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NOVEL SYNTHESIS AND PROPERTIES OF OPTICALLY PURE N-TRIFLUOROACETYLPHENYLGLYCINE HYDROXYSUCCINIMIDE ESTER

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Abstract – Phenylglycine is non-proteinogenic α -amino acid, and its partial structure is found in some biologically active compounds. C-Terminal modification of N-acyl-protected phenylglycine sometimes causes racemization due to the fact that the phenyl ring is directly connected to the α -position of the α -amino acid skeleton. In this report, synthesis and the properties of N-trifluoroacetylphenylglycine hydroxysuccinimide ester are described.

INTRODUCTION

The phenylglycine (Phg; compound **1**, Figure 1) skeleton is widely found in natural antibiotic compounds.¹ Phg has a bulky aromatic side-chain attached directly to the α -position of the α -amino acid skeleton. The structural aspects of Phg promote racemization more than other proteinogenic aromatic α -amino acids, which have a β -methylene spacer between the aromatic moiety and α -position.² N-Hydroxysuccinimide ester (OSu ester) of α -amino acids are widely used for peptide synthesis, because

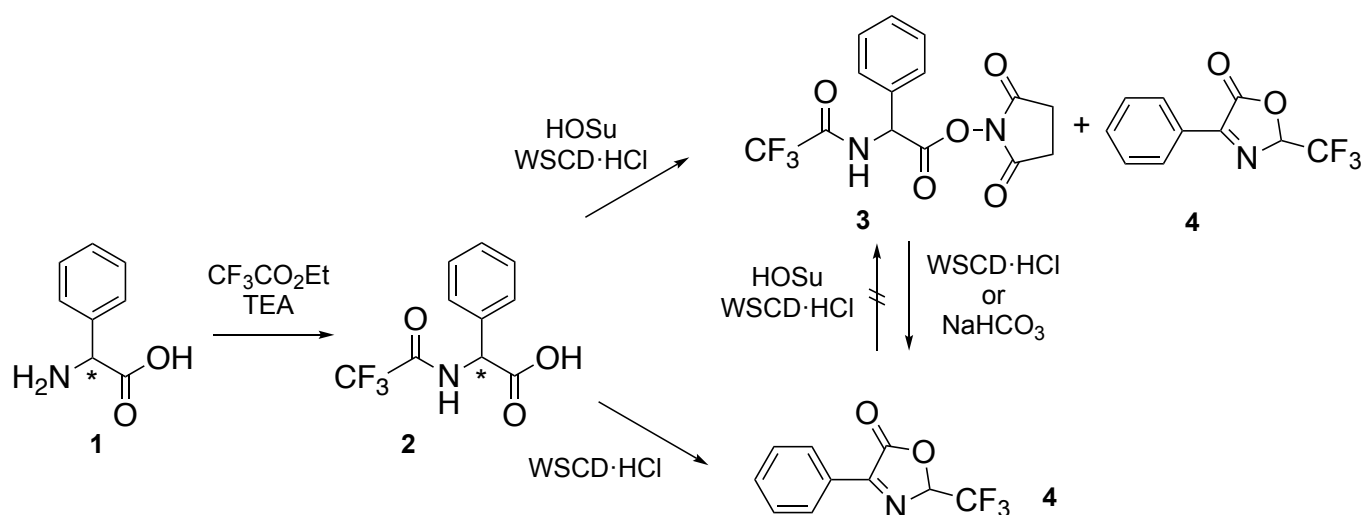
This paper is dedicated to Prof. Somsak Ruchirawat on the occasion of his 80th birthday.

of their higher storage stabilities and sufficient reactivities with amino components.³ There are several reports that N-carbamate protected Phg derivatives that were converted to their corresponding OSu ester.⁴ On the other hand, there are few reports regarding OSu ester of N-acyl-protected Phg derivatives. N-Trifluoroacetyl (TFA) protection of α -amino acid was used as a more easily removable N-acyl protective group than other N-acyl protection in peptide chemistry.⁵ This study presents details of the first synthesis of N-TFA-Phg-OSu and its utilization for peptide formation, and deprotection of protecting groups.

RESULTS AND DISCUSSION

N-Trifluoroacetyl protection of optically pure phenylglycine was conducted with ethyl trifluoroacetate in the presence of tetramethylguanidine, which is rare in the use of trifluoroacetylation, based on previous reports.⁶ Triethylamine (TEA), which is a common base for trifluoroacetylation,⁷ can be utilized in a stereo-retention manner as indicated by optical rotation analysis. TFA-Phg-OH (**2**) was treated with HOSu (1.1 eq) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide monohydrochloride (WSCD·HCl, 1.0 eq), which is the standard method for synthesizing succinimide ester of N-TFA α -amino acids,⁸ at rt for 1 h. The reaction mixture was worked up with saturated aqueous NaHCO₃ to recover the starting material (30%), and two major products were detected in the organic layer. The two components were

Table 1. Synthesis of TFA-Phg-OSu **3** from Phg **1** with various conditions



Entry	HOSu (eq)	WSCD·HCl (eq)	work up	2 (%)	3 (%)	4 (%)
1	1.1	1.0	sat NaHCO ₃	30	30	40
2	1.1	1.0	5% NaHCO ₃	30	60	10
3	1.1	1.5	5% NaHCO ₃	0	35	65
4	1.1	1.0	-	34	59	7

separated by hexane treatment. The hexane insoluble compound was identified as the desired TFA-Phg-OSu (**3**, 30%), and the byproduct (oxazolone **4**,⁹ 40%) was isolated from the hexane soluble fraction (Table 1, Entry 1).

Due to the byproduct **4**, which seemed to increase during the work-up described above, the reaction mixture was treated with 5% aqueous NaHCO₃ to recover the starting material **2**. The proportion of the desired product **3** was increased to 60% (Table 1, Entry 2). The reaction with a slight excess of WSCD·HCl (1.5 eq) afforded oxazolone **4** (65%, Table 1, Entry 3) as the major product. These results indicate that the formation of oxazolone **4** was promoted with a high pH treatment and a large amount of WSCD·HCl. The oxazolone **4** was generated quantitatively from the treatment of the isolated TFA-Phg-OSu **3** with WSCD·HCl (1.0 eq) in CH₂Cl₂ at rt or partitioned between CH₂Cl₂ and saturated aqueous NaHCO₃. On the other hand, compound **3** cannot be synthesized from oxazolone **4** in the presence of HOSu and WSCD·HCl (Table 1). The reaction mixture was directly analyzed to elucidate the proportions for each component. The results indicated that TFA-Phg-OSu **3** was major product and oxazolone **4** was less than 10% (Table 1, entry 4), with proportions that were identical with the 5% aqueous NaHCO₃ treatment.

TFA-Phg-OSu **3**, which was synthesized with 1.1 eq of HOSu and 1.0 eq WSCD·HCl, afforded optical rotation as +31 and –30 for L- and D-isomers, respectively. Further analysis of the optical rotation of **3** with acid hydrolysis of these succinimide esters, which were identified as Phg **1**, revealed +22 and –21 for L- and D-isomers, respectively. The observed values were lower than the optically pure Phg (–155 for D-isomer¹⁰). These results indicated that the racemization might have occurred during the formation of TFA-Phg-OSu **3** from TFA-Phg-OH **2**.

The higher optical rotation values of **3** were preliminary observed with the amounts of HOSu in a dependent manner. Table 2 emphasizes the proportion of the reaction mixture that were analyzed with various amount of HOSu in the presence of 1.0 eq of WSCD·HCl at rt for 1.5 h. The conversion rates for

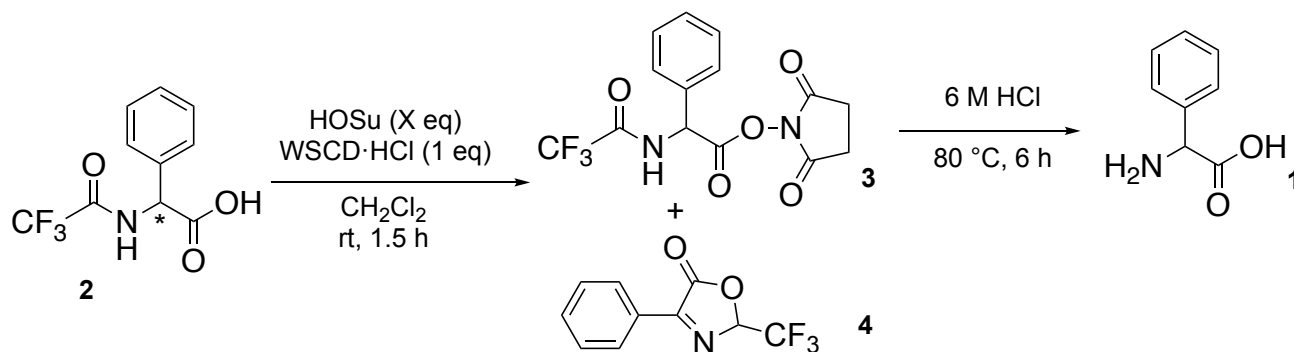
Table 2. Proportion of the reaction mixture of the equivalent of compound D-**2** and WSCD·HCl (1.0 eq) with various amounts of HOSu at rt for 1.5 h

Entry	HOSu (eq)	2 (%)	3 (%)	4 (%)
1	1.5	34	59	7
2	2	28	62	10
3	5	43	52	5
4	7.5	44	52	4
5	10	46	50	4

TFA-Phg-OSu **3** from **2** were slightly lower for the reactions with up to 10 eq of HOSu, but the oxazolone **4** formations maintained less than 5% (Table 2, Entries 3–5).

The synthesized **3** with various amounts of HOSu and acid hydrolyzed compounds were subjected to optical rotation analysis (Table 3). The optical rotation of **3** increased in a HOSu dependent manner up to 7.5 eq for both isomers. The analysis of acid hydrolyzed **3**, as unprotected Phg **1**, also had the same tendency as the analysis of **3**, and the optical rotation values were identical to the report of optical pure Phg. The results indicated the excess HOSu, which could be an intermolecular nucleophilic reaction and might play a role in the competition of the intramolecular reaction (oxazolone formation) to carboxylic acid–carbodiimide complex in a stereo-retentive manner.

Table 3. Synthesis of TFA-Phg-OSu **3** with various amounts of HOSu. The optical rotations of synthesized **3** and acid hydrolyzed **3** as Phg **1**

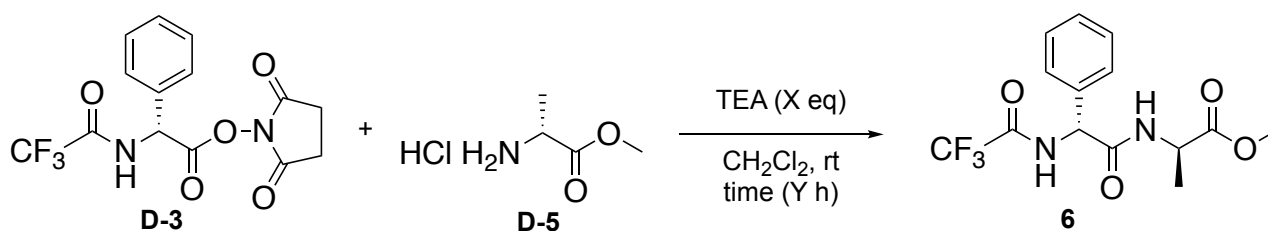


Entry	2	HOSu	[α] _D	
			3	Hydrolyzed 3
1	L-	1.1	+31	+22
	D-		-30	-21
2	L-	2	+40	+37
	D-		-41	-38
3	L-	5	+72	+115
	D-		-70	-113
4	L-	7.5	+86	+155
	D-		-87	-156
5	L-	10	+85	+154
	D-		-86	-155
			(c = 1, CHCl ₃)	(c = 1, 1M HCl)

The optically pure TFA-D-Phg-OSu (D-**3**) was subjected to D-Ala-OMe·HCl (D-**5**, 1.2 eq) at rt in CH₂Cl₂ for the formation of the peptide TFA-Phg-Ala-OMe **6**. TEA, one of the most common bases for peptide synthesis, was then used to proceed with the reaction. For a typical reaction, two diastereomer peptides, namely TFA-D-Phg-D-Ala-OMe (D, D)-**6** and TFA-L-Phg-D-Ala-OMe (L, D)-**6** were observed.

The presence of the enantiomeric position of these peptides could be distinguished by the difference of methyl ester signals on $^1\text{H-NMR}$ at a chemical shift $\delta = 3.69$ ppm for (D, D)-**6** and $\delta = 3.76$ ppm for (L, D)-**6**. The reaction with 1.5 eq TEA afforded the product **6** quantitatively, but part of the peptide ($\sim 30\%$) consisted of (L,D)-**6** (Table 4, Entry 1). The reaction with 1 eq TEA could not prevent racemization (Table 4, Entry 2). The control experiment with treatment D-**3** with 1 eq TEA, followed by the addition of D-**5** afforded diastereomeric mixture (Table 4, Entry 3). These results indicated the TFA-D-Phg-OSu (D-**3**) was converted to oxazolone under basic conditions; then, the intermediate was utilized for peptide formation to afford a diastereomeric mixture. The peptide formation was subjected with a lower amount of TEA and D-**5** in advance. Then, D-**3** was added to the mixture. Even though the formation of (L, D)-**6** was below 15%, the chemical yield was extremely lower (less than 40%, Table 4, Entries 4~8).

Table 4. Peptide formations with TFA-D-Phg-OSu (D-**3**) and D-Ala-OMe HCl (D-**5**) in the presence of TEA. * D-**3** was treated with TEA for an hour, then D-**5** was added to the rm for 1 h.



Entry	TEA (eq)	Time (h)	Chemical yield (%)	(D, D)- 6 (%)	(L, D)- 6 (%)
1	1.5	1	100	70	30
2	1	1	94	72	28
3	1	1 + 1*	90	50	50
4	0.5	1	39	85	15
5	0.25	1	26	88	12
6	0.1	1	12	87	13
7	0.05	1	8	89	11
8	0.05	8	10	90	10

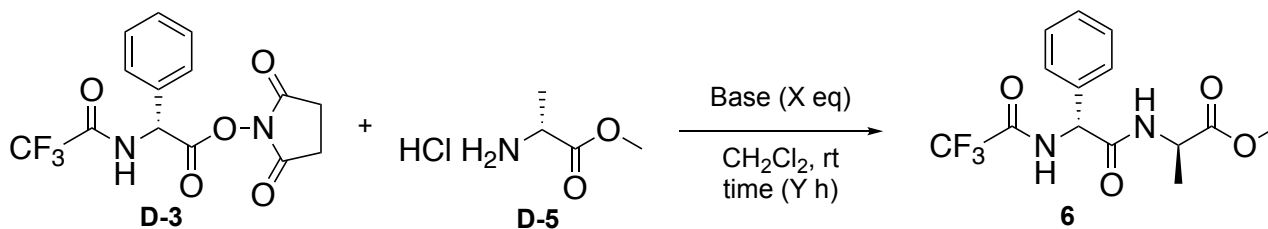
* D-**3** was treated with TEA for 1 h, then D-**5** was added to the rm for 1 h.

Based on the TEA utilization as the base for peptide formation of TFA-Phg-Ala-OMe **6**, the racemization may have been promoted by a homogeneous reaction mixture to compete the formation of oxazolone from **3** and maintain a nucleophilicity of **5**.

For further understanding the minimalization of oxazolone and effectiveness to maintain nucleophilicity of **5**, several alkaline salts were subjected for the peptide formation in heterogeneous reaction conditions

(Table 5). The heterogeneous reaction mixtures of D-**3** and D-**5** with K_2CO_3 for 1 h, the optimized reaction time for the reaction with TEA, afforded low chemical yields (Table 5, Entries 1, 3 and 7). The reaction proceeded effectively after 8 h without any increasement of unretarded optical active peptide formation (less than 15%, Table 5 Entries 2, 4 and 5). The amount of K_2CO_3 up to 1 eq was acceptable for higher chemical yield and less (L, D)-**6** formation. Under sodium bicarbonate condition, the partial hydrolysis of succinimide D-**3** was observed after 8 h (Table 5, Entry 8). The effective reaction for sodium carbonate was observed with excess amount (2.5 eq, Table 5, Entry 11). The utilization of basic salt for the peptide formation of TFA-Phg-OSu (D-**3**) in a heterogeneous reaction mixture decreased the possibility of unretarded optical active peptide formation. The diastereomer **6** at Phg moiety was synthesized from optically pure **3** and **5** in the presence of K_2CO_3 at rt for 8 h with less than 15%. The optically pure **3** and **5** were subjected to the established condition with sodium carbonate (Table 6).

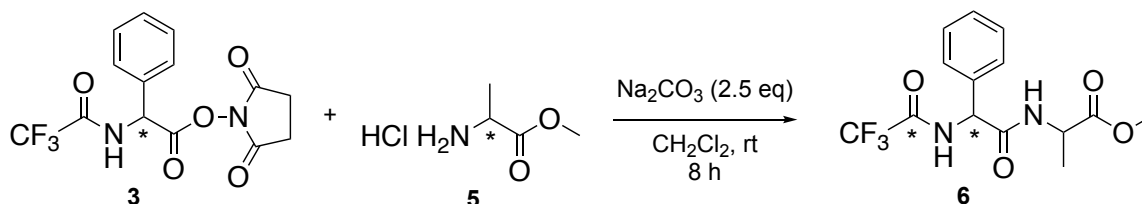
Table 5. Peptide formations with TFA-D-Phg-OSu (D-**3**) and D-Ala-OMe HCl (D-**5**) in the presence of basic salt for heterogeneous reactions



Entry	Base	eq	Time (h)	Chemical yield (%)	(D, D)- 6 (%)	(L, D)- 6 (%)
1	K_2CO_3	0.25	1	10	88	12
2	K_2CO_3	0.25	8	36	88	28
3	K_2CO_3	0.5	1	16	90	10
4	K_2CO_3	0.5	8	85	84	16
5	K_2CO_3	1	8	84	86	14
6	$NaHCO_3$	0.5	8	13	84	16
7	$NaHCO_3$	1	1	13	90	10
8	$NaHCO_3$	1	8	68*	86	14
9	Na_2CO_3	0.5	8	23	90	10
10	Na_2CO_3	1	8	28	88	12
11	Na_2CO_3	2.5	8	92	85	15

* Hydrolysis of **3** was observed

Table 6. Peptide formations with optically pure TFA-Phg-OSu (**3**) and Ala-OMe·HCl (**5**) in the presence of sodium carbonate for heterogeneous reactions

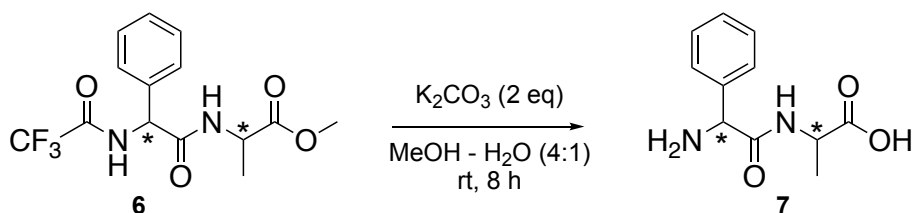


Entry	3	5	Chemical yield (%)	6 (%)	
1	D-	D-	92	(D, D)- 85	(L, D)- 15
2	D-	L-	88	(D, L)- 87	(L, L)- 13
3	L-	L-	90	(L, L)- 86	(D, L)- 14
4	L-	D-	87	(L, D)- 85	(D, D)- 15

The four optically pure diastereomer of TFA-Phg-Ala-OMe **6** were subjected to deprotection with K_2CO_3 in aqueous methanol. The unretarded optical active counterpart of Phg-Ala **7**, which could be separatable by silica column chromatography, was observed to be less than 12% (Table 7).

Activation of the C-terminal of N-acyl-protected Phg was considered that the oxazolone formation with intramolecular manner might be preferred because the aromatic ring was connected at the α -position directly. N-Trifluoroacetyl protection is well known to prevent racemization via formation of oxazolone for common α -amino acid.⁹ But there are few reports for Phg derivatives. The unique structure of Phg promotes racemization during activation of the C-terminal even though N-trifluoroacetyl protection.

Table 7. Deprotection of TFA-Phg-Ala-OMe derivatives (**6**) with K_2CO_3 in aqueous methanolic solution



Entry	TFA-Phg-Ala-OMe 6	Phg-Ala 7			
			Yield (%)		Yield (%)
1	(D, D)	(D, D)	62	(L, D)	9
2	(D, L)	(D, L)	63	(L, L)	12
3	(L, L)	(L, L)	70	(D, L)	11
4	(L, D)	(L, D)	68	(D, D)	11

The detailed analysis and minimum racemization of the Phg moiety in this study can contribute to the understanding in of the Phg peptide chemistry.

EXPERIMENTAL

General Remarks. All reagents used were of analytical grade. NMR spectra were measured by an EX 270 spectrometer (JEOL, Tokyo, Japan). Optical rotations were measured at 23 °C on a JASCO DIP370 polarimeter (JASCO, Tokyo, Japan). HRMS-ESI spectra were obtained with a Waters UPLC ESI-TOF mass spectrometer (Waters, Milford, CT, USA).

2-Phenyl-2-(2,2,2-trifluoroacetamido)acetic acid (TFA-Phg-OH, **2**)

Triethylamine (8.30 mL, 59.5 mmol) was added to a suspension of optically pure phenylglycine (6.00 g, 39.7 mmol) in MeOH (66 mL) on ice. After stirring for 15 min, ethyl trifluoroacetate (6.14 mL, 51.6 mmol) was added. The suspension was turned to solution with stirring at rt for overnight. After concentration, the residue was dissolved in H₂O (100 mL) and acidified with concentrated HCl 4 mL, then extracted with EtOAc. The organic layer was washed with brine, dried by MgSO₄, filtered, and concentrated with oil pump to afford a colorless solid.

L-2: 9.24 g (94%), [α]_D +171 (*c* 1.0, MeOH), HRMS-ESI (*m/z*) [M+Na]⁺ calcd for C₁₀H₈F₃NO₃Na⁺ 270.0354, found 270.0368. **D-2**: 9.16 g (93%), [α]_D -171 (*c* 1.0, MeOH), HRMS-ESI (*m/z*) [M+Na]⁺ calcd for C₁₀H₈F₃NO₃Na⁺ 270.0354, found 270.0368. ¹H-NMR (270 MHz, acetone-*d*₆) δ : 8.97 (1H, s), 7.52 (2H, dd, *J* = 7.7, 1.8 Hz), 7.41 (3H, ddt, *J* = 17.0, 10.5, 3.7 Hz), 5.63 (1H, d, *J* = 7.3 Hz). ¹³C-NMR (67.5 MHz, acetone-*d*₆) δ : 170.7, 157.3 (q, ²*J*_{CF} = 37.8 Hz), 136.2, 129.6, 129.5, 128.9, 116.9 (q, ¹*J*_{CF} = 287.2 Hz), 57.7.

2,5-Dioxopyrrolidin-1-yl 2-phenyl-2-(2,2,2-trifluoroacetamido)acetate (TFA-Phg-OSu, **3**) and 4-Phenyl-2-(trifluoromethyl)oxazol-5(2*H*)-one (**4**, oxazolone)

To a solution of optically pure TFA-Phg-OH **2** (1.2 g, 4.85 mmol) in CH₂Cl₂ (144 mL) was added *N*-hydroxysuccinimide (4.19 g, 36.4 mmol) and then WSCD·HCl (930.7 mg, 4.85 mmol) in CH₂Cl₂ on ice. The suspension was turned to solution with stirring at rt for 1.5 h. The solution was concentrated before being diluted with CHCl₃ and washed with water, with brine, with a solution of 50 mM aqueous NaHCO₃ and brine. The organic layer was then dried over MgSO₄ and concentrated by rotary evaporation. The residue was washed with *n*-hexane to give **2** as colorless solid. The hexane soluble compound was concentrated to afford **4**⁹ as pale yellow amorphous mass

L-2: 0.741 g (46%), [α]_D +86 (*c* 1.0, CHCl₃), HRMS-ESI (*m/z*) [M+Na]⁺ calcd for C₁₄H₁₁F₃N₂O₅Na⁺ 367.0518, found 367.0505. **D-2**: 0.859 g (53%), [α]_D -87 (*c* 1.0, CHCl₃), HRMS-ESI (*m/z*) [M+Na]⁺ calcd for C₁₄H₁₁F₃N₂O₅Na⁺ 367.0518, found 367.0515. ¹H-NMR (acetone-*d*₆) δ : 9.52 (1H, s), 7.63 (2H, dd, *J* = 6.4, 2.8 Hz), 7.47 (3H, dd, *J* = 4.9, 1.6 Hz), 6.12 (1H, d, *J* = 7.6 Hz), 2.88 (4H, s), ¹³C-NMR (acetone-*d*₆)

δ : 170.0, 166.6, 157.5 (q, $^2J_{CF} = 38.0$ Hz), 134.0, 130.3, 129.8, 129.6, 116.8 (q, $^1J_{CF} = 285.5$ Hz), 56.0, 26.3. **4**: 0.043 g (4%), HRMS-ESI (m/z) $[M+H]^+$ calcd for $C_{10}H_7F_3NO_2^+$ 230.0423, found 230.0445, 1H -NMR ($CDCl_3$) δ : 8.35 (2H, d, $J = 7.3$ Hz), 7.57 (1H, t, $J = 7.4$ Hz), 7.45 (2H, t, $J = 7.4$ Hz), 6.18 (1H, q, $J = 4.1$ Hz), ^{13}C -NMR ($CDCl_3$) δ : 162.7, 160.6, 133.9, 129.1, 129.0, 127.2, 120.4 (q, $^1J_{CF} = 282.1$ Hz), 92.3 (q, $^2J_{CF} = 35.2$ Hz).

Hydrolysis of TFA-Phg-OSu **3** (as **1**)

TFA-Phg-OSu **3** (83.6 mg, 0.25 mmol) was added 6 M HCl (1 mL). The suspension was heated at 80 °C for 6 h. After concentration, the residue was subjected to column chromatography (MeCN : MeOH : H₂O = 4 : 1 : 1) to give colorless solid. From L-3: 0.044 g (46%), $[\alpha]_D +155$ (c 1, 1M HCl). From D-3: 0.046 g (53%), $[\alpha]_D -156$ (c 1, 1M HCl).

Methyl (2-phenyl-2-(2,2,2-trifluoroacetamido)acetyl)alaninate (TFA-Phg-Ala-OMe) **6**

A) TEA method

Triethylamine (0.141 mL, 1.01 mmol) was added to a suspension of optically pure D-Ala-OMe·HCl **D-5** (338.8 mg, 2.43 mmol) in CH_2Cl_2 (30 mL). After stirring for 10 min, a solution of TFA-D-Phg-OSu **3** (700.0 mg, 2.02 mmol) in CH_2Cl_2 (60 mL) and then WSCD·HCl (387.8 mg, 2.02 mmol) in CH_2Cl_2 (30 mL) were added on ice. The suspension was turned to solution with stirring at rt for 1 h. The solvent was removed by rotary evaporation and purified by column chromatography (EtOAc : hexane = 1 : 3, then $CHCl_3$: MeOH = 15 : 1) to afford (D, D)-**6** (222.2 mg) and (L, D)-**6** (39.0 mg) as colorless solid.

B) Na₂CO₃ method

Na₂CO₃ (119.0 mg, 1.1 mmol) was added to a suspension of optically pure Ala-OMe·HCl **5** (75.0 mg, 0.54 mmol) in CH_2Cl_2 (7 mL). After the reaction mixture was stirred at rt for 10 min, a solution of optically pure TFA-Phg-OSu **3** (155.0 mg, 0.45 mmol) in CH_2Cl_2 (20 mL) on ice. The suspension was stirred at rt for 8 h. The solvent was removed by rotary evaporation and purified by column chromatography (EtOAc : hexane = 1 : 3, then $CHCl_3$: MeOH = 15 : 1) to afford **6** as colorless solid.

D-**3** + D-**5**; (D, D)-**6** (117.1 mg), (L, D)-**6** (20.5 mg)

D-**3** + L-**5**; (D, L)-**6** (111.8 mg), (L, D)-**6** (19.8 mg)

L-**3** + L-**5**; (L, L)-**6** (115.7 mg), (D, L)-**6** (18.8 mg)

L-**3** + D-**5**; (L, D)-**6** (111.0 mg), (L, D)-**6** (19.5 mg)

Methyl ((*R*)-2-phenyl-2-(2,2,2-trifluoroacetamido)acetyl)-D-alaninate (TFA-D-Phg-D-Ala-OMe, (**D**, **D**)-**6**): $[\alpha]_D -139$ (c 1, $CHCl_3$), HRMS-ESI (m/z) $[M+H]^+$ calcd for $C_{14}H_{16}F_3N_2O_4^+$ 333.1057, found 333.1086, 1H -NMR ($CDCl_3$) δ : 7.40 (5H, s), 5.41 (1H, d, $J = 6.6$ Hz), 4.58-4.48 (1H, m), 3.69 (3H, s), 1.43 (3H, d, $J = 7.3$ Hz), ^{13}C -NMR (acetone-*d*₆) δ : 173.1, 168.8, 156.9 (q, $^2J_{CF} = 37.4$ Hz), 137.4, 129.4, 129.2, 128.9, 116.9 (q, $^1J_{CF} = 288$ Hz), 57.6, 52.4, 49.2, 17.5.

Methyl ((*S*)-2-phenyl-2-(2,2,2-trifluoroacetamido)acetyl)-L-alaninate (TFA-L-Phg-L-Ala-OMe, (**L, L**)-**6**): $[\alpha]_D +132$ (c 1, CHCl₃), HRMS-ESI (m/z) $[M+H]^+$ calcd for C₁₄H₁₆F₃N₂O₄⁺ 333.1057, found 333.1075, ¹H-NMR (CDCl₃) δ : 7.40 (5H, s), 5.41 (1H, d, $J = 6.3$ Hz), 4.58-4.48 (1H, m), 3.69 (3H, s), 1.43 (3H, d, $J = 7.3$ Hz), ¹³C-NMR (acetone-*d*₆) δ : 173.1, 168.8, 156.9 (q, $^2J_{CF} = 37.1$ Hz), 137.4, 129.4, 129.2, 128.9, 116.9 (q, $^1J_{CF} = 288$ Hz), 57.6, 52.2, 49.2, 17.5.

Methyl ((*R*)-2-phenyl-2-(2,2,2-trifluoroacetamido)acetyl)-L-alaninate (TFA-D-Phg-L-Ala-OMe, (**D, L**)-**6**): $[\alpha]_D -113$ (c 1, CHCl₃), HRMS-ESI (m/z) $[M+H]^+$ calcd for C₁₄H₁₆F₃N₂O₄⁺ 333.1057, found 333.1032, ¹H-NMR (CDCl₃) δ : 7.39 (5H, s), 5.41 (1H, d, $J = 6.3$ Hz), 4.63-4.53 (1H, m), 3.76 (3H, s), 1.30 (3H, d, $J = 7.3$ Hz), ¹³C-NMR (acetone-*d*₆) δ : 173.2, 169.0, 156.8 (q, $^2J_{CF} = 37.2$ Hz), 137.8, 129.5, 129.3, 128.6, 116.9 (q, $^1J_{CF} = 287$ Hz), 57.8, 52.4, 49.2, 17.5.

Methyl ((*S*)-2-phenyl-2-(2,2,2-trifluoroacetamido)acetyl)-D-alaninate (TFA-L-Phg-D-Ala-OMe, (**L, D**)-**6**): $[\alpha]_D +122$ (c 1, CHCl₃), HRMS-ESI (m/z) $[M+H]^+$ calcd for C₁₄H₁₆F₃N₂O₄⁺ 333.1057, found 333.1030, ¹H-NMR (CDCl₃) δ : 7.38 (5H, s), 5.46 (1H, d, $J = 6.6$ Hz), 4.62-4.51 (1H, m), 3.76 (3H, s), 1.30 (3H, d, $J = 7.3$ Hz), ¹³C-NMR (acetone-*d*₆) δ : 173.2, 169.1, 156.8 (q, $^2J_{CF} = 37.2$ Hz), 137.8, 129.5, 129.3, 128.6, 116.9 (q, $^1J_{CF} = 287$ Hz), 57.8, 52.4, 49.2, 17.5.

(2-Amino-2-phenylacetyl)alanine (Phg-Ala) **7**

K₂CO₃ (110.6 mg, 0.80 mmol) in H₂O (0.8 mL) was added to a suspension of optically pure TFA-Phg-Ala-OMe **6** (132.9 mg, 0.4 mmol) in MeOH (3.2 mL). After stirring at rt for 8 h, the solvent was removed by rotary evaporation and D₂O was added. The supernatant was concentrated and purified by column chromatography (MeCN : MeOH : H₂O = 4 : 1 : 0.55) to give colorless solid.

((*R*)-2-Amino-2-phenylacetyl)-D-alanine (D-Phg-D-Ala, (**D, D**)-**7**): $[\alpha]_D -82$ (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 (5H, s), 5.01 (s, 1H), 4.16 (1H, q, $J = 7.0$ Hz), 1.20 (4H, d, $J = 7.3$ Hz), ¹³C-NMR (D₂O) δ : 178.0, 168.5, 132.4, 131.0, 130.2, 128.9, 57.1, 50.7, 17.5.

((*S*)-2-Amino-2-phenylacetyl)-L-alanine (L-Phg-L-Ala, (**L, L**)-**7**): $[\alpha]_D +48$ (c 1, 2.5M HCl), HRMS-ESI (m/z) $[M+H]^+$ calcd for C₁₁H₁₅N₂O₃⁺ 233.1077, found 233.1088, ¹H-NMR (D₂O) δ : 7.37 (5H, s), 5.00 (s, 1H), 4.17 (1H, q, $J = 7.3$ Hz), 1.21 (3H, d, $J = 7.3$ Hz). ¹³C-NMR (D₂O) δ : 177.9, 168.5, 132.4, 131.0, 130.2, 128.9, 57.1, 50.7, 16.9.

((*R*)-2-Amino-2-phenylacetyl)-L-alanine (D-Phg-L-Ala, (**D, L**)-**7**): $[\alpha]_D -71$ (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 (5H, s), 5.00 (1H, s), 4.00 (1H, q, $J = 7.5$ Hz), 1.10 (3H, d, $J = 7.3$ Hz), ¹³C-NMR (D₂O) δ : 180.2, 168.4, 132.7, 131.0, 130.3, 128.5, 57.2, 51.9, 17.4.

((*S*)-2-Amino-2-phenylacetyl)-D-alanine (L-Phg-D-Ala, (**L, D**)-**7**): $[\alpha]_D +104$ (c 1, 2.5M HCl), HRMS-ESI (m/z) $[M+H]^+$ calcd for C₁₁H₁₅N₂O₃⁺ 233.1077, found 233.1068, ¹H-NMR (D₂O) δ : 7.33 (5H,

s), 3.98 (1H, q, $J = 7.3$ Hz), 1.07 (3H, d, $J = 7.6$ Hz). $^{13}\text{C-NMR}$ (D_2O) δ : 180.1, 168.4, 132.7, 131.0, 130.3, 128.5, 57.2, 51.8, 17.4.

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REFERENCES

1. R. S. Al Toma, C. Brieke, M. J. Cryle, and R. D. Süßmuth, *Nat. Prod. Rep.*, 2015, **32**, 1207.
2. M. Sato, T. Tatsuno, and H. Matsuo, *Yakugaku Zasshi*, 1970, **90**, 1160; K. C. Nicolaou, C. N. C. Boddy, S. Bräse, and N. Winssinger, *Angew. Chem. Int. Ed.*, 1999, **38**, 2096.
3. G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Am. Chem. Soc.*, 1964, **86**, 1839; M. M. Joullie and K. M. Lassen, *ARKIVOC*, 2010, **viii**, 189.
4. V. Čaplar, L. Frkanec, N. Š. Vujičić, and M. Žinić, *Chem. Eur. J.*, 2010, **16**, 3066; M. I. Burguete, F. Galindo, M. A. Izquierdo, J.-E. O'Connor, G. Herrera, S. V. Luis, and L. Vigarà, *Eur. J. Org. Chem.*, 2010, 5967; J. Escorihuela, M. I. Burguete, G. Ujaque, A. Lledós, and S. V. Luis, *Org. Biomol. Chem.*, 2016, **14**, 11125; C. Ma, M. Chen, W. Chu, J. Tao, D. Kong, M. Zhang, and W. Feng, *Molecules*, 2019, **24**, 2185.
5. T. J. Curphey, *J. Org. Chem.*, 1979, **44**, 2805.
6. R. Di Santo, R. Costi, A. Roux, M. Artico, O. Befani, T. Meninno, E. Agostinelli, P. Palmegiani, P. Turini, R. Cirilli, R. Ferretti, B. Gallinella, and F. La Torre, *J. Med. Chem.*, 2005, **48**, 4220; S. B. Jensen, R. Di Santo, A. K. Olsen, K. Pedersen, R. Costi, R. Cirilli, and P. Cumming, *J. Med. Chem.*, 2008, **51**, 1617.
7. J. Deblander, S. Van Aeken, J. Jacobs, N. De Kimpe, and K. Abbaspour Tehrani, *Eur. J. Org. Chem.*, 2009, 4882.
8. Z. P. Tachrim, K. Oida, H. Ikemoto, F. Ohashi, N. Kurokawa, K. Hayashi, M. Shikanai, Y. Sakihama, Y. Hashidoko, and M. Hashimoto, *Molecules*, 2017, **22**, 1748; Z. P. Tachrim, K. Oida, F. Ohashi, H. Wakasa, H. Ikemoto, N. Kurokawa, Y. Sakihama, Y. Hashidoko, T. Suzuki, and M. Hashimoto, *Heterocycles*, 2018, **97**, 877; N. Kurokawa, Y. Tokoro, Z. P. Tachrim, H. Wakasa, Y. Sakihama, Y. Hashidoko, and M. Hashimoto, *ARKIVOC*, 2019, **v**, 42.
9. M. Hugener and H. Heimgartner, *Helv. Chim. Acta*, 1989, **72**, 172.
10. T. Hayakawa and K. Harada, *Bull. Chem. Soc. Jpn.*, 1965, **38**, 1354.