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PREPARATION AND SYNTHETIC APPLICATIONS OF *N*-(α,β-UNSATURATED ACYL)-α-AMINO ACID DERIVATIVES

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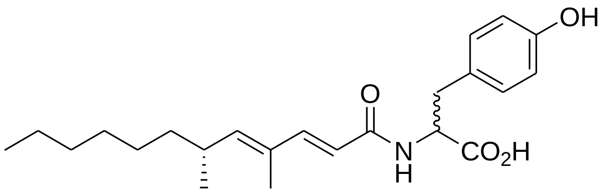
Submitted in honor of the 75th anniversary of Professor Emeritus Keiichiro Fukumoto.

Abstract – *N*-(α,β-Unsaturated acyl)-α-amino acids, amides and esters are structural motifs of many biologically active natural products. An alternate and advantageous approach for the synthesis of *N*-(α,β-unsaturated acyl)-α-amino acid derivatives is developed via acylation of unprotected α-amino acids with stable crystalline *N*-(α,β-unsaturated acyl)benzotriazole. The proposed methodology provides a new synthesis for compound (**9**) which is a precursor to a novel cytotoxic agent.

INTRODUCTION

N-(α,β-Unsaturated acyl)-α-amino acids, amides and esters are structural motifs of natural products such as gymnastatin N (**1**), its 12-hydroxy analog (**2**)[1] and gymnastatin H (**3**)[2]. *Cis*- and *trans*-tryptophan 4-aminocinnamamides have been isolated from the myxomycete *Fuligo aurea*.[3]



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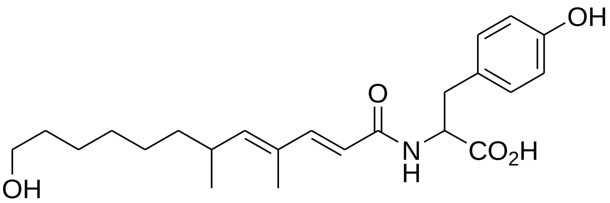

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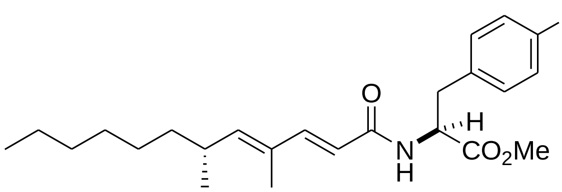

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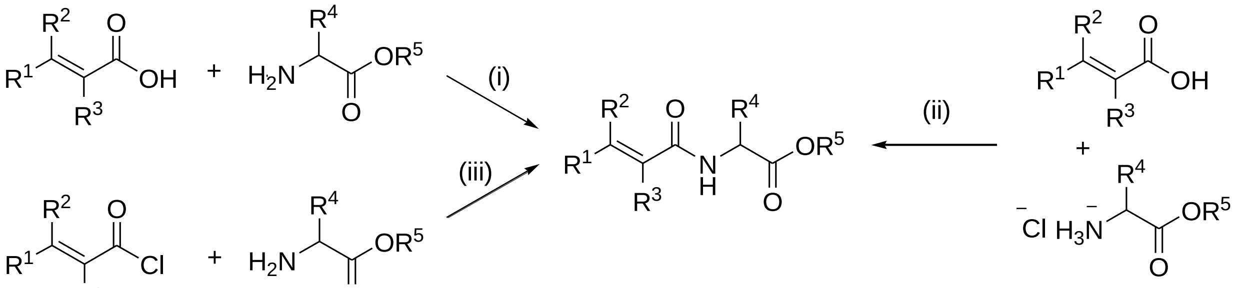
N-(α,β-Unsaturated acyl)-α-amino acids and esters have been reported to be biologically active as anticancer agents,[1] aldose reductase inhibitors,[4] rat kidney enzyme inhibitors,[5] pseudo analogs of the naturally occurring antibiotic sparsomycin,[6] chromogenic substrates of proteolytic enzymes,[7] lysozyme adsorption agents,[8] HIV-1 integrase inhibitors[9] and inhibitors of mammalian collagenase.[10] *N*-(α,β-unsaturated acyl)-α-amino acids are intermediates in the synthesis of prodrugs for cancer treatment,[11] the antibiotic Reutericyclin,[12] cell adhesive agents[13] used for the synthesis of cyclic-peptide based uPA inhibitors,[14] homo-poly(*N*-acryl α-amino acid) of biological interests[15] and 4-hydroxybenzylidene-1-methyl-2-propenyl-imidazolinone (HBMPI) or 4-hydroxybenzylidene-1-methyl-2-penta-1,3-diene-1-yl-imidazolinone (HBMPDI) models to study the chromophore within Discosoma Red (a red fluorescent protein).[16]

Published preparations for *N*-(α,β-unsaturated acyl)-α-amino acids (Scheme 1) from α,β-unsaturated acid and carboxyl - protected α-amino acid units include: (i) coupling mediated by DCC,[11,17] 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride (EDCI)[9,13] benzotriazol-1-yloxytris (dimethylamino)phosphonium hexafluorophosphate (BOP)[18] and *tert*-butyl 3-(3,4-dihydrobenzotriazine-4-on)yl carbonate (Boc-ODhbt)[19] (ii) via anhydride using isobutyl chloroformate;[4] (iii) reactions of α,β-unsaturated acid chlorides with carboxyl - protected or unprotected α-amino acids.[12,14,15,20-26]



(i) DCC or EDCI or BOP or Boc-ODhbt; (ii) *iso*-butyl chloroformate then amino ester hydrochloride (iii) base

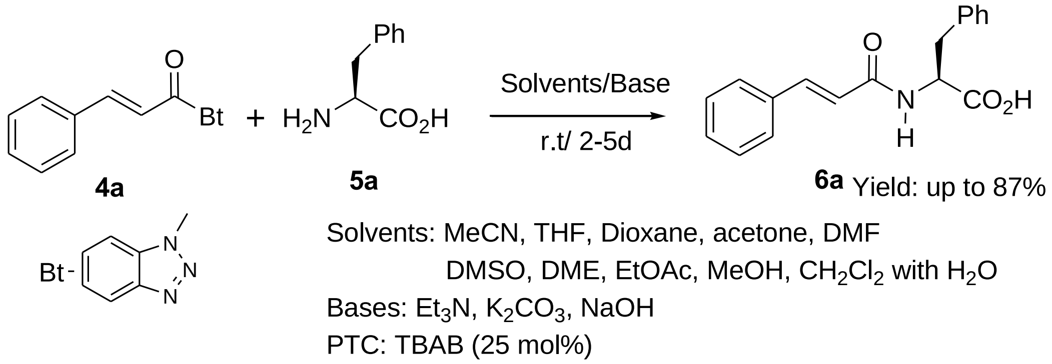
Scheme 1. Literature methods for the preparation of *N*-(α,β-unsaturated acyl)-α-amino acids and esters.

We now report an alternate, advantageous approach to *N*-(α,β-unsaturated acyl)-α-amino acid derivatives by the acylation of α-amino acids with stable crystalline *N*-(α,β-unsaturated acyl)benzotriazole acylating agents:[27] advantages include the use of stable, crystalline acylating agents and free amino acids in aqueous solutions.

RESULTS AND DISCUSSION

To generalize the preparation of *N*-(α,β-unsaturated acyl)-α-amino acids by the *N*-acylation of α-amino acids with *N*-(α,β-unsaturated acyl)benzotriazoles, we optimized conditions for the reaction of

N-cinnamoylbenzotriazole (**4a**) with phenylalanine (**5a**) with respect to solvent, base and phase-transfer catalyst (Scheme 2). The solvent combination of 1, 2-dimethoxyethane (DME)/water was identified as preferred. In the presence of triethylamine as base, the reaction was complete in 48-72 hr in DME/H_2O at 20 ^oC whereas in MeCN/H_2O, 120 hrs were required for approximately the same yield. The use of other amines or bases such as K_2CO_3 and NaOH in place of NEt_3 gave similar results. Tetrabutylammonium bromide (25 mol %) as phase transfer catalyst in CH_2Cl_2/H_2O had no effect with K_2CO_3 or NaOH systems. We used the above conditions (DME/H_2O/Et_3N at rt) for the *N*-acylation of amino acids (**5a-f**) as described below. Other methods were less useful: although microwave irradiation (60-100 W/75 ^oC for 75 min.), increased the rate of reaction of **4a** with **5b** this gave a mixture of the expected product (**6ab**) and cinnamic acid; the acylation of **5a** with **4a** in the presence of aqueous LiOH (1N) in DME at room temperature was complete in 120 hr but also gave a mixture of the expected product (**6aa**) and cinnamic acid.



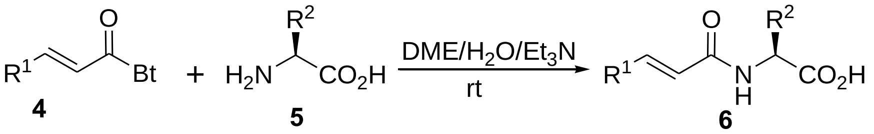
Solvents: MeCN, THF, Dioxane, acetone, DMF
DMSO, DME, EtOAc, MeOH, CH_2Cl_2 with H_2O
Bases: Et_3N, K_2CO_3, NaOH
PTC: TBAB (25 mol%)

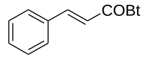
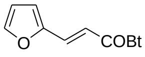
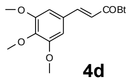
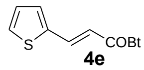
Scheme 2

The *N*-(α,β-unsaturated-acyl)benzotriazoles (**4a-e**) required were prepared in 80-90% yields from the corresponding carboxylic acid by reaction with benzotriazole and thionyl chloride.[27] Using reagents (**4a-e**) a variety of (L)-α-amino acids (**5a-f**) were acylated to give products (**6aa-ea**) in 34-87% yields (Table 1). Reactions of acylating agent **4a** with amino acids (**5a-c**) were complete in about 48 hr (entries 1-3, Table 1) but for amino acids (**5d, 5e**) the reactions required 84 hr and 72 hr respectively (entries 4, 5, Table 1). Acylation of sterically hindered L-valine **5f** with **4a** required 340 hr for complete reaction (entry 6, Table 1). Furylacryloylbenzotriazole **4b** reacted with amino acids (**5a-5f**) in 24-48 hr (entries 7-12, Table 1).

Acylating agent **4c** containing a C≡C bond reacted faster (entries 13-15, Table 1) than the corresponding C=C compound (**4a**). This method has also been successfully applied to prepare **6df** and **6ea** in good yields under mild conditions (entries 16-17, Table 1). Analysis by HPLC of compound **6ea** confirmed the absence of racemization: a single peak was obtained for the enantiomer **6ea** at 4.0 min. whereas the racemate gave two peaks at 4.0 and 4.8 min.

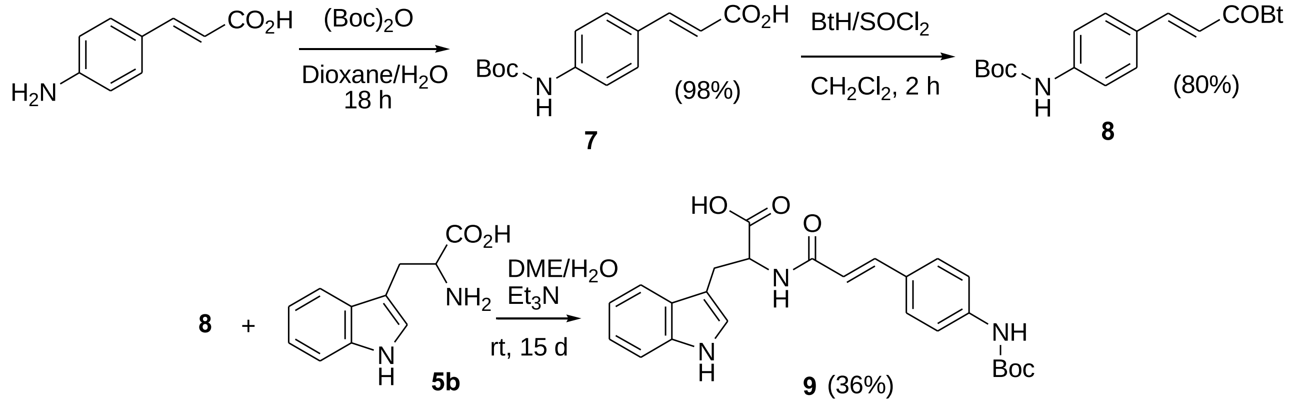
Table 1. Preparation of *N*-(α,β-unsaturated acyl)-α-amino acids (**6**)



Entry	4	5	6	Time (hr)	Yield (%)[a]	Mp (°C)	$[\alpha]_D^{25}$	Lit. Mp (°C)	Lit. Yield (%)
1	 **4a**	L-Phe **(5a)**	**6aa**	56	87	191.0-193.0	-	198.0-199.5	(75)[26]
2	**4a**	L-Trp **(5b)**	**6ab**	52	50	228.0-230.0	-	-	-
3	**4a**	Gly **(5c)**	**6ac**	48	44	198.0-201.0	-	194.7-197.3	(86)[29]
4	**4a**	L-Ala **(5d)**	**6ad**	84	40	193.4-194.5	+10.8	-	-
5	**4a**	L-Ile **(5e)**	**6ae**	72	46	80.0-82.5	-	-	-
6	**4a**	L-Val **(5f)**	**6af**	340	53	192.0-194.0	+18.2	187.0-188.0	(77)[26]
7	 **4b**	L-Phe **(5a)**	**6ba**	48	54	138.0-140.0	-15.0	119.0-121.0	(53)[30]
8	**4b**	L-Trp **(5b)**	**6bb**	24	52	65.0	-	98.0-100.0	(63)[30]
9	**4b**	Gly **(5c)**	**6bc**	48	34	221.0-223.0	-	245.0	(58)[18]
10	**4b**	L-Ala **(5d)**	**6bd**	48	63	143.0-146.5	-	112.0-113.0	(48)[30]
11	**4b**	L-Ile **(5e)**	**6be**	24	42	45.0-50.0[b]		215.0	(71)[18]
12	**4b**	L-Val **(5f)**	**6bf**	48	61	96.0-98.0	+5.2	136.0-138.0	(55)[30]
13	Ph—≡—COBt **4c**	L-Phe **(5a)**	**6ca**	22	38	112.5-114.0	-47.0	-	-
14	**4c**	L-Trp **(5b)**	**6cb**	35	69	142.2-144.0	-39.5	-	-
15	**4c**	L-Ile **(5e)**	**6ce**	19	70	-	+24.5	-	-
16	 **4d**	L-Val (5f)	**6df**	60	52	198.0-200.0	+28.2	196.0-198.0	(36)[31]
17	 **4e**	L-Phe **(5a)**	**6ea**	48	61	164.0-165.0	-31.1	172.0-173.0	(-)[32]

[a] Isolated yield. [b] On heating, the compound swells at 45 °C and becomes a transparent glassy material, no definite temperature of melting can be detected.

Preparation of Boc-protected *trans*-tryptophan 4-aminocinnamamide (**9**),[3] is outlined in Scheme 3. The amino group of *p*-aminocinnamic acid was protected to give **7** (98%)[28] which was converted into the corresponding acylbenzotriazole **8** (80%).[27] On reaction of **8** with tryptophan (**5b**) the desired product **9** was obtained in 36% yield.



Scheme 3

CONCLUSIONS

Using our acyl-benzotriazole methodology, air stable crystalline aromatic and heteroaromatic substituted *N*-(α,β-unsaturated acyl)benzotriazoles react with free amino acids in aqueous solution at rt to afford potentially bioactive *N*-(α,β-unsaturated acyl)-α-amino acids derivatives in moderate to good yields without racemization. This methodology provides a new synthesis of compound (**9**) which is a precursor for a novel cytotoxic agent.[3]

EXPERIMENTAL

Melting points were determined on a capillary point apparatus equipped with the digital thermometer and are uncorrected. NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 with TMS as internal standard for ^1H NMR (300 MHz) or a solvent as internal standard for ^{13}C NMR (75 MHz). Elemental and mass spectroscopy analyses were performed by the analytical laboratories, Department of Chemistry, University of Florida. Mass Analysis was performed using a ThermoFinnigan-LCQ ion trap mass spectrometer (San Jose, CA) in electrospray ionization (ESI) mode. Optical rotation values were measured with the use of sodium D line on Perkin-Elmer 241 polarimeter. HPLC analyses were performed on Shimadzu LC -20AT system using chirobiotic T column (4.6 X 250 mm), detection at 254 nm, flow rate 0.5 mL/min. and methanol:water (1:1) as solvent system. Reagents obtained commercially were used without further purification. Column chromatography was conducted with silica gel (200-425 mesh).

General method for the preparation of *N*-(α,β-unsaturated acyl)-α-amino acids (6): A solution of cinnamoylbenzotriazole (0.125 g, 0.5 mmol) in DME (30 mL) was added at rt to a solution of phenylalanine (0.083 g, 0.5 mmol) in DME (14 mL) and water (6 mL) in the presence of triethylamine (0.17 mL, 1.2 mmol). The reaction mixture was then stirred at rt for 56 h; 6 N HCl (1 mL) was added and the solvent was removed under reduced pressure. The residue was extracted with EtOAc (20 mL), washed with 6 N HCl (5 mL) and brine (10 mL), and then dried over $MgSO_4$ (anhyd.). Filtration and evaporation of the solvent under reduced pressure gave (2*S*)-3-phenyl-2-{[(*E*)-3-phenyl-2-propenoyl]amino}-propanoic acid (**6aa**) (0.13 g, 87%) as yellow microcrystals. Mp 191-193 °C (Lit.,[26] mp 198-199.5 °C); ^{1}H NMR (DMSO-d_6): δ 2.89-2.97 (m, 1H), 3.10-3.17 (m, 1H), 4.54-4.62 (m, 1H), 6.71 (d, *J* = 15.9 Hz, 1H), 7.18-7.31 (m, 5H), 7.37-7.42 (m, 4H), 7.54-7.57 (m, 2H), 8.45 (d, *J* = 8.1 Hz, 1H); ^{13}C NMR (DMSO-d_6): 36.8, 53.7, 121.7, 126.5, 127.6, 128.3, 129.0, 129.1, 129.6, 134.8, 137.6, 139.3, 164.9, 173.1. HRMS $[M+H]^+$ Calcd for $C_{18}H_{18}NO_3$: 296.1281. Found: 296.1270

(2*S*)-3-(1*H*-Indol-3-yl)-2{[(*E*)-3-phenyl-2-propenoyl]amino}propanoic acid (6ab): (50%) brown microcrystals (MeOH/water); mp 228-230 °C; ^{1}H NMR (DMSO-d_6): δ 3.03-3.27 (m, 2H), 4.59-4.66 (m, 1H), 6.73 (d, *J* = 15.9 Hz, 1H), 6.95-6.99 (m, 1H), 7.03-7.08 (m, 1H), 7.15-7.18 (m, 1H), 7.31-7.41 (m, 5H), 7.53-7.56 (m, 3H), 8.39 (d, *J* = 7.7 Hz, 1H), 10.85 (s, 1H), 12.74 (br s, 1H); ^{13}C NMR (DMSO-d_6): 27.2, 53.1, 109.8, 111.4, 118.2, 118.4, 120.9, 121.8, 123.6, 127.2, 127.5, 128.9, 129.5, 134.8, 136.1, 139.1, 164.9, 173.4. Anal. Calcd for $C_{20}H_{18}N_2O_3$: C, 71.84; H, 5.43; N, 8.38. Found: C, 71.62; H, 5.56; N, 8.44.

2-{[(*E*)-3-Phenyl-2-propenoyl]amino}acetic acid (6ac): (44%) white microcrystals (EtOAc/hexane); mp 198-201 °C (Lit.,[29] mp 194.7-197.3 °C); ^{1}H NMR (DMSO-d_6): δ 3.88 (d, *J* = 5.8 Hz, 2H), 6.72 (d, *J* = 15.7 Hz, 1H), 7.35-7.47 (m, 4H), 7.56-7.59 (m, 2H), 8.43 (t, *J* = 5.8 Hz, 1H), 12.63 (br s, 1H); ^{13}C NMR (DMSO-d_6): 40.8, 121.7, 127.6, 129.0, 129.6, 134.8, 139.2, 165.3, 171.4. Anal. Calcd for $C_{11}H_{11}NO_3$: C, 64.38; H, 5.40; N, 6.83. Found: C, 64.43; H, 5.34; N, 6.91.

(2*S*)-2-{[(*E*)-3-Phenyl-2-propenoyl]amino}propanoic acid (6ad): (40%) white microcrystals (EtOAc/hexane); mp 193.4-194.5 °C; ^{1}H NMR (DMSO-d_6): δ 1.32 (d, *J* = 7.1 Hz, 3H), 4.30-4.35 (m, 1H), 6.71 (d, *J* = 15.9 Hz, 1H), 7.35-7.46 (m, 4H), 7.55-7.57 (m, 2H), 8.42 (d, *J* = 6.9 Hz, 1H); ^{13}C NMR (DMSO-d_6): 17.3, 47.7, 121.7, 127.5, 129.0, 129.5, 134.8, 139.1, 164.6, 174.2. $[\alpha]_D^{25}$ +10.8 (*c* 1.0, EtOH). HRMS $[M+H]^+$ Calcd for $C_{12}H_{14}NO_3$: 220.0968. Found: 220.0948.

(2*S*,3*S*)-3-Methyl-2-{[(*E*)-3-phenyl-2-propenoyl]amino}pentanoic acid (6ae): (46%) pale yellow microcrystals (EtOAc/hexane); mp 80.0-82.5 °C; ^{1}H NMR (DMSO-d_6): δ 0.88-0.91 (m, 6H), 1.20-1.30 (m, 1H), 1.39-1.50 (m, 1H), 1.75-1.90 (m, 1H), 4.31-4.36 (m, 1H), 6.86 (d, *J* = 15.7 Hz, 1H), 7.40-7.57 (m, 6H), 8.22 (d, *J* = 8.2 Hz, 1H), 12.63 (br s, 1H); ^{13}C NMR (DMSO-d_6): 11.4, 15.7, 24.8, 36.6, 56.5, 122.0, 127.6, 129.0, 129.6, 135.0, 139.2, 165.1, 173.1. Anal. Calcd for $C_{15}H_{19}NO_3$: C, 68.94; H, 7.33. Found: C, 67.12; H, 7.43.

(2*S*)-3-Methyl-2-{[(*E*)-3-phenyl-2-propenoyl]amino}butanoic acid (6af): (53%) white microcrystals (EtOAc/hexane); mp 192.0−194.0 °C (Lit.,[26] mp 187−188 °C); ^{1}H NMR (DMSO-*d*$_6$): δ 0.92 (d, *J* = 6.6 Hz, 6H), 2.08-2.14 (m, 1H), 4.27-4.32 (m, 1H), 6.87 (d, *J* = 15.7 Hz, 1H), 7.37-7.46 (m, 4H), 7.55-7.57 (m, 2H), 8.21 (d, *J* = 8.2 Hz, 1H), 12.65 (br s, 1H); ^{13}C NMR (DMSO-*d*$_6$): 18.0, 19.2, 29.9, 57.3, 121.9, 127.5, 129.0, 129.5, 134.9, 139.1, 165.1, 173.0. $[\alpha]_D^{25}$ +18.2 (*c* 1.0, EtOH). Anal. Calcd for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66. Found: C, 67.37; H, 7.42; N, 6.00.

(2*S*)-2-{[(*E*)-3-(2-Furyl)-2-propenoyl)amino]-3-phenylpropanoic acid (6ba): (54%) yellow microcrystals (EtOAc/hexane); mp 138-140 °C (Lit.,[30] mp 119-121 °C); ^{1}H NMR (DMSO-*d*$_6$): δ 2.85-2.95 (m, 1H), 3.08-3.14, (m, 1H), 4.50-4.58 (m, 1H), 6.47 (d, *J* = 15.7 Hz, 1H), 6.56-6.60 (m, 1H), 6.76-6.77 (m, 1H), 7.16-7.25 (m, 6H), 7.77 (s, 1H), 8.50 (d, *J* = 8.0 Hz, 1H), 12.79 (br s, 1H); ^{13}C NMR (DMSO-*d*$_6$): 36.9, 53.8, 112.5, 114.1, 118.9, 126.5, 126.6, 128.3, 129.1, 137.7, 144.9, 150.9, 164.8, 173.1. $[\alpha]_D^{25}$ -15.0 (*c* 1.0, EtOH). Anal. Calcd for $C_{16}H_{15}NO4.EtOAc$ (4:1): C, 66.44; H, 5.58; N, 4.56. Found: C, 66.07; H, 5.32; N, 5.00.

(2*S*)-2-{[(*E*)-3-(2-Furyl)-2-propenoyl]amino}-3-(1*H*-indole-3-yl)propanoic acid (6bb): (52%) yellow microcrystals (EtOAc/hexane); mp 65 °C (Lit.,[30] mp 98-100 °C); ^{1}H NMR (DMSO-*d*$_6$): δ 3.01-3.26 (m, 2H), 4.50-4.70 (m, 1H), 6.51 (d, *J* = 15.8 Hz, 1H), 6.58 (s, 1H), 6.76 (s, 1H), 6.96-7.00 (m, 1H), 7.04-7.08 (m, 1H), 7.15-7.22 (m, 2H), 7.33 (d, *J* = 7.7 Hz, 1H), 7.55 (d, *J* = 7.4 Hz, 1H), 7.77 (s, 1H), 8.47 (d, *J* = 7.1 Hz, 1H), 10.85 (s, 1H); ^{13}C NMR (DMSO-*d*$_6$): 27.3, 53.2, 109.9, 111.4, 112.4, 114.0, 118.2, 118.4, 119.1, 121.0, 123.6, 126.5, 127.2, 136.1, 144.9, 151.0, 164.8, 173.5. HRMS $[M+H]^+$ Calcd for $C_{18}H_{17}N_2O_4$: 325.1183. Found: 325.1200

2-{[(*E*)-3-(2-Furyl)-2-propenoyl]amino}acetic acid (6bc): (34%) white microcrystals (EtOAc/hexane); mp 221-223 °C (Lit.,[18] mp 245 °C); ^{1}H NMR (DMSO-*d*$_6$): δ 3.86 (d, *J* = 6.0 Hz, 2H), 6.48 (d, *J* = 15.4 Hz, 1H), 6.58-6.60 (m, 1H), 6.79 (d, *J* = 3.3 Hz, 1H), 7.25 (d, *J* = 15.7 Hz, 1H), 7.78 (s, 1H), 8.49 (t, *J* = 6.0 Hz, 1H), 12.61 (br s, 1H); ^{13}C NMR (DMSO-*d*$_6$): 40.8, 112.4, 114.1, 118.8, 126.6, 144.9, 150.9, 165.2, 171.4. Anal. Calcd for $C_9H_9NO_4$: C, 55.38; H, 4.65; N, 7.18. Found: C, 55.17; H, 4.60; N, 7.13.

(2*S*)-2-{[(*E*)-3-(2-Furyl)-2-propenoyl]amino}propanoic acid (6bd): (63%) yellow microcrystals (EtOAc/hexane); mp 143−146.5 °C (Lit.,[30] mp 112-113 °C); ^{1}H NMR (DMSO-*d*$_6$): δ 1.30 (d, *J* = 7.1 Hz, 3H), 4.28-4.33 (m, 1H), 6.47 (d, *J* = 15.7 Hz, 1H), 6.58-6.59 (m, 1H), 6.78 (d, *J* = 3.3 Hz, 1H), 7.32 (d, *J* = 15.7 Hz, 1H), 7.78 (s, 1H), 8.45 (d, *J* = 7.1 Hz, 1H), 12.55 (br s, 1H); ^{13}C NMR (DMSO-*d*$_6$): 17.3, 47.7, 112.4, 114.0, 118.9, 126.5, 144.9, 150.9, 164.5, 174.2. Anal. Calcd for $C_{10}H_{11}NO_4$: C, 57.41; H, 5.30; N, 6.70. Found: C, 57.20; H, 5.43; N, 7.09.

(2*S*)-2-{[(*E*)-3-(2-Furyl)-2-propenoyl]amino}propanoic acid (6be): (42%) yellow microcrystals (EtOAc/hexane); mp 45-50 °C (Lit.,[18] mp 215 °C); ^{1}H NMR (DMSO-d_6): δ 0.83-0.89 (m, 6H), 1.16-1.26 (m, 1H), 1.38-1.47 (m, 1H), 1.75-1.86 (m, 1H), 4.28-4.33 (m,1H), 6.57-6.59 (m, 1H), 6.63 (d, *J* = 15.7 Hz, 1H), 6.76-6.77 (m, 1H), 7.23 (d, *J* = 15.7 Hz, 1H), 7.78 (s, 1H), 8.29 (d, *J* = 8.5 Hz, 1H), 12.63 (s, 1H); ^{13}C NMR (DMSO-d_6): 11.3, 15.7, 24.8, 36.5, 56.5, 112.4, 113.9, 119.2, 126.5, 144.9, 151.0, 165.0, 173.1. Anal. Calcd for $C_{13}H_{17}NO_4$: C, 62.14; H, 6.82; N, 5.57. Found: C, 62.43; H, 7.13; N, 5.47.

(2*S*)-2-{[(*E*)-3-(2-Furyl)-2-propenoyl]amino}-3-methylbutanoic acid (6bf): (61%) yellow microcrystals (EtOAc/hexane); mp 96-98 °C (Lit.,[30] mp 136-138 °C); ^{1}H NMR (DMSO-d_6): δ 0.93-0.95 (m, 6H), 2.0-2.20 (m, 1H), 4.28-4.32 (m, 1H), 6.63-6.71 (m, 2H), 6.81-6.82 (m, 1H), 7.27 (d, *J* = 15.5 Hz, 1H), 7.83 (s, 1H), 8.31 (d, *J* = 8.4 Hz, 1H), 12.68 (br s, 1H); ^{13}C NMR (DMSO-d_6): 18.0, 19.2, 29.9, 57.4, 112.4, 113.9, 119.2, 126.5, 144.9, 151.0, 165.1, 173.1. $[\alpha]_D^{25}$ +5.2 (*c* 1.0, EtOH). Anal. Calcd for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90. Found: C, 60.04; H, 7.05; N, 5.65.

(2*S*)-3-Phenyl-2-[(3-phenyl-propynoyl)amino]propanoic acid (6ca): (38%) white microcrystals (EtOAc/hexane); mp 112.5-114 °C; ^{1}H NMR (DMSO-d_6): δ 2.88-2.95 (m, 1H), 3.10-3.14 (m, 1H), 4.40-4.49 (m, 1H), 7.20-7.40 (m, 5H), 7.40-7.60 (m, 5H), 9.18 (d, *J* = 7.4 Hz, 1H), 12.89 (br s, 1H); ^{13}C NMR (DMSO-d_6): 36.1,53.9, 83.6, 83.8, 119.7, 126.5, 128.3, 129.0, 130.4, 132.2, 137.6, 172.4. $[\alpha]_D^{25}$ -47.0 (*c* 1.0, EtOH). Anal. Calcd for $C_{18}H_{15}NO_3$: C, 73.70; H, 5.15; N, 4.78. Found: C, 73.38; H, 5.33; N; 4.71.

(2*S*)-3-(1*H*-Indol-3-yl)-2-[(3-phenyl-2-propynoyl)amino]propanoic acid (6cb): (69%) pale yellow microcrystals (EtOAc/hexane); mp 142.2-144.0 °C; ^{1}H NMR (DMSO-d_6): δ 3.07-3.21 (m, 2H), 4.40-4.60 (m, 1H), 6.99 (t, *J* = 7.1 Hz, 1H), 7.07 (t, *J* = 7.7 Hz, 1H), 7.18 (s, 1H), 7.33-7.36 (m, 1H), 7.45-7.47 (m, 3H), 7.53-7.60 (m, 3H), 9.17 (d, *J* = 7.7 Hz, 1H), 10.88 (s, 1H), 12.76 (br s, 1H); ^{13}C NMR (DMSO-d_6): 26.7, 53.4, 83.8, 109.9, 111.5, 118.1, 118.5, 119.8, 121.1, 123.7, 127.0, 129.0, 130.4, 132.2, 132.6, 136.2, 152.4, 172.8. $[\alpha]_D^{25}$ -39.5 (*c* 1.0, EtOH). HRMS $[M+H]^+$ Calcd for $C_{20}H_{17}N_2O_3$: 333.1234. Found: 333.1230

(2*S*, 3*S*)-3-Methyl-2-[(3-phenyl-2-propynoyl)amino]pentanoic acid (6ce): (70%) yellow gel; ^{1}H NMR (DMSO-d_6): δ 0.85-0.90 (m, 6H), 1.10-1.30 (m, 1H), 1.30-1.50 (m, 1H), 1.70-1.90 (m, 1H), 4.20-4.30 (m, 1H), 7.40-7.50 (m, 3H), 7.60 (d, *J* = 5.8 Hz, 2H), 9.05 (d, *J* = 7.4 Hz, 1H), 12.7 (br s, 1H); ^{13}C NMR (DMSO-d_6): 11.3, 15.6, 24.8, 35.9, 57.0, 83.8, 83.9, 119.9, 129.0, 130.3, 132.2, 152.6, 172.5. $[\alpha]_D^{25}$ +24.5

(*c* 1.0, EtOH). Anal. Calcd for $C_{15}H_{17}NO_3$: C, 69.48; H, 6.61; N, 5.40. Found: C, 69.22; H, 6.64; N, 5.63.

(2*S*)-3-Methyl-2-{[(*E*)-3-(3,4,5-trimethoxyphenyl)-2-propenoyl]amino}butanoic acid (6df): (52%) pale yellow microcrystals (EtOAc/hexane); mp 198-200 °C (Lit.,[31] mp 196-198 °C); ^{1}H NMR (DMSO-d_6): δ 0.92 (d, *J* = 4.1 Hz, 6H), 2.1-2.30 (m, 1H), 3.68 (m, 3H), 3.81 (m, 6H), 4.20-4.30 (m, 1H), 6.79-6.90 (m, 3H), 7.37 (d, *J* = 14.8 Hz, 1H), 8.13 (d, *J* = 7.4 Hz, 1H), 12.65 (br s, 1H); ^{13}C NMR (DMSO-d_6): 18.0, 19.2, 30.0, 55.8, 57.3, 60.1, 104.9, 121.3, 130.6, 138.6, 139.2, 153.1, 165.2, 173.1. $[\alpha]_D^{25}$ +28.2 (*c* 1.0, EtOH). Anal. Calcd for $C_{17}H_{23}NO_6$: C, 60.52; H, 6.87; N, 4.15. Found: C, 60.64; H, 7.33; N, 4.45.

(2*S*)-3-Phenyl-2-{[(*E*)-3-(2-thienyl)-2-propenoyl]amino}propanoic acid (6ea): (61%) white microcrystals (EtOAc/hexane); mp 164-165 °C (Lit.,[32] mp 172-173 °C); ^{1}H NMR (DMSO-d_6): δ 2.85-2.93 (m, 1H), 3.08-3.13 (m, 1H), 4.45-4.62 (m, 1H), 6.44 (d, *J* = 15.4 Hz, 1H), 7.08-7.11 (m, 1H), 7.20-7.31 (m, 5H), 7.32-7.38 (m, 1H), 7.53 (d, *J* = 15.4 Hz, 1H), 7.60-7.61 (m, 1H), 8.43 (d, *J* = 8.0 Hz, 1H), 12.79 (br s, 1H); ^{13}C NMR (DMSO-d_6): 36.8, 53.7, 120.3, 126.5, 128.1, 128.3, 128.4, 129.1, 130.9, 132.3, 137.6, 139.8, 164.7, 173.1. $[\alpha]_D^{25}$ -31.1 (*c* 1.0, EtOH). Anal. Calcd for $C_{16}H_{15}NO_3S$: C, 63.77; H, 5.02; N, 4.65. Found: C, 63.41; H, 5.04; N, 4.99. HPLC analyses: Rt (E, S): 4.0 min., Rt (E, R): 4.8 min.

Preparation of (*E*)-3-{4-[(*tert*-butoxycarbonyl)amino]phenyl}-2-propenoic acid (7): *p*-Aminocinnamic acid (1.63 g, 10 mmol) was dissolved in dioxane (15 mL) and 1 M aqueous NaOH (10 mL) and stirred at 0 °C for 30 min. A solution of di-*t*-butyldicarbonate (2.4 g, 11 mmol) in dioxane (10 mL) was added at 0 °C and the mixture was stirred at 0 °C for 18 h. The reaction mixture was acidified with citric acid and extracted with EtOAc (3 × 30 mL). The combined organic layer was dried over anhydrous Na_2SO_4. Filtration and evaporation of solvent under reduced pressure gave **7** (2.33 g, 98%) as yellow microcrystals (from EtOAc/hexane). Mp 196-197.5 °C (lit.,[28] mp 204 °C); ^{1}H NMR (DMSO-d_6): δ 1.49 (s, 9H), 6.41 (d, *J* = 16.1 Hz, 1H), 7.51-7.56 (m, 3H), 7.59-7.62 (m, 2H), 9.62 (br s, 1H), 12.25 (s, 1H); ^{13}C NMR (DMSO-d_6): 28.1, 79.5, 116.8, 118.0, 127.9, 129.1, 141.6, 143.8, 152.6, 167.9. Anal. Calcd for $C_{14}H_{17}NO_4$: C, 63.86; H, 6.51; N, 5.32. Found: C, 63.55; H, 6.76; N, 5.04.

Preparation of *tert*-butyl *N*-{4-[(*E*)-3-(1*H*-1,2,3-benzotriazole-1-yl)-3-oxo-1-propenyl]phenyl}-carbamate (**8**): To a solution of benzotriazole (0.60 g, 5.06 mmol) in CH_2Cl_2 (20 mL) was added $SOCl_2$ (0.16 g, 1.37 mmol) at rt with stirring. After 30 min., *tert*-Boc-aminocinnamic acid (0.36 g, 1.37 mmol) was added in one portion and stirring was continued for 2 h. The yellow precipitate was filtered off and washed with CH_2Cl_2 (2 × 50 mL). The filtrate was washed with aqueous 2 N NaOH (3 × 60 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure to give **8** in 80% yield as yellow needles (from $CHCl_3$/hexane); mp 124-126 °C; ^{1}H NMR ($CDCl_3$): δ 1.54 (s, 9H), 6.97 (s, 1H), 7.48-7.55

(m, 3H), 7.65-7.71 (m, 3H), 8.05 (d, *J* = 12.9 Hz, 2H), 8.15 (d, *J* = 8.5 Hz, 1H), 8.42 (d, *J* = 8.2 Hz, 1H); ^{13}C NMR ($CDCl_3$): 28.3, 81.1, 113.8, 114.8, 118.3, 120.1, 126.1, 128.6, 130.2, 130.2, 131.5, 141.7, 146.3, 148.4, 152.3, 164.1. Anal. Calcd for $C_{20}H_{20}N_4O_3$: C, 65.92; H, 5.53; N, 15.37. Found: C, 65.65; H, 5.48; N, 15.20.

Preparation of (*S*)-[{(*E*)-3-{4-[(*tert*-Butoxycarbonyl)amino]phenyl}-2-propenoyl)amino]-3-(1*H*-indol-3-yl)propanoic acid (9): A solution of *tert*-butyl *N*-{4-[(*E*)-3-(1*H*-1,2,3-benzotriazole-1-yl) -3-oxo-1-propenyl]phenyl}carbamate (0.091 g, 0.25 mmol) in DME (14 mL) was added at rt to a solution of L-tryptophan (0.051 g, 0.25 mmol) in DME (1.5 mL), water (1.65 mL) and Et_3N (0.168 mL, 1.2 mmol). The reaction mixture was then stirred at rt for 15d. 6 N HCl (4 mL) was then added and the solvent was removed under reduced pressure. The residue was extracted with EtOAc (20 mL), washed with brine (10 mL), and then dried over $MgSO_4$. After removal of the solvent the crude product was purified by column chromatography (silica gel) using EtOAc/hexane (6:4) to give the product **9** in 36% yield. Yellow microcrystals (from EtOAc/hexane); mp 145 ^{o}C; ^{1}H NMR (DMSO-d_6): δ 1.51 (s, 9H), 3.06-3.13 (m, 2H), 4.60-4.65 (m, 1H), 6.62 (d, *J* = 15.7 Hz, 1H), 7.01 (t, *J* = 7.2 Hz, 1H), 7.09 (t, *J* = 7.6 Hz, 1H), 7.19 (s, 1H), 7.31-7.38 (m, 2H), 7.46-7.54 (m, 4H), 7.57-7.60 (m, 1H), 8.35 (d, *J* = 7.7 Hz, 1H), 9.61 (s, 1H), 10.89 (s, 1H), 12.71(br s, 1H); ^{13}C NMR ($CDCl_3$): 27.0, 28.3, 53.6, 81.1, 109.4, 111.4, 117.9, 118.4, 119.6, 122.1, 123.5, 127.7, 128.9, 129.1, 136.1, 139.9, 141.7, 152.2, 167.1, 174.5. HRMS $[M+Na]^+$ Calcd for $C_{25}H_{27}N_3O_5Na$: 472.1843. Found: 472.1838.

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