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Original article

Design and synthesis of new *N*-(fluorenyl-9-methoxycarbonyl) (Fmoc)-dipeptides as anti-inflammatory agents

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ABSTRACT

Twenty-four new dipeptide analogs (**1**–**24**) of aurantiamide acetate were designed, synthesized, and assayed for effects on superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB. Among them, seven *N*-(fluorenyl-9-methoxycarbonyl) (Fmoc)-dipeptides (**6**, **9**, **12**, **14**, **17**, **18** and **20**) showed potent inhibitory effects. Compounds **9** and **18** showed the most selective effects against human neutrophil elastase release, with IC_{50} values of 0.8 ± 0.1 and 1.7 ± 0.6 μM, respectively, and were 130-fold more potent than phenylmethylsulfonyl fluoride (PMSF), the positive control, in this anti-inflammatory assay. These two compounds could be developed as new lead anti-inflammatory agents.

1. Introduction

Human neutrophils are active phagocytes that act as a crucial component of immunity [1]. They play important roles in the host defense against microorganisms and in the pathogenesis of various diseases, such as rheumatoid arthritis [1–3], ischemia-reperfusion injury, chronic obstructive pulmonary disease (COPD), and asthma [4–6]. In response to diverse stimuli, activated neutrophils secrete a series of cytotoxins, including superoxide anion (O_2^-), which is a precursor of other reactive oxygen species (ROS), as well as granule protease and bioactive lipids [1,2,7]. Therefore, it is essential to control respiratory burst and degranulation in physiological conditions, while potentiating these functions in infected tissues and organs [1]. Only a few agents are now used to modulate pro-inflammatory responses of neutrophils; therefore, research and development of new generation anti-inflammatory agents continue.

In previous reports, aurantiamide acetate (Fig. 1), a dipeptide composed of *N*-benzoylphenylalanine and phenylalaninol acetate, was isolated from *Polygonum chinensis*, and showed inhibitory effects on O_2^- generation induced by formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) in human neutrophils [8]. Additionally, its analog aurantiamide (Fig. 1), a major component of *Zanthoxylum dissitum* and *Aspergillus penicilloides* [9,10], exhibited anti-bacterial [11,12], anti-inflammatory [10], antioxidant [13], and anti-HIV effects [9].

Other research has shown that several *N*-Fmoc amino acids exert anti-inflammatory activity by recruitment of neutrophils into the inflammatory site [14,15]. In addition, these compounds also increased intracellular Ca^{2+} concentrations in Madin Darby canine kidney (MDCK) renal tubular cells [16].

Therefore, we evaluated eight L-Fmoc amino acids for inhibitory effects on superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB. The results revealed that most of the tested L-configured *N*-Fmoc aromatic amino acids were equipotent in both assays and exhibited non-selective inhibitory activities (Table 1).

Accordingly, we designed and synthesized a series of aurantiamide acetate analogs in an attempt to obtain new anti-inflammatory agents. As shown in Fig. 2, the points of synthetic modification included (1) the *N*-terminal substitution, (2) the amino acid residue (part A); and (3) the configuration (*S*- or *R*-) of phenylalaninol. All newly synthesized analogs were assayed for effects on superoxide anion generation and elastase release by fMLP-activated human neutrophils. Herein the synthesis, bioactivity, and anti-inflammatory structure–activity relationships are described.

2. Chemistry

The synthetic routes to the compounds are shown in Schemes 1–3. The compounds were prepared by liquid-phase peptide synthesis via a previously described pathway (Scheme 1). In this

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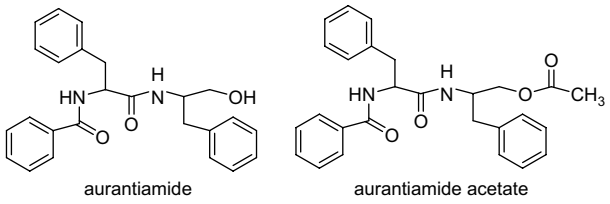


Fig. 1. Structures of aurantiamide and aurantiamide acetate.

Table 1
Inhibitory effects of Fmoc amino acids on superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB.

Compound	Superoxide anion	Elastase
	IC_{50} (μM)[a] or (Inh %)	IC_{50} (μM)[a] or (Inh %)
Fmoc-L-phenylalanine	14.1 ± 3.3	14.2 ± 1.3
Fmoc-L-*O*-*t*-butyltyrosine	5.5 ± 0.7	5.6 ± 0.9
Fmoc-L-tyrosine	2.4 ± 0.3***	2.9 ± 0.1***
Fmoc-L-trityl-histidine	0.5 ± 0.0	0.6 ± 0.0
Fmoc-L-tryptophan	3.3 ± 0.3	(45.4 ± 1.7)**
Fmoc-L-naphthalanine	1.2 ± 1.4	2.1 ± 0.2
Fmoc-L-valine	(29.4 ± 2.8)***	(14.5 ± 1.7)**
Fmoc-L-isoleucine	12.8 ± 0.8	(39.8 ± 5.4)**
DPI[b]	0.7 ± 0.4	
PMSF[b]		130.9 ± 29.1

Percentage of inhibition (Inh %) at 10 μg/mL. Results are presented as mean ± S.E.M. ($n = 3$–4). $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the control value.
[a] Concentration necessary for 50% inhibition (IC_{50}).
[b] DPI and PMSF were used as positive controls.

method, *S*- or *R*-phenylalaninol reacted with an *N*-protected amino acid in the presence of coupling agents *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate (HBTU) and diisopropylethylamine (DIEA) in CH_2Cl_2 to give an intermediate protected dipeptide alcohol. The synthetic acetate analogs were prepared from this intermediate by esterification, followed by condensation with acetic anhydride in pyridine to obtain the desired products as shown in Scheme 1. For **9** and **10**, the *tert*-butyl protecting group was removed by treatment with TFA/TIS/H_2O (95/2.5/2.5) to give **11** and **12** (Scheme 2). Moreover, the 9-fluorenylmethoxycarbonyl

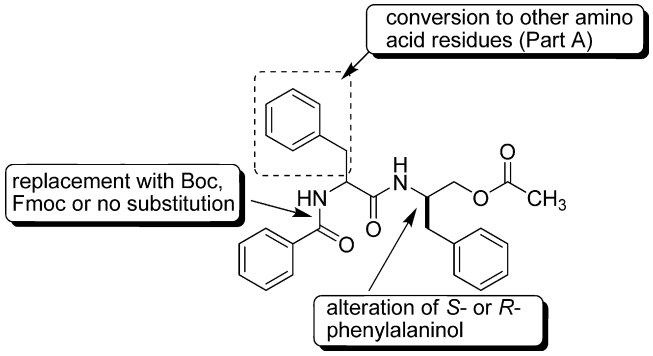


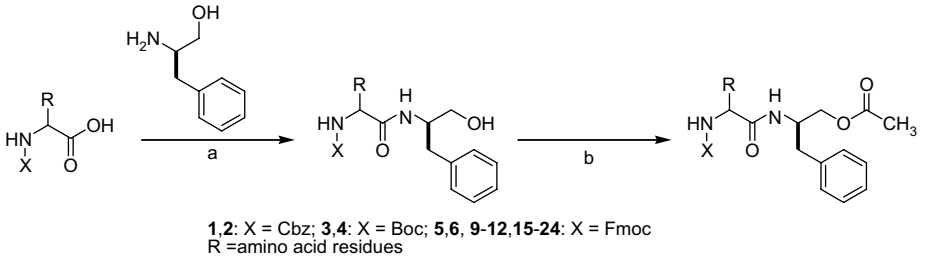
Fig. 2. Design of aurantiamide acetate analogs.

(Fmoc) group of **5**, **6**, **11** and **12** was removed by treatment with 20% piperidine in CH_2Cl_2 to afford **7**, **8**, **13** and **14**, respectively (Scheme 3). All compounds (**1**–**24**, Table 2) were >99% pure and were characterized by spectroscopic data, as shown in Section 5.

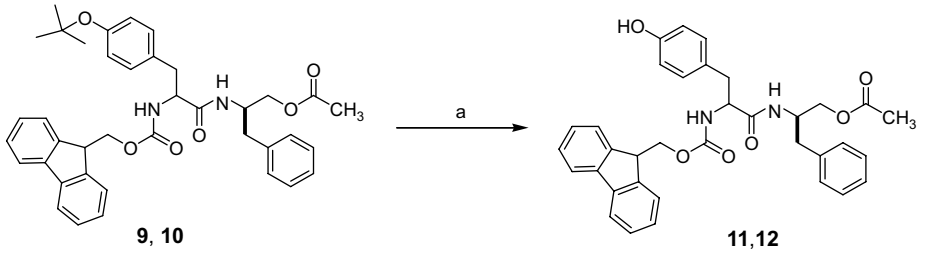
3. Results and discussion

Compounds **1**–**24** were evaluated for anti-inflammatory activity based on effects against superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB. These assay systems were performed using established protocols [14], and are widely used to identify potential anti-inflammatory compounds. Table 3 lists the results for the test compounds, as well as diphenyleneiodonium (DPI) and phenylmethylsulfonyl fluoride (PMSF), included as positive controls for superoxide anion generation and elastase release, respectively.

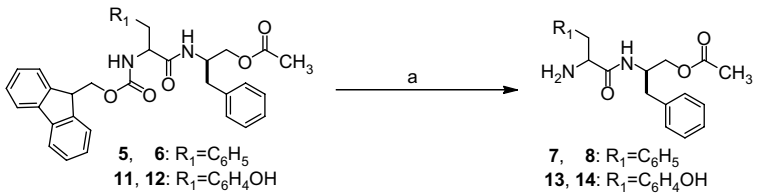
Among phenylalanine–phenylalaninol analogs **1**–**8**, compounds **1**, **2**, **3** and **6** showed 39.3%, 40.9%, 42.1% and 31.3% inhibition, respectively, in the superoxide anion assay at a concentration of 10 μg/mL, while only compound **6** inhibited fMLP-induced elastase release (IC_{50} 12.9 ± 3.7 μM). The results indicated that replacement of benzyloxycarbonyl (Cbz, **1** and **2**) by Fmoc (**5** and **6**) on the N-terminus increased inhibitory effects on elastase release, but slightly decreased inhibition of superoxide anion generation. Furthermore, when the N-terminal substitution was *t*-Boc (**3** and **4**)



Scheme 1. Reagents: (a) HBTU: *O*-benzotriazole-*N*,*N*,*N*′,*N*′′-tetramethyl-uronium-hexafluoro-phosphate, DIEA: diisopropylethylamine, CH_2Cl_2, 6 h, room temperature. (b) Acetic anhydride/pyridine, 1 h, room temperature.



Scheme 2. Reagents: (a) TFA/TIS/H_20 = 95/2.5/2.5, 0.5 h, room temperature.

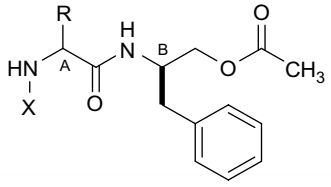


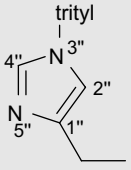
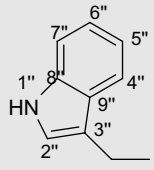
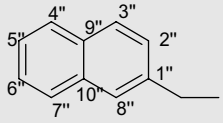
Scheme 3. Reagents: (a) 20% piperidine/CH_2Cl_2, 1 h, room temperature.

or hydrogen (**7** and **8**), the effects on neutrophil elastase release also decreased compared to **6**, while effects on superoxide anion generation were not significant, except for **3**. Therefore, an Fmoc group at the N-terminus appears important for inhibiting neutrophil elastase release induced by fMLP.

In a continuing search for more potent analogs, we evaluated the anti-inflammatory effects of *N*-Fmoc synthetic dipeptides containing various amino acids other than phenylalanine.

Table 2
Aurantiamide acetate derivatives.



Compound	R	X	Configuration	
			A	B
1	$CH_2C_6H_5$	Cbz	*S*	*S*
2			*S*	*R*
3	$CH_2C_6H_5$	*t*-Boc	*S*	*S*
4			*S*	*R*
5	$CH_2C_6H_5$	Fmoc	*S*	*S*
6			*S*	*R*
7	$CH_2C_6H_5$	H	*S*	*S*
8			*S*	*R*
9	$CH_2C_6H_4OC(CH_3)_3$	Fmoc	*S*	*S*
10			*S*	*R*
11	$CH_2C_6H_4OH$	Fmoc	*S*	*S*
12			*S*	*R*
13	$CH_2C_6H_4OH$	H	*S*	*S*
14			*S*	*R*
15		Fmoc	*S*	*S*
16			*S*	*R*
17		Fmoc	*S*	*S*
18			*S*	*R*
19		Fmoc	*S*	*S*
20			*S*	*R*
21	isopropyl	Fmoc	*S*	*S*
22			*S*	*R*
23	*sec*-butyl	Fmoc	*S*	*S*
24			*S*	*R*

Among the sixteen Fmoc dipeptides (**5**, **6**, **9**–**12**, and **15**–**24**), none showed significant activity against superoxide anion generation, except for **6**, which showed a slight inhibitory effect (31.3% inhibition at 10 μg/mL). However, dipeptides **6**, **9**, **12**, **17**, **18** and **20** did show selective inhibition of human neutrophil elastase release. Analogs **9** (Fmoc *O*-*t*-butyltyrosine) and **18** (Fmoc tryptophan) demonstrated the highest potency, with IC_{50} values of 0.8 and 1.7 μM, respectively. Compounds **12** (Fmoc tyrosine) and **14** (tyrosine) were less potent (IC_{50} 6.5 and 3.7 μM, respectively) than **9**, but slightly more potent than **6** (Fmoc phenylalanine) against elastase release. These findings suggest that anti-inflammatory activity in the analogs is increased by addition of an OH or *O*-*t*-butyl substituent at the *para*-position of the phenyl ring.

Furthermore, comparison of the anti-inflammatory data of *N*-Fmoc amino acids and *N*-Fmoc dipeptides (**5**, **6**, **9**–**12**, and **15**–**24**) (Tables 1 and 3, respectively) indicated that introduction of an L- or D-phenylalaninol acetate residue in the latter compounds abolished inhibition of superoxide anion generation. Therefore, even though the *N*-Fmoc amino acids were also generally more potent against human neutrophil elastase release than the corresponding *N*-Fmoc

Table 3
Inhibitory effects of dipeptides on superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB.

Compound	Superoxide anion	Elastase
	IC_{50} (μM)[a] or (Inh %)	IC_{50} (μM)[a] or (Inh %)
1	(39.3 ± 4.1)***	(27.2 ± 6.4)*
2	(40.9 ± 2.9)***	(35.4 ± 4.1)***
3	(42.1 ± 7.9)**	(18.9 ± 5.5)*
4	(4.7 ± 1.5)*	(11.0 ± 1.8)**
5	(2.8 ± 5.2)	(39.5 ± 6.2)**
6	(31.3 ± 6.4)**	12.9 ± 3.7***
7	>20.0[b]*	>20.0[c]***
8	>20.0	>20.0
9	(25.9 ± 5.5)*	0.8 ± 0.1***
10	(22.3 ± 2.5)*	(31.9 ± 5.6)**
11	(-0.5 ± 6.3)	(22.8 ± 5.8)**
12	(8.0 ± 2.0)*	6.5 ± 2.0**
13	(-0.5 ± 6.3)	(22.8 ± 5.8)**
14	(8.0 ± 2.0)*	3.7 ± 1.2**
15	(9.5 ± 2.0)**	(32.0 ± 2.7)***
16	(6.6 ± 1.3)**	(-2.9 ± 3.2)
17	(24.5 ± 0.6)***	5.5 ± 0.6**
18	(18.6 ± 6.6)*	1.7 ± 0.6*
19	(5.0 ± 2.4)	(37.8 ± 6.1)**
20	(2.1 ± 2.8)	14.5 ± 0.6***
21	(-6.3 ± 0.9)**	(27.7 ± 6.5)
22	(-2.3 ± 1.3)	(34.6 ± 1.8)***
23	(2.2 ± 2.3)	(25.8 ± 5.1)**
24	(6.0 ± 4.0)	(22.5 ± 1.1)***
DPI[d]	0.7 ± 0.4	
PMSF[d]		130.9 ± 29.1

Percentage of inhibition (Inh %) at 10 μg/mL. Results are presented as mean ± S.E.M. ($n = 3$–4). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the control value.

[a] Concentration necessary for 50% inhibition (IC_{50}).

[b] These compounds elicited superoxide anion generation by human neutrophils in the absence of CB/fMLP.

[c] These compounds elicited superoxide anion generation by human neutrophils in the presence of CB.

[d] DPI and PMSF were used as positive controls.

dipeptides (except for **9**, **17**, and **18**), the inhibitory selectivity of the dipeptides for elastase release increased dramatically.

Dipeptides with both *S*- or *R*-configured phenylalaninol residues were synthesized and evaluated in the anti-inflammatory assays. Comparison of data for paired dipeptides (Table 3) showed that configuration had no general effect on superoxide anion generation. The inhibitory effects on elastase release were in the order **9** (***S***) ≒ **17** (***S***) ≒ **18** (***R***) > **12** (***R***) > **6** (***R***) > **20** (***R***), suggesting that there was no preference for either the *S*- or *R*-configuration on the anti-inflammatory activity.

4. Conclusion

Several novel aurantiamide acetate analogs of Fmoc and *t*-Boc substituted dipeptides were synthesized, and several representatives of this series were identified as highly potent anti-inflammatory agents. Our studies revealed that Fmoc substitution is important for strong inhibition of elastase release in human neutrophils. Our results also showed that synthetic dipeptides with aromatic amino acids (**9**–**12** and **17**–**20**) were more effective than those with aliphatic amino acids (**21**–**24**). In addition, Fmoc-L-tryptophan-phenylalaninol dipeptides **17** and **18** possessed enhanced and selective inhibitory effects against elastase release compared with the original Fmoc-L-tryptophan amino acid. Notably, dipeptide **9** was efficacious in the elastase assay at the nanomolar level. Furthermore, **9** and **18** were 130-fold more potent than PMSF as a positive control for elastase release. Thus, these two compounds could be developed as new lead anti-inflammatory agents.

5. Experimental

5.1. Materials and methods

Unless stated otherwise, the chemicals were acquired from commercial sources and used without further purification. All amino acids were purchased from ACROS, Sigma and Aldrich. Compound purity was checked by thin layer chromatography (TLC) on precoated silica gel plates (Merck, Kieselgel 60 F_{254}, layer thickness 0.25 mm). Melting points were determined on a Melt-Temp II apparatus, optical rotations were measured with JASCO P-1020 digital polarimeter, UV spectra were obtained on a Hitachi 200-20 spectrophotometer in CH_3CN, and CD spectra were measured on a JASCO J-810 spectrometer in CH_3CN. NMR spectra, which contained 1D (^{1}H, ^{13}C, DEPT) and 2D (COSY, TOCSY, HSQC, HMBC and NOESY), were performed in 400 and 100 MHz for ^{1}H and ^{13}C, respectively. ^{1}H NMR: C_5D_5N as solvent, $\delta = 7.21$, 7.59, 8.72 ppm and ^{13}C NMR: C_5D_5N as solvent, $\delta = 123.5$, 135.3, 149.8 ppm. Low-resolution ESI-MS spectra were obtained on an API 3000™ (Applied Biosystems) in positive or negative mode (solvent: CH_3OH), high-resolution ESI-MS spectra on a Bruker Daltonics APEX II 30e spectrometer in positive or negative mode (solvent: CH_3OH).

5.1.1. General procedure for synthesis of compounds ***1*–*6*, *9*, *10* and *15*–*24***

To a solution of L-amino acid (1.0 mmol) with L- or D-phenylalaninol (1.0 mmol) in CH_2Cl_2 (10 mL), respectively, were added successively coupling agents HBTU (1.5 mmol) and DIEA (1.5 mmol). The reaction mixture was stirred for 6 h at room temperature, concentrated, esterified with acetic anhydride (150 μL/1 mmol) in pyridine (1 mL) and purified by column chromatography (Si-Gel) using $CHCl_3$ to afford the products.

*5.1.1.1. (S)-2-((S)-2-Benzamido-3-phenylpropanamido)-3-phenylpropyl acetate (**1**).* Yield: 85%, $[\alpha]_D = -26.1^\circ$ (*c* 0.1, CH_3OH). ^{1}H NMR (C_5D_5N, 400 MHz): $\delta = 2.00$ (3H, s, H-1a, b, c), 2.99 (2H, dd, $J = 2.8$, 2.8 Hz, H-10a, b), 3.32 (1H, dd, $J = 7.6$, 7.6 Hz, H-11a), 3.51 (1H, dd, $J = 7.6$, 7.6 Hz, H-11b), 4.25 (2H, dd, $J = 5.6$, 4.8 Hz, H-4a, b), 4.78–4.86 (1H, m, H-8), 5.49 (1H, q, $J = 15.6$ Hz, H-5), 7.11 (1H, t, $J = 3.8$ Hz, Ar CH), 7.18–7.31 (7H, m, Ar CH), 7.33–7.44 (7H, m, Ar CH), 9.28 (1H, d, $J = 8.4$ Hz, NH-6), 9.35 (1H, d, $J = 8.8$ Hz, NH-9). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 20.7$ (C-1), 37.5 (C-10), 38.8 (C-11), 50.2 (C-5), 55.7 (C-8), 65.4 (C-4), 126.7 (C-4′), 126.9(C-4″), 128.1, 128.6, 131.5 (Cbz Ar CH), 128.8 (C-2′, 6′, 2″, 6″), 129.8 (C-3′, 5′, 3″, 5″), 138.3 (C-1″), 138.4 (C-1′), 167.8 (Cbz CO), 170.7 (C-2), 172.1 (C-7).

*5.1.1.2. (R)-2-((S)-2-Benzamido-3-phenylpropanamido)-3-phenylpropyl acetate (**2**).* Yield: 89%, $[\alpha]_D = +30.3^\circ$ (*c* 0.1, CH_3OH). ^{1}H NMR (C_5D_5N, 400 MHz): $\delta = 1.90$ (3H, s, H-1a, b, c), 2.97 (2H, dd, $J = 2.8$, 2.8 Hz, H-10a, b), 3.28 (1H, dd, $J = 7.6$, 7.6 Hz, H-11a), 3.44 (1H, dd, $J = 6.8$, 6.4 Hz, H-11b), 4.26 (2H, dd, $J = 6.0$, 6.0 Hz, H-4a, b), 4.33 (1H, dd, $J = 4.8$, 4.4 Hz, H-8), 4.80–4.88 (1H, m, H-5), 5.48 (1H, q, $J = 15.6$ Hz, H-5), 7.12 (1H, t, $J = 3.6$ Hz, Ar CH), 7.18–7.27 (7H, m, Ar CH), 7.30–7.44 (7H, m, Ar CH), 9.26 (1H, d, $J = 8.4$ Hz, NH-6), 9.35 (1H, d, $J = 8.8$ Hz, NH-9). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 20.5$ (C-1), 37.7 (C-10), 38.8 (C-11), 50.1 (C-5), 55.7 (C-8), 65.3 (C-4), 126.9 (C-4′), 126.9(C-4″), 128.1, 128.8, 131.5 (Cbz Ar CH), 128.9 (C-2′, 6′, 2″, 6″), 129.8 (C-3′, 5′, 3″, 5″), 138.3 (C-1″), 138.5 (C-1′), 167.8 (Cbz CO), 170.6 (C-2), 172.1 (C-7).

*5.1.1.3. (S)-2-((S)-2-(*Tert*-butoxycarbonylamino)-3-phenylpropanamido)-3-phenylpropyl acetate (**3**).* Yield: 92%, $[\alpha]_D = -9.3^\circ$ (*c* 0.1, CH_3OH). ^{1}H NMR (C_5D_5N, 400 MHz): $\delta = 1.44$ (9H, s, *t*-Boc CH_3), 1.98 (3H, s, H-1), 2.97 (2H, t, $J = 8.2$ Hz, H-10a, b), 3.19 (1H, dd, $J = 7.6$, 7.6 Hz, H-11a), 3.44 (1H, dd, $J = 7.2$, 7.2 Hz, H-11b), 4.20 (2H, t, $J = 6.0$ Hz, H-4a, b), 4.74–4.79 (1H, m, H-8), 4.95 (1H, q, $J = 15.8$ Hz, H-5), 7.18–7.31 (10 H, m, Ar CH), 8.17 (1H, d, $J = 8.4$ Hz, NH-6), 9.07 (1H, d, $J = 8.8$ Hz, NH-9). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 20.7$ (C-1), 28.4 (*t*-Boc CH_3), 37.5 (C-10), 39.5 (C-11), 50.0 (C-5), 56.7 (C-8), 65.2 (C-4), 78.6 (*t*-Boc quat C), 126.8 (C-4′, 4″), 128.6 (C-2′, 6′), 128.8 (C-2″, 6″), 129.7 (C-3′, 5′), 129.9 (C-3″, 5″), 138.4 (C-1′, 1″), 156.3 (Cbz CO), 170.6 (C-2), 172.3 (C-7).

*5.1.1.4. (R)-2-((S)-2-(*Tert*-butoxycarbonylamino)-3-phenylpropanamido)-3-phenylpropyl acetate (**4**).* Yield: 90%, $[\alpha]_D = +28.0^\circ$ (*c* 0.1, CH_3OH). ^{1}H NMR (C_5D_5N, 400 MHz): $\delta = 1.40$ (9H, s, *t*-Boc CH_3), 1.92 (3H, s, H-1), 2.93 (2H, d, $J = 6.6$ Hz, H-10a, b), 3.16 (1H, dd, $J = 7.6$, 7.6 Hz, H-11a), 3.36 (1H, dd, $J = 6.8$, 6.8 Hz, H-11b), 4.25 (2H, dddd, $J = 6.0$, 6.0, 4.8, 4.8 Hz, H-4a, b), 4.79–4.84 (1H, m, H-8), 4.95 (1H, q, $J = 15.0$ Hz, H-5), 7.18–7.33 (10 H, m, Ar CH), 8.10 (1H, d, $J = 8.4$ Hz, NH-6), 9.03 (1H, d, $J = 8.0$ Hz, NH-9). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 20.5$ (C-1), 28.4 (*t*-Boc CH_3), 37.7 (C-10), 39.4 (C-11), 49.9 (C-5), 56.6 (C-8), 65.3 (C-4), 78.6 (*t*-Boc quat C), 126.8 (C-4′, 4″), 128.6 (C-2′, 6′), 128.9 (C-2″, 6″), 129.7 (C-3′, 5′), 129.9 (C-3″, 5″), 138.4 (C-1′), 138.5 (C-1″), 156.3 (Cbz CO), 170.6 (C-2), 172.3 (C-7).

*5.1.1.5. (S)-2-((S)-2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-3-phenylpropan-amido)-3-phenylpropyl acetate (**5**).* Yield: 84%, $[\alpha]_D = -9.0^\circ$ (*c* 0.1, CH_3OH). UV λ_{max}^{MeOH} nm (log ε): 300 (2.83), 288 (2.84), 264 (3.37), 211 (3.71). ^{1}H NMR (C_5D_5N, 400 MHz): $\delta = 1.99$ (3H, s, H-1a, b, c), 2.99 (2H, t, $J = 7.6$ Hz, H-10a, b), 3.24 (1H, dd, $J = 8.0$, 7.6 Hz, H-11a), 3.48 (1H, dd, $J = 7.6$, 7.6 Hz, H-11b), 4.23 (2H, t, $J = 4.4$ Hz, H-4a, b), 4.27 (1H, t, $J = 7.0$ Hz, Fmoc CH), 4.44 (1H, dd, $J = 7.2$, 7.2 Hz, Fmoc CH_2), 4.59 (1H, dd, $J = 7.2$, 7.2 Hz, Fmoc CH_2), 4.78–4.83 (1H, m, H-8), 5.04 (1H, q, $J = 15.6$ Hz, H-5), 7.15 (1H, t, $J = 7.4$ Hz, Ar CH), 7.22–7.32 (11H, m, Ar CH, Fmoc Ar CH), 7.41 (2H, t, $J = 7.4$ Hz, Fmoc Ar CH), 7.62 (2H, d, $J = 7.6$ Hz, Fmoc Ar CH), 7.85 (2H, d, $J = 7.2$ Hz, Fmoc Ar CH), 9.12 (1H, d, $J = 8.4$ Hz, NH-6), 9.22 (1H, d, $J = 8.4$ Hz, NH-9). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 20.7$ (C-1), 37.5 (C-10), 39.3 (C-11), 47.7 (Fmoc CH), 50.1 (C-5), 57.3 (C-8), 65.3 (C-4), 66.7 (Fmoc CH_2), 120.4, 125.6, 125.7, 127.5, 128.0 (Fmoc Ar CH), 126.7 (C-4′), 126.9 (C-4″), 128.7 (C-2′, 6′), 128.8 (C-2″, 6″), 129.7 (C-3′, 5′), 129.8 (C-3″, 5″), 138.4 (C-1′, 1″), 141.6, 144.6 (Fmoc Ar quat

C), 157.0 (Fmoc CO), 170.6 (C-2), 172.2 (C-7). HRESI-MS *m/z* 585.2520 $[M+Na]^+$ (calcd for $C_{35}H_{34}N_2O_5Na$ 585.2522).

*5.1.1.6. (R)-2-((S)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-phenylpropan-amido)-3-phenylpropyl acetate (**6**).* Yield: 86%, $[\alpha]_D = +17.5^\circ$ (*c* 0.1, CH_3OH). UV λ_{max}^{MeOH} nm (log ε): 300 (2.83), 288 (2.84), 264 (3.37), 211 (3.71). ^1H NMR (C_5D_5N, 400 MHz): $\delta = 1.89$ (3H, s, H-1a, b, c), 2.95 (2H, dd, $J = 2.4$, 2.4 Hz, H-10a, b), 3.20 (1H, dd, $J = 8.0$, 8.0 Hz, H-11a), 3.39 (1H, dd, $J = 6.4$, 6.4 Hz, H-11b), 4.23–4.27 (2H, m, H-4a, b), 4.31 (1H, dd, $J = 4.4$, 4.4 Hz, Fmoc CH), 4.39 (1H, dd, $J = 7.2$, 7.2 Hz, Fmoc CH_2), 4.57 (1H, dd, $J = 7.2$, 6.8 Hz, Fmoc CH_2), 4.81–4.89 (1H, m, H-8), 5.04 (1H, q, $J = 15.6$ Hz, H-5), 7.22–7.32 (12H, m, Ar CH, Fmoc Ar CH), 7.40 (2H, t, $J = 7.4$ Hz, Fmoc Ar CH), 7.60 (2H, d, $J = 7.6$ Hz, Fmoc Ar CH), 7.84 (2H, d, $J = 7.6$ Hz, Fmoc Ar CH), 9.07 (1H, d, $J = 8.4$ Hz, NH-6), 9.17 (1H, d, $J = 8.4$ Hz, NH-9). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 20.5$ (C-1), 37.7 (C-10), 39.2 (C-11), 47.7 (Fmoc CH), 50.0 (C-5), 57.3 (C-8), 65.3 (C-4), 66.6 (Fmoc CH_2), 120.4, 125.6, 125.7, 127.5, 128.0 (Fmoc Ar CH), 126.9 (C-4′, 4″), 128.7 (C-2′, 6′), 128.9 (C-2″, 6″), 129.7 (C-3′, 5′), 129.9 (C-3″, 5″), 138.4 (C-1′), 138.5 (C-1″), 141.6, 144.6 (Fmoc Ar quat C), 157.0 (Fmoc CO), 170.6 (C-2), 172.2 (C-7). HRESI-MS *m/z* 585.2522 $[M+Na]^+$ (calcd for $C_{35}H_{34}N_2O_5Na$ 585.2522).

*5.1.1.7. (S)-2-((S)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-(4-tert-butoxy-phenyl)propanamido)-3-phenylpropyl acetate (**9**).* Yield: 90%, $[\alpha]_D = -35.8^\circ$ (*c* 0.1, CH_3OH). UV λ_{max}^{MeOH} nm (log ε): 299 (3.81), 289 (3.78), 264 (4.38), 214 (4.56). ^1H NMR (C_5D_5N, 400 MHz): $\delta = 1.25$ (9H, s, *t*-butyl CH_3), 2.01 (3H, s, H-1a, b, c), 3.00 (2H, t, $J = 6.6$ Hz, H-10a, b), 3.23 (1H, dd, $J = 7.6$, 7.6 Hz, H-11a), 3.48 (1H, dd, $J = 6.8$, 6.8 Hz, H-11b), 4.22–4.29 (3H, br m, H-4a, b, Fmoc CH), 4.44 (1H, dd, $J = 7.6$, 7.2 Hz, Fmoc CH_2), 4.59 (1H, dd, $J = 7.2$, 7.2 Hz, Fmoc CH_2), 4.80–4.86 (1H, m, H-8), 5.02 (1H, q, $J = 15.8$ Hz, H-5), 7.01 (2H, d, $J = 8.0$ Hz, Ar CH), 7.15 (1H, t, $J = 7.2$ Hz, Ar CH), 7.26 (4H, dd, $J = 3.2$, 3.2 Hz, Ar CH), 7.33 (2H, t, $J = 7.8$ Hz, Fmoc Ar CH), 7.41 (2H, t, $J = 7.4$ Hz, Fmoc Ar CH), 7.64 (2H, d, $J = 7.6$ Hz, Fmoc Ar CH), 7.85 (2H, d, $J = 7.6$ Hz, Fmoc Ar CH), 9.09 (1H, d, $J = 8.8$ Hz, NH-6), 9.24 (1H, d, $J = 8.0$ Hz, NH-9). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 20.7$ (C-1), 28.8 (*t*-butyl CH_3), 37.5 (C-10), 38.5 (C-11), 47.7 (Fmoc CH), 50.1 (C-5), 57.4 (C-8), 65.2 (C-4), 66.7 (Fmoc CH_2), 78.0 (*t*-butyl quat C), 120.4, 125.6, 125.7, 127.5, 128.0 (Fmoc Ar CH), 124.3 (C-2″, 6″), 126.7 (C-4′), 128.8 (C-2′, 6′), 129.7 (C-3′, 5′), 130.3 (C-3″, 5″), 133.0 (C-1″), 138.4 (C-1′), 141.6, 144.6 (Fmoc Ar quat C), 154.6 (C-4″), 157.0 (Fmoc CO), 170.7 (C-2), 172.3 (C-7). HRESI-MS *m/z* 657.2936 $[M+Na]^+$ (calcd for $C_{39}H_{42}N_2O_6Na$ 657.2940).

*5.1.1.8. (R)-2-((S)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-(4-tert-butoxy-phenyl)propanamido)-3-phenylpropyl acetate (**10**).* Yield: 93%, $[\alpha]_D = +15.5^\circ$ (*c* 0.1, CH_3OH). UV λ_{max}^{MeOH} nm (log ε): 299 (3.81), 289 (3.78), 264 (4.38), 214 (4.56). ^1H NMR (C_5D_5N, 400 MHz): $\delta = 1.24$ (9H, s, *t*-butyl CH_3), 1.89 (3H, s, H-1a, b, c), 2.96 (2H, dd, $J = 7.2$, 7.2 Hz, H-10a, b), 3.19 (1H, dd, $J = 8.0$, 8.0 Hz, H-11a), 3.39 (1H, dd, $J = 6.8$, 6.8 Hz, H-11b), 4.23–4.31 (3H, m, H-4a, b, Fmoc CH), 4.40 (1H, dd, $J = 7.2$, 7.2 Hz, Fmoc CH_2), 4.57 (1H, dd, $J = 7.2$, 6.8 Hz, Fmoc CH_2), 4.84–4.88 (1H, m, H-8), 5.02 (1H, q, $J = 15.8$ Hz, H-5), 7.04 (2H, d, $J = 8.4$ Hz, Ar CH), 7.25 (4H, dd, $J = 8.0$, 7.6 Hz, Ar CH), 7.33 (3H, d, $J = 4.4$ Hz, Ar CH, Fmoc Ar CH), 7.40 (2H, t, $J = 7.6$ Hz, Fmoc Ar CH), 7.62 (2H, d, $J = 7.6$ Hz, Fmoc Ar CH), 7.84 (2H, d, $J = 7.6$ Hz, Fmoc Ar CH), 9.08 (1H, d, $J = 8.4$ Hz, NH-6), 9.20 (1H, d, $J = 8.0$ Hz, NH-9). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 20.5$ (C-1), 28.8 (*t*-butyl CH_3), 37.8 (C-10), 38.7 (C-11), 47.7 (Fmoc CH), 50.0 (C-5), 57.4 (C-8), 65.3 (C-4), 66.6 (Fmoc CH_2), 78.0 (*t*-butyl quat C), 120.4, 125.6, 125.7, 127.5, 128.0 (Fmoc Ar CH), 124.3 (C-2″, 6″), 126.8 (C-4′), 128.9 (C-2′, 6′), 129.7 (C-3′, 5′), 130.4 (C-3″, 5″), 133.0 (C-1″), 138.4 (C-1′), 141.6, 144.6 (Fmoc Ar quat C), 154.6 (C-4″), 157.0 (Fmoc CO), 170.7 (C-2), 172.3 (C-7). HRESI-MS *m/z* 657.2936 $[M+Na]^+$ (calcd for $C_{39}H_{42}N_2O_6Na$ 657.2940).

*5.1.1.9. (S)-2-((S)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-(1-trityl-1H-imidazol-4-yl)propanamido)-3-phenylpropyl acetate (**15**).* Yield: 88%, $[\alpha]_D = -3.4^\circ$ (*c* 0.1, CH_3OH). UV λ_{max}^{MeOH} nm (log ε): 299 (3.62), 289 (3.63), 265 (br, 4.17), 255 (4.28), 246 (4.12), 214 (4.53). ^1H NMR (C_5D_5N, 400 MHz): $\delta = 1.93$ (3H, s, H-1a, b, c), 3.05 (2H, dddd, $J = 7.6$, 7.6, 7.2, 6.8 Hz, H-10a, b), 3.37 (1H, dd, $J = 8.4$, 8.4 Hz, H-11a), 3.52 (1H, dd, $J = 5.2$, 4.8 Hz, H-11b), 4.27 (2H, dd, $J = 6.8$, 6.0 Hz, H-4a, b), 4.35 (1H, dd, $J = 4.8$, 4.4 Hz, Fmoc CH), 4.42 (1H, dd, $J = 7.6$, 7.2 Hz, Fmoc CH_2), 4.50 (1H, dd, $J = 7.6$, 7.6 Hz, Fmoc CH_2), 4.81–4.87 (1H, m, H-8), 5.19–5.24 (1H, m, H-5), 6.93 (1H, s, histidine CH), 7.15 (1H, t, $J = 7.2$ Hz, Ar CH), 7.22–7.29 (19H, m, Ar CH, trityl CH), 7.34 (1H, s, histidine CH), 7.39 (2H, t, $J = 7.6$ Hz, Fmoc Ar CH), 7.65 (2H, d, $J = 7.6$ Hz, Fmoc Ar CH), 7.84 (2H, d, $J = 7.6$ Hz, Fmoc Ar CH), 8.87 (1H, d, $J = 8.0$ Hz, NH-6), 9.12 (1H, d, $J = 8.0$ Hz, NH-9). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 20.6$ (C-1), 32.2 (C-11), 37.7 (C-10), 47.7 (Fmoc CH), 50.2 (C-5), 56.2 (C-8), 65.1 (C-4), 66.9 (Fmoc CH_2), 75.4 (Trityl quat C), 119.8, 129.8 (histidine CH), 120.4, 125.8, 127.5, 128.0 (Fmoc Ar CH), 126.8 (C-4′), 128.2 (C-2′, 6′), 128.4, 130.1, 138.4, 138.8, 143.1 (trityl Ar CH), 128.8 (C-3′, 5′), 138.3 (C-1′), 141.6, 144.6 (Fmoc Ar quat C), 157.1 (Fmoc CO), 170.7 (C-2), 172.5 (C-7). HRESI-MS *m/z* 817.6645 $[M+Na]^+$ (calcd for $C_{51}H_{46}N_4O_5Na$ 817.6650).

*5.1.1.10. (R)-2-((S)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-(1-trityl-1H-imidazol-4-yl)propanamido)-3-phenylpropyl acetate (**16**).* Yield: 88%, $[\alpha]_D = +1.3^\circ$ (*c* 0.1, CH_3OH). UV λ_{max}^{MeOH} nm (log ε): 299 (3.62), 289 (3.63), 265 (br, 4.17), 255 (4.28), 246 (4.12), 214 (4.53). ^1H NMR (C_5D_5N, 400 MHz): $\delta = 1.94$ (3H, s, H-1a, b, c), 3.02 (2H, d, $J = 7.2$ Hz, H-10a, b), 3.24 (1H, dd, $J = 8.2$, 7.6 Hz, H-11a), 3.42 (1H, dd, $J = 5.2$, 4.8 Hz, H-11b), 4.28 (2H, dd, $J = 7.0$, 7.0 Hz, H-4a, b), 4.36 (1H, dd, $J = 4.8$, 4.4 Hz, Fmoc CH), 4.44 (1H, dd, $J = 7.6$, 7.2 Hz, Fmoc CH_2), 4.48 (1H, dd, $J = 7.6$, 7.6 Hz, Fmoc CH_2), 4.82–4.88 (1H, m, H-8), 5.22 (1H, q, $J = 15.8$ Hz, H-5), 6.77 (1H, s, histidine CH), 7.11 (1H, t, $J = 7.2$ Hz, Ar CH), 7.24–7.42 (22H, m, Ar CH, Trityl CH, histidine CH, Fmoc Ar CH), 7.64 (2H, d, $J = 7.6$ Hz, Fmoc Ar CH), 7.83 (2H, d, $J = 7.6$ Hz, Fmoc Ar CH), 8.75 (1H, d, $J = 8.0$ Hz, NH-6), 9.12 (1H, d, $J = 8.0$ Hz, NH-9). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 20.1$ (C-1), 31.6 (C-11), 37.4 (C-10), 47.2 (Fmoc CH), 49.7 (C-5), 55.6 (C-8), 65.1 (C-4), 66.5 (Fmoc CH_2), 75.0 (trityl quat C), 119.3, 129.2 (histidine CH), 119.8, 125.3, 126.2, 128.3 (Fmoc Ar CH), 127.0 (C-4′), 128.2 (C-2′, 6′), 128.4, 130.1, 138.4, 138.8, 143.1 (trityl Ar CH), 128.8 (C-3′, 5′), 138.3 (C-1′), 141.6, 144.6 (Fmoc Ar quat C), 157.1 (Fmoc CO), 170.1 (C-2), 171.8 (C-7). HRESI-MS *m/z* 817.6652 $[M+Na]^+$ (calcd for $C_{51}H_{46}N_4O_5Na$ 817.6650).

*5.1.1.11. (S)-2-((S)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-(1H-indol-3-yl)propanamido)-3-phenylpropyl acetate (**17**).* Yield: 90%, $[\alpha]_D = -1.2^\circ$ (*c* 0.1, CH_3OH). UV λ_{max}^{MeOH} nm (log ε): 264 (4.27), 209 (4.86). ^1H NMR (C_5D_5N, 400 MHz): $\delta = 1.93$ (3H, s, H-1a, b, c), 2.95 (2H, dddd, $J = 7.6$, 7.2, 6.8, 6.8 Hz, H-10a, b), 3.60 (1H, dd, $J = 7.2$, 6.8 Hz, H-11a), 3.77 (1H, dd, $J = 7.6$, 7.6 Hz, H-11b), 4.20 (2H, d, $J = 4.8$ Hz, H-4a, b), 4.29 (1H, t, $J = 7.2$ Hz, Fmoc CH), 4.47 (1H, dd, $J = 7.2$, 7.2 Hz, Fmoc CH_2), 4.60 (1H, dd, $J = 7.2$, 7.2 Hz, Fmoc CH_2), 4.80–4.85 (1H, m, H-8), 5.20 (1H, q, $J = 15.2$ Hz, H-5), 7.13 (2H, q, $J = 16.0$ Hz, Trp Ar CH), 7.25 (5H, d, $J = 7.2$, Ar CH), 7.29 (2H, t, $J = 7.0$ Hz, Trp Ar CH), 7.39 (2H, t, $J = 7.6$ Hz, Fmoc Ar CH), 7.43 (1H, s, Trp CH), 7.54 (1H, d, $J = 8.4$ Hz, Fmoc Ar CH), 7.63 (2H, d, $J = 7.6$ Hz,), 7.85 (3H, t, $J = 8.0$ Hz, Fmoc Ar CH), 9.07 (1H, d, $J = 8.0$ Hz, NH-6), 9.22 (1H, d, $J = 8.4$ Hz, NH-9), 11.9 (1H, s, Trp NH). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 20.6$ (C-1), 29.4 (C-11), 37.5 (C-10), 47.7 (Fmoc CH), 50.1 (C-5), 56.9 (C-8), 65.2 (C-4), 66.7 (Fmoc CH_2), 111.3, 111.9, 114.2, 119.2, 121.7, 124.3, 128.6, 137.5 (Trp Ar CH), 120.4, 125.7, 125.8, 127.5, 128.0 (Fmoc Ar CH), 126.8 (C-4′), 128.8 (C-2′, 6′), 129.7 (C-3′, 5′), 138.5 (C-1′), 141.6, 144.6 (Fmoc Ar quat C), 157.1 (Fmoc CO), 170.7 (C-2), 172.5 (C-7). HRESI-MS *m/z* 624.2470 $[M+Na]^+$ (calcd for $C_{37}H_{325}N_3O_5Na$ 624.2474).

*5.1.1.12. (*R*)-2-((S)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-(1H-indol-3-yl)propanamido)-3-phenylpropyl acetate (**18**).* Yield: 90%, $[\alpha]_D = +9.9^\circ$ (*c* 0.1, CH_3OH). UV λ_{max}^{MeOH} nm (log ε): 264 (4.42), 209 (4.96). ^{1}H NMR (C_5D_5N, 400 Hz): $\delta = 1.88$ (3H, s, H-1a, b, c), 2.89 (2H, d, $J = 6.8$ Hz, H-10a, b), 3.55 (1H, dd, $J = 7.6$, 7.2 Hz, H-11a), 3.70 (1H, dd, $J = 6.8$, 6.8 Hz, H-11b), 4.22 (2H, d, $J = 4.8$ Hz, H-4a, b), 4.28 (1H, t, $J = 7.2$ Hz, Fmoc CH), 4.43 (1H, dd, $J = 7.2$, 7.2 Hz, Fmoc CH_2), 4.57 (1H, dd, $J = 7.2$, 7.2 Hz, Fmoc CH_2), 4.85–4.90 (1H, m, H-8), 5.18 (1H,q, $J = 15.6$ Hz, H-5), 7.17 (2H, q, $J = 13.2$ Hz, Trp Ar CH), 7.22-7.38 (8H, m, Ar CH, Trp Ar CH), 7.40 (2H, t, $J = 7.6$ Hz, Fmoc Ar CH), 7.41 (1H, s, Trp CH), 7.55 (1H, d, $J = 8.0$ Hz, Fmoc Ar CH), 7.61 (2H, d, $J = 7.6$ Hz,), 7.83 (2H, d, $J = 7.6$ Hz, Fmoc Ar CH), 7.89 (1H, d, $J = 8.0$ Hz, Fmoc Ar CH), 9.06 (1H, d, $J = 8.4$ Hz, NH-6), 9.20 (1H, d, $J = 8.4$ Hz, NH-9), 11.9 (1H, s, Trp NH). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 20.5$ (C-1), 29.5 (C-11), 37.6 (C-10), 47.7 (Fmoc CH), 49.9 (C-5), 57.1 (C-8), 65.2 (C-4), 66.6 (Fmoc CH_2), 111.4, 112.0, 121.7, 124.0, 128.6, 135.8 (Trp Ar CH), 120.4, 125.7, 125.8, 127.5, 128.0 (Fmoc Ar CH), 126.8 (C-4′), 128.8 (C-2′, 6′), 129.7 (C-3′, 5′), 138.5 (C-1′), 141.6, 144.6 (Fmoc Ar quat C), 157.1 (Fmoc CO), 170.6 (C-2), 172.8 (C-7). HRESI-MS *m*/*z* 624.2471 $[M + Na]^+$ (calcd for $C_{37}H_{325}N_3O_5Na$ 624.2474).

*5.1.1.13. (S)-2-((S)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-(naphthalen-2-yl) propanamido)-3-phenylpropyl acetate (**19**).* Yield: 83%, $[\alpha]_D = -20.0^\circ$ (*c* 0.1, CH_3OH). UV λ_{max}^{MeOH} nm (log ε): 264 (3.38), 224 (5.11), 210 (5.04). ^{1}H NMR (C_5D_5N, 400 MHz): $\delta = 1.82$ (3H, s, H-1a, b, c), 2.98 (2H, dddd, $J = 7.2$, 7.2, 7.2, 6.8 Hz, H-10a, b), 3.39 (1H, dd, $J = 7.6$, 7.6 Hz, H-11a), 3.63 (1H, dd, $J = 8.0$, 7.6 Hz, H-11b), 4.22 (2H, d, $J = 5.2$, H-4a, b), 4.26 (1H, t, $J = 7.0$ Hz, Fmoc CH), 4.45 (1H, dd, $J = 7.2$, 7.2 Hz, Fmoc CH_2), 4.58 (1H, dd, $J = 7.6$, 7.2 Hz, Fmoc CH_2), 4.78–4.82 (1H, m, H-8), 5.14 (1H, q, $J = 15.8$ Hz, H-5), 7.15 (2H, q, $J = 15.4$ Hz, Ar CH), 7.21–7.25 (4H, m, Ar CH), 7.29 (2H, d, $J = 7.2$ Hz, Fmoc Ar CH), 7.39 (2H, t, $J = 7.6$ Hz, Fmoc Ar CH), 7.46 (3H, m, naphthylalanine Ar CH), 7.60 (2H, d, $J = 7.6$ Hz, Fmoc Ar CH), 7.76 (3H, d, $J = 7.6$ Hz, naphthylalanine Ar CH), 7.84 (2H, d, $J = 7.2$ Hz, Fmoc Ar CH), 9.26 (1H, d, $J = 8.4$ Hz, NH-6), 9.33 (1H, d, $J = 8.4$ Hz, NH-9). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 20.5$ (C-1), 37.5 (C-10), 39.5 (C-11), 47.7 (Fmoc CH), 50.2 (C-5), 57.4 (C-8), 65.2 (C-4), 66.7 (Fmoc CH_2), 120.4, 125.5, 125.8, 127.4, 128.0 (Fmoc Ar CH), 126.3 (C-4′, 4″), 128.2 (C-2′, 6′), 128.4 (C-2″, 6″), 132.8 (C-3′, 5′), 134.0 (C-3″, 5″), 138.4 (C-1′, 1″), 141.6, 144.5 (Fmoc Ar quat C), 157.0 (Fmoc CO), 170.5 (C-2), 172.1 (C-7). HRESI-MS *m*/*z* 635.2519 $[M + Na]^+$ (calcd for $C_{39}H_{36}N_2O_5Na$ 635.2522).

*5.1.1.14. (*R*)-2-((S)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-(naphthalen-2-yl) propanamido)-3-phenylpropyl acetate (**20**).* Yield: 85%, $[\alpha]_D = +7.9^\circ$ (*c* 0.1, CH_3OH). UV λ_{max}^{MeOH} nm (log ε): 264 (3.22), 224 (5.04), 210 (4.86). ^{1}H NMR (C_5D_5N, 400 MHz): $\delta = 1.88$ (3H, s, H-1a, b, c), 2.92 (2H, dddd, $J = 7.2$, 7.2, 7.2, 6.8 Hz, H-10a, b), 3.36 (1H, dd, $J = 8.0$, 7.6 Hz, H-11a), 3.55 (1H, dd, $J = 6.8$, 6.8 Hz, H-11b), 4.24 (2H, m, H-4a, b), 4.30 (1H, dd, $J = 7.2$, 6.4 Hz, Fmoc CH), 4.39 (1H, dd, $J = 7.2$, 6.8 Hz, Fmoc CH_2), 4.55 (1H, dd, $J = 7.2$, 6.8 Hz, Fmoc CH_2), 4.83–4.88 (1H, m, H-8), 5.14 (1H, q, $J = 15.6$ Hz, H-5), 7.17–7.24 (8H, m, Fmoc Ar CH, Ar CH, naphthylalanine Ar CH), 7.38 (2H, t, $J = 7.6$ Hz, Fmoc Ar CH), 7.44 (3H, m, naphthylalanine Ar CH), 7.56 (2H, d, $J = 7.6$ Hz, Fmoc Ar CH), 7.76 (3H, m, naphthylalanine Ar CH), 7.82 (2H, d, $J = 7.2$ Hz, Fmoc Ar CH), 9.22 (1H, d, $J = 8.8$ Hz, NH-6), 9.26 (1H, d, $J = 8.0$ Hz, NH-9). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 20.5$ (C-1), 37.6 (C-10), 39.4 (C-11), 47.6 (Fmoc CH), 50.0 (C-5), 57.3 (C-8), 65.2 (C-4), 66.6 (Fmoc CH_2), 120.4, 125.5, 125.7, 127.4, 128.0 (Fmoc Ar CH), 126.8, 128.8, 129.7, 138.5 (Ar CH), 125.9, 126.4, 128.3, 128.5, 132.8, 134.0 (naphthylalanine Ar CH), 141.6, 144.6 (Fmoc Ar quat C), 157.0 (Fmoc CO), 170.6 (C-2), 172.3 (C-7). HRESI-MS *m*/*z* 635.2518 $[M + Na]^+$ (calcd for $C_{39}H_{36}N_2O_5Na$ 635.2522).

*5.1.1.15. (S)-2-((S)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-methylbutan-amido)-3-phenylpropyl acetate (**21**).* Yield: 92%, $[\alpha]_D = -5.9^\circ$ (*c* 0.1, CH_3OH). UV λ_{max}^{MeOH} nm (log ε): 263 (4.24), 208 (4.84). ^{1}H NMR (C_5D_5N, 400 MHz): $\delta = 1.07$ (6H, dd, $J = 3.6$, 3.2 Hz, H-12, 13), 2.03 (3H, s, H-1a, b, c), 2.33–2.41 (1H, m, H-11), 3.00 (2H, t, $J = 6.4$ Hz, H-10a, b), 4.32 (3H, dddd, $J = 7.2$, 6.8, 6.8, 6.8 Hz, H-8, Fmoc CH_2, Fmoc CH), 4.42 (1H, dd, $J = 4.4$, 4.4 Hz, H-4a), 4.55 (1H, ddd, $J = 4.0$, 2.8, 2.4 Hz, H-4b), 4.71 (1H, dd, $J = 7.2$, 7.2 Hz, Fmoc CH_2), 4.86–4.74 (1H, m, H-5), 7.22–7.29 (3H, m, Ar CH), 7.32 (2H, d, $J = 7.2$ Hz, Fmoc Ar CH), 7.40 (2H, ddd, $J = 4.0$, 3.6, 3.6 Hz, Ar CH), 7.69 (2H, dd, $J = 2.4$, 2.0 Hz, Fmoc Ar CH), 7.85 (2H, d, $J = 7.6$ Hz, Fmoc Ar CH), 8.89 (1H, d, $J = 9.2$ Hz, NH-6), 9.21 (1H, d, $J = 8.4$ Hz, NH-9). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 18.9$ (C-12), 19.6 (C-13), 20.7 (C-1), 31.5 (C-11), 37.8 (C-10), 47.8 (Fmoc CH), 49.9 (C-5), 61.7 (C-8), 65.3 (C-4), 66.6 (Fmoc CH_2), 120.4, 125.7, 127.5, 128.0 (Fmoc Ar CH), 126.8 (C-4′), 128.8 (C-2′, 6′), 129.7 (C-3′, 5′), 138.3 (C-1′), 141.7, 144.5, 144.7 (Fmoc Ar quat C), 157.4 (Fmoc CO), 170.7 (C-2), 172.4 (C-7). HRESI-MS *m*/*z* 537.2518 $[M + Na]^+$ (calcd for $C_{31}H_{34}N_2O_5Na$ 537.2522).

*5.1.1.16. (R)-2-((S)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-methylbutan-amido)-3-phenylpropyl acetate (**22**).* Yield: 94%, $[\alpha]_D = +13.3^\circ$ (*c* 0.1, CH_3OH). UV λ_{max}^{MeOH} nm (log ε): 263 (4.16), 208 (4.64). ^{1}H NMR (C_5D_5N, 400 MHz): $\delta = 0.95$ (6H, dd, $J = 6.8$, 6.8 Hz, H-12, 13), 1.91 (3H, s, H-1a, b, c), 2.24–2.32 (1H, m, H-11), 3.04 (2H, d, $J = 7.2$ Hz, H-10a, b), 4.30–4.37 (3H, m, H-8, Fmoc CH_2, Fmoc CH), 4.52 (2H, m, H-4a, b), 4.68 (1H, dd, $J = 7.6$, 7.6 Hz, Fmoc CH_2), 4.89–4.97 (1H, m, H-5), 7.23–7.27 (3H, m, Ar CH), 7.32 (2H, t, Fmoc Ar CH), 7.37–7.42 (4H, m, Fmoc Ar CH, Ar CH), 7.67 (2H, t, $J = 6.4$ Hz, Fmoc Ar CH), 7.84 (2H, d, $J = 7.6$ Hz, Fmoc Ar CH), 8.82 (1H, d, $J = 8.8$ Hz, NH-6), 9.12 (1H, d, $J = 8.4$ Hz, NH-9). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 18.5$ (C-12), 19.7 (C-13), 20.5 (C-1), 31.6 (C-11), 37.8 (C-10), 47.8 (Fmoc CH), 49.9 (C-5), 61.6 (C-8), 65.7 (C-4), 66.5 (Fmoc CH_2), 120.4, 125.6, 125.7, 127.5, 128.0 (Fmoc Ar CH), 126.8 (C-4′), 128.9 (C-2′, 6′), 129.7 (C-3′, 5′), 138.6 (C-1′), 141.6, 144.5, 144.7 (Fmoc Ar quat C), 157.4 (Fmoc CO), 170.6 (C-2), 172.4 (C-7). HRESI-MS *m*/*z* 537.2520 $[M + Na]^+$ (calcd for $C_{31}H_{34}N_2O_5Na$ 537.2522).

*5.1.1.17. (9H-Fluoren-9-yl)methyl (2S,3S)-1-((S)-1-hydroxy-3-phenylpropan-2-ylamino)-3-methyl-1-oxopentan-2-ylcarbamate (**23**).* Yield: 89%, $[\alpha]_D = -7.4^\circ$ (*c* 0.1, CH_3OH). UV λ_{max}^{MeOH} nm (log ε): 263 (3.59), 207 (4.22). ^{1}H NMR (C_5D_5N, 400 MHz): $\delta = 0.79$ (3H, t, $J = 7.4$ Hz, H-13a, b, c), 1.06 (3H, d, $J = 6.8$ Hz, H-14a, b, c), 1.31 (1H, quint, H-12a), 1.70–1.77 (1H, m, H-12b), 2.05 (3H, s, H-1a, b, c), 2.14–2.19 (1H, m, H-11), 3.01 (2H, t, $J = 6.6$ Hz, H-10a, b), 4.32 (2H, ddd, $J = 7.2$, 6.8, 6.8 Hz, Fmoc CH_2, Fmoc CH), 4.43 (1H, dd, $J = 4.4$, 4.4 Hz, H-8), 4.57 (2H, dd, $J = 9.2$, 8.0 Hz, H-4a, b), 4.72 (1H, dd, $J = 7.2$, 7.2 Hz, Fmoc CH_2), 4.88–4.92 (1H, m, H-5), 7.14 (1H, t, $J = 7.2$ Hz, Ar CH), 7.23–7.28 (4H, m, Ar CH), 7.33 (2H, d, $J = 7.2$ Hz, Fmoc Ar CH), 7.40 (2H, ddd, $J = 3.6$, 3.6, 3.6 Hz, Fmoc Ar CH), 7.69 (2H, dd, $J = 3.6$, 3.6 Hz, Fmoc Ar CH), 7.85 (2H, d, $J = 7.2$ Hz, Fmoc Ar CH), 8.90 (1H, d, $J = 9.2$ Hz, NH-6), 9.24 (1H, d, $J = 8.4$ Hz, NH-9). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 11.0$ (C-13), 15.8 (C-14), 20.7 (C-1), 25.3 (C-12), 37.3 (C-10), 37.8 (C-11), 47.8 (Fmoc CH), 49.9 (C-5), 60.4 (C-8), 65.3 (C-4), 66.6 (Fmoc CH_2), 120.4, 125.7, 127.5, 128.0 (Fmoc Ar CH), 126.8, 128.8, 129.7, 138.3 (Ar CH), 141.7, 144.5, 144.8 (Fmoc Ar quat C), 157.3 (Fmoc CO), 170.7 (C-2), 172.5 (C-7). HRESI-MS *m*/*z* 551.2520 $[M + Na]^+$ (calcd for $C_{32}H_{36}N_2O_5Na$ 551.2522).

*5.1.1.18. (9H-Fluoren-9-yl)methyl (2S,3S)-1-((R)-1-hydroxy-3-phenylpropan-2-ylamino)-3-methyl-1-oxopentan-2-ylcarbamate (**24**).* Yield: 89%, $[\alpha]_D = +6.4^\circ$ (*c* 0.1, CH_3OH). UV λ_{max}^{MeOH} nm (log ε): 263 (3.80), 207 (4.38). ^{1}H NMR (C_5D_5N, 400 MHz): $\delta = 0.75$ (3H, t, $J = 7.4$ Hz, H-13a, b,c), 0.90 (3H, d, $J = 6.8$ Hz, H-14a, b, c), 1.22 (1H, quint, H-12a), 1.61–1.68 (1H, m, H-12b), 1.91 (3H, s, H-1a, b, c), 2.04–2.08 (1H, m, H-11), 3.05 (2H, t, $J = 7.6$ Hz, H-10a, b), 4.30–4.38 (3H, m, H-8, Fmoc CH_2, Fmoc CH), 4.50–4.57 (2H, m, H-4a, b), 4.69 (1H, dd, $J = 7.2$, 7.2 Hz, Fmoc CH_2), 4.92–4.96 (1H, m, H-5), 7.14 (1H, t, $J = 7.2$ Hz, Ar CH), 7.23-7.24 (3H, m, Ar CH), 7.33 (2H, d, $J = 7.4$ Hz, Fmoc Ar CH), 7.40 (4H, m,

Fmoc Ar CH, Ar CH), 7.67 (2H, t, $J = 7.2$ Hz, Fmoc Ar CH), 7.84 (2H, d, $J = 7.6$ Hz, Fmoc Ar CH), 8.84 (1H, d, $J = 9.2$ Hz, NH-6), 9.14 (1H, d, $J = 8.4$ Hz, NH-9). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 11.1$ (C-13), 15.8 (C-14), 20.6 (C-1), 25.2 (C-12), 37.5 (C-10), 37.7 (C-11), 47.8 (Fmoc CH), 49.9 (C-5), 60.4 (C-8), 65.7 (C-4), 66.5 (Fmoc CH_2), 120.4, 125.6, 125.8, 127.5, 128.0 (Fmoc Ar CH), 126.8, 128.8, 129.7, 138.7 (Ar CH), 141.2, 144.5, 144.8 (Fmoc Ar quat C), 157.3 (Fmoc CO), 170.6 (C-2), 172.5 (C-7). HRESI-MS m/z 551.2520 $[M + Na]^+$ (calcd for $C_{32}H_{36}N_2O_5Na$ 551.2522).

*5.1.2. General procedure for synthesis of compounds **11** and **12***

To a solution of compounds **9** and **10** in TFA/TIS/H_2O = 95/2.5/2.5 (100 μL/mg), respectively, was stirred at room temperature for 1 h. The solvent was evaporated under vacuum and the reaction mixture was purified by suction (MeOH as solvent) to give the desired products.

*5.1.2.1. (S)-2-((S)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-(4-hydroxyphenyl) propanamido)-3-phenylpropyl acetate (**11**).* Yield: 93%, $[\alpha]_D = -12.3°$ (*c* 0.1, CH_3OH). UV λ_{max}^{MeOH} nm (log ε): 264 (3.88), 208 (4.50). ^{1}H NMR (C_5D_5N, 400 MHz): 2.04 (3H, s, H-1a, b, c), 3.01 (2H, dddd, $J = 7.6$, 7.2, 7.2, 6.8 Hz, H-10a, b), 3.23 (1H, dd, $J = 7.6$, 7.6 Hz, H-11a), 3.46 (1H, dd, $J = 7.6$, 7.2 Hz, H-11b), 4.22–4.31 (3H, m, H-4a, b, Fmoc CH), 4.44 (1H, dd, $J = 7.2$, 7.2 Hz, Fmoc CH_2), 4.61 (1H, dd, $J = 7.2$, 6.8 Hz, Fmoc CH_2), 4.81–4.85 (1H, m, H-8), 5.04 (1H,q, $J = 15.4$ Hz, H-5), 7.09 (2H, d, $J = 8.0$ Hz, Ar CH), 7.15 (1H, t, $J = 7.4$ Hz, Ar CH), 7.23–7.28 (2H, m, Ar CH), 7.31 (2H, d, $J = 7.2$ Hz, Fmoc Ar CH), 7.39 (2H, t, $J = 7.4$ Hz, Fmoc Ar CH), 7.64 (2H, dd, $J = 4.0$, 3.6 Hz, Fmoc Ar CH), 7.84 (2H, d, $J = 8.0$ Hz, Fmoc Ar CH), 9.09 (1H, d, $J = 8.4$ Hz, NH-6), 9.26 (1H, d, $J = 8.0$ Hz, NH-9). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 20.7$ (C-1), 37.5 (C-10), 38.6 (C-11), 47.7 (Fmoc CH), 50.0 (C-5), 57.7 (C-8), 65.3 (C-4), 66.7 (Fmoc CH_2), 116.1 (C-2″, 6″), 120.4, 125.6, 125.8, 127.5, 128.0 (Fmoc Ar CH), 126.7 (C-4′), 128.4 (C-1″), 128.8 (C-2′, 6′), 128.9 (C-2″, 6″), 129.7 (C-3′, 5′), 131.0 (C-3″, 5″), 138.5 (C-1′), 141.6, 144.6 (Fmoc Ar quat C), 157.0 (Fmoc CO), 157.6 (C-4″), 170.7 (C-2), 172.4 (C-7). HRESI-MS m/z 601.2317 $[M + Na]^+$ (calcd for $C_{35}H_{34}N_2O_6Na$ 601.2314).

*5.1.2.2. (R)-2-((S)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-(4-hydroxyphenyl) propanamido)-3-phenylpropyl acetate (**12**).* Yield: 85%, $[\alpha]_D = +15.3°$ (*c* 0.1, CH_3OH). UV λ_{max}^{MeOH} nm (log ε): 263 (3.81), 208 (4.34). ^{1}H NMR (C_5D_5N, 400 MHz): 1.90 (3H, s, H-1a, b, c), 2.99 (2H, t, $J = 7.6$ Hz, H-10a, b), 3.20 (1H, dd, $J = 8.0$, 8.0 Hz, H-11a), 3.38 (1H, dd, $J = 6.8$, 6.4 Hz, H-11b), 4.24–4.32 (3H, m, H-4a, b; Fmoc CH), 4.39 (1H, dd, $J = 7.2$, 6.8 Hz, Fmoc CH_2), 4.59 (1H, dd, $J = 7.2$, 7.2 Hz, Fmoc CH_2), 4.84–4.89 (1H, m, H-8), 5.02 (1H,q, $J = 15.6$ Hz, H-5), 7.10 (2H, d, $J = 8.0$ Hz, Ar CH), 7.22–7.27 (3H, m, Ar CH), 7.31 (2H, d, $J = 7.2$ Hz, Fmoc Ar CH), 7.38 (2H, t, $J = 7.4$ Hz, Fmoc Ar CH), 7.62 (2H, d, $J = 7.6$ Hz, Fmoc Ar CH), 7.83 (2H, d, $J = 7.2$ Hz, Fmoc Ar CH), 9.02 (1H, d, $J = 8.4$ Hz, NH-6), 9.18 (1H, d, $J = 8.4$ Hz, NH-9). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 20.5$ (C-1), 37.7 (C-10), 38.6 (C-11), 47.7 (Fmoc CH), 50.0 (C-5), 57.6 (C-8), 65.3 (C-4), 66.6 (Fmoc CH_2), 116.1 (C-2″, 6″), 120.4, 125.6, 125.8, 127.5, 128.0 (Fmoc Ar CH), 126.8 (C-4′), 128.5 (C-1″), 128.9 (C-2′, 6′), 129.8 (C-3′, 5′), 130.3 (C-3″, 5″), 131.0 (C-3″, 5″), 138.5 (C-1′), 141.6, 144.6 (Fmoc Ar quat C), 157.0 (Fmoc CO), 157.7 (C-4″), 170.6 (C-2), 172.4 (C-7). HRESI-MS m/z 601.2318 $[M + Na]^+$ (calcd for $C_{35}H_{34}N_2O_6Na$ 601.2314). HRESI-MS m/z 624.2317 $[M + Na]^+$ (calcd for $C_{37}H_{34}N_3O_5Na$ 624.2314).

*5.1.3. General procedure for synthesis of compounds **7**, **8**, **13** and **14***

To a solution of compounds **5**, **6**, **11** and **12** in 20% piperidine/CH_2Cl_2 (1 mL), respectively, was stirred at room temperature for 1 h. The solvent was evaporated under vacuum and the reaction mixture was purified by column chromatography on silica gel to give the desired products.

*5.1.3.1. (S)-2-((S)-2-Amino-3-phenylpropanamido)-3-phenylpropyl acetate (**7**).* Yield: 80%, $[\alpha]_D = -6.5°$ (*c* 0.1, CH_3OH). ^{1}H NMR (C_5D_5N, 400 MHz): $\delta = 1.99$ (3H, s, H-1a, b, c), 2.99 (2H, t, $J = 7.6$ Hz, H-10a, b), 3.20 (1H, dd, $J = 8.0$, 8.0 Hz, H-11a), 3.43 (1H, dd, $J = 7.6$, 7.6 Hz, H-11b), 3.94 (2H, d, $J = 6.4$, H-4a, b), 3.98 (1H, dd, $J = 5.2$, 5.2 Hz, H-8), 4.89 (1H, m, H-5), 7.20–7.29 (8H, m, Ar CH), 7.42 (2H, d, $J = 8.0$ Hz, Ar CH), 8.70 (1H,d, $J = 8.4$ Hz, NH-3). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 20.7$ (C-1), 37.6 (C-10), 41.5 (C-11), 53.2 (C-5), 57.1 (C-8), 63.0 (C-4), 126.5 (C-4′), 126.8 (C-4″), 128.7 (C-2′, 6′), 128.8 (C-2″, 6″), 129.9 (C-3′, 5′, 3″, 5″), 138.9 (C-1′), 139.6 (C-1″), 170.6 (C-2), 174.0 (C-7).

*5.1.3.2. (R)-2-((S)-2-Amino-3-phenylpropanamido)-3-phenylpropyl acetate (**8**).* Yield: 80%, $[\alpha]_D = +5.9°$ (*c* 0.1, CH_3OH). ^{1}H NMR (C_5D_5N, 400 MHz): $\delta = 1.89$ (3H, s, H-1a, b, c), 2.93 (1H, dd, $J = 8.4$, 8.4 Hz, H-10a), 3.11 (1H, dd, $J = 7.2$, 6.8 Hz, H-10b), 3.22 (1H, dd, $J = 7.2$, 6.8 Hz, H-11a), 3.40 (1H, dd, $J = 4.8$, 4.4 Hz, H-11b), 3.85 (2H, dd, $J = 4.8$, 4.4 Hz, H-4a, b), 3.93 (1H, dd, $J = 5.2$, 4.4 Hz, H-5), 4.70 (1H, m, H-8), 7.22–7.30 (8H, m, Ar CH), 7.42 (2H, d, $J = 8.4$ Hz, Ar CH), 8.54 (1H, d, $J = 8.4$ Hz, NH-3). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 20.5$ (C-1), 37.7 (C-10), 41.9 (C-11), 53.1 (C-5), 57.3 (C-8), 63.0 (C-4), 126.5 (C-4′), 126.7 (C-4″), 128.7 (C-2′, 6′), 128.8 (C-2″, 6″), 129.9 (C-3′, 5′), 130.0 (C-3″, 5″), 139.2 (C-1′), 139.6 (C-1″), 170.6 (C-2), 174.6 (C-7).

*5.1.3.3. (S)-2-((S)-2-Amino-3-(4-hydroxyphenyl)propanamido)-3-phenylpropyl acetate (**13**).* Yield: 82%, ^{1}H NMR (C_5D_5N, 400 MHz): $\delta = 1.98$ (3H, s, H-1a, b, c), 2.89 (1H, d, $J = 8.4$, 8.4 Hz, H-11a), 2.97 (2H, t, $J = 8.6$ Hz, H-10a, b), 3.31 (1H, dd, $J = 4.8$, 4.8 Hz, H-11b), 3.84 (1H, dd, $J = 4.8$, 4.8 Hz, H-8), 4.26 (2H, dddd, $J = 6.0$, 5.6, 4.8, 4.8 Hz, H-4a, b), 4.76–4.85 (2H, m, H-5, 8), 7.04 (2H, d, $J = 8.0$ Hz, Ar CH), 7.23–7.32 (7H, m, Ar CH), 8.55 (1H, d, $J = 8.8$ Hz, NH-6). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 20.6$ (C-1), 28.8 (*t*-butyl CH_3), 37.7 (C-10), 41.2 (C-11), 49.5 (C-5), 57.3 (C-8), 65.4 (C-4), 78.1 (*t*-butyl quat C), 124.3 (C-2″, 6″), 126.8 (C-4′), 128.8 (C-2′, 6′), 129.7 (C-3′, 5′), 130.3 (C-3″, 5″), 133.7 (C-1″), 138.4 (C-1′), 154.5 (C-4″), 170.7 (C-2), 175.1 (C-7). HRESI-MS m/z 356.2520 $[M + Na]^+$ (calcd for $C_{20}H_{24}N_2O_4Na$ 356.2522).

*5.1.3.4. (R)-2-((S)-2-Amino-3-(4-hydroxyphenyl)propanamido)-3-phenylpropyl acetate (**14**).* Yield: 83%, $\delta = 1.89$ (3H, s, H-1a, b, c), 2.93 (1H, dd, $J = 8.8$, 8.6 Hz, H-11a), 3.18 (2H, t, $J = 8.6$ Hz, H-10a, b), 3.39 (1H, dd, $J = 4.6$, 4.4 Hz, H-11b), 3.83 (1H, dd, $J = 4.8$, 4.8 Hz, H-8), 3.92 (2H, t, $J = 4.2$ Hz, H-4a, b), 4.68–4.72 (2H, m, H-5, 8), 7.06 (2H, d, $J = 8.4$ Hz, Ar CH), 7.25 (4H, dd, $J = 8.0$, 7.6 Hz, Ar CH), 7.33 (1H, d, $J = 6.6$ Hz, Ar CH), 7.43 (2H, d, $J = 7.4$ Hz, Fmoc Ar CH), 8.46 (1H, d, $J = 8.8$ Hz, NH-6). ^{13}C NMR (C_5D_5N, 100 MHz): $\delta = 20.5$ (C-1), 37.8 (C-10), 41.2 (C-11), 50.0 (C-5), 57.4 (C-8), 65.4 (C-4), 124.3 (C-2″, 6″), 126.8 (C-4′), 128.9 (C-2′, 6′), 129.7 (C-3′, 5′), 130.4 (C-3″, 5″), 133.8 (C-1″), 138.4 (C-1′), 154.6 (C-4″), 170.7 (C-2), 174.9 (C-7). HRESI-MS m/z 356.2520 $[M + Na]^+$ (calcd for $C_{20}H_{24}N_2O_4Na$ 356.2522).

5.2. Biological assay

5.2.1. Preparation of human neutrophils

Blood was taken from healthy human donors (20–32 years old) by venipuncture, using a protocol approved by the institutional review board at Chang Gung Memorial Hospital. Neutrophils were isolated with a standard method of dextran sedimentation prior to centrifugation in a Ficoll Hypaque gradient and hypotonic lysis of erythrocytes [1,17]. Purified neutrophils that contained >98% viable cells, as determined by the trypan blue exclusion method, were resuspended in calcium (Ca^{2+})-free HBSS buffer at pH 7.4, and were maintained at 4 °C before use.

5.2.2. Measurement of $O_2^{\bullet-}$ generation

The assay of $O_2^{\bullet-}$ generation was based on the SOD-inhibitable reduction of ferricytochrome *c* [1,17]. In brief, after supplementation with 0.5 mg/mL ferricytochrome *c* and 1 mM Ca^{2+}, neutrophils were equilibrated at 37 °C for 2 min and incubated with drugs for 5 min. Cells were activated with 100 nM FMLP for 10 min. When

FMLP was used as a stimulant, CB (1 μg/mL) was incubated for 3 min before activation by the peptide (FMLP/CB). Changes in absorbance with the reduction of ferricytochrome *c* at 550 nm were continuously monitored in a double-beam, six-cell positioner spectrophotometer with constant stirring (Hitachi U-3010, Tokyo, Japan). Calculations were based on differences in the reactions with and without SOD (100 U/mL) divided by the extinction coefficient for the reduction of ferricytochrome *c* ($\varepsilon = 21.1$/mM/10 mm).

5.2.3. Measurement of elastase release

Degranulation of azurophilic granules was determined by elastase release as described previously [17]. Experiments were performed using MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide as the elastase substrate. Briefly, after supplementation with MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide (100 μM), neutrophils (5×10^5/mL) were equilibrated at 37 °C for 2 min and incubated with drugs for 5 min. Cells were activated by100 nM FMLP and 0.5 μg/mL CB, and changes in absorbance at 405 nm were continuously monitored to assay elastase release. The results are expressed as the percent of the initial rate of elastase release in the FMLP/CB-activated, drug-free control system.

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