A Conventional New Procedure for *N*-Acylation of Unprotected Amino Acids

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The preparation of amide derivatives (4) by *N*-acylation of unprotected α-amino acids is easily achieved *via* readily available benzotriazolyl carboxylates (2a—d) or succinimidyl carboxylates (2e—f). These intermediates (2) are prepared from reaction of carboxylic acids (1) with 1-hydroxybenzotriazole (HO-Bt) or *N*-hydroxysuccinimide (HO-Su) in the presence of equimolar amounts of 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride (WSCI). The overall yields of the target compounds (4) were excellent, and this two-stage procedure could be applicable as an alternative procedure for one-pot reaction.

Key words unprotected amino acid; β-aminoalanine; *N*-acylation; 1-hydroxybenzotriazole; *N*-hydroxysuccinimide; 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide

Preparation of *N*-acyl derivatives of amino acids from acyl halides (usually chlorides) and amino acids is well known in organic synthesis, the so-called Schotten–Baumann reaction.[1)] Many of the coupling reagents for the formation of amide bonds in the synthesis of biologically active compounds have been reported.[2)] Recently, a few effective coupling reagents for *N*-acylation of unprotected amino acids have been reported.[3—7)] For instance, the coupling reaction utilizing *N*-acyl benzotriazole derivatives and unprotected amino acids proceeds under mild reaction conditions.[7)]

Recently, we reported that *N*-acyl derivatives of β-aminoalanines were synthesized under Schotten–Baumann reaction conditions with acyl halides.[8,9)] For further structural modification of *N*-acyl derivatives of β-aminoalanines in the search for biologically active compounds, we were in need of a conventional method for the synthesis of some *N*-acyl β-aminoalanines. In the course of our studies, we found that the combination of 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride (WSCI)[3,10)] and 1-hydroxybenzotriazole (HO-Bt)[3)] or 1-hydroxysuccinimide (HO-Su)[3)] for such coupling reactions is effective to obtain the *N*-acylated derivatives from unprotected β-aminoalanines. We report herein a simple conventional method for the *N*-acylation of unprotected amino acids.

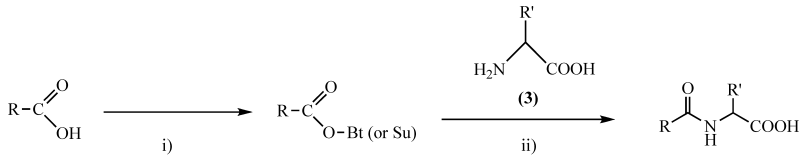
Results and Discussion

As shown in Tables 1 and 2, the use of equimolar amounts of WSCI[10)] gave almost quantitative yields of the desired activated carboxylic acid (**2**), and this procedure may be efficiently exploited in the case of starting acids with an amine functionality [see entries 2, 3 (Table 1) and entries 2, 3, and 5 (Table 2), respectively].

The structures of the activated derivatives (**2a**—**d**) were easily confirmed from the spectroscopic and elemental analysis. Thus IR spectra of compounds **2a**—**d** showed a characteristic absorption band at 1809—1781 cm^{-1} ascribable to the C=O group in the molecules. In the ^{1}H-NMR spectra, four aromatic protons of benzotriazole ring were observed at δ 7.26—8.14 ppm as multiplets. Regarding the benzotriazole ring in compounds **2a**—**d**, six ^{13}C-signals were observed at δ 108.3—143.6 ppm elucidating the representative structure (**2**).

Other assignments of NMR data of the products are given in Experimental. In the case of derivatives (**2e**—**f**), IR spectra showed three absorption bands at 1821—1730 cm^{-1}, ascribable to the two C=O groups in the succinimide ring and a C=O for –COON= groups. Other physical data (FAB-MS and elemental analysis) were in good agreement with the structures (**2**) (see Experimental). These assignments and correlation of ^{1}H- and ^{13}C-NMR signals were supported by 2D spectroscopic analysis.

Table 2 shows the results of reactions of these activated carboxylic acid derivatives (**2**) with unprotected amino acids (**3**). The structures were confirmed by spectroscopic and physical data of the target amide derivatives (**4**). Thus IR spectra (KBr) showed two typical absorption bands at



2a– d : Bt
2e– f : Su

Bt = benzotriazol-1-yl
Su = succinimid-1-yl
WSCI = 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride

i) Bt-OH (or Su-OH), WSCI; ii) aqueous K_2CO_3

Chart 1

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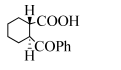
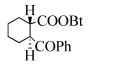
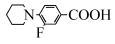
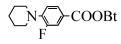
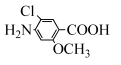
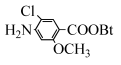
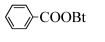
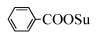
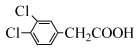
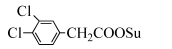
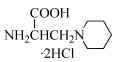
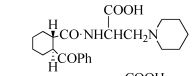
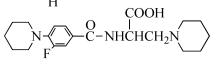
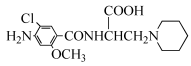
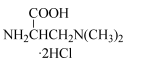
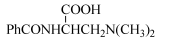
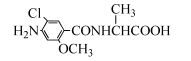
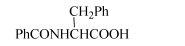
Table 1. Preparation of Activated Carboxylic Acids

Entry	R–COOH (**1**)		Additive	Solvent	t_R	Activated carboxylic acid (**2**)		Yield (%)[a]	mp °C (Solvent)
1		**1a**	HO-Bt	CH_2Cl_2	40 min		**2a**	98	118—124 (AcOEt–hexane)
2		**1b**	HO-Bt	DMF	10 min		**2b**	97	102—103 (iso-Pr_2O)
3		**1c**	HO-Bt	DMF	3 h		**2c**	99	169—170 (dec.) (CH_3CN)
4		**1d**	HO-Bt	CH_2Cl_2	2 min		**2d**	97	84—85 (dec.) (iso-Pr_2O)
5		**1d**	HO-Su	DMF	10 min		**2e**	97	136—138 (dec.)[b] (AcOEt–iso-Pr_2O)
6		**1e**	HO-Su	CH_2Cl_2	20 min		**2f**	99	141—142 (dec.) (iso-PrOH)

a) The isolated products showed one spot by TLC analysis. *b*) Lit.[14] mp 134—136 °C

Table 2. Preparation of *N*-Acylamino Acids from Compounds (**2**)

Entry	Compound (**2**)	Amino acid (**3**)		Solvent	t_R	Product (**4**)		Yield (%)[a]	mp °C (Solvent)
1	**2a**		(**3a**)	CH_2Cl_2/EtOH	5 h		(**4a**)	89[b]	151 (dec.)
2	**2b**	**3a**		CH_3CN	0.5 h		(**4b**)	78[b]	149 (dec.)
3	**2c**	**3a**		CH_2Cl_2	1 d		(**4c**)	86[b]	157 (dec.)
4	**2d**		(**3b**)	CH_2Cl_2	5 h		(**4d**)	76[b]	186 (dec.) (EtOH)
5	**2c**	Ala		DMF	10 min		(**4e**)	90	236 (dec.) (CH_3CN)
6	**2e**	Phe		CH_2Cl_2	2 h		(**4f**)	97	184—185[c] (CH_3COOH)
7	**2f**	Ala		DMF	10 min		(**4g**)	89	169—171 (CH_3CN)

a) The isolated products showed one spot by TLC analysis. *b*) Purified by column chromatography (SiO_2). *c*) Lit.[1] mp 184—185 °C.

1686—1613 and 3292–3194 cm^{-1} attributable to the amide C=O and amide –NH– groups, respectively. In addition to these observations, compounds **4a**, **4c**, and **4d** showed at *ca.* 1600 cm^{-1} ascribable to a carboxylate ($-COO^-$) group, which apparently indicates that these compounds require the twitter ion structure in the solid state.[8] In all ^{1}H-NMR spectra, a characteristic Cα-H proton for α-amino acids could be observed at δ 4.20—5.10 ppm. Other protons were also in good agreement with the represented structures of **4**. In the ^{13}C-NMR spectra of the products **4**, three ^{13}C signals were observed at δ 163.0—175.5, 171.4—176.8, and 47.6—54.4 ppm, ascribable to a –CON= amide, a –COOH, and a Cα carbon, respectively. Full assignments of these data of structures **4** are recorded in Experimental. Other physical data (FAB-MS and elemental analysis) of these compounds are also in good agreement with the representative structure (**4**).

In our experimental results, some of the advantages of combination of HO-Bt (or HO-Su) and WSCI are that the procedure by two-stage coupling reaction is quite conventional and gave isolable stable activated derivatives in excellent yields, and can effect rapid coupling. Coupling reactions with the reported reagents are frequently moisture sensitive, and include some difficulties of isolation and purification processes due to the formation of by-products from the coupling reagents.[3,7]

Preparation of the coupling products by the above procedure gave excellent results in terms of the yield or easy preparation procedure of overall process. In addition, this two-stage procedure is accessible for a new procedure for one-pot amide formation based on activation of carboxylic acids *in situ*. For example, the preparation of **4f** by one-pot reaction starting with **1d** using either HO-Bt or Su-OH can be achieved in excellent yield (see Method A and B in Experimental).[11] The molecular modification by this conventional procedure has a wide range of applications for *N*-acylation of unprotected amino acids. Further synthetic and biological studies on related compounds are in progress.

Experimental

Melting points are uncorrected. IR spectra were measured by Shimadzu FTIR-8100 spectrometer. ^{1}H- and ^{13}C-NMR spectra were obtained on a JEOL JNM A-500 (500 MHz for ^{1}H, 125 MHz for ^{13}C) at 35 °C unless otherwise noted. Chemical shifts are expressed as δ ppm downfield from an internal tetramethylsilane (TMS). Signal assignments were confirmed by ^{1}H--^{1}H correlation spectroscopy (COSY), ^{1}H–^{13}C heteronuclear multiple-quantum coherence (HMQC), or ^{1}H–^{13}C heteronuclear multiple-bond connectivity (HMBC) spectra. FAB-MS were obtained by JEOL JMS-HX110 mass spectrometer. The preparation of racemic β-aminoalanines (**3a**, **b**) as starting materials has already been described in our previous paper.[12] The following abbreviations in brackets were used: cyclohexane ring (Cyhx), piperidine ring (Ppd), pyrrolidine ring (Pyr), and benzotriazole group (Bt).

Isolation of Activated Carboxylic Acid (2). General Procedure At room temperature, 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride (WSCI) (5.5 mmol) was added to a solution of carboxylic acid (**1**) (5.0 mmol) and 1-hydroxybenzotriazole monohydrate (HO-Bt) (5.5 mmol) or 1-hydroxysuccinimide (HO-Su) (5.5 mmol) in dichloromethane (CH_2Cl_2) or dimethylformamide (DMF) and the resulting mixture was stirred until the starting material disappeared. In case of entries 1, 4, and 6 (Table 1), the reaction mixture was washed with water. After drying over Na_2SO_4, evaporation of the solvent gave the desired products (**2a**,**d**, **f**) in almost quantitative yields. In the case of entries 2, 3, and 5 (Table 1), after addition of water the precipitates were collected by filtration or the resulting mixture was extracted with diethyl ether, washed with brine, and dried over Na_2SO_4. Evaporation of the solvent afforded good purity of the compounds (**2b**, **c**, **e**). The products could be used for the next stage without further purification.

1*H*-Benzotriazol-1-yl *trans*-2-Benzoylcyclohexanecarboxylate (2a) FAB-MS (positive) *m/z*: 350 $(M+H)^+$. IR (KBr) cm^{-1}: 1802, 1678. ^{1}H-NMR ($CDCl_3$) δ: 1.56—1.60 (3H, m, Cyhx H-3, H-4, H-5), 1.78—1.84 (1H, m, Cyhx H-6), 1.94—1.99 (1H, m, Cyhx H-4), 2.08—2.14 (1H, m, Cyhx H-5), 2.19—2.25 (1H, m, Cyhx H-3), 2.37—2.44 (1H, m, Cyhx H-6), 3.26 (1H, dd, *J*=11.0, 4.5 Hz, Cyhx H-1), 3.94—3.98 (1H, m, Cyhx H-2), 7.26—7.41 (1H, m, Bt Ar-H), 7.47—7.60 (4H, m, Bt Ar-H×2 and *m*-Ar-H), 7.85 (1H, d, *J*=8.0 Hz, *p*-Ar-H), 7.93 (2H, dd, *J*=8.5, 1.0 Hz, *o*-Ar-H), 8.02 (1H, d, *J*=8.0 Hz, Bt Ar-H). ^{13}C-NMR ($CDCl_3$) δ: 25.3, 25.5 (Cyhx C-4, C-5), 29.1 (Cyhx C-6), 30.0 (Cyhx C-3), 42.4 (Cyhx C-1), 47.9 (Cyhx C-2), 108.8 (Bt Ar-C), 120.2 (Bt Ar-C), 124.7 (Bt Ar-C), 128.4, 128.6, 128.7 (*o*, *m*-Ar-C and Bt Ar-C), 133.4 (*p*-Ar-C), 135.5 (Bt Ar-C), 135.7 (Ar C-1), 143.4 (Bt Ar-C), 171.7 (–COO), 202.1 (COPh). *Anal.* Calcd for $C_{20}H_{19}N_3O_3$: C, 68.75; H, 5.48; N, 12.03. Found: C, 68.72; H, 5.53; N, 12.03.

1*H*-Benzotriazol-1-yl 4-(1-Piperidinyl)-3-fluorobenzoate (2b) FAB-MS (positive) *m/z*: 341 $(M+H)^+$. IR (KBr) cm^{-1}: 1782, 1611. ^{1}H-NMR ($CDCl_3$) δ: 1.66—1.69 (2H, m, Ppd H-4), 1.73—1.78 (4H, m, Ppd H-3, H-5), 3.30—3.33 (4H, m, Ppd H-2, H-6), 7.00 (1H, t, *J*=8.5 Hz, Ar H-2), 7.27—7.45 (2H, m, Bt Ar-H), 7.52—7.55 (1H, m, Bt Ar-H), 7.84 (1H, dd, *J*=4.0, 2.0 Hz, Ar H-5), 7.94—7.97 (1H, m, Ar H-6), 8.08 (1H, d, *J*=8.0 Hz, Bt Ar-H). ^{13}C-NMR ($CDCl_3$) δ: 24.2 (Ppd C-4), 25.8 (Ppd C-3, C-5), 50.9 (Ppd C-2 , C-6), 108.4 (Bt Ar-C), 114.8 (Ar C-1), 117.9 (Ar C-2, *J*=4.0 Hz), 118.4 (Ar C-5, *J*=24.0 Hz), 120.5 (Bt Ar-C), 124.7 (Bt Ar-C), 128.2 (Ar C-6), 128.6 (Bt Ar-C), 129.0 (Bt Ar-C), 143.6 (Bt Ar-C), 146.9 (Ar C-4, *J*=7.0 Hz), 153.6 (Ar C-3, *J*=247.0 Hz), 161.9 (CO). *Anal.* Calcd for $C_{18}H_{17}N_4O_2F$: C, 63.52; H, 5.03; N, 16.46. Found: C, 63.64; H, 5.16; N, 16.34.

1*H*-Benzotriazol-1-yl 4-Amino-5-chloro-2-methoxybenzoate (2c) FAB-MS (positive) *m/z*: 319 $(M+H)^+$. IR (KBr) cm^{-1}: 3422, 3322, 1786, 1636. ^{1}H-NMR (DMSO-d_6) δ: 3.83 (3H, s, OCH_3), 6.46 (1H, s, Ar H-3), 6.58 (2H, br s, NH_2), 7.51, 7.64 (each 1H, dd, *J*=7.0, 1.0 Hz, Bt Ar-H), 7.78 (1H, d, *J*=8.0 Hz, Bt Ar-H), 8.03 (1H, s, Ar H-6), 8.14 (1H, d, *J*=8.0 Hz, Bt Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 55.9 (OCH_3), 97.1 (Ar C-3), 99.0 (Ar C-1), 108.8 (Ar C-5), 109.8 (Bt Ar-C), 119.7 (Bt Ar-C), 125.0 (Bt Ar-C), 127.7 (Bt Ar-C), 128.6 (Bt Ar-C), 132.6 (Ar C-6), 142.7 (Bt Ar-C), 152.6 (Ar C-4), 159.4 (Ar C-2), 161.6 (–COO). *Anal.* Calcd for $C_{14}H_{11}N_4O_3Cl$: C, 52.76; H, 3.48; N, 17.58. Found: C, 52.80; H, 3.60; N, 17.63.

1*H*-Benzotriazol-1-yl Benzoate (2d) FAB-MS (positive) *m/z*: 239 $(M+H)^+$. IR (KBr) cm^{-1}: 1809, 1781. ^{1}H-NMR ($CDCl_3$) δ: 726—7.28 (2H, m, Bt Ar H), 7.53—7.57 (1H, m, Bt Ar H), 7.59—7.63 (2H, m, Ar H-3, H-5), 7.76—7.79 (1H, m, Ar H-4), 8.09—8.11 (1H, m, Bt Ar), 8.27—8.30 (2H, m, Ar H-2, H-6). ^{13}C-NMR ($CDCl_3$) δ: 108.3 (Bt Ar-C), 120.6 (Bt Ar-C), 124.8 (Bt Ar-C), 124.9 (Ar C-1), 128.7 (Bt Ar-C), 128.9 (Bt Ar-C), 129.2 (Ar C-3, C-5), 130.7 (Ar C-2, C-6), 135.5 (Ar C-4), 143.6 (Bt Ar-C), 162.8 (–COO). *Anal.* Calcd for $C_{13}H_9N_3O_2$: C, 65.27; H, 3.79; N, 17.56. Found: C, 65.47; H, 3.98; N, 17.64.

Pyrrolidine-2,5-dion-1-yl Benzoate (2e) FAB-MS (positive) *m/z*: 220 $(M+H)^+$. IR (KBr) cm^{-1}: 1794, 1768, 1732. ^{1}H-NMR ($CDCl_3$) δ: 2.89 (4H, s, Pyr), 7.50—7.53 (2H, m, Ar H-3, H-5), 7.66—7.69 (1H, m, Ar H-4), 8.13—8.15 (2H, m, Ar H-2, H-6). ^{13}C-NMR ($CDCl_3$) δ: 25.7 (Pyr C-3, C-4), 125.2 (Ar C-1), 128.8 (Ar C-3, C-5), 130.5 (Ar C-2, C-6), 134.9 (Ar C-4), 161.9 (–COO), 169.1 (Pyr C-2, C-5). *Anal.* Calcd for $C_{11}H_9NO_4$: C, 60.27; H, 4.14; N, 6.39. Found: C, 60.24; H, 4.24; N, 6.34.

Pyrrolidine-2,5-dion-1-yl 3,4-Dichlorophenylacetate (2f) FAB-MS (positive) *m/z*: 302 $(M+H)^+$. IR (KBr) cm^{-1}: 1821, 1784, 1730. ^{1}H-NMR ($CDCl_3$) δ: 2.83 (4H, s, Pyr), 3.89 (2H, s, CH_2Ph), 7.18—7.19 (1H, m, Ar H-6), 7.43—7.46 (2H, m, Ar H-2, H-5). ^{13}C-NMR ($CDCl_3$) δ: 25.6 (Pyr C-3, C-4), 36.7 (CH_2Ph), 128.6 (Ar C-6), 130.8 (Ar C-5), 131.3 (Ar C-2), 131.4 (Ar C-4), 132.3 (Ar C-3), 132.9 (Ar C-1), 165.9 (–COO), 168.7 (Pyr C-2, C-5). *Anal.* Calcd for $C_{12}H_9NO_4Cl_2$: C, 47.71; H, 3.00; N, 4.64. Found: C, 47.75; H, 3.10; N, 4.66.

Preparation of 2-[(2-Benzoylcyclohexane)carboxamido]-3-(piperidin-1-yl)propanoic Acid (4a) A solution of β-aminoalanine dihydrochloride (**3a**) (0.35 g, 1.43 mmol) in potassium bicarbonate (K_2CO_3) (0.99 g, 7.17 mmol) in H_2O (*ca.* 8 ml) was added to a solution of activated carboxylic acid (**2a**) (0.5 g, 1.43 mmol) in CH_2Cl_2, then EtOH (10 ml) was added to this solution. After stirring for 5 h at room temperature, the reaction mixture was concentrated under reduced pressure and the residue triturated with EtOH to give a solid material. After separation of this product by filtration, the filtrate was concentrated, and purification of the product by silica gel column with ethanol as solvent afford **4a** (0.49 g, 89%). FAB-MS (positive) *m/z*: 387 $(M+H)^+$. IR (KBr) cm^{-1}: 3363, 3262, 1665, 1640, 1607. ^{1}H-NMR ($CDCl_3$) δ: 1.25—2.12 (14H, m, Ppd H-3, H-4, H-5 and Cyhx H-3, H-4, H-5, H-6), 2.72—2.87 (1H, m, Cyhx H-1), 2.92—3.35 (6H, m, Ppd H2, H-6 and CH_2-1-piperidinyl), 3.62—3.67 (1H, m, Cyhx H-6), 4.26—4.30 (1H, m, NHCH-COOH), 5.0—7.0 (1H, br, COOH), 7.47 (1H, dd, *J*=15.0, 7.5 Hz, CONH), 7.50—7.56 (3H, m, *m*, and *p*-Ar-H), 7.96—7.98 (2H, m, *o*-Ar-H). ^{13}C-NMR ($CDCl_3$) δ: 22.1, 22.2, 22.8, 25.5, 25.6, 29.8, 30.0 (Cyhx C-3—C-6 and Ppd C-3—C-5), 46.2 (Cyhx C-1), 46.9 (Cyhx C-2), 49.2 (CONHCHCOOH), 53.9 (×2) (Ppd C-2, C-6), 56.9 (CH_2-1-piperidinyl), 128.5 (×3), 128.6 (Ar C-2, C-3, C-5, C-6), 132.7 (Ar C-4), 136.5 (Ar C-1), 172.3 (COOH), 175.4 (CONH), 203.2 (COPh). *Anal.* Calcd for $C_{22}H_{30}N_2O_4\cdot H_2O$: C, 65.32; H, 7.97; N, 6.93. Found: C, 65.25; H, 8.00; N, 6.92.

Preparation of 2-[3-Fluoro-4-(piperidin-1-yl)benzamido]-3-(piperidin-1-yl)propanoic Acid (4b) A solution of β-aminoalanine dihydrochloride (**3a**) (0.43 g, 1.76 mmol) and K_2CO_3 (1.21 g, 8.76 mmol) in H_2O (*ca.* 10 ml) was added to a solution of activated carboxylic acid (**2b**) (0.6 g, 1.76 mmol) in acetonitrile (CH_3CN), and the mixture was stirred for 0.5 h. After concentration under reduced pressure, 1 N-hydrochloric acid (1 N-HCl) was added to make the solution pH *ca.* 5. Evaporation of the solvent gave a viscous residue, and then trituration of this product with EtOH afforded solid material. After separation of this material by filtration, the filtrate was concentrated and purified through a silica gel column (ethanol→ethanol/ammonia as solvent) to give **4b** (0.52 g, 78%). FAB-MS (positive) *m/z*: 378 $(M+H)^+$. IR (KBr) cm^{-1}: 3390, 3260, 1650, 1617. ^{1}H-NMR (DMSO-d_6) δ: 1.43—1.46 (2H, m, Ppd H-4), 155—1.67 [10H, m, (Ppd H-3×2, H-5×2 and H-4)×2], 2.69—2.94 [10H, m, (Ppd H-2×2, H-6×2)×2 and CH_2-1-piperidinyl], 4.50—4.51 (1H, m, NHCHCOOH), 4.65 (1H, br, COOH), 7.05 (1H, t, *J*=8.5 Hz, Ar H-2), 7.58—7.63 (2H, m, Ar H-5, H-6), 8.24 (1H, d, *J*=7.0 Hz, NH). ^{13}C-NMR (DMSO-d_6) δ: 22.7, 23.6 (Ppd C-4), 24.5 (×2), 25.4 (×2) (Ppd C-3, C-5), 48.6 (CHCOOH), 51.8 (×4) (Ppd C-2, C-6), 64.8 (CH_2-1-piperidinyl), 114.8, (Ar C-5), 118.3 (Ar C-2), 124.0 (Ar C-6), 126.7 (Ar C-1), 143.0 (Ar C-4), 153.7 (d, *J*=244.1 Hz, Ar C-3), 164.5 (CONH), 172.4 (COOH). *Anal.* Calcd for $C_{20}H_{28}N_3O_3F\cdot 0.3H_2O$: C, 62.74; H, 7.53; N, 10.98. Found: C, 62.73; H, 7.70; N, 10.75.

Preparation of 2-(4-Amino-5-chloro-2-methoxy)benzamido-3-(piperidin-1-yl)propanoic Acid (4c) Reaction of activated carboxylic acid (**2c**) (0.5 g, 1.57 mmol) with β-aminoalanine dihydrochloride (**3a**) (0.39 g, 1.59 mmol) was carried out in the same manner described above in the preparation of **4b**. The isolated crude product was purified by column chromatography (SiO_2/ethanol) to give (**4c**) (0.48 g, 86%). FAB-MS (positive) *m/z*: 356 $(M+H)^+$. IR (KBr) cm^{-1}: 3460, 3368, 3206, 1680, 1624, 1590. ^{1}H-NMR (DMSO-d_6) δ: 1.48 (2H, t, *J*=5.5 Hz, Ppd H-4), 1.62—1.63 (4H, m, H-3, Ppd H-5), 2.80—2.84 (3H, m, Ppd H-2, H-6 and CH_AH_B-1-piperidinyl), 2.91—2.94 (2H, m, Ppd H-2, H-6), 3.15 (1H, dd, *J*=12.0, 6.0 Hz, CH_AH_B-1-piperidinyl), 3.86 (3H, s, OCH_3), 4.0—5.6 (1H, br, COOH), 4.30—4.32 (1H, m, NHCHCOOH), 5.97 (2H, s, NH_2), 6.52 (1H, s, Ar H-3), 7.73 (1H, s, Ar H-6), 8.58 (1H, d, *J*=5.0 Hz, NH). ^{13}C-NMR (DMSO-d_6) δ: 22.3 (Ppd C-4), 24.2 (Ppd C-3, C-5), 48.7 (NHCHCOOH), 52.6 (Ppd C-2, C-6), 56.0 (OCH_3), 57.3 (CH_2-1-piperidinyl), 97.6 (Ar C-3), 109.0 (Ar C-1), 109.6 (Ar C-5), 131.5 (Ar C-6), 148.7 (Ar C-4), 157.7 (Ar

C-2), 163.3 (CONH), 171.9 (COOH). *Anal.* Calcd for $C_{16}H_{22}N_3O_4Cl \cdot 1.3H_2O$: C, 50.67; H, 6.54; N, 11.08. Found: C, 50.80; H, 6.36; N, 11.01.

Preparation of 2-Benzamido-3-(dimethylamino)propanoic Acid (4d) A solution of β-aminoalanine dihydrochloride (**3b**) (0.239 g, 1.0 mmol) and K_2CO_3 (0.691 g, 5.0 mmol) in H_2O (*ca.* 4 ml) was added to the solution of activated carboxylic acid (**2d**) (0.239 g, 1.0 mmol) in CH_2Cl_2. After stirring for 5 h at 30 °C, the reaction mixture was concentrated under reduced pressure and the residue triturated with EtOH to give a solid material. After separation of this product by filtration, the filtrate was concentrated and eluted by a silica gel column with methanol as solvent. The collected product was neutralized by addition of HCl/EtOH (*ca.* pH=7) and dried under reduced pressure. The residue was dissolved into EtOH and insoluble material removed by filtration, and evaporation of the filtrate gave **4d** (0.18 g, 76%). Analytical sample was obtained by recrystallization from EtOH. FAB-MS (positive) *m/z*: 237 $(M+H)^+$. IR (KBr) cm^{-1}: 3395, 1653, 1620. ^{1}H-NMR (D_2O) δ: 3.01 (6H, s, $CH_3\times2$), 3.50 (1H, dd, J=13.0, 8.0 Hz, CH_AH_B–N=), 3.65 (1H, dd, J=13.0, 6.5 Hz, CH_AH_B–N=), 4.83 (1H, dd, J=8.0, 6.5 Hz, NHCHCOOH), 7.57 (2H, t, J=8.0 Hz, *m*-Ar-H), 7.66 (1H, t, J=7.0 Hz, *p*-Ar-H). 7.86—7.88 (2H, m, *o*-Ar-H). ^{13}C-NMR (D_2O) δ: 46.2 ($CH_3\times2$), 53.0 (CONHCHCOOH), 61.7 (CH_2N=), 130.2 (Ar C-3, C-5), 131.7 (Ar C-2, C-6), 135.4 (Ar C-4), 135.6 (Ar C-1), 173.4 (CONH), 176.8 (COOH). *Anal.* Calcd for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83; N, 11.86. Found: C, 60.92; H, 6.86; N,11.89.

Preparation of 2-(4-Amino-5-chloro-2-methoxy)benzamidopropanoic Acid (4e) A solution of DL-alanine (0.20 g, 2.25 mmol) and K_2CO_3 (0.95 g, 6.88 mmol) in H_2O (*ca.* 7 ml) was added to a solution of activated carboxylic acid (**2c**) (0.72 g, 2.26 mmol) in DMF, then the mixture was stirred for 10 min at room temperature. After concentration of the solvent, the residue was acidified with 1 N-HCl and the precipitated material collected by filtration. The precipitated materials were washed with AcOEt (100 ml) to give a high purity of **4e** (0.55 g, 90%). Analytical sample was obtained by recrystallization from acetonitrile. FAB-MS (positive) *m/z*: 273 $(M+H)^+$. IR (KBr) cm^{-1}: 3472, 3391, 3320, 3196, 1719, 1624. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (3H, d, J=7.0 Hz, CH_3), 2.50 (3H, s, OCH_3), 4.38—4.44 (1H, m, CONHCH), 5.95 (2H, s, NH_2), 6.51 (1H, s, Ar H-3), 7.71 (1H, s, Ar H-6), 8.19 (1H, s, CONH), 12.5 (1H, br, COOH). ^{13}C-NMR (DMSO-d_6) δ: 17.9 (CH_3), 47.9 (NHCHCOOH), 56.0 (OCH_3), 97.6 (Ar C-3), 109.1 (Ar C-1), 109.7 (Ar C-5), 131.5 (Ar C-6), 148.7 (Ar C-4), 157.5 (Ar C-2), 163.0 (CONH), 174.1 (COOH). *Anal.* Calcd for $C_{11}H_{13}N_2O_4Cl$: C, 48.45; H, 4.81; N, 10.27. Found: C, 48.55; H, 4.84; N, 10.49.

Compound (**4e**) was also obtained from the reaction of **2g**[13] with DL-alanine. Thus a solution of DL-alanine (0.20 g, 2.25 mmol) and K_2CO_3 (0.95 g, 6.88 mmol) in H_2O (8 ml) was added to a solution of activated carboxylic acid (**2g**) (0.67 g, 2.25 mmol) in DMF, EtOH (10 ml) was added to the solution, then the mixture was stirred for 0.5 h at room temperature. After concentration of the solvent, the residue was acidified with 1 N-HCl and the precipitated material collected by filtration to give **4e** (0.56 g, 92%).

Preparation of 2-Benzamido-3-phenylpropanoic Acid (4f) A solution of DL-phenylalanine (0.38 g, 2.30 mmol) and K_2CO_3 (0.95 g, 6.88 mmol) in H_2O (*ca.* 7 ml) was added to the solution of activated carboxylic acid (**2e**) (0.5 g, 2.28 mmol) in CH_2Cl_2 and stirred for 2 h. After removal of the solvent under reduced pressure, the residue was acidified with 1 N-HCl. The precipitate was extracted with AcOEt, washed with brine, then dried over Na_2SO_4. Evaporation of the solvent gave a high purity of **4f** (0.6 g, 97%). Analytical sample was obtained from recrystallization from acetic acid. FAB-MS (positive) *m/z*: 270 $(M+H)^+$. IR (KBr) cm^{-1}: 3328, 1721, 1613. ^{1}H-NMR ($CDCl_3$) δ: 3.24—3.27 (1H, m, CH_AH_B-Ph), 3.34—3.38 (1H, m, CH_AH_B-Ph), 5.06—5.10 (1H, m, CONHCHCOOH), 6.60 (1H, d, J=7.5 Hz, NH), 6.95 (1H, br, COOH), 7.19—7.68 (10H, m, Ar-H). ^{13}C-NMR ($CDCl_3$) δ: 37.3 (CH_2Ph), 53.7 (NHCHCOOH), 127.1, 127.3, 128.7 (×2), 129.4, 132.0 (Ar C2—C5), 133.5, 135.6, (Ar-C1), 167.8 (CONH), 174.8 (COOH). *Anal.* Calcd for $C_{16}H_{15}NO_3 \cdot 0.1H_2O$: C, 70.89; H, 5.65; N, 5.17. Found: C, 70.85; H, 5.74; N, 5.06.

Preparation of 2-[2-(3,4-Dichlorophenyl)acetamido]propanoic Acid (4g) A solution of DL-alanine (0.147 g, 1.65 mmol) and K_2CO_3 (0.69 g, 5.00 mmol) in H_2O (*ca.* 6 ml) was added to the solution of activated carboxylic acid (**2f**) (0.50 g, 1.66 mmol) in DMF and stirred for 10 min at room temperature. Precipitated material by addition of water was collected by filtration to afford **4g** (0.40 g, 89%). Analytical sample was obtained by recrystallization from CH_3CN. FAB-MS (positive) *m/z*: 276 $(M+H)^+$. IR (KBr) cm^{-1}: 3295, 1748, 1612. ^{1}H-NMR (DMSO-d_6) δ: 1.27 (3H, s, CH_3), 3.17 (1H, br, COOH), 3.49 (2H, s, CH_2Ph), 4.20 (1H, t, J=7.3 Hz, CONHCH), 7.24—7.26 (1H, m, Ar-H), 7.53—7.55 (2H, m, Ar-H), 8.40 (1H, d, J=7.3 Hz, NH). ^{13}C-NMR (DMSO-d_6) δ: 17.1 (CH_3), 40.5 (CH_2Ph), 47.6 (CONHCHCOOH), 129.0 129.4, 130.1, 130.5, 130.9 (Ar-C), 137.3 (Ar C-1), 168.9 (CONH), 173.9 (COOH). *Anal.* Calcd for $C_{11}H_{11}NO_3Cl_2$: C,47.85; H, 4.02; N, 5.07. Found: C, 47.98; H, 4.03; N, 5.10.

One-Pot Synthesis of 4f from 1d. (Method A) A combination of consecutive steps that saves time and makes the procedure conventional was devised. The procedure can be depicted as follows: To a stirred solution of carboxylic acid (**1d**) (0.5 g, 4.10 mmol) in CH_2Cl_2 (5 ml) was added HO-Su (0.52 g, 4.51 mmol) and WSCI (0.86 g, 4.51 mmol) at 35 °C and the resulting mixture was stirred for 10 min. To this solution was added a solution of DL-phenylalanine (0.677 g, 4.10 mol) and K_2CO_3 (3.40 g, 24.6 mmol) in H_2O (*ca.* 25 ml) then the mixture was kept stirred at 35 °C for 3 h. After removal of the solvent under reduced pressure, the residue was acidified with 1 N-HCl. The precipitate was extracted with AcOEt, washed with water and brine, then dried over Na_2SO_4. Evaporation of the solvent gave (**4f**) (1.05 g, 95%).

(Method B) To a stirred solution of carboxylic acid (**1d**) (0.16 g, 1.33 mmol) in CH_2Cl_2 (3 ml) was added HO-Bt (0.27 g, 1.74 mmol) and WSCI (0.33 g, 1.74 mmol) at 35 °C and the resulting mixture was stirred for 10 min. To this solution was added a solution of DL-phenylalanine (0.22 g, 1.33 mmol) and K_2CO_3 (1.10 g, 7.97 mmol) in H_2O (*ca.* 8 ml) then the mixture was kept stirred at 35 °C for 3 h. After removal of the solvent under reduced pressure, the residue was acidified with concentrated HCl. The precipitate was extracted with AcOEt, washed with concentrated HCl, water, and brine, then dried over Na_2SO_4. Evaporation of the solvent gave (**4f**) (0.30 g, 84%).

References and Notes

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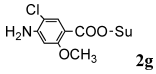
13) This activated intermediate (**2g**) was obtained in a similar manner as that described for the general procedure of compound (**2**). Compound (**2g**); mp 238 °C (dec.) (CH_3CN). FAB-MS (positive) *m/z*: 298 $(M+H)^+$. IR (KBr) cm^{-1}: 1796, 1773, 1720. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 2.83 (4H, s, Pyr), 3.78 (3H, s, OCH_3), 6.52 (1H, s, Ar H-3), 6.60 (2H, br s, NH_2), 7.73 (1H, s, Ar H-6). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 25.4 (×2) (Pyr C-3, C-4), 55.8 (OCH_3), 97.3 (Ar C-3), 100.0 (Ar C-1), 108.4 (Ar C-5), 132.6 (Ar C-6), 152.0 (Ar C-4), 158.6 (Ar C-2), 161.0 (–COO), 170.6 (×2) (Pyr C-2, C-5). *Anal.* Calcd for $C_{12}H_{11}N_2O_5Cl$: C, 48.26; H, 3.71; N, 9.38. Found: C, 48.25; H, 3.78; N, 9.51.



2g

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