Fast and Convenient Synthesis of α-N-Protected Amino Acid Hydrazides

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Abstract: A fast, simple and convenient synthesis of α-N-protected amino acid hydrazides is reported. The procedure involves the reaction between hydrazine monohydrate and the mixed anhydride obtained from an α-N-protected amino acid and ethyl chloroformate. When methylhydrazine was used, the reaction showed a good selectivity, yielding a mixture of 1-acyl-1-methylhydrazine and 1-acyl-2-methylhydrazine in a ratio 87:13. In contrast, when the reaction was carried out with phenylhydrazine, only 1-acyl-2-phenylhydrazine was obtained. No significant difference in reactivity was observed with the different amino acids and N-protection introduced.

Key words: amino acids, hydrazides, regioselectivity, anhydrides, protecting groups

Amino acid hydrazides are important derivatives in peptide synthesis in that they may be used not only as carboxy-activating groups when transformed into the corresponding acid azides[1] or phenyldiimides,[2] but also as carboxy-protecting groups when they are obtained from alkoxycarbonylhydrazines[3] or phenylhydrazine.[4] Moreover, they are involved in the synthesis of azapeptides,[5] a class of backbone-modified peptides that has become important in pharmaceutical chemistry.

Among all the reported procedures, the most commonly cited synthesis of α-amino acid hydrazides involves the reaction of hydrazine with the methyl,[6] ethyl[7] (used also in connection with monoalkylhydrazines)[3c,8] or hydroxychloropropyl[9] ester of the α-amino protected acids or peptides.[3d,g,i,10] Esters have also been employed in the α-amino free form in combination with hydrazine[11] and phenylhydrazine.[12] All these methods require long reaction times (from several hours to three days) and the availability of esters which are more expensive than the corresponding α-amino acids.

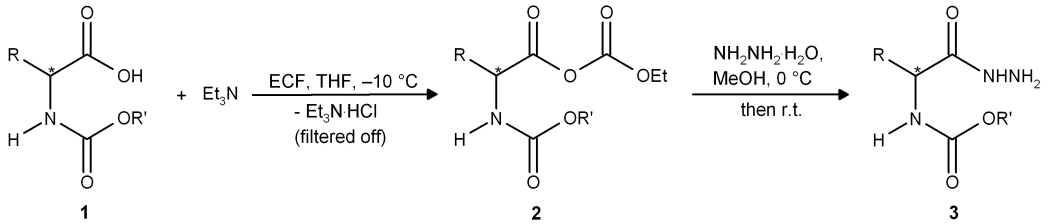
Other synthetic methods utilize hydrazine in connection with amino protected carboxy activated derivatives, such as acid chlorides,[11] and trimethylsilyl[13] or hydroxysuccinimide[14] esters. These procedures are unsatisfactory to some extent, such as the use of strict reaction conditions and/or toxic reagents.

The reaction of α-*N*-acyl-amino acids with the less reactive phenylhydrazine is carried out in the presence of papain,[2a–c,4a,15] DCC–1-hydroxybenzotriazole[4c] or starting from the corresponding acid chloride.[16]

Surprisingly, to the best of our knowledge, the mixed carboxylic–carbonic anhydrides, prepared from N-protected amino acids and alkyl chloroformates, have been used exclusively in combination with alkoxycarbonylhydrazides.[3d,f,g,i] Since these mixed anhydrides can be easily and rapidly obtained, we decided to utilize them in connection with hydrazine.

The simple and convenient synthesis of α-N-protected amino acid hydrazides **3** here reported, involves two steps (Scheme 1; Table 1): the first step of the reaction was the preparation of the mixed anhydride **2** which was obtained by reacting the triethylammonium salt of α-N-protected amino acid **1** with ethyl chloroformate (ECF) in THF at –10 °C. After a few minutes, $Et_3N{\cdot}HCl$ was filtered off and the filtrate, second step, was slowly added to a solution of $NH_2NH_2{\cdot}H_2O$ in MeOH at 0 °C. The resulting mixture was stirred at room temperature affording, after workup, **3** in 80–90% yield.

In order to optimize the reaction conditions, we examined the following parameters using **1ea** as a model substrate: amount of $NH_2NH_2{\cdot}H_2O$, order of addition of ECF, amount of ECF and Et_3N, and reaction time required to accomplish the two steps. On the basis of this study, the synthesis of the mixed anhydride **2ea** was carried out by



Scheme 1

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Table 1 Identity of Compounds **1–3**

Compounds **1–3**	R	R′	Compounds **1–3**	R	R′
aa	Me_2CH	Et	**ea**	$PhCH_2$	Et
ab	Me_2CH	Bn	**eb**	$PhCH_2$	Bn
ba	Me_2CHCH_2	Et	**fa**	Ph	Et
ca	$NH_2COCH_2CH_2$	Et	**ga**	1*H*-indole-3-CH_2	Et
cb	$NH_2COCH_2CH_2$	Bn	**gc**	1*H*-indole-3-CH_2	*t*-Bu
da	$CH_3SCH_2CH_2$	Et			

adding a solution of **1ea** and Et_3N (1.05 equivalents) in THF to a solution of ECF (1.05 equivalents) in THF at –10 °C. The reaction mixture was stirred ten additional minutes at –10 °C, $Et_3N{\cdot}HCl$ was filtered off and the filtrate was slowly added to a solution of $NH_2NH_2{\cdot}H_2O$ (2.0 equivalents) in MeOH at 0 °C. The reaction mixture was allowed to warm up to r.t. and stirred for one hour furnishing, after work-up, the hydrazide **3ea** in a spectroscopically pure form without any further purification in a 91% overall yield.

Following this procedure we prepared all the hydrazides **3** depicted in the Table 1.

The protection of the α-amino function of tyrosine via ECF produced *N*,*O*-dicarbethoxytyrosine (**1ha**)[17] but, when **1ha** was used to prepare the corresponding hydrazide **3ha**, ^1H NMR analysis of the crude reaction mixture showed the presence of two compounds: **3ha**, and the corresponding O-deprotected hydrazide **3ha′** promoted by the action of excess NH_2NH_2. Thus, to improve the conversion of **3ha** into **3ha′**, two additional equivalents of $NH_2NH_2{\cdot}H_2O$ were added at the end of the second step of the reaction and the stirring was continued for two hours at room temperature.

The results obtained and some properties of **3** are reported in Table 2.

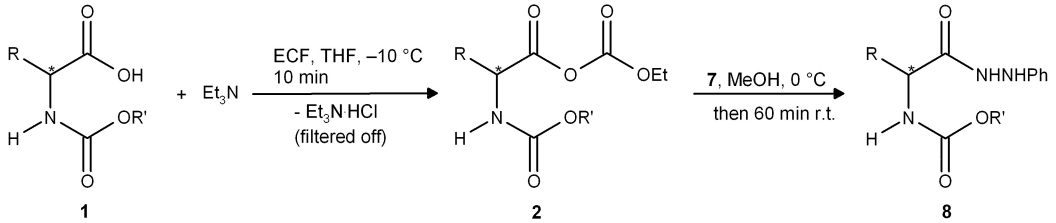
Since several authors reported[18] that the acylation of methylhydrazine (**4**) with acid anhydrides produces chiefly 1-acyl-1-methylhydrazines, while acylation with esters gives chiefly 1-acyl-2-methylhydrazines, we tested our methodology by reacting **1ea** with **4**. The ^1H NMR analysis of the reaction mixture after work up allowed us to distinguish the two isomers by means of their *N*-methyl signals: 1-(*N*-ethoxycarbonyl-L-phenylalanyl)-1-methylhydrazine (**5**) showed two singlets, indicative of the *syn*–*anti* isomerism characteristic of amides, at δ = 3.10 and 3.05, and 1-(*N*-ethoxycarbonyl-L-phenylalanyl)-2-methylhydrazine (**6**), a singlet at δ = 2.41. The integration of these peaks evidenced a good selectivity towards the formation of **5** in a ratio 87:13.

Table 2 Yields and Some Properties of Hydrazides **3** Prepared

Product[a]	Yield (%)[b]	Mp (°C) (Lit.)	$[\alpha]_D^{20}$ (*c*, MeOH)
3aa	87	160	–15.5 (1.0)
3ab	93	178 (178)[6b]	–12.6 (1.0)
3ba	86	74 (56–59)[6i]	+17.8 (1.1)
3ca	80	118	–13.7 (1.1)
3cb	81	180 (173)[6f]	–22.7 (1.1)
3da	92	77	–11.8 (1.0)
3ea	91	124	+15.0 (1.0)
3eb	90	165 (164–166)[9]	+10.3 (1.0)
3fa	89	132	–75.0 (1.0)
3ga	90	155	+19.0 (1.0)
3gc	89	150	+14.7 (1.0)
3ha′	90	142	+20.0 (1.0)

[a] Satisfactory microanalyses obtained: C ± 0.16, H ± 0.10, N ± 0.14.
[b] Yields refer to pure isolated products.

When our procedure was carried out with the less reactive phenylhydrazine (**7**), only the 1-acyl-2-phenylhydrazines **8** were obtained in good yields (Scheme 2, Tables 3 and 4) using 1.5 mol equivalents of **7**.



Scheme 2

Table 3 Identity of Compounds **1**, **2**, and **8**

Compounds **1**, **2**, **8**	R	R′	Compounds **1**, **2**, **8**	R	R′
aa	Me_2CH	Et	**ca**	$NH_2COCH_2CH_2$	Et
bb	Me_2CHCH_2	Bn	**ea**	$PhCH_2$	Et

We did not observe any significant difference in reactivity among the different N-protecting groups introduced into the α-amino function. The HPLC analysis of the hydrazides **3** and **8** using a chiral column showed that no racemization occurred during the reaction. When the hydrazides **3** were analysed by GC-MS using a chiral column, we observed, in addition to the L-enantiomer of **3**, a peak whose spectrum was consistent with the product of thermal cyclization of the hydrazide, namely 5-substituted-1,2,4-triazinane-3,6-dione, generated in the injector of the gas chromatograph. The amount of this compound increased with the rising temperature of the injector.

Table 4 Yields and Some Properties of Phenylhydrazides **8** Prepared

Product[a]	Yield (%)[b]	Mp (°C)	$[\alpha]_D^{20}$ (*c*, MeOH)
8aa	80	190	–20.0 (1.0)
8bb	84	138	–18.3 (1.0)
8ca	89	220	–20.8 (1.0)
8ea	82	187	–13.5 (1.0)

[a] Satisfactory microanalyses obtained: C ± 0.11, H ± 0.13, N ± 0.14.
[b] Yields refer to pure isolated products.

Data pertinent to the synthesized hydrazides **3** and **8** are collected on Table 5.

To summarize, we have presented here a fast, simple, and versatile method for the preparation of α-N-protected amino acid hydrazides, an important class of organic compounds.

Table 5 Spectroscopic Data of Hydrazides **3** and **8**

Product	IR (KBr) (cm^{-1})	^{1}H NMR δ, *J* (Hz)	^{13}C NMR δ	MS *m*/*z* (%)
3aa	3307, 2976, 2964, 1674, 1649, 1542, 1443, 1296, 1246, 1047, 931, 776, 676, 580	0.95 [d, 6 H, *J* = 6.7, $CH(CH_3)_2$], 1.25 (t, 3 H, *J* = 7.0, CH_2CH_3), 2.08 [app sext, 1 H, *J* = 6.7, $CH(CH_3)_2$], 3.80 (br s, 2 H, NH_2), 3.90–4.02 (m, 1 H, *CH), 4.12 (q, 2 H, *J* = 7.0, CH_2), 5.60 (br d, 1 H, *J* = 9.1, *CHNH), 8.18 (br s, 1 H, $NHNH_2$)[a]	14.5, 19.2, 30.8, 59.2, 61.3, 156.7, 172.3[a]	203 (M^+, 1), 172 (18), 144 (100), 129 (21), 116 (13), 98 (20), 72 (63), 70 (10), 55 (26)
3ab	3316, 3251, 2937, 1685, 1658, 1635, 1539, 1295, 1244, 1037, 705, 656, 594	0.93 and 0.94 [2 d, 6 H, *J* = 6.8, 6.8, $CH(CH_3)_2$], 2.09 [app sext, 1 H, *J* = 6.8, $CH(CH_3)_2$], 3.69 (br s, 2 H, NH_2), 3.93 (dd, 1 H, *J* = 8.5, 7.0, *CH), 5.05 and 5.13 (AB system, 2 × d, 2 H, *J* = 12.6, CH_2), 5.51 (br d, 1 H, *J* = 8.5, *CHNH), 7.32 (app s, 5 H, H_{arom}), 7.69 (br s, 1H, $NHNH_2$)[a]	18.2, 18.9, 30.1, 59.0, 65.2, 127.4, 127.5, 128.1, 155.8, 170.2[a]	265 (M^+, 1), 234 (3), 162 (2), 129 (7), 122 (11), 108 (16), 107 (13), 91 (100), 79 (9), 77 (5), 72 (8)
3ba	3307, 2959, 1689, 1653, 1606, 1539, 1286, 1250, 1045, 677	0.92 and 0.94 [2 d, 6 H, *J* = 5.9, 5.9, $CH(CH_3)_2$], 1.23 (t, 3 H, *J* = 7.0, CH_2CH_3), 1.46–1.78 [m, 3 H, $CH_2CH(CH_3)_2$], 4.00–4.41 (m, 5 H, OCH_2, NH_2, *CH), 5.95 (br d, 1 H, *J* = 8.9, *CHNH), 8.70 (br s, $NHNH_2$)[a]	14.4, 21.6, 22.7, 24.5, 41.4, 51.8, 61.0, 156.4, 173.2[a]	217 (M^+, 1), 186 (22), 172 (4), 158 (100), 102 (27), 86 (24), 74 (11), 72 (22), 58 (26), 44 (72), 43 (52), 41 (31)
3ca	3422, 3301, 1692, 1653, 1616, 1531, 1408, 1253, 1060, 779, 667	1.13 (t, 3 H, *J* = 7.0, CH_3), 1.62–1.90 (m, 2 H, *$CHCH_2$), 1.98–2.18 (m, 2 H, CH_2CO), 3.83–4.03 (m, 3 H, *CH, OCH_2), 4.40 (br s, 2 H, $NHNH_2$), 6.67 (br s, 1 H, *CHNH), 7.05 (br s, 1 H, $NHNH_2$), 7.29 (br s, 2 H, NH_2CO)[b]	14.5, 27.9, 31.4, 53.2, 59.8, 155.9, 170.9, 173.9[b]	232 (M^+, 1), 216 (6), 215 (5), 187 (2), 173 (4), 170 (5), 128 (22), 99 (60), 98 (43), 84 (100)
3cb	3552, 3432, 3314, 1678, 1665, 1635, 1539, 1277, 1251, 1052, 749, 680	1.60–1.98 (m, 2 H, *$CHCH_2$), 2.03–2.21 (m, 2 H, CH_2CO), 3.87–4.04 (m, 1 H, *CH), 4.10 (br s, 2 H, $NHNH_2$), 5.01 (app s, 2 H, $PhCH_2$), 6.69 (br s, 1 H, *CHNH), 7.22 (br s, 2 H, $CONH_2$), 7.23–7.51 (m, 5 H, H_{arom}), 9.06 (br s, 1 H, $NHNH_2$)[b]	27.9, 31.4, 53.2, 65.4, 127.6, 127.7, 128.3, 136.9, 155.7, 170.8, 173.7[b]	294 (M^+, 1), 277 (2), 261 (1), 191 (2), 174 (5), 169 (17), 108 (19), 107 (13), 91 (100), 84 (10), 79 (15), 44 (10)
3da	3299, 2980, 2918, 1691, 1653, 1644, 1539, 1285, 1251, 1056, 680	1.24 (t, 3 H, *J* = 7.0, CH_2CH_3), 1.81–2.15 (m, 2 H, *$CHCH_2$), 2.09 (s, 3 H, CH_3S), 2.55 (app t, 2 H, *J* = 7.0, CH_2S), 3.91–4.55 (m, 5 H, *CH, OCH_2, NH_2), 6.33 (br d, 1 H, *J* = 8.8, *CHNH), 8.30 (br s, 1 H, $NHNH_2$)[a]	14.3, 15.1, 29.9, 31.6, 52.2, 61.1, 156.4, 172.1[a]	235 (M^+, 17), 204 (30), 176 (89), 161 (100), 128 (15), 61 (16)

Table 5 Spectroscopic Data of Hydrazides **3** and **8** (continued)

Product	IR (KBr) (cm^{-1})	^1H NMR δ, *J* (Hz)	^{13}C NMR δ	MS *m*/*z* (%)
3ea	3295, 2979, 1693, 1657, 1538, 1290, 1265, 1246, 1051, 751, 703, 675	1.18 (t, 3 H, *J* = 7.2, CH_3), 2.99 and 3.07 (AB of ABX, 2 H, *J* = 14.1, 7.3, 7.0, $PhCH_2$), 3.66 (br s, 2 H, NH_2), 4.05 (q, 2 H, *J* = 7.1, OCH_2), 4.42 (app q, 1 H, *J* = 7.0, *CH), 5.77 (d, 1 H, *J* = 8.8, *CHN*H*), 7.12–7.36 (m, 5 H, H_{arom}), 8.09 (br s, 1 H, N*H*NH_2)[a]	14.4, 38.5, 54.8, 61.3, 126.9, 128.6, 129.1, 136.3, 156.2, 171.8[a]	251 (M^+, 1), 220 (19), 192 (92), 177 (8), 160 (20), 148 (18), 131 (21), 120 (100), 103 (19), 91 (51)
3eb	3316, 1688, 1654, 1609, 1535, 1264, 1250, 1041, 745, 697	3.03 (d, 2 H, *J* = 7.1, *CHCH_2), 3.83 (br s, 2 H, NH_2), 4.39 (app q, 1 H, *J* = 7.3, *CH), 4.99 and 5.07 (AB system, 2 × d, 2 H, *J* = 12.2, OCH_2), 5.60 (d, 1 H, *J* = 8.5, *CHN*H*), 7.09–7.38 (m, 10 H, H_{arom}), 7.64 (br s, 1 H, N*H*NH_2)[a]	38.4, 55.0, 67.1, 127.1, 127.9, 128.2, 128.5, 128.7, 129.1, 135.9, 136.1, 155.9, 171.6[a]	313 (M^+, 1), 282 (2), 205 (1), 178 (2), 177 (3), 131 (5), 122 (18), 120 (21), 108 (15), 107 (10), 91 (100)
3fa	3307, 2983, 1685, 1653, 1540, 1256, 1052, 696	1.22 (t, 3 H, *J* = 7.1, CH_3), 3.89 (br s, 2 H, NH_2), 4.10 (q, 2 H, *J* = 7.1, CH_2), 5.31 (d, 1 H, *J* = 7.4, *CH), 6.22 (br d, 1 H, *J* = 7.4, *CHN*H*), 7.26–7.48 (m, 5 H, H_{arom}), 8.07 (br s, 1 H, N*H*NH_2)[a]	14.4, 57.3, 61.4, 127.0, 128.4, 128.9, 137.5, 156.1, 171.0[a]	237 (M^+, 1), 206 (10), 178 (100), 147 (16), 134 (16), 118 (15), 106 (76), 104 (28), 79 (32), 77 (21)
3ga	3348, 3278, 1700, 1653, 1646, 1558, 1539, 1534, 1522, 1258, 750, 651	1.10 (t, 3 H, *J* = 6.8, CH_3), 2.90 and 3.05 (AB of ABX, 2 H, *J* = 14.4, 8.8, 5.0, *CHCH_2), 3.33 (br s, 2 H, NH_2), 3.90 (q, 2 H, *J* = 6.8, OCH_2), 4.13–4.33 (m, 1 H, *CH), 6.92–7.17 (m, 4 H, H_{arom}, *CHN*H*), 7.29–7.38 (m, 1 H, H_{arom}), 7.56–7.66 (m, 1 H, H_{arom}), 9.14 (s, 1 H, N*H*NH_2), 10.75 (s, 1 H, NH_{arom})[b]	14.4, 27.9, 53.9, 59.6, 110.0, 111.1, 118.1, 118.3, 120.7, 123.6, 127.2, 135.9, 171.0[b]	290 (M^+, 1), 244 (2), 201 (3), 170 (7), 158 (1), 143 (1), 130 (100)
3gc	3345, 3319, 3221, 2982, 1685, 1675, 1654, 1526, 1367, 1249, 1170, 1025, 731, 643	1.31 [s, 9 H, $C(CH_3)_3$], 2.90 and 3.04 (AB of ABX, 2 H, *J* = 14.4, 5.3, 5.3, *CHCH_2), 4.11–4.30 (m, 3 H, *CH, NH_2), 6.64 (br d, 1 H, *J* = 7.3, *CHN*H*), 6.91–7.17 (m, 3 H, H_{arom}), 7.28–7.37 (m, 1 H, H_{arom}), 7.54–7.66 (m, 1 H, H_{arom}), 9.07 (s, 1 H, N*H*NH_2), 10.74 (br s, 1 H, NH_{arom})[b]	28.0, 39.9, 53.7, 77.8, 110.0, 111.1, 118.0, 118.3, 120.7, 123.5, 127.3, 135.9, 154.9, 171.1[b]	318 (M^+, 1), 262 (1), 201 (4), 170 (6), 159 (3), 130 (100), 117 (2), 57 (1), 41 (1)
3ha′	3305, 3279, 1688, 1653, 1542, 1516, 1291, 1248, 1046, 837, 650	1.09 (t, 3 H, *J* = 7.0, CH_3), 2.63 and 2.78 (AB of ABX, 2 H, *J* = 13.8, 9.7, 5.0, *CHCH_2), 3.39 (br s, 2 H, NH_2), 3.89 (q, 2 H, *J* = 7.0, OCH_2), 4.03–4.14 (m, 1 H, *CH), 4.20 (br s, 1 H, OH), 6.60–6.69 (m, 2 H, H_{arom}), 6.98–7.08 (m, 2 H, H_{arom}), 7.10 (br d, 1 H, *J* = 8.5, *CHN*H*), 9.08 (br s, 1 H, N*H*NH_2)[b]	14.5, 37.0, 59.7, 114.8, 128.0, 130.0, 155.7, 170.9[b]	267 (M^+, 1), 221 (1), 208 (12), 178 (37), 177 (16), 160 (13), 147 (82), 136 (24), 107 (100), 91 (14)
8aa	3296, 3256, 2960, 1691, 1657, 1541, 1497, 1298, 1251, 1045, 772, 660	0.91 and 0.92 [2 d, 6 H, *J* = 6.7, 6.7, $CH(CH_3)_2$], 1.19 (t, 3 H, *J* = 7.0, CH_2CH_3), 2.00 [app sext, 1 H, *J* = 7.0, $CH(CH_3)_2$], 3.86 (app t, 1 H, *J* = 8.2, *CH), 4.03 (q, 2 H, *J* = 7.0, CH_2), 6.63 (m, 3 H, H_{arom}), 6.98–7.20 (m, 3 H, H_{arom}, *CHN*H*), 7.65 (br s, 1 H, NHPh), 9.71 (s, 1 H, N*H*NHPh)[b]	14.5, 18.3, 19.1, 29.9, 59.0, 59.7, 112.2, 118.3, 128.4, 149.2, 156.1, 171.1[b]	279 (M^+, 12), 233 (2), 172 (9), 144 (91), 134 (11), 116 (17), 108 (100), 93 (11), 92 (15), 77 (15), 72 (38)
8bb	3385, 3306, 3257, 2957, 1696, 1659, 1541, 1496, 1267, 1251, 1239,1035, 752, 699	0.91 [app t, 6 H, *J* = 6.7, $CH(CH_3)_2$], 1.36–1.70 [m, 3 H, $(CH_3)_2CHCH_2$], 4.07–4.22 (m, 1 H, *CH), 5.02 and 5.10 (AB system, 2 × d, 2 H, *J* = 13.8, OCH_2), 6.63–6.78 (m, 3 H, H_{arom}), 7.04–7.18 (m, 2 H, H_{arom}), 7.30–7.49 (m, 6 H, H_{arom}, *CHN*H*), 7.64 (br s, 1 H, NHPh), 9.78 (s, 1 H, N*H*NHPh)[b]	21.5, 22.7, 24.1, 40.5, 51.8, 65.3, 112.1, 118.3, 127.5, 127.6, 128.2, 128.4, 136.9, 149.2, 155.8, 172.0[b]	355 (M^+, 1), 247 (20), 198 (3), 176 (2), 134 (14), 108 (66), 107 (100), 86 (69), 79 (18), 77 (23)
8ca	3410, 3315, 3259, 1684, 1653, 1540, 1496, 1270, 1258, 1062, 888, 768, 693	1.18 (t, 3 H, *J* = 7.0, CH_3), 1.62–2.02 (m, 2 H, *CHCH_2), 2.05–2.25 (m, 2 H, CH_2CO), 3.91–4.09 (m, 3 H, *CH, OCH_2), 6.61–6.83 (m, 4 H, H_{arom}, *CHN*H*), 6.94–7.23 (m, 5 H, H_{arom}, NH_2, NHPh), 10.21 (br s, 1 H, N*H*NHPh)[b]	14.5, 27.5, 31.4, 53.2, 59.8, 114.5, 121.4, 128.8, 149.2, 155.8, 171.6, 173.6[b]	308 (M^+, 22), 291 (15), 262 (6), 201 (6), 174 (28), 173 (17), 134 (13), 128 (35), 108 (100), 93 (40), 84 (88), 77 (10)
8ea	3301, 3242, 3034, 2983, 1689, 1657, 1535, 1496, 1289, 1251, 1047, 752, 699	1.18 (t, 3 H, *J* = 7.2, CH_3), 3.06 (app d, 2 H, *J* = 7.3, $PhCH_2$), 4.05 (q, 2 H, *J* = 7.2, OCH_2), 4.57 (app q, 1 H, *J* = 7.8, *CH), 5.52 (d, 1 H, *J* = 8.5, *CHN*H*), 6.04 (br d, 1 H, *J* = 3.0, NHPh), 6.42–6.57 (m, 1 H, H_{arom}), 6.78–6.94 (m, 1 H, H_{arom}), 6.93–7.35 (m, 8 H, H_{arom}), 8.37 (br d, 1 H, *J* = 3.0, N*H*NHPh)[b]	14.4, 38.4, 54.8, 61.5, 113.5, 121.1, 127.1, 128.8, 129.0, 129.4, 136.1, 147.4, 156.4, 171.3[b]	327 (M^+, 43), 281 (10), 236 (8), 220 (4), 192 (57), 147 (30), 134 (18), 131 (13), 120 (100), 108 (78), 91 (14), 77 (9)

[a] Solvent: $CDCl_3$.
[b] Solvent: DMSO-d_6.

The optical purities of hydrazides 3 and 8 were determined by HPLC analysis with a Waters M-45 apparatus using a Daicel Chiracel OD column (0.46 cm I.D. × 25 cm; eluent: hexane–*i*-PrOH, 50:50; flow rate, 0.5 mL/min; UV detector, 254 nm). Direct inlet mass spectra (DI-MS) were obtained with a Fisons TRIO 2000 gas chromatograph–mass spectrometer, working in the positive ion 70 eV electron impact mode. Spectra were recorded in the range 35–450 u. Temperatures between 100 and 200 °C were found suitable to volatilise all the compounds into the ion source. Chiral GC-MS analyses were performed on the same instrument using a Chrompack Chirasil-val-L column (26 m × 0.22 mm I.D., 0.14 μm film thickness). IR spectra were obtained with a Nicolet FT-IR Magna 550 spectrophotometer using the KBr technique for solids and recorded in the range 4000–400 cm^{-1}. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC-F 200 spectrometer at 200 and 50 MHz, respectively, using $CDCl_3$ at r.t. or DMSO-d_6 at 40 °C as solvents. NMR peak locations are reported as δ values from TMS. Some ^1H multiplets are characterized by the term *app* (apparent): this refers only to their appearance and may be an oversimplification. Optical rotations were determined at 20 °C (concentration in g/100 mL of solvent) using a POLAX-D polarimeter purchased from ATAGO (Japan). Elemental analyses were performed with a Carlo Erba Mod. 1106 elemental analyser. Mps were determined with an automatic Mettler (Mod. FP61) mp apparatus and are not corrected. All N-protected amino acids **1** were prepared by traditional procedures.[19] All solvents and reagents were purchased from Aldrich Chemical Company and used without further purification. ECF is ethyl chloroformate. The bp of the petroleum ether used was 40–60 ºC.

α-N-Protected Hydrazides 3; General Procedure

To a stirred solution of α-N-protected amino acid **1** (2.64 mmol) in THF (4 mL) was slowly added Et_3N (0.28 g, 2.77 mmol). After 5 min, the resulting mixture was slowly added to a solution of ECF (0.30 g, 2.77 mmol) in THF (4 mL) at –10 °C and the stirring was continued for 10 min at –10 °C. Then Et_3N·HCl formed was filtered off and washed with THF (2 × 3 mL) and the filtrate was slowly added to a solution of $NH_2NH_2·H_2O$ (0.26 g, 5.28 mmol) in MeOH (7 mL) at 0 °C. The mixture was allowed to warm up to r.t. and stirred for 1 h. The solvents were removed under reduced pressure. The residue was dissolved in EtOAc (20 mL), washed successively with sat. aq $NaHCO_3$ (3 mL) and sat. brine (3 mL), and dried (Na_2SO_4). After filtration and evaporation of the solvent in vacuo the hydrazide **3** remained in analytically pure form (Tables 2 and 5).

α-N-Protected Phenylhydrazides 8; General Procedure

To a stirred solution of α-N-protected amino acid **1** (2.64 mmol) in THF (4 mL) was slowly added Et_3N (0.28 g, 2.77 mmol). After 5 min, the resulting mixture was slowly added to a solution of ECF (0.30 g, 2.77 mmol) in THF (4 mL) at –10 °C, and the stirring was continued for 10 min at –10 °C. Then Et_3N·HCl formed was filtered off and washed with THF (2 × 3 mL) and the filtrate was slowly added to a solution of phenylhydrazine (**7**) (0.43 g, 3.96 mmol) in MeOH (7 mL) at 0 °C. The mixture was allowed to warm up to r.t. and stirred for 1 h. The solvents were removed under reduced pressure. The residue was washed with hexane–Et_2O (7:3, 3 mL) and recrystallized from petroleum ether–EtOAc to provide the hydrazide **8** (Tables 4 and 5).

Reaction of *N*-Ethoxycarbonyl-L-phenylalanine (1ea) with Methylhydrazine

To a stirred solution of **1ea** (0.63 g, 2.66 mmol) in THF (4 mL) was slowly added Et_3N (0.28 g, 2.79 mmol). After 5 min, the resulting mixture was slowly added to a solution of ECF (0.30 g, 2.79 mmol) in THF (4 mL) at –10 °C and the stirring was continued for 10 min at –10 °C. Then the Et_3N·HCl formed was filtered off and washed with THF (2 × 3 mL) and the filtrate was slowly added to a solution of methylhydrazine (0.24 g, 5.32 mmol) in MeOH (7 mL) at 0 °C. The mixture was allowed to warm up to r.t. and stirred for 1 h. The solvents were removed under reduced pressure and the residue was dissolved in EtOAc (25 mL). The organic mixture was washed successively with sat. aq $NaHCO_3$ (3 mL) and sat. brine (3 mL), dried (Na_2SO_4), filtered and the solvent was evaporated in vacuo. The ^1H NMR spectrum (CD_3OD) of the residue (0.62 g, 88%) evidenced the presence of a mixture of 1-(*N*-ethoxycarbonyl-L-phenylalanyl)-1-methylhydrazine (**5**) and 1-(*N*-ethoxycarbonyl-L-phenylalanyl)-2-methylhydrazine (**6**) in a ratio 87:13 as measured by the integration of the *N*-methyl signals of **5** at δ = 3.10 and 3.05 and **6** at δ = 2.41.

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References

(1) Klausner, Y. S.; Bodanszky, M. *Synthesis* **1974**, 549.
(2) (a) Milne, H. B.; Kilday, W. *J. Org. Chem.* **1965**, *30*, 64. (b) Milne, H. B.; Most, C. F. *J. Org. Chem.* **1968**, *33*, 169. (c) Milne, H. B.; Carpenter, F. H. *J. Org. Chem.* **1968**, *33*, 4476.
(3) (a) Hofmann, K.; Magee, M. Z.; Lindenmann, A. *J. Am. Chem. Soc.* **1950**, *72*, 2814. (b) Hofmann, K.; Lindenmann, A.; Magee, M. Z.; Khan, N. H. *J. Am. Chem. Soc.* **1952**, *74*, 470. (c) Gutmann, H.; Hegedues, B.; Zeller, P. Ger. Pat. 1089390, **1960**; *Chem. Abstr.* **1962**, *56*, 4860. (d) Schroeder, E. *Ann. Chem.* **1965**, *688*, 250. (e) Wuensch, E.; Volk, A. *Chem. Ber.* **1965**, *98*, 797. (f) Wuensch, E.; Drees, F.; Jentsch, J.; Volk, A. *Chem. Ber.* **1965**, *98*, 803. (g) Wang, Y.; Chien, S.-C.; Lu, J.-Y. *Huaxue Xuebao* **1966**, *32*, 252; *Chem. Abstr.* **1967**, *66*, 46577. (h) Poddubnaya, N. A.; Balandina, G. N.; Tambieva, O. A. *Zh. Obshch. Khim.* **1971**, *41*, 436; *Chem. Abstr.* **1971**, *75*, 20968. (i) Beacham, J.; Dupuis, G.; Finn, F. M.; Storey, H. T.; Yanaihara, C.; Yanaihara, N.; Hofmann, K. *J. Am. Chem. Soc.* **1971**, *93*, 5526.
(4) (a) Kullmann, W. *Biochem. Biophys. Res. Commun.* **1979**, *91*, 693. (b) Semenov, A. N.; Lomonosova, I. V.; Berezin, V. I.; Titov, M. I. *Biotechnol. Bioeng.* **1993**, *42*, 1137. (c) Semenov, A. N.; Lomonosova, I. V. *Int. J. Pept. Protein Res.* **1994**, *43*, 113.
(5) (a) Dutta, A. S.; Morley, J. S. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1712. (b) Gante, J. *Synthesis* **1989**, 405. (c) Gante, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699. (d) Venkatraman, S.; Kong, J.-S.; Nimkar, S.; Wang, Q. M.; Aubé, J.; Hanzlik, R. P. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 577.
(6) (a) Hegedüs, B. *Helv. Chim. Acta* **1948**, *31*, 737. (b) Nyman, M. A.; Herbst, R. M. *J. Org. Chem.* **1950**, *15*, 108. (c) Harris, J. I.; Fruton, J. D. *J. Biol. Chem.* **1951**, *191*, 143. (d) Schlögl, K.; Korger, G. *Monatsh.* **1951**, *82*, 799. (e) Schlögl, K.; Derkosch, J.; Wawersich, E. *Monatsh. Chem.* **1954**, *85*, 607. (f) Sondheimer, E.; Holley, R. W. *J. Am. Chem. Soc.* **1954**, *76*, 2816. (g) F. Hoffman La Roche & Co. A.-G; Neth. Pat. Appl. 6414901, **1965**; *Chem. Abstr.* **1966**, *64*, 3685. (h) Tilak, M. A.; Hoffmann, J. A. *J. Org. Chem.* **1977**, *42*, 2098. (i) Moutevelis-Manakakis, P.; Photaki, I. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2277.

(7) (a) Radina, L. B.; Khvorova, K. M.; Pushkareva, Z. V. *Zh. Obshch. Khim.* **1964**, *34*, 2140; *Chem. Abstr.* **1964**, *61*, 9576. (b) Szarvasi, E.; Fontaine, L. Ger. Pat. 2200375, **1972**; *Chem. Abstr.* **1973**, *78*, 16024.
(8) F. Hoffman La Roche & Co. A.-G.; Brit. Pat. 836332, **1960**; *Chem. Abstr.* **1961**, *55*, 408.
(9) Jeschkeit, H. Ger. (East) Pat. 59793, **1968**; *Chem. Abstr.* **1969**, *70*, 106892.
(10) (a) Zahn, H.; Schnabel, E. *Ann.* **1957**, *604*, 62. (b) Schroeder, E. *Ann. Chem.* **1965**, *681*, 231. (c) Fujino, M.; Shinagawa, S.; Kawai, K. Eur. Pat. Appl. 5248, **1979**; *Chem. Abstr.* **1982**, *96*, 143328. (d) Fujino, M.; Shinagawa, S.; Kawai, K. U. S. Pat. 4277394, **1981**; *Chem. Abstr.* **1982**, *96*, 143328.
(11) Ueda, T.; Kato, S.; Toyoshima, S. Japan Pat. 8070, **1957**; *Chem. Abstr.* **1958**, *52*, 13783.
(12) Verardo, G.; Toniutti, N.; Gorassini, A.; Giumanini, A. G. *Eur. J. Org. Chem.* **1999**, 2943.
(13) Krysin, E. P.; Karel'skii, V. N.; Antonov, A. A.; Rostovskaya, G. E. *Khim. Prir. Soedin.* **1979**, 684; *Chem. Abstr.* **1981**, *94*, 175509.
(14) Sham, H. L.; Rosenbrook, W.; Kati, W.; Betebenner, D. A.; Wideburg, N. E.; Saldivar, A.; Plattner, J. J.; Norbeck, D. W. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1081.
(15) Cerovsky, V.; Jost, K. *Collect. Czech. Chem. Commun.* **1985**, *50*, 878.
(16) Cabezas, M. J.; del Campo, C.; Llama, E.; Sinisterra, J. V.; Gaertner, H. *J. Mol. Catal.* **1992**, *71*, 261.
(17) Paulus, W. *Z. Ernaehrungswiss.* **1968**, *9*, 11.
(18) (a) Hinman, R. L.; Fulton, D. *J. Am. Chem. Soc.* **1958**, *80*, 1895. (b) Theuer, W. J.; Moore, J. A. *J. Org. Chem.* **1964**, *29*, 3734. (c) Condon, F. E. *J. Org. Chem.* **1972**, *37*, 3608.
(19) (a) Chen, F. M. F.; Benoiton, N. L. *Can. J. Chem.* **1987**, *65*, 1224. (b) Vogel, A. In *Vogel's Textbook of Practical Organic Chemistry*, 5th ed.; Longman Scientific & Technical: New York, **1989**, 762. (c) Vogel, A. In *Vogel's Textbook of Practical Organic Chemistry*, 5th ed.; Longman Scientific & Technical: New York, **1989**, 786.