

Article

Stereochemical Stability of Phenylglycine in Peptide Synthesis: Stereoretentive Coupling and Deprotection Strategies

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Abstract

Phenylglycine (Phg) is a nonproteinogenic α -amino acid found in various bioactive molecules. The C-terminal activation of *N*-acyl Phg is often accompanied by oxazolone-mediated racemization, arising from the direct attachment of the phenyl ring to the α -carbon. After peptide bond formation with another chiral amino acid, this stereochemical erosion is observed as Phg-site epimerization and diastereomer formation. *N*-acyl activated esters, particularly *N*-hydroxysuccinimide (OSu) esters, are widely used for peptide bond formation with proteinogenic α -amino acids. Our previous study on *N*-trifluoroacetyl phenylglycine (TFA-Phg-OH) revealed that Phg-site epimer formation could still occur when TFA-Phg-OSu was employed as an acyl donor for coupling with amino acid ester hydrochlorides (AA-OMe·HCl) in the presence of a soluble organic base. To address these issues, in this study, we report a base-limited one-pot coupling of TFA-Phg-OH with α -amino acid ester hydrochlorides (AA-OR·HCl; R = Me or *tert*-Bu) using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSCD·HCl), which effectively suppresses Phg epimerization. The resulting TFA-Phg-AA-OR dipeptides (AA = Ala, Val, Leu, Met, Phg) were all obtained at a >60% yield with a diastereomeric excess (de) $\geq$ 98.5%. Notably, reducing the amount of triethylamine further minimized epimer formation, while Ba(OH)₂·8H₂O and trifluoroacetic acid enabled stereoretentive deprotection of the *N*-TFA group and *tert*-butyl ester, respectively. This workflow provides practical access to both protected and deprotected Phg-AA motifs, thereby facilitating the preparation of unprotected Phg-containing peptide building blocks.

Keywords: phenylglycine (Phg); Phg epimerization; diastereomeric excess; carbodiimide coupling; trifluoroacetyl (TFA) protection; Ba(OH)₂ deprotection; liquid-phase peptide synthesis

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1. Introduction

Phenylglycine (Phg) is a nonproteinogenic α -amino acid bearing a benzylic stereogenic center, with Phg motifs found in peptide natural products and other bioactive scaffolds [1]. The α -aryl substitution of Phg makes its α -center particularly susceptible to configurational change during C-terminal activation and subsequent acyl-transfer processes [2,3]. In medicinal and process chemistry, enantiopure Phg derivatives serve as valuable chiral building blocks, notably as side-chain precursors in semisynthetic β -lactam antibiotics, where strict stereochemical fidelity is essential for downstream performance [4,5]. When a D- or L-Phg residue is incorporated into a peptide or dipeptide that contains another stereogenic amino acid residue, inversion at the Phg α -center generates an epimeric diastereomer rather than a pair of enantiomers. Therefore, the stereochemical outcome of Phg-containing peptide products is more appropriately described in terms of Phg epimerization (%) or diastereomeric excess (de, %) [6–8]. *N*-protected activated esters, particularly *N*-hydroxysuccinimide (OSu) esters, are widely used for peptide bond formation with proteinogenic α -amino acids in both solution-phase synthesis and bioconjugation [2,3,9]. These esters are typically generated *via* carbodiimide-mediated activation under mild conditions and offer a practical balance between reactivity and handling stability [3,9]. However, for *N*-acyl Phg derivatives, C-terminal activation is frequently accompanied by configurational erosion at the α -center, which is commonly attributed to oxazolone formation and/or base-promoted enolization intermediates [6,10]. WSCD·HCl was employed as the carbodiimide coupling reagent, as it provides efficient activation of the carboxyl group while generating a water-soluble urea byproduct, thereby facilitating aqueous workup and offering practical advantages in terms of cost and handling [10,11].

In our previous study on the *N*-trifluoroacetyl phenylglycine OSu ester (TFA-Phg-OSu), conventional preparation conditions were found to promote configurational erosion at the Phg α -center. Stereoretentive synthesis of TFA-Phg-OSu required excess *N*-hydroxysuccinimide (HOSu; >7.5 equiv) and WSCD·HCl (1.0 equiv). Moreover, when TFA-Phg-OSu was employed as an acyl donor for coupling with amino acid ester hydrochlorides (AA-OMe·HCl), Phg epimerization remained observable in the presence of soluble organic bases. In contrast, heterogeneous inorganic base conditions were beneficial, limiting the formation of the undesired Phg epimer to $\leq 15\%$ under the reported conditions. Importantly, alkaline deprotection steps were also identified as an additional trigger for stereochemical erosion, indicating that stereoretention must be controlled across both coupling and deprotection processes rather than treated as a single-step issue [10].

To address these limitations, we investigated a direct one-pot coupling strategy using TFA-Phg-OH and amino acid ester hydrochlorides (AA-OR·HCl; R = Me or *tert*-Bu) under carbodiimide activation (WSCD·HCl) with a limited amount of base. This approach was designed to suppress racemization by avoiding the isolation of TFA-Phg-OSu and minimizing exposure to basic conditions during coupling. In addition, orthogonal deprotection of the resulting dipeptides was examined to enable practical access to unprotected Phg-containing peptides.

2. Materials and Methods

2.1. General Remarks

All reagents were of analytical grade and were used as received unless otherwise noted. WSCD·HCl and L- and D-Phg were purchased from TCI (Tokyo, Japan). NMR spectra were recorded on JEOL EX-270 and ECZ-400 spectrometers (JEOL, Tokyo, Japan). Chemical shifts (δ) are reported in ppm relative to residual solvent signals. Optical rotations were measured at 23 °C using a JASCO DIP-370 polarimeter (JASCO, Tokyo, Japan).

HRMS-ESI spectra were obtained using a Waters UPLC ESI-TOF mass spectrometer (Waters, Milford, CT, USA).

2.2. Determination of Diastereomeric Excess

The stereochemical outcome of each coupling and deprotection reaction was evaluated through ^1H NMR analysis using diagnostic resonances of the two Phg epimers (diastereomers). In this study, the Phg α -H resonance was used as the primary diagnostic signal whenever it was sufficiently separated from overlapping resonances. The integral of the signal corresponding to the retained Phg configuration was defined as retained, and the integral of the signal corresponding to the epimerized Phg configuration was defined as epimer (see Supplementary Materials). When the Phg α -H signal was not sufficiently resolved, a separated reporter resonance from the other amino acid ester moiety was used instead, typically the OMe or Ot-Bu signal. This substitution is valid because inversion at Phg in a dipeptide generates diastereomeric products that place the ester substituent in different magnetic environments. NMR-based stereochemical analysis commonly relies on the integration of separated diagnostic resonances after the two stereochemical forms have become magnetically nonequivalent [10,12]. The percentage of Phg epimerization was calculated using the formula shown in Figure 1.

$$de = \frac{A - B}{A + B} \times 100\%$$

Figure 1. Formula for calculating the diastereomeric excess (de) of the peptide. *de*: diastereomeric excess; A: signal intensity of the dominant enantiomer (integral); B: signal intensity of the inferior enantiomer (integral).

2.3. Synthesis

2.3.1. 2-Phenyl-2-(2,2,2-trifluoroacetamido)acetic acid (TFA-Phg-OH, 1)

Triethylamine (4.14 mL, 29.6 mmol) was added to a suspension of L- or D-phenylglycine (3.00 g, 19.85 mmol) in MeOH (50 mL) at 0 °C. After stirring for 15 min, ethyl trifluoroacetate (3.06 mL, 25.8 mmol) was added dropwise at the same temperature. The reaction mixture was then stirred at room temperature overnight until a homogeneous solution was obtained.

The solvent was removed under reduced pressure, and the residue was dissolved in H₂O (100 mL). The solution was acidified with concentrated HCl (4 mL) and stirred for 15 min, followed by extraction with EtOAc. The organic layer was washed with brine, dried over MgSO₄, filtered, and concentrated to afford a white solid. If necessary, the product was further dried under high vacuum overnight.

D-1: 4.87 g, 19.7 mmol, 99.3% yield, $[\alpha]_D -174$ (c 1, MeOH). ^1H -NMR (400 MHz, ACETONE-D₆) δ 9.01 (s, 1H), 7.53–7.36 (m, 5H), 5.63–5.61 (m, 1H). ^{13}C -NMR (101 MHz, ACETONE-D₆) δ 169.9, 156.4 (q, $^2J_{\text{CF}} = 37.6$ Hz), 135.5, 128.9, 128.8, 128.1, 116.1 (q, $^1J_{\text{CF}} = 287$ Hz), 56.9.

L-1: 4.82 g, 19.5 mmol, 98.3% yield, $[\alpha]_D +171$ (c 1, MeOH). ^1H -NMR (400 MHz, ACETONE-D₆) δ 9.00 (s, 1H), 7.53–7.37 (m, 5H), 5.63–5.61 (m, 1H). ^{13}C -NMR (101 MHz, ACETONE-D₆) δ 170.7, 156.4 ($^2J_{\text{CF}} = 37.6$ Hz), 136.3, 129.7, 129.6, 129.0, 116.1 ($^1J_{\text{CF}} = 287$ Hz), 57.8.

2.3.2. Methyl (2-phenyl-2-(2,2,2-trifluoroacetamido)acetyl)-alaninate (TFA-Phg-Ala-OMe, 3)

Triethylamine (28.2 μ L, 0.202 mmol, 0.5 equiv) was added to a suspension of alanine methyl ester hydrochloride (Ala-OMe-HCl, 0.486 mmol) in CH_2Cl_2 (6 mL), and the mixture was stirred at room temperature for 10 min. A solution of TFA-Phg-OH (100 mg, 0.405 mmol) in CH_2Cl_2 (12 mL) was added, followed by WSCD-HCl (77.6 mg, 0.405 mmol) at 0 $^\circ\text{C}$. The reaction mixture was stirred at room temperature for 2 h and monitored using TLC. The reaction was quenched with brine, and the mixture was extracted with EtOAc. The organic layer was dried over MgSO_4 , filtered, and concentrated under reduced pressure. The crude product was purified through silica gel column chromatography (EtOAc/hexane = 1:3 *v/v*) to afford the corresponding dipeptide.

(D, D)-3: $[\alpha]_D -139$ (c 1, CHCl_3), HRMS-ESI (*m/z*) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{14}\text{H}_{16}\text{F}_3\text{N}_2\text{O}_4^+$ 333.1057, found 333.1032. $^1\text{H-NMR}$ (400 MHz, CHLOROFORM- D_3) δ : 7.40 (s, 5H), 5.41 (d, $J = 6.6$ Hz, 1H), 4.53 (t, $J = 7.0$ Hz, 1H), 3.69 (s, 3H), 1.43 (d, $J = 7.3$ Hz, 3H). $^{13}\text{C-NMR}$ (ACETONE- D_6) δ : 173.1, 168.8, 156.9 (q, $^2J_{\text{CF}} = 37.4$ Hz), 137.4, 129.4, 129.2, 128.9, 116.9 (q, $^1J_{\text{CF}} = 288$ Hz), 57.6, 52.4, 49.2, 17.5.

(L, L)-3: $[\alpha]_D +132$ (c 1, CHCl_3), HRMS-ESI (*m/z*) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{14}\text{H}_{16}\text{F}_3\text{N}_2\text{O}_4^+$ 333.1057, found 333.1075. $^1\text{H-NMR}$ (400 MHz, CHLOROFORM- D_3) δ : 7.40 (s, 5H), 5.41 (d, $J = 6.3$ Hz, 1H), 4.53 (t, $J = 7.2$ Hz, 1H), 3.69 (s, 3H), 1.43 (d, $J = 7.3$ Hz, 3H). $^{13}\text{C-NMR}$ (ACETONE- D_6) δ : 173.1, 168.8, 156.9 (q, $^2J_{\text{CF}} = 37.1$ Hz), 137.4, 129.4, 129.2, 128.9, 116.9 (q, $^1J_{\text{CF}} = 288$ Hz), 57.6, 52.2, 49.2, 17.5.

(D, L)-3: $[\alpha]_D -113$ (c 1, CHCl_3), HRMS-ESI (*m/z*) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{14}\text{H}_{16}\text{F}_3\text{N}_2\text{O}_4^+$ 333.1057, found 333.1032. $^1\text{H-NMR}$ (400 MHz, CHLOROFORM- D_3) δ : 7.39 (s, 5H), 5.41 (d, $J = 6.3$ Hz, 1H), 4.57 (t, $J = 7.2$ Hz, 1H), 3.76 (s, 3H), 1.30 (d, $J = 7.3$ Hz, 3H). $^{13}\text{C-NMR}$ (ACETONE- D_6) δ : 173.2, 169.0, 156.8 (q, $^2J_{\text{CF}} = 37.2$ Hz), 137.8, 129.5, 129.3, 128.6, 116.9 (q, $^1J_{\text{CF}} = 287$ Hz), 57.8, 52.4, 49.2, 17.5.

(L, D)-3: $[\alpha]_D +122$ (c 1, CHCl_3), HRMS-ESI (*m/z*) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{14}\text{H}_{16}\text{F}_3\text{N}_2\text{O}_4^+$ 333.1057, found 333.1030. $^1\text{H-NMR}$ (400 MHz, CHLOROFORM- D_3) δ : 7.38 (s, 5H), 5.46 (d, $J = 6.6$ Hz, 1H), 4.57 (t, $J = 7.2$ Hz, 1H), 3.76 (s, 3H), 1.30 (d, $J = 7.3$ Hz, 3H). $^{13}\text{C-NMR}$ (ACETONE- D_6) δ : 173.2, 169.1, 156.8 (q, $^2J_{\text{CF}} = 37.2$ Hz), 137.8, 129.5, 129.3, 128.6, 116.9 (q, $^1J_{\text{CF}} = 287$ Hz), 57.8, 52.4, 49.2, 17.5.

2.3.3. tert-Butyl (2-phenyl-2-(2,2,2-trifluoroacetamido)acetyl)-alaninate (TFA-Phg-Ala-Ot-Bu, 12)

Triethylamine (56.4 μ L, 0.405 mmol, 0.5 equiv) was added in two portions to a suspension of the appropriate alanine tert-butyl ester hydrochloride (Ala-Ot-Bu-HCl (D/L), 176.4 mg, 0.971 mmol) in 12 mL CH_2Cl_2 , and the mixture was stirred at room temperature for ca. 10 min. A solution of the corresponding TFA-Phg-OH (D/L, 200 mg, 0.809 mmol) in 24 mL CH_2Cl_2 was then combined with the above mixture, and WSCD-HCl (155.8 mg, 0.809 mmol) in 12 mL CH_2Cl_2 was added on ice. The reaction mixture was stirred at room temperature for 2 h with TLC monitoring. Saturated brine and AcOEt were added, and the organic layer was separated, dried over MgSO_4 , filtered, and concentrated under reduced pressure. The residue was purified through silica gel column chromatography (EtOAc: hexane = 1:3 *v/v*) to afford the corresponding TFA-Phg-Ala-OtBu (**12**).

(D, D)-12: $[\alpha]_D -47$ (c 1, MeOH), HRMS-ESI (*m/z*) $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{18}\text{H}_{25}\text{F}_3\text{N}_2\text{NaO}_4^+$ 397.1346, found 397.1340. $^1\text{H-NMR}$ (400 MHz, CHLOROFORM- D_3) δ : 8.05 (d, $J = 6.6$ Hz, 1H), 7.31–7.44 (5H), 6.63 (d, $J = 7.0$ Hz, 1H), 5.55 (d, $J = 7.0$ Hz, 1H), 4.39 (m, 1H), 1.38 (d, $J = 7.0$ Hz, 3H), 1.36 (s, 9H). $^{13}\text{C-NMR}$ (101 MHz, CHLOROFORM- D_3) δ : 171.1, 167.8, 156.6 (q, $^2J_{\text{CF}} = 37.8$ Hz), 136.0, 129.3, 129.1, 127.4, 115.8 (q, $^1J_{\text{CF}} = 287$ Hz), 82.5, 57.1, 49.4, 27.9, 18.3.

(L, L)-12: $[\alpha]_D +73$ (c 1, MeOH), HRMS-ESI (*m/z*) $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{18}\text{H}_{25}\text{F}_3\text{N}_2\text{NaO}_4^+$ 397.1346, found 397.1348. $^1\text{H-NMR}$ (400 MHz, CHLOROFORM- D_3) δ : 7.94 (d, $J = 6.1$ Hz,

1H), 7.41–7.35 (m, 5H), 6.43 (d, $J = 6.9$ Hz, 1H), 5.47 (d, $J = 6.7$ Hz, 1H), 4.38 (m, 1H), 1.39 (d, $J = 7.2$ Hz, 3H), 1.37 (s, 9H). ^{13}C -NMR (101 MHz, CHLOROFORM- D_3) δ 171.1, 167.7, 156.5 (q, $^2J_{\text{CF}} = 37.8$ Hz), 135.9, 129.4, 129.2, 127.4, 115.7 (q, $^1J_{\text{CF}} = 287.79$ Hz), 82.5, 57.2, 49.5, 27.9, 18.4.

(D, L)-**12**: $[\alpha]_{\text{D}} -104$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{18}\text{H}_{25}\text{F}_3\text{N}_2\text{O}_4^+$ 397.1346, found 397.1339. ^1H -NMR (400 MHz, CHLOROFORM- D_3) δ 7.96 (d, $J = 6.1$ Hz, 1H), 7.39–7.35 (m, 5H), 6.39 (d, $J = 7.0$ Hz, 1H), 5.46 (d, $J = 6.6$ Hz, 1H), 4.43 (m, 1H), 1.45 (s, 9H), 1.25 (d, $J = 7.0$ Hz, 3H). ^{13}C -NMR (101 MHz, CHLOROFORM- D_3) δ 171.5, 167.8, 156.4 (q, $^2J_{\text{CF}} = 37.8$ Hz), 136.2, 129.4, 129.1, 127.4, 115.7 (q, $^1J_{\text{CF}} = 288$ Hz), 82.8, 57.1, 49.3, 28.0, 18.1.

(L, D)-**12**: $[\alpha]_{\text{D}} +123$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{18}\text{H}_{25}\text{F}_3\text{N}_2\text{NaO}_4^+$ 397.1346, found 397.1338. ^1H -NMR (400 MHz, CHLOROFORM- D_3) δ 7.98 (d, $J = 6.1$ Hz, 1H), δ 7.39–7.37 (m, 5H), 6.43 (d, $J = 7.0$ Hz, 1H), 5.47 (d, $J = 6.7$ Hz, 1H), 4.44 (m, 1H), 1.45 (s, 9H), 1.25 (d, $J = 7.2$ Hz, 3H). ^{13}C -NMR (101 MHz, CHLOROFORM- D_3) δ 171.6, 167.8, 156.4 (q, $^2J_{\text{CF}} = 37.8$), 136.3, 129.4, 129.1, 127.4, 115.7 (q, $^1J_{\text{CF}} = 288$ Hz), 82.7, 57.1, 49.3, 28.0, 18.1.

2.3.4. Methyl (2-phenyl-2-(2,2,2-trifluoroacetamido)acetyl)-valinate (TFA-Phg-Val-OMe, 13)

Triethylamine (28.2 μL , 0.203 mmol) was added to a suspension of valine methyl ester hydrochloride (Val-OMe-HCl (D/L), 83.4 mg, 0.486 mmol) in 12 mL CH_2Cl_2 . After stirring for 10 min, a solution of TFA-Phg-OH (D/L, 100 mg, 0.405 mmol) in 15 mL CH_2Cl_2 and then one of WSCD-HCl (77.6 mg, 0.405 mmol) in 6 mL CH_2Cl_2 were added on ice. The suspension was turned into a solution through stirring at room temperature for 1 h. The reaction mixture was extracted with saturated brine and EtOAc, resulting in an organic layer. The organic solution was dried with MgSO_4 and filtered, and the crude product was obtained using rotary evaporation and purified through column chromatography (EtOAc: hexane = 1:3 v/v) to give a colorless solid.

(D, D)-**13**: $[\alpha]_{\text{D}} -59$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{16}\text{H}_{19}\text{F}_3\text{N}_2\text{O}_4^+$ 361.1370, found 361.1370. ^1H -NMR (400 MHz, CHLOROFORM- D_3) δ 7.85 (d, $J = 6.1$ Hz, 1H), 7.38 (s, 5H), 6.20 (d, $J = 8.3$ Hz, 1H), 5.51 (d, $J = 6.6$ Hz, 1H), 4.48 (dd, $J = 8.3, 4.8$ Hz, 1H), 3.64 (s, 3H), 2.17 (m, 1H), 0.94 (d, $J = 6.9$ Hz, 3H), 0.88 (d, $J = 6.9$ Hz, 3H). ^{13}C -NMR (101 MHz, CHLOROFORM- D_3) δ 171.4, 168.5, 156.6 (q, $^2J_{\text{CF}} = 37.8$ Hz), 135.6, 129.4, 129.3, 127.4, 115.7 (q, $^1J_{\text{CF}} = 288$ Hz), 58.0, 57.2, 52.3, 31.2, 18.9, 17.8.

(L, L)-**13**: $[\alpha]_{\text{D}} +61$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{16}\text{H}_{19}\text{F}_3\text{N}_2\text{O}_4^+$ 361.1370, found 361.1377. ^1H -NMR (400 MHz, CHLOROFORM- D_3) δ 7.89 (d, $J = 6.2$ Hz, 1H), 7.38 (s, 5H), 6.28 (d, $J = 8.5$ Hz, 1H), 5.55 (d, $J = 6.6$ Hz, 1H), 4.48 (dd, $J = 8.5, 4.8$ Hz, 1H), 3.64 (s, 3H), 2.17 (m, 1H), 0.94 (d, $J = 6.9$ Hz, 3H), 0.88 (d, $J = 6.9$ Hz, 3H). ^{13}C -NMR (101 MHz, CHLOROFORM- D_3) δ 171.4, 168.4, 156.6 (q, $^2J_{\text{CF}} = 37.8$ Hz), 135.6, 129.4, 129.3, 127.5, 115.7 (q, $^1J_{\text{CF}} = 288$ Hz), 58.0, 57.2, 52.4, 31.2, 18.9, 17.8.

(D, L)-**13**: $[\alpha]_{\text{D}} -113$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{16}\text{H}_{19}\text{F}_3\text{N}_2\text{O}_4^+$ 361.1370, found 361.1378. ^1H -NMR (400 MHz, CHLOROFORM- D_3) δ 7.97 (d, $J = 5.9$ Hz, 1H), 7.42–7.35 (m, 5H), 6.33 (d, $J = 9.0$ Hz, 1H), 5.51 (d, $J = 6.2$ Hz, 1H), 4.54 (dd, $J = 9.0, 5.0$ Hz, 1H), 3.75 (s, 3H), 2.07 (m, 1H), 0.68 (d, $J = 6.9$ Hz, 3H), 0.64 (d, $J = 6.9$ Hz, 3H). ^{13}C -NMR (101 MHz, CHLOROFORM- D_3) δ 171.8, 168.3, 156.4 (q, $^2J_{\text{CF}} = 37.8$ Hz), 136.4, 129.5, 129.3, 127.3, 115.7 (q, $^1J_{\text{CF}} = 288$ Hz), 57.5, 57.4, 52.6, 31.4, 18.8, 17.2.

(L, D)-**13**: $[\alpha]_{\text{D}} +109$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{16}\text{H}_{19}\text{F}_3\text{N}_2\text{O}_4^+$ 361.1370, found 361.1373. ^1H -NMR (400 MHz, CHLOROFORM- D_3) δ 7.94 (d, $J = 5.9$ Hz, 1H), 7.42–7.34 (m, 5H), 6.22 (d, $J = 9.0$ Hz, 1H), 5.47 (d, $J = 6.4$ Hz, 1H), 4.54 (dd, $J = 9.0, 4.8$ Hz, 1H), 3.75 (s, 3H), 2.06 (m, 1H), 0.68 (d, $J = 6.7$ Hz, 3H), 0.63 (d, $J = 6.9$ Hz, 3H).

^{13}C -NMR (101 MHz, CHLOROFORM- D_3) δ 171.8, 168.3, 156.4 (q, $^2J_{\text{CF}} = 37.8$ Hz), 136.4, 129.4, 129.2, 127.2, 115.7 (q, $^1J_{\text{CF}} = 288$ Hz), 57.4, 57.3, 52.5, 31.4, 18.7, 17.1.

2.3.5. tert-Butyl (2-phenyl-2-(2,2,2-trifluoroacetamido)acetyl)-valinate (TFA-Phg-Val-Ot-Bu, 14).

Triethylamine (28.2 μ L, 0.203 mmol) was added to a suspension of valine tert-butyl ester hydrochloride (Val-Ot-Bu·HCl (D/L), 102 mg, 0.486 mmol) in 12 mL CH₂Cl₂. After stirring for 10 min, a solution of TFA-Phg-OH (D/L, 100 mg, 0.405 mmol) in 12 mL CH₂Cl₂ and then one of WSCD·HCl (77.6 mg, 0.405 mmol) in 6 mL CH₂Cl₂ were added on ice. The suspension was turned into a solution through stirring at room temperature for 1 h. The reaction mixture was extracted with saturated brine and EtOAc, resulting in an organic layer. The organic solution was dried with MgSO₄ and filtered, and the crude product was obtained using rotary evaporation and purified through column chromatography (EtOAc: hexane = 1:3 *v/v*) to give a colorless solid.

(D, D)-**14**: $[\alpha]_D -44$ (c 1, MeOH), HRMS-ESI (*m/z*) [*M* + *H*]⁺ calcd for C₁₉H₂₆F₃N₂O₄⁺ 403.1839, found 403.1847. ¹H-NMR (400 MHz, CHLOROFORM-D) δ 7.99 (d, *J* = 6.6 Hz, 1H), 7.40–7.34 (m, 5H), 6.36 (d, *J* = 8.3 Hz, 1H), 5.56 (d, *J* = 6.7 Hz, 1H), 4.34 (dd, *J* = 8.5, 5.0 Hz, 1H), 2.12 (m, 1H), 1.33 (s, 9H), 0.94 (d, *J* = 6.9 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H). ¹³C-NMR (101 MHz, CHLOROFORM-D) δ 169.8, 168.3, 156.6 (q, ²*J*_{CF} = 37.8 Hz), 135.9, 129.4, 129.2, 127.3, 115.7 (q, ¹*J*_{CF} = 288 Hz), 82.4, 58.5, 57.2, 31.5, 27.9, 18.8, 17.8.

(L, L)-**14**: $[\alpha]_D +48.4$ (c 1, MeOH), HRMS-ESI (*m/z*) [*M* + *H*]⁺ calcd for C₁₉H₂₆F₃N₂O₄⁺ 403.1839, found 403.1845. ¹H-NMR (400 MHz, CHLOROFORM-D) δ 7.92 (d, *J* = 6.1 Hz, 1H), 7.39–7.36 (m, 5H), 6.22 (d, *J* = 8.5 Hz, 1H), 5.50 (d, *J* = 6.6 Hz, 1H), 4.34 (dd, *J* = 8.5, 4.8 Hz, 1H), 2.13 (m, 1H), 1.34 (s, 9H), 0.94 (d, *J* = 6.9 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H). ¹³C-NMR (101 MHz, CHLOROFORM-D) δ 169.7, 168.2, 156.5 (q, ²*J*_{CF} = 37.8 Hz), 135.8, 129.3, 129.2, 127.3, 115.7 (q, ¹*J*_{CF} = 288 Hz), 82.4, 58.4, 57.2, 31.4, 27.8, 18.8, 17.7.

(D, L)-**14**: $[\alpha]_D -99$ (c 1, MeOH), HRMS-ESI (*m/z*) [*M* + *H*]⁺ calcd for C₁₉H₂₆F₃N₂O₄⁺ 403.1839, found 403.1847. ¹H-NMR (400 MHz, CHLOROFORM-D) δ 7.93 (d, *J* = 5.4 Hz, 1H), 7.41–7.36 (m, 5H), 6.02 (d, *J* = 8.6 Hz, 1H), 5.39 (d, *J* = 6.1 Hz, 1H), 4.43 (q, *J* = 4.4 Hz, 1H), 2.05 (m, 1H), 1.46 (s, 9H), 0.67 (d, *J* = 6.9 Hz, 3H), 0.62 (d, *J* = 7.0 Hz, 3H). ¹³C-NMR (101 MHz, CHLOROFORM-D) δ 170.3, 168.1, 156.3 (q, ²*J*_{CF} = 37.8 Hz), 136.5, 129.4, 129.2, 127.2, 115.7 (q, ¹*J*_{CF} = 288 Hz), 82.7, 57.7, 57.4, 31.6, 28.0, 18.6, 17.0.

(L, D)-**14**: $[\alpha]_D +106.8$ (c 1, MeOH), HRMS-ESI (*m/z*) [*M* + *H*]⁺ calcd for C₁₉H₂₆F₃N₂O₄⁺ 403.1839, found 403.1845. ¹H-NMR (400 MHz, CHLOROFORM-D) δ 7.96 (d, *J* = 5.4 Hz, 1H), 7.40–7.34 (m, 5H), 6.09 (d, *J* = 8.6 Hz, 1H), 5.42 (d, *J* = 6.1 Hz, 1H), 4.43 (q, *J* = 4.4 Hz, 1H), 2.05 (m, 1H), 1.46 (s, 9H), 0.67 (d, *J* = 6.9 Hz, 3H), 0.62 (d, *J* = 6.9 Hz, 3H). ¹³C-NMR (101 MHz, CHLOROFORM-D) δ 170.3, 168.1, 156.3 (q, ²*J*_{CF} = 37.8 Hz), 136.5, 129.4, 129.2, 127.2, 115.7 (q, ¹*J*_{CF} = 288 Hz), 82.7, 57.7, 57.4, 31.6, 28.0, 18.6, 17.0.

2.3.6. Methyl (2-phenyl-2-(2,2,2-trifluoroacetamido)acetyl)-leucinate (TFA-Phg-Leu-OMe, 15)

Triethylamine (28.2 μ L, 0.203 mmol) was added to a suspension of leucine methyl ester hydrochloride (Leu-OMe·HCl (D/L), 89 mg, 0.486 mmol) in 12 mL CH₂Cl₂. After stirring for 10 min, a solution of TFA-Phg-OH (D/L, 100 mg, 0.405 mmol) in 15 mL CH₂Cl₂ and then one of WSCD·HCl (77.6 mg, 0.405 mmol) in 6 mL CH₂Cl₂ were added on ice. The suspension was turned into a solution through stirring at room temperature for 1 h. The reaction mixture was extracted with saturated brine and EtOAc, resulting in an organic layer. The organic solution was dried with MgSO₄ and filtered, and the crude product was obtained using rotary evaporation and purified through column chromatography (EtOAc: hexane = 1:3 *v/v*) to give a colorless solid.

(D, D)-**15**: $[\alpha]_D -53.2$ (c 1, MeOH), HRMS-ESI (*m/z*) [*M* + *H*]⁺ calcd for C₁₇H₂₂F₃N₂O₄⁺ 375.1526, found 375.1544. ¹H-NMR (400 MHz, CHLOROFORM-D) δ 7.90 (d, *J* = 6.4 Hz, 1H), 7.38 (s, 5H), 6.15 (d, *J* = 8.0 Hz, 1H), 5.50 (d, *J* = 6.6 Hz, 1H), 4.57 (m, 1H), 3.63 (s, 3H), 1.67–1.59 (m, 2H), 1.51 (m, 1H), 0.94 (d, *J* = 6.2 Hz, 3H), 0.92 (d, *J* = 6.2 Hz, 3H). ¹³C-NMR

(101 MHz, CHLOROFORM-D) δ 172.4, 168.2, 156.5 (q, $^2J_{CF}$ = 37.8 Hz), 135.5, 129.3, 129.2, 127.5, 115.6 (q, $^1J_{CF}$ = 288 Hz), 57.0, 52.4, 51.4, 41.1, 24.8, 22.7, 21.8.

(L, L)-**15**: $[\alpha]_D^{+54}$ (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{17}H_{22}F_3N_2O_4^+$ 375.1526, found 375.1553. 1H -NMR (400 MHz, CHLOROFORM-D) δ 7.90 (d, J = 6.4 Hz, 1H), 7.38 (s, 5H), 6.16 (d, J = 8.0 Hz, 1H), 5.51 (d, J = 6.7 Hz, 1H), 4.57 (m, 1H), 3.63 (s, 3H), 1.67–1.59 (m, 2H), 1.52 (m, 1H), 0.94 (d, J = 6.0 Hz, 3H), 0.92 (d, J = 6.2 Hz, 3H). ^{13}C -NMR (101 MHz, CHLOROFORM-D) δ 172.4, 168.2, 156.5 (q, $^2J_{CF}$ = 37.8 Hz), 135.5, 129.3, 129.2, 127.5, 115.7 (q, $^1J_{CF}$ = 288 Hz), 57.0, 52.4, 51.4, 41.1, 24.8, 22.7, 21.8.

(D, L)-**15**: $[\alpha]_D^{-134.6}$ (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{17}H_{22}F_3N_2O_4^+$ 375.1526, found 375.1547. 1H -NMR (400 MHz, CHLOROFORM-D) δ 7.95 (d, J = 6.1 Hz, 1H), 7.39–7.35 (m, 5H), 6.24 (d, J = 8.3 Hz, 1H), 5.47 (d, J = 6.4 Hz, 1H), 4.59 (m, 1H), 3.75 (s, 3H), 1.53 (m, 1H), 1.42 (m, 1H), 1.20 (m, 1H), 0.74 (d, J = 6.0 Hz, 6H). ^{13}C -NMR (101 MHz, CHLOROFORM-D) δ 172.8, 168.2, 156.4 (q, $^2J_{CF}$ = 37.8 Hz), 136.1, 129.3, 129.2, 127.2, 115.6 (q, $^1J_{CF}$ = 288 Hz), 57.1, 52.6, 51.2, 41.0, 24.6, 22.6, 21.5.

(L, D)-**15**: $[\alpha]_D^{+130.3}$ (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{17}H_{22}F_3N_2O_4^+$ 375.1526, found 375.1542. 1H -NMR (400 MHz, CHLOROFORM-D) δ 7.94 (d, J = 5.9 Hz, 1H), 7.39–7.35 (m, 5H), 6.21 (d, J = 8.3 Hz, 1H), 5.46 (d, J = 6.4 Hz, 1H), 4.59 (m, 1H), 3.75 (s, 3H), 1.55 (m, 1H), 1.40 (m, 1H), 1.20 (m, 1H), 0.74 (d, J = 6.7 Hz, 6H). ^{13}C -NMR (101 MHz, CHLOROFORM-D) δ 172.8, 168.1, 156.4 (q, $^2J_{CF}$ = 38.1 Hz), 136.1, 129.3, 129.2, 127.2, 115.6 (q, $^1J_{CF}$ = 288 Hz), 57.1, 52.6, 51.2, 41.1, 24.6, 22.6, 21.5.

2.3.7. tert-Butyl (2-phenyl-2-(2,2,2-trifluoroacetamido)acetyl)-leucinate (TFA-Phg-Leu-Ot-Bu, **16**)

Triethylamine (28.2 μ L, 0.203 mmol) was added to a suspension of the appropriate leucine tert-butyl ester hydrochloride (Leu-Ot-Bu-HCl (D/L), 108.7 mg, 0.486 mmol) in 6 mL CH_2Cl_2 . After stirring for ca. 10 min, a solution of the corresponding TFA-Phg-OH (D/L, 100 mg, 0.405 mmol) in 12 mL CH_2Cl_2 and then one of WSCD-HCl (77.6 mg, 0.405 mmol) in 6 mL CH_2Cl_2 were added on ice. The reaction mixture was stirred at room temperature for 1 h for the homochiral pairs [(D, D) and (L, L)] or for 2 h for the heterochiral pairs [(D, L) and (L, D)] with TLC monitoring. The reaction mixture was extracted with saturated brine and EtOAc, and the organic layer was separated, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified through silica gel column chromatography (EtOAc/hexane = 1:3 v/v) to afford the corresponding TFA-Phg-Leu-Ot-Bu (**16**) as a colorless solid.

(D, D)-**16**: $[\alpha]_D^{-44}$ (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{20}H_{28}F_3N_2O_4^+$ 417.1996, found 417.1994. 1H -NMR (400 MHz, CHLOROFORM-D) δ 7.92 (d, J = 6.2 Hz, 1H), 7.39–7.36 (m, 5H), 6.11 (d, J = 8.2 Hz, 1H), 5.46 (d, J = 6.6 Hz, 1H), 4.44 (m, 1H), 1.60 (m, 2H), 1.50 (m, 1H), 1.33 (s, 9H), 0.94 (d, J = 6.4 Hz, 6H). ^{13}C -NMR (101 MHz, CHLOROFORM-D) δ 170.8, 167.9, 156.5 (q, $^2J_{CF}$ = 37.8 Hz), 135.8, 129.4, 129.3, 127.6, 115.7 (q, $^1J_{CF}$ = 288 Hz), 82.3, 57.2, 52.3, 41.6, 27.9, 25.0, 22.7, 22.1.

(L, L)-**16**: $[\alpha]_D^{+34}$ (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{20}H_{28}F_3N_2O_4^+$ 417.1996, found 471.2005. 1H -NMR (400 MHz, CHLOROFORM-D) δ 7.90 (d, J = 6.1 Hz, 1H), 7.38–7.37 (m, 5H), 6.07 (d, J = 8.2 Hz, 1H), 5.44 (d, J = 6.4 Hz, 1H), 4.44 (m, 1H), 1.62 (m, 2H), 1.49 (m, 1H), 1.34 (s, 9H), 0.94 (d, J = 6.4 Hz, 6H). ^{13}C -NMR (101 MHz, CHLOROFORM-D) δ 171.2, 168.4, 156.9 (q, $^2J_{CF}$ = 37.8 Hz), 136.2, 129.5, 129.3, 127.7, 116.1 (q, $^1J_{CF}$ = 288 Hz), 82.5, 57.2, 52.6, 41.7, 28.1, 25.2, 22.9, 22.4.

(D, L)-**16**: $[\alpha]_D^{-118}$ (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{20}H_{28}F_3N_2O_4^+$ 417.1996, found 417.1998. 1H -NMR (400 MHz, CHLOROFORM-D) δ 7.94 (d, J = 5.8 Hz, 1H), 7.39–7.35 (m, 5H), 6.02 (d, J = 8.3 Hz, 1H), 5.39 (d, J = 6.1 Hz, 1H), 4.48 (m, 1H), 1.51 (m, 1H), 1.46 (s, 9H), 1.34 (m, 1H), 1.22 (m, 1H), 0.75 (d, J = 6.7 Hz, 6H). ^{13}C -NMR (101 MHz,

CHLOROFORM-D) δ 171.4, 167.8, 156.3 (q, $^2J_{CF}$ = 37.8 Hz), 136.2, 129.3, 129.1, 127.3, 115.6 (q, $^1J_{CF}$ = 288 Hz), 82.6, 57.3, 51.8, 41.4, 27.9, 24.7, 22.6, 21.8.

(L, D)-**16**: $[\alpha]_D$ +115.4 (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{20}H_{28}F_3N_2O_4^+$ 417.1996, found 471.1994. 1H -NMR (400 MHz, CHLOROFORM-D) δ 7.94 (d, J = 5.8 Hz, 1H), 7.39–7.35 (m, 5H), 6.03 (d, J = 8.3 Hz, 1H), 5.39 (d, J = 6.2 Hz, 1H), 4.48 (m, 1H), 1.51 (m, 1H), 1.46 (s, 9H), 1.35 (m, 1H), 1.22 (m, 1H), 0.75 (d, J = 6.5 Hz, 6H). ^{13}C -NMR (101 MHz, CHLOROFORM-D) δ 171.4, 167.8, 156.3 (q, $^2J_{CF}$ = 37.8 Hz), 136.2, 129.3, 129.1, 127.3, 115.7 (q, $^1J_{CF}$ = 288 Hz), 82.6, 57.3, 51.8, 41.4, 27.9, 24.7, 22.6, 21.8.

2.3.8. Methyl (2-phenyl-2-(2,2,2-trifluoroacetamido)acetyl)-methionate (TFA-Phg-Met-OMe, 17)

Triethylamine (28.2 μ L, 0.202 mmol) was added to a suspension of the appropriate methionine methyl ester hydrochloride (Met-OMe-HCl (D/L), 97.1 mg, 0.486 mmol) in 6 mL CH_2Cl_2 , and the mixture was stirred at room temperature for ca. 10 min. A solution of the corresponding TFA-Phg-OH (D/L, 100 mg, 0.405 mmol) in 12 mL CH_2Cl_2 was then combined with the above mixture, and WSCD-HCl (77.6 mg, 0.405 mmol) in 6 mL CH_2Cl_2 was added on ice. The reaction mixture was stirred at room temperature for 1 h for the homochiral pairs [(D, D) and (L, L)] or for 2 h for the heterochiral pairs [(D, L) and (L, D)] with TLC monitoring. Saturated brine and AcOEt were then added, and the organic layer was separated, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified through silica gel column chromatography (EtOAc/hexane = 1:3 v/v) to afford the corresponding TFA-Phg-Met-OMe (**17**).

(D, D)-**17**: $[\alpha]_D$ -36.6 (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{16}H_{20}F_3N_2O_4S^+$ 393.1090, found 393.1108. 1H -NMR (400 MHz, CHLOROFORM-D) δ 7.82 (d, J = 6.1 Hz, 1H), 7.39 (s, 5H), 6.47 (d, J = 7.5 Hz, 1H), 5.45 (d, J = 6.4 Hz, 1H), 4.67 (m, 1H), 3.67 (s, 3H), 2.49 (m, 2H), 2.18 (m, 1H), 2.07 (s, 3H), 2.00 (m, 1H). ^{13}C -NMR (101 MHz, CHLOROFORM-D) δ : 171.5, 168.4, 156.7 (q, $^2J_{CF}$ = 37.8 Hz), 135.6, 129.5, 129.4, 127.5, 115.6 (q, $^1J_{CF}$ = 288 Hz), 57.2, 52.7, 52.3, 30.9, 30.0, 15.5.

(L, L)-**17**: $[\alpha]_D$ +39.6 (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{16}H_{20}F_3N_2O_4S^+$ 393.1090, found 393.1095. 1H -NMR (400 MHz, CHLOROFORM-D) δ 7.82 (d, J = 5.9 Hz, 1H), 7.40 (s, 5H), 6.46 (d, J = 7.5 Hz, 1H), 5.44 (d, J = 6.4 Hz, 1H), 4.67 (m, 1H), 3.67 (s, 3H), 2.49 (m, 1H), 2.17 (m, 1H), 2.07 (s, 3H), 2.00 (m, 1H). ^{13}C -NMR (101 MHz, CHLOROFORM-D) δ 171.5, 168.4, 156.4 (q, $^2J_{CF}$ = 37.8 Hz), 135.6, 129.4, 129.4, 127.5, 115.6 (q, $^1J_{CF}$ = 285 Hz), 57.2, 52.7, 52.3, 30.9, 30.0, 15.5.

(D, L)-**17**: $[\alpha]_D$ -76.4 (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{16}H_{20}F_3N_2O_4S^+$ 393.1090, found 393.1111. 1H -NMR (400 MHz, CHLOROFORM-D) δ 7.93 (d, J = 6.1 Hz, 1H), 7.39 (m, 5H), 6.58 (d, J = 8.0 Hz, 1H), 5.47 (d, J = 6.4 Hz, 1H), 4.73 (m, 1H), 3.76 (s, 3H), 2.16 (m, 2H), 2.06 (m, 1H), 1.91–1.83 (m, 4H). ^{13}C -NMR (101 MHz, CHLOROFORM-D) δ 171.9, 168.4, 156.5 (q, $^2J_{CF}$ = 38.0 Hz), 136.3, 129.5, 129.3, 127.2, 115.7 (q, $^1J_{CF}$ = 288 Hz), 57.2, 52.9, 51.8, 30.9, 29.5, 15.3.

(L, D)-**17**: $[\alpha]_D$ +103.2 (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{16}H_{20}F_3N_2O_4S^+$ 393.1090, found 393.1096. 1H -NMR (400 MHz, CHLOROFORM-D) δ 7.91 (d, J = 6.1 Hz, 1H), 7.41–7.36 (m, 5H), 6.56 (d, J = 7.8 Hz, 1H), 5.46 (d, J = 6.4 Hz, 1H), 4.73 (m, 1H), 3.76 (s, 3H), 2.16 (m, 2H), 2.06 (m, 1H), 1.91–1.83 (m, 4H). ^{13}C -NMR (101 MHz, CHLOROFORM-D) δ 171.9, 168.5, 156.5 (q, $^2J_{CF}$ = 37.8 Hz), 136.3, 129.4, 129.3, 127.2, 115.7 (q, $^1J_{CF}$ = 288 Hz), 57.2, 52.9, 51.8, 30.9, 29.8, 15.3.

2.3.9. Methyl (2-phenyl-2-(2,2,2-trifluoroacetamido)acetyl)-phenylalaninate (TFA-Phg-Phg-OMe, 18)

Triethylamine (28.2 μ L, 0.202 mmol) was added to a suspension of the appropriate phenylglycine methyl ester hydrochloride (Phg-OMe-HCl (D/L), 98.0 mg, 0.486 mmol) in

6 mL CH₂Cl₂, and the mixture was stirred at room temperature for ca. 10 min. A solution of the corresponding TFA-Phg-OH (D/L, 100 mg, 0.405 mmol) in 15 mL CH₂Cl₂ was then combined with the above mixture, and WSCD-HCl (77.6 mg, 0.405 mmol) in 6 mL CH₂Cl₂ was added on ice. The reaction mixture was stirred at room temperature for 1 h for the homochiral pairs [(D, D) and (L, L)] or for 2 h for the heterochiral pairs [(D, L) and (L, D)] with TLC monitoring. Saturated brine and AcOEt were then added, and the organic layer was separated, dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified through silica gel column chromatography (EtOAc: hexane = 1:3 v/v) to afford the corresponding TFA-Phg-Phg-OMe (**18**).

(D, D)-**18**: [α]_D -127 (c 1, MeOH), HRMS-ESI (*m/z*) [*M* + *H*]⁺ calcd for C₁₉H₁₈F₃N₂O₄⁺ 395.1213, found 395.1206. ¹H-NMR (400 MHz, CHLOROFORM-D) δ 7.78 (d, *J* = 5.4 Hz, 1H), 7.43–7.29 (m, 10H), 6.64 (d, *J* = 6.2 Hz, 1H), 5.45 (dd, *J* = 9.1, 6.6 Hz, 2H), 3.67 (s, 3H), 7.77 (s, 1H), 7.41–7.30 (m, 10H), 6.63 (d, *J* = 6.2 Hz, 1H), 5.43 (dd, *J* = 9.1, 6.6 Hz, 2H), 3.66 (s, 3H). ¹³C-NMR (101 MHz, CHLOROFORM-D) δ 170.5, 167.8, 156.4 (q, ²*J*_{CF} = 38.1 Hz), 135.7, 135.6, 129.6, 129.5, 129.3, 129.1, 127.6, 127.3, 115.7 (q, ¹*J*_{CF} = 288 Hz), 57.2, 57.1, 53.1.

(L, L)-**18**: [α]_D +143 (c 1, MeOH), HRMS-ESI (*m/z*) [*M* + *H*]⁺ calcd for C₁₉H₁₈F₃N₂O₄⁺ 395.1213, found 395.1212. ¹H-NMR (400 MHz, CHLOROFORM-D) δ 7.77 (d, *J* = 5.4 Hz, 1H), 7.42–7.29 (m, 10H), 6.67 (d, *J* = 6.2 Hz, 1H), 5.45 (dd, *J* = 6.2, 4.5 Hz, 2H), 3.68 (s, 3H). ¹³C-NMR (101 MHz, CHLOROFORM-D) δ 170.5, 167.8, 156.4 (q, ²*J*_{CF} = 38.1 Hz), 135.7, 135.6, 129.6, 129.5, 129.3, 129.1, 127.6, 127.3, 115.7 (q, ¹*J*_{CF} = 288 Hz), 57.2, 57.1, 53.1.

(D, L)-**18**: [α]_D -6 (c 1, MeOH), HRMS-ESI (*m/z*) [*M* + *H*]⁺ calcd for C₁₉H₁₈F₃N₂O₄⁺ 395.1213, found 395.1210. ¹H-NMR (400 MHz, CHLOROFORM-D) δ 7.87 (d, *J* = 6.4 Hz, 1H), 7.35–7.04 (m, 10H), 6.92 (d, *J* = 6.7 Hz, 1H), 5.59 (d, *J* = 6.6 Hz, 1H), 5.51 (d, *J* = 6.9 Hz, 1H), 3.70 (s, 3H). ¹³C-NMR (101 MHz, CHLOROFORM-D) δ 170.7, 167.7, 156.5 (q, ²*J*_{CF} = 38.7 Hz), 135.8, 135.5, 129.4, 128.9, 127.5, 127.4, 126.9, 126.8, 115.7 (q, ¹*J*_{CF} = 287 Hz), 57.0, 56.9, 53.3.

(L, D)-**18**: [α]_D +4 (c 1, MeOH), HRMS-ESI (*m/z*) [*M* + *H*]⁺ calcd for C₁₉H₁₈F₃N₂O₄⁺ 395.1213, found 395.1206. ¹H-NMR (400 MHz, CHLOROFORM-D) δ 7.92 (d, *J* = 6.6 Hz, 1H), 7.34–7.05 (m, 10H), 7.02 (d, *J* = 6.9 Hz, 1H), 5.62 (d, *J* = 6.7 Hz, 1H), 5.51 (d, *J* = 6.9 Hz, 1H), 3.70 (s, 3H). ¹³C-NMR (101 MHz, CHLOROFORM-D) δ 170.7, 167.8, 156.5 (q, ²*J*_{CF} = 37.8 Hz), 135.8, 135.5, 129.4, 129.2, 128.9, 128.8, 127.5, 126.9, 115.7 (q, ¹*J*_{CF} = 288 Hz), 57.0, 56.9, 53.2.

2.3.10. (2-Amino-2-phenylacetyl)alanine (Phg-Ala, **19**)

TFA-Phg-Ala-OMe (33.2 mg, 0.10 mmol) was dissolved in 1.1 mL anhydrous MeOH, and a solution of Ba(OH)₂·8H₂O (157.75 mg, 0.50 mmol) in 1.0 mL H₂O was added. The reaction mixture was stirred at room temperature for 1 h and then centrifuged (3500 rpm, 10 min). The supernatant was collected, adjusted to pH 6–7 with 1 M H₂SO₄, and centrifuged again. The resulting supernatant was concentrated under reduced pressure, centrifuged, and concentrated again. The crude product was purified through column chromatography using CH₃CN/MeOH/H₂O (4:1:0.4 v/v/v) to afford H-Phg-Ala-OH (**19**).

(D, D)-**19**: [α]_D -82 (c 1, 2.5M HCl), HRMS-ESI (*m/z*) [*M* + *H*]⁺ calcd for C₁₁H₁₅N₂O₃⁺ 233.1077, found 233.1095. ¹H-NMR (D₂O) δ : 7.38 (s, 5H), 5.01 (s, 1H), 4.16 (q, *J* = 7.0 Hz, 1H), 1.20 (d, *J* = 7.3 Hz, 3H). ¹³C-NMR (D₂O) δ : 178.0, 168.5, 132.4, 131.0, 130.2, 128.9, 57.1, 50.7, 17.5.

(L, L)-**19**: [α]_D +48 (c 1, 2.5M HCl), H HRMS-ESI (*m/z*) [*M* + *H*]⁺ calcd for C₁₁H₁₅N₂O₃⁺ 233.1077, found 233.1088. ¹H-NMR (D₂O) δ : 7.37 (s, 5H), 5.00 (s, 1H), 4.17 (q, *J* = 7.3 Hz, 1H), 1.21 (d, *J* = 7.3 Hz, 3H). ¹³C-NMR (D₂O) δ : 177.9, 168.5, 132.4, 131.0, 130.2, 128.9, 57.1, 50.7, 16.9.

(D, L)-**19**: [α]_D -71 (c 1, 2.5M HCl), HRMS-ESI (*m/z*) [*M* + *H*]⁺ calcd for C₁₁H₁₅N₂O₃⁺ 233.1077, found 233.1056. ¹H-NMR (D₂O) δ : 7.36 (s, 5H), 5.00 (s, 1H), 4.00 (q, *J* = 7.5 Hz,

1H), 1.10 (d, $J = 7.3$ Hz, 3H). ^{13}C -NMR (D_2O) δ : 180.2, 168.4, 132.7, 131.0, 130.3, 128.5, 57.2, 51.9, 17.4.

(L, D)-**19**: $[\alpha]_{\text{D}} +104$ (c 1, 2.5M HCl), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{11}\text{H}_{15}\text{N}_2\text{O}_3^+$ 233.1077, found 233.1068. ^1H -NMR (D_2O) δ : 7.33 (s, 5H), 5.00 (s, 1H), 3.98 (q, $J = 7.3$ Hz, 1H), 1.07 (d, $J = 7.6$ Hz, 3H). ^{13}C -NMR (D_2O) δ : 180.1, 168.4, 132.7, 131.0, 130.3, 128.5, 57.2, 51.8, 17.4.

2.3.11. (2-Amino-2-phenylacetyl)valine (Phg-Val, 20)

The corresponding TFA-Phg-Val-OMe (36.03 mg, 0.10 mmol) was dissolved in 1.1 mL anhydrous MeOH, and a solution of $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ (157.8 mg, 0.50 mmol) in 1 mL H_2O was added. The reaction mixture was stirred at room temperature for 1 h and then centrifuged (3500 rpm, 10 min). The supernatant was collected, adjusted to pH 6–7 with 1 M H_2SO_4 , and centrifuged again to remove BaSO_4 . The resulting supernatant was concentrated under reduced pressure, centrifuged, and concentrated again. The crude product was purified through column chromatography using $\text{CH}_3\text{CN}/\text{MeOH}/\text{H}_2\text{O}$ (4:1:0.4 $v/v/v$) to afford Phg-Val (**20**). The same procedure was applied to the preparation of all four stereoisomers.

(D, D)-**20**: $[\alpha]_{\text{D}} -42$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{13}\text{H}_{19}\text{N}_2\text{O}_3^+$ 251.1390, found 251.1399. ^1H -NMR (400 MHz, D_2O) δ 7.37–7.33 (m, 5H), 4.86 (s, 1H), 3.94 (d, $J = 5.9$ Hz, 1H), 1.95 (m, 1H), 0.79 (d, $J = 6.9$ Hz, 3H), 0.76 (d, $J = 6.9$ Hz, 3H). ^{13}C -NMR (101 MHz, D_2O) δ 178.0, 170.8, 135.0, 129.5, 129.4, 127.7, 61.3, 57.5, 30.5, 18.9, 17.4.

(L, L)-**20**: $[\alpha]_{\text{D}} +40$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{13}\text{H}_{19}\text{N}_2\text{O}_3^+$ 251.1390, found 251.1397. ^1H -NMR (400 MHz, D_2O) δ 7.37–7.33 (m, 5H), 4.89 (s, 1H), 3.93 (d, $J = 6.1$ Hz, 1H), 1.92 (m, 1H), 0.78 (d, $J = 6.9$ Hz, 3H), 0.75 (d, $J = 6.9$ Hz, 3H). ^{13}C -NMR (101 MHz, D_2O) δ 178.1, 171.8, 136.1, 129.3, 129.2, 127.5, 61.1, 57.9, 30.6, 18.8, 17.4.

(D, L)-**20**: $[\alpha]_{\text{D}} -121$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{13}\text{H}_{19}\text{N}_2\text{O}_3^+$ 251.1390, found 251.1396. ^1H -NMR (400 MHz, D_2O) δ 7.38–7.34 (m, 5H), 4.93 (s, 1H), 3.96 (d, $J = 5.6$ Hz, 1H), 1.91 (m, 1H), 0.57 (d, $J = 6.9$ Hz, 3H), 0.53 (d, $J = 6.7$ Hz, 3H). ^{13}C -NMR (101 MHz, D_2O) δ 178.4, 170.5, 134.8, 129.6, 129.4, 127.5, 60.9, 57.2, 30.5, 18.8, 16.9.

(L, D)-**20**: $[\alpha]_{\text{D}} +45$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{13}\text{H}_{19}\text{N}_2\text{O}_3^+$ 251.1390, found 251.1402. ^1H -NMR (400 MHz, D_2O) δ 7.38–7.34 (m, 5H), 4.95 (s, 1H), 3.96 (d, $J = 5.6$ Hz, 1H), 1.91 (m, 1H), 0.57 (d, $J = 6.9$ Hz, 3H), 0.53 (d, $J = 6.7$ Hz, 3H). ^{13}C -NMR (101 MHz, D_2O) δ 178.4, 170.2, 134.5, 129.7, 129.4, 127.5, 60.9, 57.1, 30.5, 18.8, 16.9.

2.3.12. (2-Amino-2-phenylacetyl)leucine (Phg-Leu, 21)

The corresponding TFA-Phg-Leu-OMe (37.4 mg, 0.10 mmol) was dissolved in 1.1 mL anhydrous MeOH, and a solution of $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ (157.75 mg, 0.50 mmol) in 1 mL H_2O was added. The reaction mixture was stirred at room temperature for 1 h and then centrifuged (3500 rpm, 10 min). The supernatant was collected, adjusted to pH 6–7 with 1 M H_2SO_4 , and centrifuged again to remove BaSO_4 . The resulting supernatant was concentrated under reduced pressure, centrifuged, and concentrated again. The crude product was purified through column chromatography using $\text{CH}_3\text{CN}/\text{MeOH}/\text{H}_2\text{O}$ (4:1:0.4 $v/v/v$) to afford Phg-Leu (**21**). The same procedure was applied to the preparation of all four stereoisomers.

(D, D)-**21**: $[\alpha]_{\text{D}} -36$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{14}\text{H}_{21}\text{N}_2\text{O}_3^+$ 265.1547, found 265.1563. ^1H -NMR (400 MHz, D_2O) δ 7.37–7.31 (m, 5H), 4.72 (s, 1H), 4.07 (m, 1H), 1.46 (m, 3H), 0.77 (d, $J = 6.2$ Hz, 3H), 0.73 (d, $J = 6.2$ Hz, 3H). ^{13}C -NMR (101 MHz, D_2O) δ 179.5, 171.7, 136.1, 129.3, 129.3, 127.6, 57.9, 54.2, 40.6, 24.6, 22.4, 20.8.

(L, L)-**21**: $[\alpha]_{\text{D}} +30$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{14}\text{H}_{21}\text{N}_2\text{O}_3^+$ 265.1547, found 265.1556. ^1H -NMR (400 MHz, D_2O) δ 7.36–7.32 (m, 5H), 4.74 (s, 1H), 4.08

(m, 1H), 1.48 (m, 3H), 0.78 (d, $J = 6.1$ Hz, 3H), 0.74 (d, $J = 6.2$ Hz, 3H). ^{13}C -NMR (101 MHz, D_2O) δ 179.5, 171.5, 135.9, 129.3, 127.6, 57.8, 54.3, 40.6, 24.6, 22.4, 20.8.

(D, L)-**21**: $[\alpha]_D -121$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{14}\text{H}_{21}\text{N}_2\text{O}_3^+$ 265.1547, found 265.1563. ^1H -NMR (400 MHz, D_2O) δ 7.37–7.33 (m, 5H), 4.82 (s, 1H), 4.05 (m, 1H), 1.39 (m, 2H), 1.06 (m, 1H), 0.61 (d, $J = 6.6$ Hz, 3H), 0.55 (d, $J = 6.6$ Hz, 3H). ^{13}C -NMR (101 MHz, D_2O) δ 180.0, 171.2, 135.7, 129.4, 129.3, 127.3, 57.4, 54.0, 40.4, 24.4, 22.4, 20.2.

(L, D)-**21**: $[\alpha]_D +122$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{14}\text{H}_{21}\text{N}_2\text{O}_3^+$ 265.1547, found 265.1563. ^1H -NMR (400 MHz, D_2O) δ 7.35–7.33 (m, 5H), 4.79 (s, 1H), 4.05 (m, 1H), 1.38 (m, 2H), 1.05 (m, 1H), 0.61 (d, $J = 6.7$ Hz, 3H), 0.55 (d, $J = 6.6$ Hz, 3H). ^{13}C -NMR (101 MHz, D_2O) δ 180.0, 171.0, 135.4, 129.4, 129.3, 127.3, 57.4, 54.0, 40.4, 24.4, 22.4, 20.2.

2.3.13. (2-Amino-2-phenylacetyl)methinine (Phg-Met, 22)

TFA-Phg-Met-OMe (39.4 mg, 0.10 mmol) was dissolved in 1.1 mL anhydrous MeOH, and a solution of $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ (157.75 mg, 0.50 mmol) in 1 mL H_2O was added. The reaction mixture was stirred at room temperature for 1 h and then centrifuged (3500 rpm, 10 min). The supernatant was separated, and the precipitate was treated with 1 M H_2SO_4 , followed by centrifugation. The resulting supernatant was combined with the original supernatant and concentrated under reduced pressure. The residue was centrifuged again, concentrated, and purified through column chromatography using $\text{CH}_3\text{CN}/\text{MeOH}/\text{H}_2\text{O}$ (4:1:0.4 v/v/v) to afford H-Phg-Met-OH (**22**).

(D, D)-**22**: $[\alpha]_D -83$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{13}\text{H}_{19}\text{N}_2\text{O}_3\text{S}^+$ 283.1111, found 283.1119. ^1H -NMR (400 MHz, D_2O) δ 7.34 (s, 5H), 4.82 (s, 1H), 4.15 (dd, $J = 8.6, 4.7$ Hz, 1H), 2.36 (m, 2H), 1.98 (m, 1H), 1.93 (s, 3H), 1.86 (m, 1H). ^{13}C -NMR (101 MHz, D_2O) δ 177.9, 171.4, 134.6, 129.2, 129.0, 127.3, 58.3, 54.5, 30.9, 29.7, 14.2.

(L, L)-**22**: $[\alpha]_D +45$ (c 1, MeOH), H HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{13}\text{H}_{19}\text{N}_2\text{O}_3\text{S}^+$ 283.1111, found 283.1166. ^1H -NMR (400 MHz, D_2O) δ 7.33 (s, 5H), 4.82 (s, 1H), 4.14 (dd, $J = 8.6, 4.6$ Hz, 1H), 2.35 (m, 2H), 1.97 (m, 1H), 1.92 (s, 3H), 1.85 (m, 1H). ^{13}C -NMR (101 MHz, D_2O) δ 177.9, 171.8, 136.2, 129.3, 129.3, 127.5, 57.9, 54.6, 30.9, 29.7, 14.2.

(D, L)-**22**: $[\alpha]_D -128$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{13}\text{H}_{19}\text{N}_2\text{O}_3\text{S}^+$ 283.1111, found 283.1109. ^1H -NMR (400 MHz, D_2O) δ 7.34 (s, 5H), 4.82 (s, 1H), 4.19 (dd, $J = 9.5, 3.9$ Hz, 1H), 2.14 (m, 1H), 2.01 (m, 1H), 1.92 (m, 1H), 1.77 (s, 3H), 1.70 (m, 1H). ^{13}C -NMR (101 MHz, D_2O) δ 178.4, 170.9, 135.9, 129.3, 129.1, 127.1, 57.7, 54.0, 30.9, 29.5, 14.0.

(L, D)-**22**: $[\alpha]_D +100$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{13}\text{H}_{19}\text{N}_2\text{O}_3\text{S}^+$ 283.1111, found 283.1111. ^1H -NMR (400 MHz, D_2O) δ 7.34 (s, 5H), 4.82 (s, 1H), 4.19 (dd, $J = 9.7, 3.8$ Hz, 1H), 2.13 (m, 1H), 1.98 (m, 2H), 1.76 (s, 3H), 1.69 (m, 1H). ^{13}C -NMR (101 MHz, D_2O) δ 178.4, 172.9, 137.6, 129.2, 129.0, 127.1, 57.8, 54.0, 30.9, 29.5, 14.0.

2.3.14. (2-Amino-2-phenylacetyl)phenylglycine (Phg-Phg, 23)

The corresponding TFA-Phg-Phg-OMe (39.4 mg, 0.10 mmol) was dissolved in 1.1 mL anhydrous MeOH, and a solution of $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ (157.75 mg, 0.50 mmol) in 1 mL H_2O was added. The reaction mixture was stirred at room temperature for 2 h and then centrifuged (3500 rpm, 10 min). The supernatant was collected, adjusted to pH 6–7 with 1 M H_2SO_4 , and centrifuged again to remove BaSO_4 . The resulting supernatant was concentrated under reduced pressure, centrifuged, and concentrated again. The crude product was purified through column chromatography using $\text{CH}_3\text{CN}/\text{MeOH}/\text{H}_2\text{O}$ (4:1:0.4 v/v/v) to afford Phg-Phg (**23**). The same procedure was applied to the preparation of all four stereoisomers.

(D, D)-**23**: $[\alpha]_D -65$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{16}\text{H}_{17}\text{N}_2\text{O}_3^+$ 285.1234, found 285.1220. ^1H -NMR (400 MHz, D_2O) δ 7.30–7.21 (m, 10H), 5.00 (s, 1H). ^{13}C -

NMR (101 MHz, METHANOL- D_4) δ 174.7, 169.9, 140.2, 137.5, 128.7, 128.5, 127.8, 127.3, 127.0, 126.9, 59.4, 58.1, 20.7.

(L, L)-**23**: $[\alpha]_D^{+72}$ (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{16}H_{17}N_2O_3^+$ 285.1234, found 285.1231. 1H -NMR (400 MHz, D_2O) δ 7.31–7.04 (m, 10H), 5.07 (s, 1H). ^{13}C -NMR (101 MHz, METHANOL- D_4) δ 176.3, 172.4, 139.7, 128.5, 127.9, 127.8, 127.1, 127.0, 127.0, 59.1, 58.8.

(D, L)-**23**: $[\alpha]_D^{-124}$ (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{16}H_{17}N_2O_3^+$ 285.1234, found 285.1230. 1H -NMR (400 MHz, D_2O) δ 7.29–7.02 (m, 10H), 5.05 (s, 1H). ^{13}C -NMR (101 MHz, METHANOL- D_4) δ 175.1, 167.5, 139.2, 134.5, 129.3, 128.9, 127.9, 127.7, 127.0, 59.5, 56.8.

(L, D)-**23**: $[\alpha]_D^{+57}$ (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{16}H_{17}N_2O_3^+$ 285.1234, found 285.1227. 1H -NMR (400 MHz, D_2O) δ 7.28–7.03 (m, 10 H), 5.04 (s, 1H). ^{13}C -NMR (101 MHz, D_2O) δ 182.6, 176.2, 140.1, 138.1, 129.2, 128.8, 128.0, 127.1, 127.0, 65.3, 59.7.

2.3.15. tert-Butyl-(2-Amino-2-phenylacetyl)alanine (Phg-Ala-Ot-Bu, 24)

The corresponding TFA-Phg-Ala-Ot-Bu (37.4 mg, 0.10 mmol) was dissolved in 1.1 mL anhydrous MeOH, and a solution of $Ba(OH)_2 \cdot 8H_2O$ (78.87 mg, 0.25 mmol) in 1.0 mL H_2O was added. The reaction mixture was stirred at room temperature for 2 h, and the progress of the reaction was monitored using TLC. The mixture was then centrifuged (12000 g, 10 min), and the supernatant was collected. The supernatant was adjusted to pH 6–7 with 1 M H_2SO_4 and centrifuged again to remove $BaSO_4$, and the resulting supernatant was concentrated under reduced pressure. The residue was centrifuged, concentrated again, and purified through column chromatography using $CH_2Cl_2/EtOAc$ (1:2 *v/v*), followed by $CHCl_3/MeOH$ (5:1 *v/v*), to afford Phg-Ala-Ot-Bu (**24**). The same procedure was applied to the preparation of all four stereoisomers.

(D, D)-**24**: $[\alpha]_D^{-52}$ (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{15}H_{23}N_2O_3^+$ 279.1703, found 279.1700. 1H -NMR (400 MHz, CHLOROFORM- D) δ 7.62 (d, $J = 3.0$ Hz, 1H), 7.44–7.31 (m, 5H), 4.79 (s, 1H), 4.47–4.36 (m, 1H), 1.41 (s, 9H), 1.32 (d, $J = 7.2$ Hz, 3H). ^{13}C -NMR (101 MHz, CHLOROFORM- D) δ 175.6, 172.2, 140.3, 129.0, 128.6, 127.3, 82.1, 59.9, 48.7, 27.9, 18.2.

(L, L)-**24**: $[\alpha]_D^{+14}$ (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{15}H_{23}N_2O_3^+$ 279.1703, found 279.1702. 1H -NMR (400 MHz, CHLOROFORM- D) δ 7.69 (d, $J = 6.7$ Hz, 1H), 7.41–7.30 (m, 5H), 4.54 (s, 1H), 4.49–4.42 (m, 1H), 1.46 (s, 9H), 1.36 (d, $J = 7.0$ Hz, 3H). ^{13}C -NMR (101 MHz, CHLOROFORM- D) δ 172.5, 172.2, 140.9, 128.9, 128.0, 126.9, 81.9, 59.8, 48.4, 28.0, 18.6.

(D, L)-**24**: $[\alpha]_D^{-110}$ (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{15}H_{23}N_2O_3^+$ 279.1703, found 279.1701. 1H -NMR (400 MHz, CHLOROFORM- D) δ 7.53 (d, $J = 6.1$ Hz, 1H), 7.43–7.30 (m, 5H), 4.54 (s, 1H), 4.51–4.43 (m, 1H), 1.44 (s, 9H), 1.38 (d, $J = 7.2$ Hz, 3H). ^{13}C -NMR (101 MHz, CHLOROFORM- D) δ 176.0, 172.1, 139.4, 128.9, 128.0, 127.1, 81.9, 59.8, 48.5, 27.9, 18.6.

(L, D)-**24**: $[\alpha]_D^{+120}$ (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{15}H_{23}N_2O_3^+$ 279.1703, found 279.1701. 1H -NMR (400 MHz, CHLOROFORM- D) δ 7.51 (d, $J = 6.9$ Hz, 1H), 7.43–7.29 (m, 5H), 4.53 (s, 1H), 4.47 (m, 1H), 1.44 (s, 9H), 1.38 (d, $J = 7.0$ Hz, 3H). ^{13}C -NMR (101 MHz, CHLOROFORM- D) δ 175.7, 172.3, 129.9, 129.4, 128.0, 127.5, 82.7, 54.9, 49.3, 27.9, 17.6.

2.3.16. tert-Butyl-(2-Amino-2-phenylacetyl)valine (Phg-Val-Ot-Bu, 25)

The corresponding TFA-Phg-Val-Ot-Bu (40.2 mg, 0.10 mmol) was dissolved in 1.1 mL anhydrous MeOH, and a solution of $Ba(OH)_2 \cdot 8H_2O$ (157.75 mg, 0.50 mmol) in 1 mL H_2O was added. The reaction mixture was stirred at room temperature for 1 h. The mixture was then centrifuged, and the supernatant was collected, adjusted to pH 6–7 with 1

M H₂SO₄, and centrifuged again to remove BaSO₄. The resulting supernatant was concentrated under reduced pressure, and the crude product was purified through column chromatography CH₂Cl₂/EtOAc (1:2 *v/v*) to afford Phg-Val-Ot-Bu (**25**). The same procedure was applied to the preparation of all four stereoisomers.

(D, D)-**25**: [α]_D -15.4 (c 1, CHCl₃), HRMS-ESI (*m/z*) [M + H]⁺ calcd for C₁₇H₂₆N₂O₃⁺ 307.2016, found 307.2012. ¹H-NMR (400 MHz, CHLOROFORM-D) δ 7.77 (d, *J* = 8.6 Hz, 1H), 7.44–7.29 (m, 5H), 4.57 (s, 1H), 4.42 (q, *J* = 4.5 Hz, 1H), 2.16 (m, 1H), 1.46 (s, 9H), 0.89 (d, *J* = 6.9 Hz, 3H), 0.85 (d, *J* = 6.9 Hz, 3H). ¹³C-NMR (101 MHz, CHLOROFORM-D) δ 173.0, 171.2, 141.1, 128.9, 128.1, 126.9, 82.0, 60.2, 57.1, 31.6, 28.1, 19.0, 17.6.

(L, L)-**25**: [α]_D +20.6 (c 1, CHCl₃), HRMS-ESI (*m/z*) [M + H]⁺ calcd for C₁₇H₂₆N₂O₃⁺ 307.2016, found 307.2013. ¹H-NMR (400 MHz, CHLOROFORM-D) δ 7.77 (d, *J* = 8.5 Hz, 1H), 7.44–7.29 (m, 5H), 4.57 (s, 1H), 4.42 (q, *J* = 4.5 Hz, 1H), 2.15 (m, 1H), 1.46 (s, 9H), 0.89 (d, *J* = 6.9 Hz, 3H), 0.85 (d, *J* = 6.9 Hz, 3H). ¹³C-NMR (101 MHz, CHLOROFORM-D) δ 173.1, 171.2, 140.3, 128.9, 128.1, 126.9, 82.0, 60.3, 57.1, 31.7, 28.1, 19.0, 17.6.

(D, L)-**25**: [α]_D -25.4 (c 1, CHCl₃), HRMS-ESI (*m/z*) [M + H]⁺ calcd for C₁₇H₂₆N₂O₃⁺ 307.2016, found 307.2016. ¹H-NMR (400 MHz, CHLOROFORM-D) δ 7.42–7.29 (m, 6H), 4.56 (s, 1H), 4.42 (q, *J* = 4.6 Hz, 1H), 2.16 (m, 1H), 1.45 (s, 9H), 0.88 (d, *J* = 6.9 Hz, 3H), 0.85 (d, *J* = 6.9 Hz, 3H). ¹³C-NMR (101 MHz, CHLOROFORM-D) δ 172.8, 171.0, 141.3, 129.0, 128.1, 127.1, 82.0, 60.0, 57.3, 31.5, 28.1, 19.0, 17.6.

(L, D)-**25**: [α]_D +29.6 (c 1, CHCl₃), HRMS-ESI (*m/z*) [M + H]⁺ calcd for C₁₇H₂₆N₂O₃⁺ 307.2016, found 307.2011. ¹H-NMR (400 MHz, CHLOROFORM-D) δ 7.42–7.29 (m, 6H), 4.56 (s, 1H), 4.42 (q, *J* = 4.6 Hz, 1H), 2.16 (m, 1H), 1.45 (s, 9H), 0.89 (d, *J* = 6.9 Hz, 3H), 0.85 (d, *J* = 6.9 Hz, 3H). ¹³C-NMR (101 MHz, CHLOROFORM-D) δ 172.9, 171.0, 141.3, 128.9, 128.1, 127.1, 82.0, 60.0, 57.3, 31.5, 28.1, 19.0, 17.6.

2.3.17. tert-Butyl-(2-Amino-2-phenylacetyl)leucine (Phg-Leu-Ot-Bu, **26**)

The corresponding TFA-Phg-Leu-Ot-Bu (41.6 mg, 0.10 mmol) was dissolved in 1.0 mL anhydrous MeOH, and a solution of Ba(OH)₂·8H₂O (157.75 mg, 0.50 mmol) in 1.0 mL H₂O was added. The reaction mixture was stirred at room temperature for 1 h. The mixture was then centrifuged, and the supernatant was collected, adjusted to pH 6–7 with 1 M H₂SO₄, and centrifuged again to remove BaSO₄. The resulting supernatant was concentrated under reduced pressure, and the crude product was purified through column chromatography CH₂Cl₂/EtOAc (1:2 *v/v*) to afford Phg-Leu-Ot-Bu (**26**). The same procedure was applied to the preparation of all four stereoisomers.

(D, D)-**26**: [α]_D -10 (c 1, MeOH), HRMS-ESI (*m/z*) [M + H]⁺ calcd for C₁₈H₂₉N₂O₃⁺ 321.2173, found 321.2176. ¹H-NMR (400 MHz, CHLOROFORM-D) δ 7.54 (d, *J* = 8.2 Hz, 1H), 7.41–7.29 (m, 5H), 4.55 (s, 1H), 4.47 (m, 1H), 1.60 (m, 3H), 1.45 (s, 9H), 0.91 (d, *J* = 6.2 Hz, 3H), 0.87 (d, *J* = 5.9 Hz, 3H). ¹³C-NMR (101 MHz, CHLOROFORM-D) δ 172.8, 172.2, 141.0, 128.9, 128.1, 126.9, 81.8, 60.0, 51.2, 41.9, 28.1, 25.1, 22.9, 22.1.

(L, L)-**26**: [α]_D +6 (c 1, MeOH), HRMS-ESI (*m/z*) [M + H]⁺ calcd for C₁₈H₂₉N₂O₃⁺ 321.2173, found 321.2175. ¹H-NMR (400 MHz, CHLOROFORM-D) δ 7.54 (d, *J* = 8.3 Hz, 1H), 7.41–7.29 (m, 5H), 4.55 (s, 1H), 4.47 (m, 1H), 1.60 (m, 3H), 1.45 (s, 9H), 0.91 (d, *J* = 6.2 Hz, 3H), 0.87 (d, *J* = 5.9 Hz, 3H). ¹³C-NMR (101 MHz, CHLOROFORM-D) δ 172.8, 172.2, 141.0, 128.9, 128.1, 126.9, 81.9, 60.0, 51.2, 41.9, 28.1, 25.0, 22.9, 22.1.

(D, L)-**26**: [α]_D -69 (c 1, MeOH), HRMS-ESI (*m/z*) [M + H]⁺ calcd for C₁₈H₂₉N₂O₃⁺ 321.2173, found 321.2170. ¹H-NMR (400 MHz, CHLOROFORM-D) δ 7.42–7.28 (m, 6H), 4.53 (s, 1H), 4.49 (m, 1H), 1.63 (m, 3H), 1.43 (s, 9H), 0.92 (d, *J* = 6.0 Hz, 6H). ¹³C-NMR (101 MHz, CHLOROFORM-D) δ 172.6, 172.1, 141.2, 128.9, 128.1, 127.2, 81.8, 59.9, 51.3, 41.9, 28.0, 25.1, 22.9, 22.1.

(L, D)-**26**: [α]_D +53 (c 1, MeOH), HRMS-ESI (*m/z*) [M + H]⁺ calcd for C₁₈H₂₉N₂O₃⁺ 321.2173, found 321.2169. ¹H-NMR (400 MHz, CHLOROFORM-D) δ 7.42–7.28 (m, 6H),

4.53 (s, 1H), 4.49 (dd, $J = 8.6, 5.1$ Hz, 1H), 1.59 (m, 3H), 1.43 (s, 9H), 0.92 (d, $J = 6.0$ Hz, 6H). ^{13}C -NMR (101 MHz, CHLOROFORM- D_3) δ 172.6, 172.1, 141.2, 128.9, 128.1, 127.2, 81.9, 59.9, 51.3, 41.9, 28.0, 25.1, 22.9, 22.1.

2.3.18. (2-Phenyl-2-(2,2,2-trifluoroacetamido)acetyl)-alaninate (TFA-Phg-Ala, 27)

The corresponding TFA-Phg-Ala-*Ot*-Bu (37.4 mg, 0.10 mmol) was treated with trifluoroacetic acid (1.0 mL, 13.06 mmol) and anhydrous 1.0 mL CH_2Cl_2 on ice. The reaction mixture was then stirred at room temperature for 1 h. The progress of the reaction was monitored using TLC. After completion, the solvent was removed under reduced pressure, and the residue was co-evaporated with fresh CH_2Cl_2 several times to remove residual TFA. The crude product was purified through silica gel column chromatography using $\text{CHCl}_3/\text{MeOH}$ (4:1 *v/v*) to afford the corresponding TFA-Phg-Ala (27). Product-containing fractions were combined and co-evaporated with MeOH several times before analysis in CD_3OD . The same procedure was applied to the preparation of all four stereoisomers of 27.

(D, D)-27: $[\alpha]_D -70$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{13}\text{H}_{14}\text{F}_3\text{N}_2\text{O}_4^+$ 319.0900, found 319.0895. ^1H -NMR (400 MHz, METHANOL- D_4) δ 7.46–7.29 (m, 5H), 5.60 (s, 1H), 4.41 (q, $J = 7.3$ Hz, 1H), 1.39 (d, $J = 7.2$ Hz, 3H). ^{13}C -NMR (101 MHz, METHANOL- D_4) δ 174.1, 169.2, 157.1 (q, $^2J_{\text{CF}} = 37.8$ Hz), 135.8, 128.4, 128.3, 127.8, 115.9 (q, $^1J_{\text{CF}} = 287$ Hz), 57.1, 16.2.

(L, L)-27: $[\alpha]_D +74$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{13}\text{H}_{14}\text{F}_3\text{N}_2\text{O}_4^+$ 319.0900, found 319.0895. ^1H -NMR (400 MHz, METHANOL- D_4) δ 7.47–7.29 (m, 5H), 5.60 (s, 1H), 4.41 (q, $J = 7.3$ Hz, 1H), 1.39 (d, $J = 7.4$ Hz, 3H). ^{13}C -NMR (101 MHz, METHANOL- D_4) δ 174.1, 169.2, 157.1 (q, $^2J_{\text{CF}} = 37.8$ Hz), 135.8, 128.4, 128.3, 127.8, 116.0 (q, $^1J_{\text{CF}} = 287$ Hz), 57.1, 16.2.

(D, L)-27: $[\alpha]_D -120$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{13}\text{H}_{14}\text{F}_3\text{N}_2\text{O}_4^+$ 319.0900, found 319.0896. ^1H -NMR (400 MHz, METHANOL- D_4) δ 7.44–7.30 (m, 5H), 5.60 (s, 1H), 4.36 (q, $J = 7.3$ Hz, 1H), 1.30 (d, $J = 7.4$ Hz, 3H). ^{13}C -NMR (101 MHz, METHANOL- D_4) δ 174.3, 169.4, 156.9 (q, $^2J_{\text{CF}} = 37.8$ Hz), 136.4, 128.6, 128.4, 127.5, 116.0 (q, $^1J_{\text{CF}} = 287$ Hz), 57.1, 16.1.

(L, D)-27: $[\alpha]_D +105$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{13}\text{H}_{14}\text{F}_3\text{N}_2\text{O}_4^+$ 319.0900, found 319.0891. ^1H -NMR (400 MHz, METHANOL- D_4) δ 7.44–7.30 (m, 5H), 5.60 (s, 1H), 4.27 (q, $J = 7.3$ Hz, 1H), 1.30 (d, $J = 7.4$ Hz, 3H). ^{13}C -NMR (101 MHz, METHANOL- D_4) δ 174.3, 169.4, 157.1 (q, $^2J_{\text{CF}} = 37.8$ Hz), 136.4, 128.6, 128.4, 127.5, 116.0 (q, $^1J_{\text{CF}} = 287$ Hz), 57.1, 16.1.

2.3.19. (2-Phenyl-2-(2,2,2-trifluoroacetamido)acetyl)-valine (TFA-Phg-Val, 28)

The corresponding TFA-Phg-Val-*Ot*-Bu (40.5 mg, 0.10 mmol) was treated with trifluoroacetic acid (1.0 mL, 13.06 mmol) and anhydrous 1.0 mL CH_2Cl_2 on ice. The reaction mixture was then stirred at room temperature for 1 h. The progress of the reaction was monitored using TLC. After completion, the solvent was removed under reduced pressure, and the residue was co-evaporated with fresh CH_2Cl_2 several times to remove residual TFA. The crude product was dissolved in D_2O and subjected to NMR analysis, and then purified through silica gel column chromatography using $\text{CHCl}_3/\text{MeOH}$ (4:1 *v/v*) to afford the corresponding TFA-Phg-Val (28). The same procedure was applied to the preparation of all four stereoisomers of 28.

(D, D)-28: $[\alpha]_D -14$ (c 1, MeOH), HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{18}\text{F}_3\text{N}_2\text{O}_4^+$ 347.1213, found 347.1221. ^1H -NMR (400 MHz, METHANOL- D_4) δ 7.46–7.29 (m, 5H), 5.66 (s, 1H), 4.24 (d, $J = 5.6$ Hz, 1H), 2.14 (m, 1H), 0.95 (d, $J = 6.9$ Hz, 3H), 0.93 (d, $J = 6.9$ Hz, 3H). ^{13}C -NMR (101 MHz, METHANOL- D_4) δ 175.3, 169.5, 157.2 (q, $^2J_{\text{CF}} = 37.6$ Hz), 135.8, 128.5, 128.3, 127.6, 116.0 (q, $^1J_{\text{CF}} = 287$ Hz), 59.1, 57.4, 30.9, 18.4, 17.0.

(L, L)-**28**: $[\alpha]_D^{+33}$ (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{15}H_{18}F_3N_2O_4^+$ 347.1213, found 347.1221. 1H -NMR (400 MHz, METHANOL- D_4) δ 7.48–7.33 (m, 5H), 5.68 (s, 1H), 4.23 (d, $J = 5.4$ Hz, 1H), 2.16 (m, 1H), 0.97 (d, $J = 6.9$ Hz, 3H), 0.94 (d, $J = 6.9$ Hz, 3H). ^{13}C -NMR (101 MHz, METHANOL- D_4) δ 176.5, 169.3, 157.2 (q, $^2J_{CF} = 37.6$ Hz), 135.9, 128.5, 128.3, 127.6, 116.0 (q, $^1J_{CF} = 287$ Hz), 59.8, 57.5, 31.1, 18.6, 17.1.

(D, L)-**28**: $[\alpha]_D^{-122}$ (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{15}H_{18}F_3N_2O_4^+$ 347.1213, found 347.1205. 1H -NMR (400 MHz, D_2O) δ 7.36–7.33 (m, 5H), 5.49 (s, 1H), 4.00 (d, $J = 5.6$ Hz, 1H), 1.93 (m, 1H), 0.61 (d, $J = 6.9$ Hz, 3H), 0.58 (d, $J = 6.9$ Hz, 3H). ^{13}C -NMR (101 MHz, D_2O) δ 178.3, 170.2, 158.5 (q, $^2J_{CF} = 38.5$ Hz), 134.9, 129.3, 127.7, 115.6 (q, $^1J_{CF} = 286$ Hz), 60.8, 58.3, 30.8, 18.8, 16.9.

(L, D)-**28**: $[\alpha]_D^{+75}$ (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{15}H_{18}F_3N_2O_4^+$ 347.1213, found 347.1235. 1H -NMR (400 MHz, D_2O) δ 7.37–7.34 (m, 5H), 5.50 (s, 1H), 4.02 (d, $J = 5.4$ Hz, 1H), 1.95 (m, 1H), 0.62 (d, $J = 6.9$ Hz, 3H), 0.59 (d, $J = 6.7$ Hz, 3H). ^{13}C -NMR (101 MHz, D_2O) δ 178.0, 170.2, 158.5 (q, $^2J_{CF} = 38.3$ Hz), 134.9, 129.3, 129.3, 127.7, 115.6 (q, $^1J_{CF} = 286$ Hz), 60.6, 58.3, 30.7, 18.8, 16.9.

2.3.20. (2-Phenyl-2-(2,2,2-trifluoroacetamido)acetyl)-leucine (TFA-Phg-Leu, 29)

The corresponding TFA-Phg-Leu-*Ot*-Bu (41.7 mg, 0.10 mmol) was treated with trifluoroacetic acid (1.0 mL, 13.06 mmol) and anhydrous 1.0 mL CH_2Cl_2 on ice. The reaction mixture was then stirred at room temperature for 1 h. The progress of the reaction was monitored using TLC. After completion, the solvent was removed under reduced pressure, and the residue was co-evaporated with fresh CH_2Cl_2 several times to remove residual TFA. The crude product was purified through silica gel column chromatography using $CHCl_3$ /MeOH (4:1 *v/v*) to afford the corresponding TFA-Phg-Leu (29). Product-containing fractions were combined and co-evaporated with $CHCl_3$ and MeOH several times before 1H NMR analysis in CD_3OD . The same procedure was applied to the preparation of all four stereoisomers of **29**.

(D, D)-**29**: $[\alpha]_D^{-39}$ (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{16}H_{20}F_3N_2O_4^+$ 361.1370, found 361.1385. 1H -NMR (400 MHz, METHANOL- D_4) δ 7.45–7.30 (m, 5H), 5.60 (s, 1H), 4.33 (dd, $J = 8.9, 5.4$ Hz, 1H), 1.69 (m, 1H), 1.58 (m, 2H), 0.92 (d, $J = 4.3$ Hz, 3H), 0.91 (d, $J = 4.5$ Hz, 3H). ^{13}C -NMR (101 MHz, METHANOL- D_4) δ 178.4, 169.1, 157.1 (q, $^2J_{CF} = 37.8$ Hz), 136.0, 128.5, 128.3, 127.6, 116.1 (q, $^1J_{CF} = 287$ Hz), 57.4, 53.2, 41.3, 24.7, 22.3, 20.9.

(L, L)-**29**: $[\alpha]_D^{+37}$ (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{16}H_{20}F_3N_2O_4^+$ 361.1370, found 361.1383. 1H -NMR (400 MHz, METHANOL- D_4) δ 7.48–7.32 (m, 5H), 5.63 (s, 1H), 4.36 (dd, $J = 8.6, 5.6$ Hz, 1H), 1.72 (m, 1H), 1.60 (m, 2H), 0.94 (d, $J = 5.1$ Hz, 3H), 0.92 (d, $J = 4.6$ Hz, 3H). ^{13}C -NMR (101 MHz, METHANOL- D_4) δ 177.8, 169.1, 157.2 (q, $^2J_{CF} = 37.8$ Hz), 135.9, 128.5, 128.3, 127.7, 116.1 (q, $^1J_{CF} = 287$ Hz), 57.4, 52.9, 41.2, 24.7, 22.2, 20.8.

(D, L)-**29**: $[\alpha]_D^{-99}$ (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{16}H_{20}F_3N_2O_4^+$ 361.1370, found 361.1375. 1H -NMR (400 MHz, METHANOL- D_4) δ 7.46–7.32 (m, 5H), 5.60 (s, 1H), 4.30 (dd, $J = 10.3, 4.6$ Hz, 1H), 1.51 (m, 2H), 1.31 (m, 1H), 0.75 (d, $J = 6.6$ Hz, 3H), 0.71 (d, $J = 6.6$ Hz, 3H). ^{13}C -NMR (101 MHz, METHANOL- D_4) δ 178.4, 169.2, 1156.8 (q, $^2J_{CF} = 37.6$ Hz), 136.7, 128.5, 128.4, 127.5, 116.0 (q, $^1J_{CF} = 286.6$ Hz), 57.4, 52.7, 40.8, 24.6, 22.2, 20.3.

(L, D)-**29**: $[\alpha]_D^{+92}$ (c 1, MeOH), HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{16}H_{20}F_3N_2O_4^+$ 361.1370, found 361.1375. 1H -NMR (400 MHz, METHANOL- D_4) δ 7.46–7.32 (m, 5H), 5.61 (s, 1H), 4.30 (dd, $J = 10.2, 4.5$ Hz, 1H), 1.52 (m, 2H), 1.30 (m, 1H), 0.74 (d, $J = 6.6$ Hz, 3H), 0.71 (d, $J = 6.6$ Hz, 3H). ^{13}C -NMR (101 MHz, METHANOL- D_4) δ 178.6, 169.3, 156.8 (q, $^2J_{CF} = 37.6$ Hz), 136.6, 128.5, 128.4, 127.5, 116.0 (q, $^1J_{CF} = 287.0$ Hz), 57.4, 52.8, 40.8, 24.6, 22.2, 20.3.

3. Results

3.1. Peptide Formation of TFA-Phg-OH (1) and Ala-OMe-HCl (2)

TFA-Phg-OH readily undergoes cyclization to the corresponding oxazolone in the presence of excess WSCD·HCl and triethylamine (TEA) [8]. Based on this observation, coupling conditions were developed using TFA-D-Phg-OH (D-1, 1.0 equiv), WSCD·HCl (1.0 equiv), and D-Ala-OMe-HCl (D-2) in CH₂Cl₂, with the free amine generated in situ under a limited amount of TEA (0.5 equiv). While the initial experiments employed a large excess of D-Ala-OMe-HCl (10 equiv), analogous to typical activated ester protocols, this condition resulted in low coupling efficiency and increased formation of the oxazolone byproduct (4) (Table 1, entry 1). Reducing the amount of D-Ala-OMe-HCl to 2.0 or 1.2 equiv significantly improved the outcome, affording TFA-D-Phg-D-Ala-OMe (3) at a ~70% yield with good stereoretention (<2% epimer formation; Table 1, entries 2 and 3). These results highlight the importance of reagent stoichiometry in suppressing oxazolone formation while maintaining efficient coupling.

Table 1. Amount-dependent optimization of TFA-Phg-Ala-OMe (3); formation from TFA-Phg-OH (1) and Ala-OMe-HCl (2).

Entry	D-2 (eq)	Peptide 3 yield (%)	de (%)	oxazolone 4 (%)
1	10	19.5	100	9.1
2	2	67.5	97.6	15.9
3	1.2	70.6	96.8	13.5

3.2. Stereochemical Effects on Coupling Efficiency

Under the optimized conditions, all four stereoisomers of TFA-Phg-Ala-OMe (3) were examined. Coupling between homochiral pairs proceeded efficiently, affording a ~70% yield within 1 h (Table 2, entries 1 and 2). In contrast, heterochiral combinations showed slower conversion (<50% at 1 h; entries 3 and 4).

Table 2. Stereoretentive synthesis of all diastereomers for TFA-Phg-Ala-OMe (3) in a time-dependent manner.

Entry	TFA-Phg 1	Ala-OMe-HCl 2	Time (h)	Peptide 3		Oxazolone 4 (%)
				Yield (%)	de (%)	
1	D	D	1	70.3	96.3	5.9
2	L	L	1	65.3	99.1	5.5
3	D	L	1	48.7	97.1	3.8
4	L	D	1	47.2	94.9	4.3
5	D	L	2	75.2	99.5	7.4
6	L	D	2	72.3	96.4	5.2

Extending the reaction time to 2 h improved the yields of heterochiral products (~71–75%) while maintaining excellent stereochemical integrity (<1% epimer formation; entries 5 and 6). These results indicate that the reaction rate is dependent on stereochemical pairing, and that reaction time must be carefully adjusted to balance conversion and Phg epimerization.

3.3. Protecting Group Effects and Substrate Scope

The generality of the optimized protocol was evaluated using a range of amino acid ester hydrochlorides. Alanine tert-butyl ester hydrochloride (Ala-Ot-Bu-HCl, 5) underwent coupling under standard conditions to afford TFA-Phg-Ala-Ot-Bu (12), showing trends consistent with the methyl ester series (Table 3, entries 1–4). As summarized in

Table 3, the coupling of TFA-Phg-OH (**1**) with various amino acid esters (Val, Leu, Met, and Phg) provided the corresponding TFA-protected dipeptides (**12–18**) in moderate to good yields (>70%), with a consistently high stereochemical purity ($\geq 98\%$ de). A minor oxazolone-type byproduct (**4**) was observed in all cases (<12%). Notably, tert-butyl esters exhibited comparable stereochemical outcomes to methyl esters, supporting their use in orthogonal protection strategies without compromising stereochemical integrity.

Table 3. Stereoretentive synthesis of all diastereomers for TFA-Phg-OH (**1**) with various H-AA-OR-HCl (**5–11**).

Entry	Product	Configuration	Reaction Time (h)	Yield (%)	de (%)	4 (%)
1	TFA-Phg-Ala- <i>Ot</i> -Bu (12)	DD	1	75.0	99.6	0.2
2		LL	1	76.8	99.9	1.3
3		DL	2	76.5	99.7	1.1
4		LD	2	76.9	99.7	1.1
5	TFA-Phg-Val-OMe (13)	DD	1	72.0	99.1	8.9
6		LL	1	81.6	99.7	5.8
7		DL	2	79.0	99.5	6.0
8		LD	2	70.9	99.9	4.1
9	TFA-Phg-Val- <i>Ot</i> -Bu (14)	DD	1	81.7	98.8	12.7
10		LL	1	79.1	99.6	9.5
11		DL	2	80.8	99.9	8.6
12		LD	2	83.3	99.4	11.7
13	TFA-Phg-Leu-OMe (15)	DD	1	74.6	99.4	10.3
14		LL	1	77.4	99.7	11.3
15		DL	2	78.2	99.5	10.7
16		LD	2	77.3	99.9	7.6
17	TFA-Phg-Leu- <i>Ot</i> -Bu (16)	DD	1	69.8	98.7	12.6
18		LL	1	79.1	98.3	12.9
19		DL	2	79.0	98.5	9.3
20		LD	2	71.5	99.8	12.2
21	TFA-Phg-Met-OMe (17)	DD	1	75.6	99.1	10.3
22		LL	1	75.9	99.8	8.7
23		DL	2	74.8	99.6	10.2
24		LD	2	80.1	99.9	12.7
25	TFA-Phg-Phg-OMe (18)	DD	1	74.8	99.8	6.1
26		LL	1	72.6	99.4	1.6
27		DL	2	75.9	98.8	4.6
28		LD	2	81.4	99.2	7.5

3.4. Deprotection Studies

3.4.1. Deprotection of TFA-Phg-AA-OMe

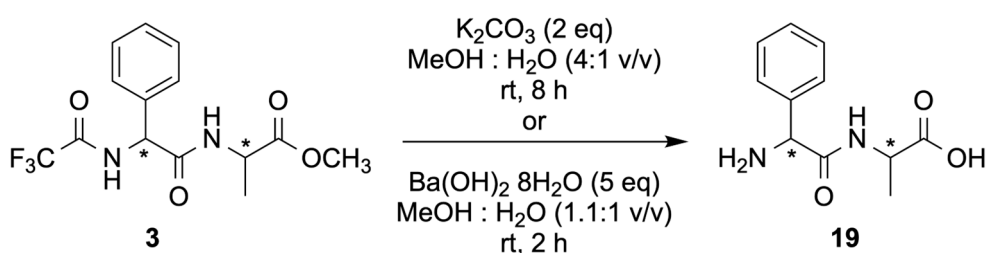
Deprotection conditions were next examined, with a focus on preserving stereochemical integrity. The treatment of TFA-Phg-Ala-OMe (**3**) with K_2CO_3 (2 equiv) in MeOH/ H_2O (4:1 *v/v*) at room temperature required >8 h for completion and resulted in significant racemization (de values of approximately 68–75%).

In contrast, $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ (5 equiv) in MeOH/ H_2O (~1.1:1 *v/v*) enabled the rapid and efficient removal of both the *N*-TFA and methyl ester groups within ~2 h, affording Phg-Ala (**19**) with substantially reduced Phg epimerization (<4%) relative to the carbonate conditions. These results demonstrate that conventional carbonate bases promote stereo-

chemical erosion, whereas $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ provides a significantly milder and more stereoretentive alternative (Table 4).

Table 4. Stereoretentive deprotection of all diastereomers, from TFA-Phg-Ala-OMe (**3**) to Phg-Ala (**19**). * denotes each amino acid stereocenter.

Entry	TFA-Phg-Ala-OMe 3	Base	9 Yield (%)	de (%)
1	(D,D)	K_2CO_3	71.0	74.6
2	(D,L)	K_2CO_3	75.0	68.0
3	(L,L)	K_2CO_3	88.0	75.0
4	(L,D)	K_2CO_3	79.0	72.2
5	(D,D)	$\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$	65.6	95.1
6	(D,L)	$\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$	74.4	93.5
7	(L,L)	$\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$	61.2	96.1
8	(L,D)	$\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$	65.2	87.1

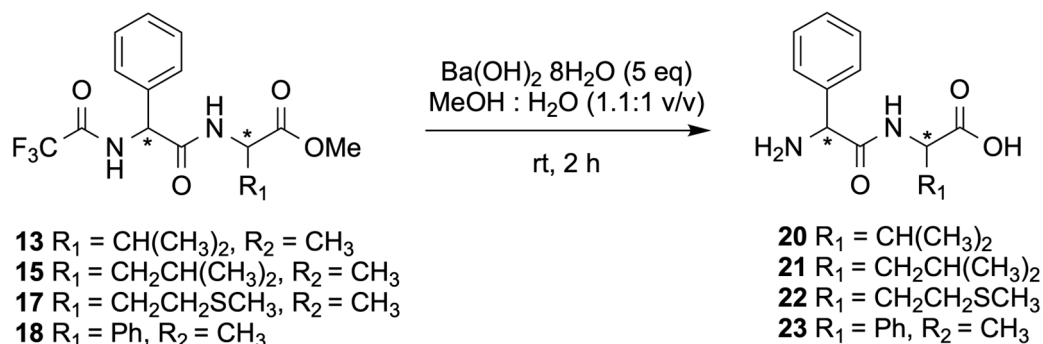


3.4.2. Scope of Deprotection

Application of the optimized $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ conditions to other methyl ester substrates furnished Phg-Val (**20**), Phg-Leu (**21**), Phg-Met (**22**), and Phg-Phg (**23**) in high yields with excellent stereochemical purity (>95% de; Table 5). These results confirm the generality of the method and highlight its suitability for preparing unprotected Phg-containing dipeptides.

Table 5. Stereoretentive deprotection of all diastereomers, from TFA-Phg-AA-OMe to Phg-AA. * denotes each amino acid stereocenter.

Entry	Starting Material	Product	Type	Yield (%)	de (%)
1	TFA-Phg-Val-OMe (13)	Phg-Val (20)	DD	98.0	99.1
2			DL	88.9	99.3
3			LL	92.7	99.5
4			LD	98.3	99.5
5	TFA-Phg-Leu-OMe (15)	Phg-Leu (21)	DD	98.0	99.0
6			DL	96.9	99.4
7			LL	96.1	99.4
8			LD	98.5	99.3
9	TFA-Phg-Met-OMe (17)	Phg-Met (22)	DD	94.1	99.7
10			DL	91.2	99.8
11			LL	94.6	99.8
12			LD	92.0	99.8
13	TFA-Phg-Phg-OMe (18)	Phg-Phg (23)	DD	95.3	99.7
14			DL	97.2	94.7
15			LL	87.2	94.6
16			LD	95.3	96.6



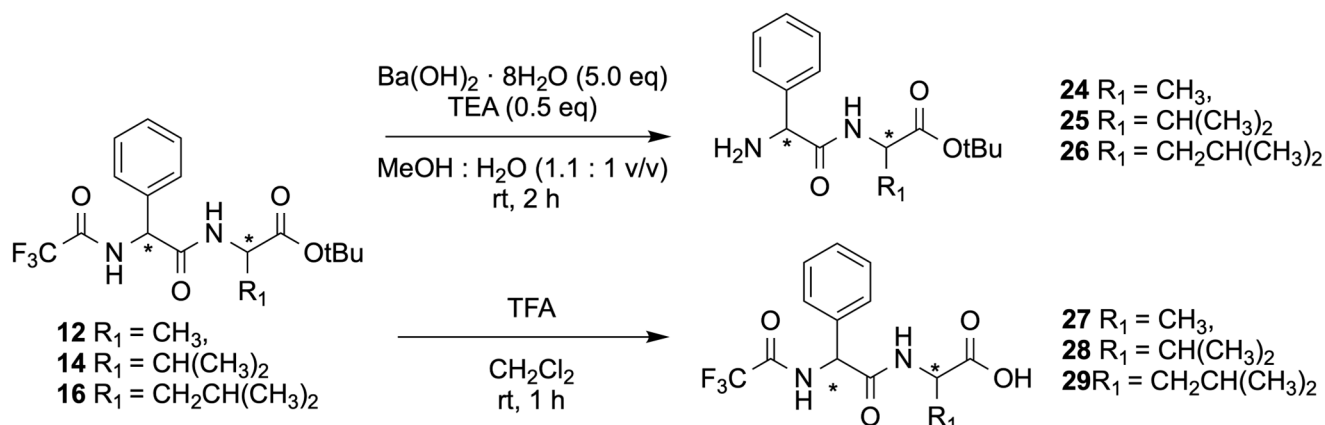
3.4.3. Selective Deprotection of TFA-Phg-AA-OtBu

Orthogonal deprotection was further demonstrated using tert-butyl ester derivatives. Treatment with $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ selectively removed the *N*-TFA group to afford Phg-AA-Ot-Bu (**24–26**) without a loss of stereochemical integrity ((a) in Table 6).

Conversely, treatment with trifluoroacetic acid in CH_2Cl_2 selectively cleaved the tert-butyl ester, affording TFA-Phg-AA (**27–29**) in good yields while preserving stereochemistry ((b) in Table 6). These complementary conditions provide a versatile platform for step-wise peptide manipulation.

Table 6. Selective deprotection of TFA-Phg-AA-Ot-Bu peptides. * denotes each amino acid stereo-center.

Entry	Starting Material	Product	Type	Yield (%)	de (%)
(a) Selective N-terminal deprotection of <i>N</i> -TFA-Phg-AA-Ot-Bu under alkaline conditions.					
1	TFA-Phg-Ala-Ot-Bu (12)	Phg-Ala-Ot-Bu (24)	DD	84.4	98.8
2			DL	78.1	99.5
3			LL	73.5	97.4
4			LD	85.7	93.3
5	TFA-Phg-Val-Ot-Bu (14)	Phg-Val-Ot-Bu (25)	DD	82.9	99.7
6			DL	97.4	93.2
7			LL	98.0	99.6
8			LD	92.8	96.1
9	TFA-Phg-Leu-Ot-Bu (16)	Phg-Leu-Ot-Bu (26)	DD	76.9	99.7
10			DL	86.3	99.6
11			LL	83.9	99.5
12			LD	83.7	99.7
(b) Selective C-terminal deprotection of <i>N</i> -TFA-Phg-AA-Ot-Bu under acidic conditions.					
1	TFA-Phg-Ala-Ot-Bu (12)	TFA-Phg-Ala (27)	DD	91.5	98.1
2			DL	85.2	98.5
3			LL	90.2	98.2
4			LD	84.0	99.9
5	TFA-Phg-Val-Ot-Bu (14)	TFA-Phg-Val (28)	DD	86.4	99.7
6			DL	95.4	93.2
7			LL	84.4	99.6
8			LD	94.2	96.1
9	TFA-Phg-Leu-Ot-Bu (16)	TFA-Phg-Leu (29)	DD	93.6	99.7
10			DL	93.3	99.7
11			LL	99.0	99.7
12			LD	94.5	99.7



4. Conclusions

The present results indicate that stereochemical erosion in TFA-Phg peptide chemistry is controlled by a competition between productive acyl transfer and epimerization at the Phg α -center. In *N*-acyl Phg derivatives, the benzylic α -proton is more acidic than in ordinary proteinogenic aromatic amino acids because the benzene ring is directly attached to the stereogenic carbon. Therefore, under basic activation conditions, deprotonation and oxazolone formation can generate an achiral or rapidly equilibrating intermediate before aminolysis, leading to Phg-site epimerization in the peptide product [6,10]. This mechanism is consistent with our previous TFA-Phg-OSu study, in which excessive WSCD·HCl, high-pH workup, and a soluble organic base during aminolysis increased oxazolone formation and diastereomer generation [10].

The present strategy complements the existing methods for inhibiting Phg epimerization. Liang et al. obtained 93–100% of the desired diastereomer using Fmoc-based solid-phase peptide synthesis (SPPS) with DEPBT or COMU and sterically less hindered bases such as TMP or DMP [9]. Elsayy et al. also showed that the racemization/epimerization of Phg readily occurs during SPPS, and that even optimized peptide sequences still contain substantial amounts of epimeric impurities [10]. Compared to these Fmoc-SPPS methods, this method uses TFA-Phg-OH, a simple carbodiimide reagent, and an amino acid ester hydrochloride in solution as coupling agents. Its main advantage is that it avoids the need for pre-prepared TFA-Phg-OSu and circumvents the problems associated with strong or excess organic bases encountered in our previous work. A limitation of this method is that it has so far been applied mainly to dipeptide units, and its applicability to longer resin-bound sequences or side-chain-functionalized Phg analogs has not yet been verified.

The one-pot protocol developed here addresses these problems by changing both the timing and the basicity of the coupling process. Instead of isolating TFA-Phg-OSu, TFA-Phg-OH is activated in the presence of the amino acid ester hydrochloride under stoichiometric WSCD·HCl and limited triethylamine. The small amount of triethylamine is sufficient to generate a reactive fraction of the free amine from AA-OR·HCl, but avoids the strongly basic homogeneous conditions that favor deprotonation at the Phg α -position. The observation that excessive amino ester hydrochloride did not improve the reaction, and that heterochiral combinations required longer reaction times, suggests that both reagent stoichiometry and stereochemical matching influence the balance between aminolysis, oxazolone formation, and epimerization. Thus, this method is not simply a change in the coupling reagent; it is a base-limited activation strategy designed around the particular lability of the Phg α -center.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/org7030027/s1>, All ^1H and ^{13}C NMR spectra are provided in the Supplementary Materials.

Author Contributions: Methodology, validation, formal analysis, investigation, Z.W., S.I., Y.F., S.Y., M.I., X.Y., D.T.A., and Z.P.T.; data curation, T.S.; conceptualization, supervision, Y.M. and M.H.; writing—original draft preparation, Z.W.; writing—review and editing, Y.M. and M.H. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data that support the findings of this study are available from the corresponding author (M.H.) upon reasonable request.

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