H); $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 169.8, 169.7, 164.3, 155.4, 137.8, 137.7, 135.6, 131.4 (q, $J$ = 32.8 Hz), 130.4, 129.5, 129.3, 128.5 (q, $J$ = 2.5 Hz), 124.5, 124.1 (q, $J$ = 5.0 Hz), 123.6 (q, $J$ = 273.4 Hz), 120.1, 119.9, 80.3, 77.1, 72.5, 56.0, 52.7, 52.5, 28.2 (3C). HRMS (ESI$^+$): calcd. for C$_{26}$H$_{30}$F$_3$N$_2$O$_8$ [M+H]$^+$: 555.1949, found 555.1950.

(L-threo)-3-{3-[4-(trifluoromethyl)benzoylamino]benzyloxy}aspartate (2a, L-TFB-TBOA)

To a stirred solution of 11a (45 mg, 0.08 mmol) in dry DCM (2 mL), in an ice-bath, was added trifluoroacetic acid (0.8 mL) dropwise. After the complete addition of trifluoroacetic acid, the ice-bath was removed and the reaction was allowed to proceed at room temperature for further 1.5 h. After completion of the starting material, solvent was removed in vacuo to provide S6a quantitatively. Compound S6a was directly used for the next step without purification.
To a stirred solution of S6a in THF/H2O (1:1, each 1 mL) was added LiOH (11.6 mg, 0.5 mmol) and the reaction mixture was stirred at room temperature for 2 h. After completion of the starting material, THF was removed in vacuo and the resulting aqueous solution was reverse extracted with EtOAc (1 mL). The aqueous layer was acidified with 1 M HCl (pH=1) until white precipitates appeared. The white precipitates were filtered through a Büchner funnel and washed with 5 mL of cold water. The obtained white solid was dried under vacuum overnight to provide the final product 2a (L-TFB-TBOA) (22 mg, two-step yield 59%, de >98%, ee >99%). 1H NMR (500 MHz, DMSO-d6): δ 10.49 (s, 1H), 8.16 (d, J = 8.1 Hz, 2H), 7.76 (s, 1H), 7.71 (d, J = 8.1 Hz, 1H), 7.35 (t, J = 7.8 Hz, 1H), 7.28 (d, J = 7.7 Hz, 1H), 4.83 (d, J = 10.6 Hz, 1H), 4.48 (d, J = 10.6 Hz, 1H), 4.13 (d, J = 9.5 Hz, 1H), 3.83 (d, J = 9.5 Hz, 1H); 13C NMR (126 MHz, DMSO-d6): δ 170.7, 168.4, 164.3, 138.7, 138.5, 138.4, 131.3 (q, J = 31.5 Hz), 128.6 (2C), 128.4, 125.4 (2C, q, J = 5.0 Hz), 124.2, 123.9 (q, J = 272.2 Hz), 120.4, 119.8, 75.1, 72.5, 53.4; 19F NMR (376 MHz, DMSO-d6): δ -61.3 (s). HRMS (ESI+): calcd. for C19H18F3N2O6 [M+H]+: 427.1112, found 427.1108. [α]D25 = -55.0 (c 0.60, DMSO). Comparison of the 1H NMR data of 2a with the 1H NMR data of chemically prepared racemic DL-erythro-S12a showed that the de of product 2a is >98%. Chiral HPLC analysis: CROWNPAK CR-I (+) 150 x 3 mm. Phase A: ACN+0.5%TFA, phase B: H2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C, tR (L-threo-2a) = 2.8 min, ee >99% (Figure S2). Following the same procedures, we prepared 2a at multigram-scale (2.3 g) from 11a (4.15 g).

(L-threo)-3-{3-[2-(trifluoromethyl)benzoylamino]benzyloxy}aspartate (2b)
Compound 2b was prepared from 11b (45 mg, 0.08 mmol) following a procedure similar to that used for 2a. The title compound was obtained as a white solid (16 mg, two-step yield 43%, de >98%, ee >99%). 1H NMR (400 MHz, DMSO-d6): δ 10.56 (s, 1H), 7.85 (d, J = 7.7 Hz, 1H), 7.79 (t, J = 7.5 Hz, 1H), 7.73 – 7.66 (m, 3H), 7.62 (d, J = 7.8 Hz, 1H), 7.34 – 7.26 (m, 2H), 4.80 (d, J = 10.5 Hz, 1H), 4.46 (d, J = 10.5 Hz, 1H), 4.11 (d, J = 9.4 Hz, 1H), 3.81 (d, J = 9.3 Hz, 1H); 13C NMR (126 MHz, DMSO-d6): δ 170.6, 168.4, 165.6, 138.6, 138.5, 136.2, 132.6, 130.0, 128.5 (2C), 126.3 (q, J = 5.0 Hz), 125.8 (q, J = 31.5 Hz), 124.1, 123.8 (q, J = 274.7 Hz), 119.5, 74.8, 72.7, 53.1; 19F NMR (376 MHz, DMSO-d6): δ -57.9 (s). HRMS (ESI+): calcd. for C19H18F3N2O6 [M+H]+: 427.1112, found 427.1106. [α]D25 = -55.0 (c 0.60, DMSO). Chiral HPLC analysis: CROWNPAK CR-I (+) 150 x 3 mm. Phase A: ACN+0.5%TFA, phase B: H2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C, tR (L-threo-2b) = 2.6 min, ee >99% (Figure S3).

(L-threo)-3-{3-[3-(trifluoromethyl)benzoylamino]benzyloxy}aspartate (2c)
Compound 2c was prepared from 11c (42 mg, 0.076 mmol) following a procedure similar to that used for 2a. The title compound was obtained as a white solid (18 mg, two-step yield
48%, de >98%, ee >99%). 1H NMR (400 MHz, DMSO-δ6): δ 10.51 (s, 1H), 8.31 (s, 1H), 8.28 (d, J = 8.0 Hz, 1H), 7.97 (d, J = 7.8 Hz, 1H), 7.79 (t, J = 7.8 Hz, 1H), 7.73 (d, J = 7.7 Hz, 2H), 7.35 (t, J = 7.8 Hz, 1H), 7.27 (d, J = 7.6 Hz, 1H), 4.81 (d, J = 10.7 Hz, 1H), 4.47 (d, J = 10.7 Hz, 1H), 4.11 (d, J = 8.9 Hz, 1H), 3.77 (d, J = 9.0 Hz, 1H); 13C NMR (126 MHz, DMSO-δ6): δ 171.1, 168.9, 164.4, 138.9, 138.8, 136.2, 132.3, 130.2, 129.6 (q, J = 32.8 Hz), 128.8, 128.6 (q, J = 3.8 Hz), 124.7 (q, J = 3.8 Hz), 124.7, 124.5 (q, J = 273.4 Hz), 121.0, 120.4, 75.4, 73.0, 53.7; 19F NMR (376 MHz, DMSO-δ6): δ -61.1 (s). HRMS (ESI+): calcd. for C19H18F3N2O6 [M+H]+: 427.1112, found 427.1112. [α]D25 = -50.7 (c 0.43, DMSO). Chiral HPLC analysis: CROWNPAK CR-I (+) 150 x 3 mm. Phase A: ACN+0.5%TFA, phase B: H2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C, tR (L-threo-2c) = 2.7 min, ee >99% (Figure S4).

### Synthesis of L-TFB-TBOA derivatives from protected meta-NH2-TBOA (13)

(L-threo)-dimethyl 2-(3-nitrobenzyloxy)-3-[(tert-butoxycarbonyl)amino]succinate (12)

To a stirred solution of compound 4 (277 mg, 1.08 mmol) in dry DMF (5 mL) was added bromomethyl-3-nitrobenzene (S7, 466 mg, 2.16 mmol) at -20 °C. After 10 min, NaH (60% in mineral oil, 43 mg, 1.08 mmol) was added to the reaction mixture and the mixture was stirred at -20 °C for further 4 h. After completion of the reaction, the reaction mixture was quenched with cold water and extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (3 x 50 mL), dried over Na2SO4 and evaporated to provide crude product 12, which was purified via flash chromatography (EtOAc/Petroleum ether, 17%, v/v) to give pure 12 as a clear oil (275 mg, 61%). 1H NMR (500 MHz, CDCl3): δ 8.16 (d, J = 8.1 Hz, 1H), 8.12 (s, 1H), 7.61 (d, J = 7.6 Hz, 1H), 7.52 (t, J = 7.9 Hz, 1H), 5.31 (d, J = 10.0 Hz, 1H), 4.92 – 4.87 (m, 2H), 4.56 (d, J = 2.4 Hz, 1H), 4.48 (d, J = 12.1 Hz, 1H), 3.79 (s, 3H), 3.74 (s, 3H), 1.42 (s, 9H); 13C NMR (126 MHz, CDCl3): δ 169.7, 169.4, 155.4, 148.4, 139.1, 133.8, 129.6, 123.2, 122.7, 80.6, 78.2, 71.7, 56.1, 53.0, 52.7, 28.3 (3C). HRMS (ESI+): calcd. for C18H24N2O9Li [M+Li]+: 419.1636, found 419.1638.
(L-threo)-dimethyl 2-(3-aminobenzyl)oxy)-3-[(tert-butoxycarbonyl)amino]succinate (13)

To a stirred solution of 12 (275 mg, 0.67 mmol) in dry MeOH (10 mL) was added Pd/C (50.0 mg, 10 wt.% loading) under nitrogen atmosphere. The reaction was stirred under H₂ atmosphere (balloon) for 25 min at room temperature. After completion of the reaction, the reaction mixture was filtered through Celite and washed with MeOH (5 mL). The filtrate was concentrated under vacuum to provide product 13 as a colorless oil (235 mg, 92%). ¹H NMR (500 MHz, CDCl₃): δ 7.10 (t, J = 7.7 Hz, 1H), 6.62 – 6.60 (m, 2H), 6.57 (d, J = 2.0 Hz, 1H), 5.37 (d, J = 9.9 Hz, 1H), 4.79 (dd, J = 10.0, 2.3 Hz, 1H), 4.72 (d, J = 11.9 Hz, 1H), 4.47 (d, J = 2.3 Hz, 1H), 4.26 (d, J = 12.0 Hz, 1H), 3.77 (s, 3H), 3.68 (s, 2H), 3.64 (s, 3H), 1.42 (s, 9H); ¹³C NMR (126 MHz, CDCl₃): δ 169.9 (2C), 155.6, 146.7, 137.8, 129.5, 118.5, 115.0, 114.9, 80.3, 76.6, 72.8, 56.1, 52.7, 52.6, 28.3 (3C). HRMS (ESI⁺): calcd. for C₁₈H₂₆F₁₅N₂O₇Li [M+Li]⁺: 389.1895, found 389.1895.

(L-threo)-dimethyl 2-[(tert-butoxycarbonyl)amino]-3-{3-[2-[(4-(trifluoromethyl)phenyl]acetamido]benzyl}oxy)succinate (15a)

To a stirred solution of 13 (80 mg, 0.21 mmol) in dry DCM (2 mL), in an ice-bath, was added TEA (64 mg, 0.63 mmol), followed by dropwise addition of acid chloride solution (14a, 100 mg in 1 mL of DCM, 0.42 mmol). After 10 min, the ice-bath was removed and the reaction was kept at room temperature for further 2 h. After completion of the reaction, the solvent was evaporated under vacuum and the resulting residue was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with 1 M aqueous HCl (30 mL), saturated aqueous NaHCO₃ solution (50 mL), brine (50 mL), and dried over Na₂SO₄. The solvent was evaporated to provide crude product, which was purified via flash chromatography (EtOAc/Petroleum ether, 30%, v/v) to give pure 15a as a clear oil (77 mg,
(L-threo)-dimethyl 2-[tert-butoxycarbonyl]amino]-3-[3-[4-(trifluoromethyl)phenyl]propanamido]benzyl]oxy)succinate (15b) 

Compound 15b was prepared from 13 (80 mg, 0.21 mmol), TEA (64 mg, 0.63 mmol) and acid chloride solution (14b, 100 mg in 1 mL of DCM, 0.45 mmol) following a procedure similar to that used for 16a. The title product was obtained as a clear oil (75 mg, 61%). 1H NMR (500 MHz, CDCl3): δ 7.65 (d, J = 8.0 Hz, 2H), 7.50 (d, J = 7.6 Hz, 1H), 6.93 (d, J = 7.6 Hz, 2H) 7.30 – 7.27 (m, 2H), 7.21 (s, 1H), 7.03 (d, J = 7.6 Hz, 1H), 5.32 (d, J = 10.0 Hz, 1H), 4.81 (dd, J = 9.9, 2.4 Hz, 1H), 4.76 (d, J = 11.9 Hz, 1H), 4.47 (d, J = 2.3 Hz, 1H), 4.33 (d, J = 11.8 Hz, 1H), 3.77 (s, 2H), 3.77 (s, 3H), 3.60 (s, 3H), 1.42 (s, 9H); 13C NMR (126 MHz, CDCl3): δ 169.8, 169.7, 168.4, 155.6, 138.6, 137.9, 137.7, 129.8 (2C), 129.2, 125.9 (2C, q, J = 272.2 Hz), 124.1 (q, J = 272.2 Hz), 124.2, 119.8, 119.6, 80.5, 77.2, 72.6, 56.1, 52.7, 52.6, 44.3, 28.2 (3C). HRMS (ESI^+): calcd. for C27H31F3N2O8Li [M+Li]^+: 575.2187, found 575.2192.

(L-threo)-3-[3-[2-[4-(trifluoromethyl)phenyl]acetamido]benzyl]oxy]aspartate (16a) 

Compound 16a was prepared from 15a (70 mg, 0.12 mmol) following a procedure similar to that used for 2a. The title compound was obtained as a white solid (26 mg, two-step yield 48%, de >98%, ee >99%). 1H NMR (400 MHz, DMSO-d6): δ 10.59 (s, 1H), 7.67 (d, J = 8.1 Hz, 2H), 7.61 – 7.56 (m, 4H), 7.23 (t, J = 7.7 Hz, 1H), 7.14 (d, J = 7.6 Hz, 1H), 4.73 (d, J = 10.9 Hz, 1H), 4.43 (d, J = 10.9 Hz, 1H), 4.17 (d, J = 7.9 Hz, 1H), 3.80 (s, 2H), 3.79 (d, J = 8.3 Hz, 1H); 13C NMR (126 MHz, DMSO-d6): δ 171.0, 168.4 (2C), 141.0, 138.9, 138.4, 130.1 (2C), 128.4, 127.3 (q, J = 31.5 Hz), 125.1 (2C, q, J = 3.8 Hz), 124.4 (q, J = 272.2 Hz), 123.2, 118.9, 118.4, 75.4, 72.4, 53.6, 42.8; 19F NMR (376 MHz, DMSO-d6): δ -60.8 (s). HRMS (ESI^+): calcd. for C20H20F3N2O6 [M+H]^+: 441.1268, found 441.1268. [α]D25 = -46.7 (c 0.45, DMSO). Comparison of the 1H NMR data of 16a with the 1H NMR data of chemically
prepared racemic DL-erythro-S12d showed that de of product 16a is >98%. Chiral HPLC analysis: CROWNPAK CR-I (+) 150 x 3 mm. Phase A: ACN+0.5%TFA, phase B: H2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C, tR (L-threo-16a) = 2.6 min, ee >99% (Figure S5).

(L-threo)-3-[[3-[[4-(trifluoromethyl)phenyl]propanamido]benzyloxy]aspartate (16b)

Compound 16b was prepared from 15b (75 mg, 0.13 mmol) following a procedure similar to that used for 2a. The title compound was obtained as a white solid (22 mg, two-step yield 38%, de >98%, ee >99%). 1H NMR (400 MHz, DMSO-d6): δ 10.23 (s, 1H), 7.63 (d, J = 8.0 Hz, 2H), 7.58 (d, J = 8.1 Hz, 1H), 7.54 (s, 1H), 7.49 (d, J = 8.0 Hz, 2H), 7.21 (t, J = 7.8 Hz, 1H), 7.10 (d, J = 7.6 Hz, 1H), 4.69 (d, J = 11.0 Hz, 1H), 4.40 (d, J = 11.0 Hz, 1H), 4.16 (d, J = 7.0 Hz, 1H), 3.72 (d, J = 7.0 Hz, 1H), 2.99 (t, J = 7.6 Hz, 2H), 2.69 (t, J = 7.6 Hz, 2H); 13C NMR (126 MHz, DMSO-d6): δ 171.3, 170.1, 168.5, 146.3, 139.0, 138.5, 129.2 (2C), 128.3, 126.7 (q, J = 31.5 Hz), 125.1 (2C, q, J = 3.8 Hz), 124.4 (q, J = 272.2 Hz), 122.9, 118.7, 118.3, 75.9, 72.1, 53.9, 37.3, 30.6; 19F NMR (376 MHz, DMSO-d6): δ -60.7 (s). HRMS (ESI+): calcd. for C21H22F3N2O6 [M+H]+: 455.1424 found 455.1425. [α]D25 = -34.3 (c 0.42, DMSO). Chiral HPLC analysis: CROWNPAK CR-I (+) 150 x 3 mm. Phase A: ACN+0.5%TFA, phase B: H2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C, tR (L-threo-16b) = 2.6 min, ee >99% (Figure S6).

5. Synthesis of DL-TFB-TBOA and its derivatives as chiral reference compounds

The chemical synthesis of compound (DL-threo)-S9 has been described elsewhere.4


To a stirred solution of compound (DL-threo)-S9 (156 mg, 0.5 mmol) in dry DMF (3 mL) was added 5a (358 mg, 1.0 mmol) at -20 °C. After 10 min, NaH (60% in mineral oil, 20 mg, 0.5 mmol) was added to the reaction mixture and the mixture was stirred at -20 °C for further 2 h. Subsequently, the reaction mixture was warmed up to 4 °C and stirred for another 2 h. After completion of the reaction, the reaction mixture was quenched with cold water and extracted with EtOAc (3 x 20 mL), washed with brine (3 x 50 mL), and
dried over Na2SO4. The solvent was evaporated under vacuum to provide crude product, which was purified via flash chromatography (EtOAc/Petroleum ether, 25%, v/v) to give (DL-threo)-S10a as a clear oil (190 mg, 64%). 1H NMR (500 MHz, CDCl3): δ 8.11 (s, 1H), 7.97 (d, J = 8.2 Hz, 2H), 7.66 (d, J = 8.1 Hz, 1H), 7.52 (s, 1H), 7.35 – 7.29 (m, 6H), 7.05 (d, J = 7.6 Hz, 1H), 5.62 (d, J = 9.8 Hz, 1H), 5.08 (d, J = 1.9 Hz, 2H), 4.89 (dd, J = 9.9, 2.4 Hz, 1H), 4.80 (d, J = 11.9 Hz, 1H), 4.54 (d, J = 2.4 Hz, 1H), 4.39 (d, J = 11.8 Hz, 1H), 3.74 (s, 3H), 3.66 (s, 3H); 13C NMR (126 MHz, CDCl3): δ 169.6, 164.6, 156.3, 138.2, 137.9, 137.9, 136.1, 133.7 (q, J = 34.0 Hz), 129.4, 128.6 (2C), 128.3, 128.0 (2C), 127.7 (2C), 125.9 (2C, q, J = 3.8 Hz), 124.6, 123.7 (q, J = 273.4 Hz), 120.3, 120.0, 77.1, 72.8, 71.0, 67.4, 56.6, 53.0, 52.7. HRMS (ESI+): calcd. for C29H28F3N2O8 [M+H]+: 589.1792, found 589.1794. In addition, a mixture of (DL-threo)-S10a and (DL-erythro)-S10a (50 mg, 16%, threo/erythro = 1:2) was obtained.

(DL-threo)-dimethyl 2-[(benzyloxycarbonyl)amino]-3-[3-[2-(trifluoromethyl)benzoyl-amino]benzyloxy]succinate. (DL-threo-S10b)

Compound (DL-threo)-S10b was prepared from (DL-threo)-S9 (156 mg, 0.5 mmol), 5b (359 mg, 1.0 mmol) and NaH (60% in mineral oil, 20 mg, 0.5 mmol) following a procedure similar to that used for (DL-threo)-S10a. The title product (DL-threo)-S10b was obtained as a clear oil (130 mg, 44%). 1H NMR (500 MHz, CDCl3): δ 8.08 (s, 1H), 7.67 (d, J = 7.5 Hz, 1H), 7.55 – 7.50 (m, 5H), 7.33 – 7.27 (m, 6H), 7.03 (d, J = 7.5 Hz, 1H), 5.62 (d, J = 9.8 Hz, 1H), 5.03 (s, 2H), 4.84 (dd, J = 9.9, 2.4 Hz, 1H), 4.76 (d, J = 11.9 Hz, 1H), 4.51 (d, J = 2.4 Hz, 1H), 4.35 (d, J = 11.9 Hz, 1H), 3.70 (s, 3H), 3.62 (s, 3H); 13C NMR (126 MHz, CDCl3): δ 169.5, 169.4, 165.9, 156.1, 137.9, 137.6, 136.1, 135.7, 132.1, 130.1, 129.2, 128.5 (2C), 128.4, 128.1, 127.9 (2C), 127.2 (q, J = 32.8 Hz), 126.5 (q, J = 5.0 Hz), 124.5, 123.6 (q, J = 273.4 Hz), 120.0, 119.8, 76.9, 72.6, 67.1, 56.4, 52.8, 52.5. HRMS (ESI+): calcd. for C29H28F3N2O8 [M+H]+: 589.1792, found 589.1792.

(DL-threo)-dimethyl 2-[(benzyloxycarbonyl)amino]-3-[3-[3-(trifluoromethyl)benzoyl- amino]benzyloxy]succinate. (DL-threo-S10c)

Compound (DL-threo)-S10c was prepared from (DL-threo)-S9 (161 mg, 0.52 mmol), 5c (370 mg, 1.04 mmol) and NaH (60% in mineral oil, 24 mg, 0.52 mmol) following a procedure similar to that used for (DL-threo)-S10a. The title product (DL-threo)-S10c was obtained as a clear oil (144 mg, 47%). 1H NMR (500 MHz, CDCl3): δ 8.13 (s, 1H), 8.06 (d, J = 7.8 Hz, 1H), 7.97 (s, 1H), 7.81 (d, J = 7.8 Hz, 1H), 7.67 – 7.61 (m, 2H), 7.52 (s, 1H), 7.37 – 7.31 (m, 6H), 7.06 (d, J = 7.6 Hz, 1H), 5.60 (d, J = 9.9 Hz, 1H), 5.09 (d, J = 2.4 Hz, 2H), 4.89 (dd, J = 9.8, 2.3 Hz, 1H), 4.82 (d, J = 11.9 Hz, 1H), 4.54 (d, J = 2.3 Hz, 1H), 4.41 (d, J = 11.9 Hz, 1H), 3.75 (s, 3H), 3.67 (s, 3H); 13C NMR (126 MHz, CDCl3): δ 169.5, 169.5, 164.3, 156.1, 137.7, 136.0, 135.6, 131.4 (q, J = 32.8 Hz), 130.4, 129.5, 129.3, 128.5, 128.5 (2C),
128.2, 128.0, 124.5, 124.1 (q, \( J = 3.8 \) Hz), 120.2, 119.9, 77.0, 72.6, 67.2, 56.4, 52.8, 52.6. HRMS (ESI\(^+\)): calcd. for \( \text{C}_{29}\text{H}_{28}\text{F}_{3}\text{N}_{2}\text{O}_{8} [\text{M+H}]^+ \): 589.1792, found 589.1794.

(DL-threo)-dimethyl 2-[(benzylxoycarbonyl)amino]-3-\{3-\{2-[4-(trifluoromethyl)phenyl]acetamido\}benzyloxy\}succinate. (DL-threo-S\textbf{10}d)

Compound (DL-threo)-S\textbf{10}d was prepared from (DL-threo)-S\textbf{9} (100 mg, 0.32 mmol), 5d (239 mg, 0.64 mmol) and NaH (60% in mineral oil, 13 mg, 0.32 mmol) following a procedure similar to that used for (DL-threo)-S\textbf{10}a. The title product (DL-threo)-S\textbf{10}d was obtained as a clear oil (60 mg, 43%). \(^1\)H NMR (500 MHz, CDCl\(_3\)): \( \delta \) 7.61 (d, \( J = 8.0 \) Hz, 2H), 7.47 – 7.44 (m, 4H), 7.34 – 7.30 (m, 6H), 7.24 (t, \( J = 7.8 \) Hz, 1H), 6.99 (d, \( J = 7.6 \) Hz, 1H), 5.59 (d, \( J = 9.9 \) Hz, 1H), 5.08 (d, \( J = 2.5 \) Hz, 2H), 4.87 (dd, \( J = 9.9, 2.4 \) Hz, 1H), 4.74 (d, \( J = 11.8 \) Hz, 1H), 4.50 (d, \( J = 2.4 \) Hz, 1H), 4.33 (d, \( J = 11.8 \) Hz, 1H), 3.73 (s, 5H), 3.60 (s, 3H); \(^{13}\)C NMR (126 MHz, CDCl\(_3\)): \( \delta \) 169.6, 169.6, 168.2, 156.2, 138.5, 137.8, 137.7, 136.1, 129.9 (2C), 129.2, 128.6(2C), 128.3, 128.1 (2C), 126.0 (2C, q, \( J = 3.8 \) Hz), 126.0, 124.3, 119.8, 119.6, 77.0, 72.7, 67.3, 56.5, 52.9, 52.7, 44.4. HRMS (ESI\(^+\)): calcd. for \( \text{C}_{30}\text{H}_{30}\text{F}_{3}\text{N}_{2}\text{O}_{8} [\text{M+H}]^+ \): 603.1949, found 603.1950. In addition, a mixture of (DL-threo)-S\textbf{10}d and (DL-erythro)-S\textbf{10}d (20 mg, 14\%, \( \text{threo/erythro} = 1:2 \)) was obtained.

(DL-threo)-dimethyl 2-[(benzylxoycarbonyl)amino]-3-\{3-\{3-[4-(trifluoromethyl)phenyl]propanamido\}benzyloxy\}succinate. (DL-threo-S\textbf{10}e)

Compound (DL-threo)-S\textbf{10}e was prepared from (DL-threo)-S\textbf{9} (125 mg, 0.40 mmol), 5e (313 mg, 0.81 mmol) and NaH (60% in mineral oil, 16 mg, 0.40 mmol) following a procedure similar to that used for (DL-threo)-S\textbf{10}a. The title product (DL-threo)-S\textbf{10}e was obtained as a clear oil (54 mg, 22\%). \(^1\)H NMR (500 MHz, CDCl\(_3\)): \( \delta \) 7.54 (s, 1H), 7.51 (d, \( J = 8.0 \) Hz, 2H), 7.35 – 7.29 (m, 9H), 7.23 (d, \( J = 7.9 \) Hz, 1H), 6.97 (d, \( J = 7.6 \) Hz, 1H), 5.62 (d, \( J = 9.8 \) Hz, 1H), 5.07 (s, 2H), 4.87 (dd, \( J = 9.8, 2.4 \) Hz, 1H), 4.73 (d, \( J = 11.8 \) Hz, 1H), 4.51 (d, \( J = 2.3 \) Hz, 1H), 4.33 (d, \( J = 11.7 \) Hz, 1H), 3.73 (s, 3H), 3.60 (s, 3H), 3.06 (t, \( J = 7.6 \) Hz, 2H), 2.64 (t, \( J = 7.6 \) Hz, 2H); \(^{13}\)C NMR (126 MHz, CDCl\(_3\)): \( \delta \) 170.0, 169.6, 169.5, 156.2, 144.9, 138.1, 137.6, 136.1, 129.2, 128.8 (2C), 128.6 (2C), 128.5 (q, \( J = 34.0 \) Hz), 128.2 (2C), 128.0 (2C), 125.5 (q, \( J = 3.8 \) Hz), 124.3 (q, \( J = 272.2 \) Hz), 124.0, 119.7, 119.5, 77.0, 72.8, 67.3, 56.5, 52.8, 52.6, 38.6, 31.1. HRMS (ESI\(^+\)): calcd. for \( \text{C}_{31}\text{H}_{32}\text{F}_{3}\text{N}_{2}\text{O}_{8} [\text{M+H}]^+ \): 617.2105, found 617.2108.
(DL-threo)-3-[3-[4-(trifluoromethyl)benzoylamino]benzyloxy]aspartate
(DL-threo-S12a, DL-TFB-TBOA)
To a stirred solution of (DL-threo)-S10a (190 mg, 0.32 mmol) in dry MeOH (10 mL) was added Pd/C (10.0 mg, 10 wt.% loading) under nitrogen atmosphere. The reaction mixture was stirred under H₂ atmosphere (balloon) for 2 h at room temperature. After completion of the reaction, the reaction mixture was filtered through Celite and washed with MeOH (5 mL). The filtrate was concentrated under vacuum to provide product (DL-threo)-S11a as a clear oil (88 mg, 61%). No purification was needed, the product (DL-threo)-S11a was directly used for the next step.

To a stirred solution of (DL-threo)-S11a (88 mg, 0.19 mmol) in THF/H₂O (1:1, each 1.5 mL) was added LiOH (24 mg, 1.0 mmol) and the reaction mixture was stirred at room temperature for 2 h. After completion of the starting material, THF was removed in vacuo and the resulting aqueous solution was reverse extracted with EtOAc (1 mL). The aqueous layer was acidified with 1 M HCl (pH=1) until white precipitates appeared. The white precipitates were filtered through a Büchner funnel and washed with 5 mL of cold water. The white solid was dried under vacuum overnight to provide the product (DL-threo)-S12a (DL-TFB-TBOA, 35 mg, two steps yield 43%). ¹H NMR (500 MHz, DMSO-d₆): δ 10.48 (s, 1H), 8.16 (d, J = 8.1 Hz, 2H), 7.92 (d, J = 8.2 Hz, 2H), 7.75 (s, 1H), 7.71 (d, J = 8.5 Hz, 1H), 7.35 (t, J = 7.8 Hz, 1H), 7.28 (d, J = 7.6 Hz, 1H), 4.83 (d, J = 10.6 Hz, 1H), 4.48 (d, J = 10.6 Hz, 1H), 4.12 (d, J = 9.5 Hz, 1H), 3.82 (d, J = 9.5 Hz, 1H); ¹³C NMR (126 MHz, DMSO-d₆): δ 170.5, 168.4, 164.3, 138.7, 138.5, 138.3, 131.3 (q, J = 32.8 Hz), 128.6 (2C), 128.4, 125.4 (2C, q, J = 5.0 Hz), 124.3, 123.9 (q, J = 273.4 Hz), 120.5, 119.9, 74.7, 72.7, 53.1; ¹⁹F NMR (376 MHz, DMSO-d₆): δ -61.3 (s). HRMS (ESI⁺): calcd. for C₁₉H₁₈F₃N₂O₆ [M+H]⁺: 427.1112, found 427.1111. Chiral HPLC analysis: CROWNPAK CR-I (+) 150 x 3 mm. Phase A: ACN+0.5%TFA, phase B: H₂O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C, tr (D-threo-S12a) = 2.4 min, tr (L-threo-S12a) = 2.8 min (Figure S2).

(DL-erythro)-3-[3-[4-(trifluoromethyl)benzoylamino]benzyloxy]aspartate (DL-erythro-S12a)
Compound (DL-erythro)-S12a was prepared from the mixture of (DL-threo)-S10a and (DL-erythro)-S10a (50 mg, 16%, threo/erythro = 1:2), following a procedure similar to that used for (DL-threo)-S12a. The title product (DL-erythro)-S12a was obtained as a white solid (7 mg, two steps yield 44%, erythro/threo = 93:7). ¹H NMR (500 MHz, DMSO-d₆): δ
10.50 (s, 1H), 8.15 (d, J = 8.2 Hz, 2H), 7.92 (d, J = 8.2 Hz, 2H), 7.73 (d, J = 9.2 Hz, 1H), 7.68 (s, 1H), 7.36 (t, J = 7.9 Hz, 1H), 7.17 (d, J = 7.7 Hz, 1H), 4.59 (d, J = 11.4 Hz, 1H), 4.45 (d, J = 11.4 Hz, 1H), 4.12 (d, J = 1.5 Hz, 1H), 4.07 (d, J = 1.3 Hz, 1H); 19F NMR (376 MHz, DMSO-d6): δ -61.3 (s). HRMS (ESI+): calcd for: C19H18F3N2O6 [M+H]+: 427.1112, found 427.1108.

(DL-threo)-3-[3-(trifluoromethyl)benzoylamino]benzyloxy]aspartate (DL-threo-S12b) Compound (DL-threo)-S12b was prepared from (DL-threo)-S10b (130 mg, 0.22 mmol) following a procedure similar to that used for (DL-threo)-S12a. The pure title product (DL-threo)-S12b was obtained as a white solid (24 mg, two steps yield 38%). 1H NMR (400 MHz, DMSO-d6): δ 10.59 (s, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.79 (t, J = 7.5 Hz, 1H), 7.72 – 7.67 (m, 2H), 7.63 (d, J = 10.4 Hz, 2H), 7.31 (t, J = 7.7 Hz, 1H), 7.25 (d, J = 7.6 Hz, 1H), 4.76 (d, J = 10.8 Hz, 1H), 4.45 (d, J = 10.8 Hz, 1H), 4.13 (d, J = 8.2 Hz, 1H), 3.73 (d, J = 8.2 Hz, 1H); 13C NMR (126 MHz, DMSO-d6): δ 170.9, 168.4, 165.6, 138.6, 138.5, 136.2, 132.6, 130.0, 128.6, 128.5, 126.3 (q, J = 5.0 Hz), 125.8 (q, J = 31.5 Hz), 124.0, 123.8 (q, J = 274.7 Hz), 119.4, 118.9, 75.4, 72.4, 53.5; 19F NMR (376 MHz, DMSO-d6): δ -57.9 (s). HRMS (ESI+): calcd. for C19H18F3N2O6 [M+H]+: 427.1112, found 427.1112. Chiral HPLC analysis: CROWNPAK CR-I (+) 150 x 3 mm. Phase A: ACN+0.5%TFA, phase B: H2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C, tR (D-threo-S12b) = 2.3 min, tR (L-threo-S12b) = 2.6 min (Figure S3).

(DL-threo)-3-[3-(trifluoromethyl)benzoylamino]benzyloxy]aspartate (DL-threo-S12c) Compound (DL-threo)-S12c was prepared from (DL-threo)-S10c (144 mg, 0.24 mmol) following a procedure similar to that used for (DL-threo)-S12a. The pure title product (DL-threo)-S12c was obtained as a white solid (25 mg, two steps yield 41%). 1H NMR (400 MHz, DMSO-d6): δ 10.56 (s, 1H), 8.31 (d, J = 10.7 Hz, 2H), 7.96 (d, J = 7.7 Hz, 1H), 7.80 – 7.73 (m, 3H), 7.34 (t, J = 7.7 Hz, 1H), 7.25 (d, J = 7.6 Hz, 1H), 4.79 (d, J = 10.8 Hz, 1H), 4.47 (d, J = 10.8 Hz, 1H), 4.13 (d, J = 8.3 Hz, 1H), 3.74 (d, J = 8.3 Hz, 1H); 13C NMR (126 MHz, DMSO-d6): δ 171.0, 168.4, 163.9, 138.6, 138.4, 135.7, 132.0, 129.7, 129.2 (q, J = 32.8 Hz), 128.3, 128.1 (q, J = 3.8 Hz), 124.4 (q, J = 32.8 Hz), 124.0 (q, J = 273.4 Hz), 124.0, 120.4, 119.9, 75.5, 72.3, 53.7; 19F NMR (376 MHz, DMSO-d6): δ -61.1 (s). HRMS (ESI+): calcd. for C19H18F3N2O6 [M+H]+: 427.1112, found 427.1110. Chiral HPLC analysis: CROWNPAK CR-I (+) 150 x 3 mm. Phase A: ACN+0.5%TFA, phase B: H2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C, tR (D-threo-S12c) = 2.3 min, tR (L-threo-S12c) = 2.7 min (Figure S4).
(DL-threo)-3-{2-[4-(trifluoromethyl)phenyl]acetamido}benzyloxy]aspartate (DL-threo-S12d)

Compound (DL-threo)-S12d was prepared from (DL-threo)-S10d (60 mg, 0.10 mmol) following a procedure similar to that used for (DL-threo)-S12a. The title product (DL-threo)-S12d was obtained as a white solid (7 mg, two steps yield 28%). 1H NMR (500 MHz, DMSO-d6): δ 10.32 (s, 1H), 7.69 (d, J = 8.0 Hz, 2H), 7.56 (d, J = 7.6 Hz, 4H), 7.26 (t, J = 8.1 Hz, 1H), 7.18 (d, J = 7.6 Hz, 1H), 4.76 (d, J = 10.6 Hz, 1H), 4.43 (d, J = 10.6 Hz, 1H), 4.09 (d, J = 9.4 Hz, 1H), 3.80 (d, J = 9.5 Hz, 1H), 3.77 (s, 2H); 19F NMR (376 MHz, DMSO-d6): δ -60.8 (s). HRMS (ESI+): calcd. for C20H20F3NO6 [M+H]+: 441.1268, found 441.1268.

Chiral HPLC analysis: CROWNPAK CR-I (+) 150 x 3 mm. Phase A: ACN+0.5%TFA, phase B: H2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C, tR (D-threo-S12d) = 2.3 min, tR (L-threo-S12d) = 2.6 min (Figure S5).

(DL-erythro)-3- {3-[4-(trifluoromethyl)phenyl]propanamido}benzyloxy]aspartate (DL-erythro-S12d)

Compound (DL-erythro)-S12d was prepared from the mixture of (DL-threo)-S10d and (DL-erythro)-S10d (20 mg, 14%, threo/erythro = 1:2) following a procedure similar to that used for (DL-threo)-S12a. The title product (DL-erythro)-S12d was obtained as a white solid (2 mg, two steps yield 35%, erythro/threo = 88:12). 1H NMR (500 MHz, DMSO-d6): δ 10.28 (s, 1H), 7.69 (d, J = 8.1 Hz, 2H), 7.56 (t, J = 8.4 Hz, 3H), 7.46 (s, 1H), 7.27 (t, J = 7.9 Hz, 1H), 7.08 (d, J = 7.7 Hz, 1H), 4.54 (d, J = 11.4 Hz, 1H), 4.40 (d, J = 11.4 Hz, 1H), 4.10 (s, 1H), 4.06 (d, J = 1.3 Hz, 1H), 3.77 (s, 2H); 19F NMR (376 MHz, DMSO-d6): δ -60.8 (s). HRMS (ESI+): calcd. for C20H20F3N2O6 [M+H]+: 441.1268, found 441.1268.

Chiral HPLC analysis: CROWNPAK CR-I (+) 150 x 3 mm. Phase A: ACN+0.5%TFA, phase B: H2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C, tR (D-erythro-S12d) = 2.3 min, tR (L-erythro-S12d) = 2.6 min (Figure S6).
**Table S1.** Comparison of the $^1$H NMR data of TFB-TBOA$^a$ and TFB-EBOA$^b$.

<table>
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<tr>
<th>Chemoenzymatic product 2a</th>
<th>Authentic reference$^c$</th>
<th>(DL-threo)-S12a</th>
<th>(DL-erythro)-S12a</th>
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<tr>
<td>L-TFB-TBOA</td>
<td>L-TFB-TBOA</td>
<td>DL-TFB-TBOA</td>
<td>DL-TFB-EBOA</td>
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<tr>
<td>10.49 (s, 1H)</td>
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<td>10.48 (s, 1H)</td>
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<td>8.16 (d, $J = 8.1$ Hz, 2H)</td>
<td>8.16 (d, $J = 8.1$ Hz, 2H)</td>
<td>8.15 (d, $J = 8.1$ Hz, 2H)</td>
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<td>7.92 (d, $J = 8.2$ Hz, 2H)</td>
<td>7.92 (d, $J = 8.2$ Hz, 2H)</td>
<td>7.92 (d, $J = 8.2$ Hz, 2H)</td>
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<tr>
<td>7.76 (s, 1H)</td>
<td>7.76 (s, 1H)</td>
<td>7.75 (s, 1H)</td>
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<td>7.28 (t, $J = 7.7$ Hz, 1H)</td>
<td>7.28 (t, $J = 7.6$ Hz, 1H)</td>
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<td>4.12 (d, $J = 9.6$ Hz, 1H)</td>
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<td>3.82 (d, $J = 9.6$ Hz, 1H)</td>
<td>3.82 (d, $J = 9.5$ Hz, 1H)</td>
<td>4.07 (d, $J = 1.3$ Hz, 1H)</td>
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</table>

[c] Authentic sample of L-TFB-TBOA was purchased from Tocris Bioscience.

**Figure S1.** Comparison of the $^1$H NMR spectra of TFB-TBOA and TFB-EBOA.
**Table S2.** Optical rotation of L-TFB-TBOA and its derivatives.

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<th>Entry</th>
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<td></td>
<td></td>
<td><strong>Authentic sample</strong> (L-TFB-TBOA)</td>
</tr>
<tr>
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<td>2a</td>
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<td>0</td>
<td>$-55.0 , (c , 0.60, \text{DMSO})$</td>
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<tr>
<td>3</td>
<td>2b</td>
<td>$o$CF$_3$</td>
<td>0</td>
<td>$-53.1 , (c , 0.43, \text{DMSO})$</td>
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<tr>
<td>4</td>
<td>2c</td>
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<td>0</td>
<td>$-50.7 , (c , 0.43, \text{DMSO})$</td>
</tr>
<tr>
<td>5</td>
<td>16a</td>
<td>$p$CF$_3$</td>
<td>1</td>
<td>$-46.7 , (c , 0.45, \text{DMSO})$</td>
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<tr>
<td>6</td>
<td>16b</td>
<td>$p$CF$_3$</td>
<td>2</td>
<td>$-34.3 , (c , 0.42, \text{DMSO})$</td>
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</table>
IV) Chiral HPLC analysis

Figure S2. Chiral HPLC analysis of product 2a. Chiral HPLC conditions: CROWNPAK CR-I (+) 150 x 3 mm, 5 um. Phase A: ACN+0.5% TFA, phase B: H2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C. This analysis showed that chemoenzymatically prepared 2a has the L-threo configuration (ee >99%), tR (D-threo) = 2.4 min, tR (L-threo) = 2.8 min.
**Figure S3.** Chiral HPLC analysis of product 2b. Chiral HPLC conditions: CROWNPAK CR-I (+) 150 x 3 mm, 5 um. Phase A: ACN+0.5% TFA, phase B: H2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C. This analysis indicated ee >99%, tR (D-threo) = 2.3 min, tR (L-threo) = 2.6 min.
Figure S4. Chiral HPLC analysis of product 2c. Chiral HPLC conditions: CROWNPAK CR-I (+) 150 x 3 mm, 5 um. Phase A: ACN+0.5% TFA, phase B: H₂O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C. This analysis indicated ee >99%, \( t_R \) (D-threo) = 2.3 min, \( t_R \) (L-threo) = 2.7 min.
Figure S5. Chiral HPLC analysis of product 16a. Chiral HPLC conditions: CROWNPAK CR-I (+) 150 x 3 mm, 5 µm. Phase A: ACN+0.5% TFA, phase B: H₂O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C. This analysis indicated ee >99, tᵣ (D-threo) = 2.3 min, tᵣ (L-threo) = 2.6 min.
Figure S6. Chiral HPLC analysis of product 16b. Chiral HPLC conditions: CROWNPAK CR-I (+) 150 x 3 mm, 5 um. Phase A: ACN+0.5% TFA, phase B: H₂O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C. This analysis indicated ee >99, tᵣ (D-threo) = 2.2 min, tᵣ (L-threo) = 2.6 min.
V) Supplementary references


