Organic Chemistry

Synthesis of functional derivatives of *N*-carboxamidomethyl- and *N*-phthalimidomethyl-α-amino acids and peptides by reaction of amides and nitriles of α-amino acids with formaldehyde and primary amides or phthalimide

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A direct method for the synthesis of functional derivatives of *N*-carboxamidomethyl- and *N*-phthalimidomethyl-α-amino acids by the reaction of nitriles and amides of α-amino acids (including peptides) with formaldehyde and NH-compounds (amides and imides) in DMF in the presence of TsOH was developed. The reactions of the compounds synthesized with acetic anhydride, tosyl chloride, and phenylalanine benzylamide in the presence of dicyclohexylcarbodiimide affording the corresponding *N*-acyl and *N*-sulfonyl derivatives or peptides containing carboxamido- and phthalimidomethyl substituents at the terminal N-atom of the peptide chain, were studied.

Key words: L-alanine benzylamide hydrochloride, L-phenylalanine benzylamide hydrochloride, glycine nitrile, peptides, tosyl chloride, acetic anhydride, dimethylformamide, para-toluene sulfonic acid, glycylglycine, dicyclohexylcarbodiimide, amides, phthalimide.

Earlier we have developed a method for the synthesis of esters of *N*-carboxamidomethyl- and *N*-phthalimidomethyl-α-amino acids by the reaction of esters of α-amino acids with formaldehyde and NH-compounds.[1–3] It has been shown that the compounds formed react with aqueous alkali and ammonia with the retention of the $N-CH_2-N$ group and the formation of *N*-carboxamidomethyl-α-amino acids and their amides involving dipeptides containing amidomethyl substituents at the terminal *N*-atom of the peptide chain.[4] *N*-Amidomethyl and *N*-phthalimidomethyl derivatives of α-amino acids and peptides are structural analogs of the corresponding natural compounds[5,6] and possible carriers of therapeutically useful compounds to microbial cells.[7,8] Therefore, it seemed reasonable to develop alternative methods for the synthesis of functionally substituted *N*-carboxamidomethyl- and *N*-phthalimidomethyl-α-amino acids and peptides by the direct condensation of amides and nitriles of α-amino acids with formaldehyde and primary amides or imides.

Aminoacetonitrile hydrochloride (glycine nitrile **1**), L-alanine benzylamide and L-phenylalanine benzylamide hydrochlorides (**2** and **3**), L-phenylalanine benzylamide (**4**), ethyl *N*-(L-phenylalanine)glycinate hydrochloride

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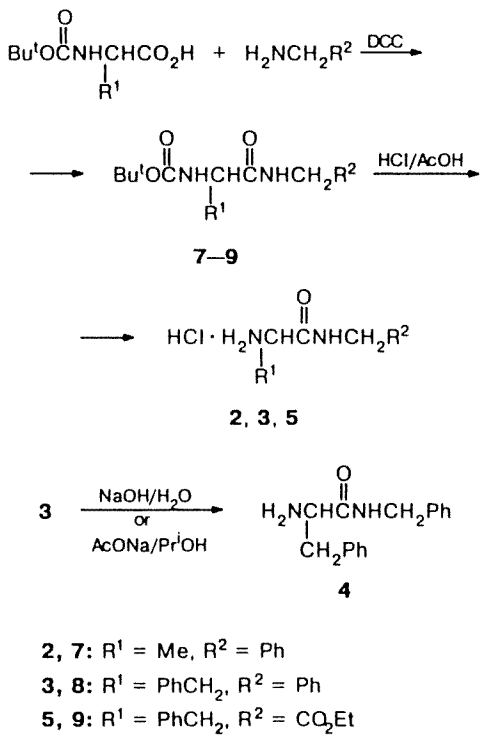
(5), and glycylglycine (6) were used as initial derivatives of amino acids. The hitherto unknown compounds **2**, **3**, and **5** were obtained according to Scheme 1 that involves condensation of *N*-*tert*-butoxycarbonyl derivatives of α-amino acids with the corresponding amines in the presence of dicyclohexylcarbodiimide (DCC) followed by the removal of the protective group from amides **7**—**9** with HCl in glacial acetic acid.

Scheme 1



7—**9**

2, 3, 5

4

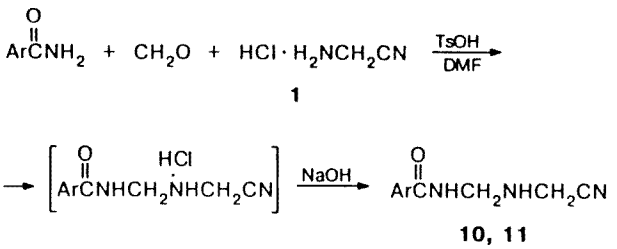
2, 7: R^1 = Me, R^2 = Ph
3, 8: R^1 = $PhCH_2$, R^2 = Ph
5, 9: R^1 = $PhCH_2$, R^2 = CO_2Et

Free base **4** was isolated from hydrochloride **3** by the action of NaOH in water or sodium acetate in propan-2-ol.

It has been found that aminoacetonitrile hydrochloride does not react with formaldehyde and aryl amides in alcoholic and aqueous-alcoholic media typical of the Mannich reaction. However, we succeeded in carrying out the condensation under conditions similar to those for the synthesis of hydrochlorides of esters of *N*-carboxamidomethyl-α-amino acids[3] by involving anhydrous paraformaldehyde in DMF in the reaction in the presence of a catalytic amount of TsOH. The reactions with aminoacetonitrile hydrochloride, formaldehyde, and *p*-nitro- and *o*-fluorobenzamides gave the corresponding nitriles of *N*-(carboxamidomethyl)glycine (**10**, **11**) in 30 and 13 % yields (Scheme 2).

The course of the reaction in the case of amides of α-amino acids could be complicated by the side process of imidazolidone formation.[9—12] In fact, the reaction of amide **4** with paraformaldehyde in the presence of benzylamide afforded a complex mixture of products. We were able to isolate 3,5-dibenzylimidazolidone-4 (**12**) in 19 % yield from this mixture. This compound was also obtained when the reaction was carried out in the absence of benzylamide (Scheme 3).

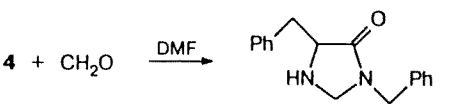
Scheme 2



10, 11

10: Ar = *p*-$NO_2C_6H_4$
11: Ar = *o*-FC_6H_4

Scheme 3



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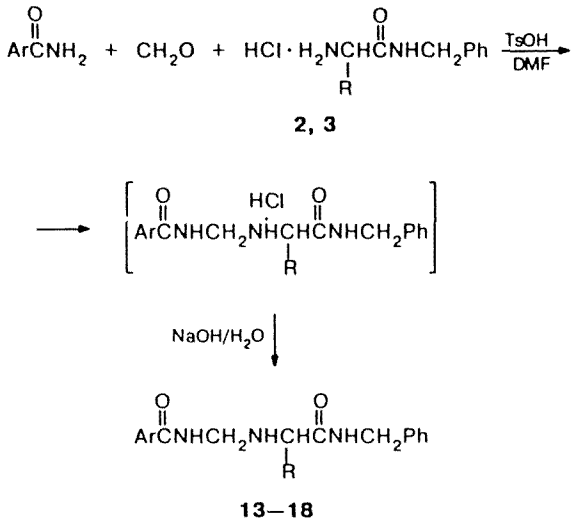
Using salts of amides of α-amino acids allows one to direct the process toward the formation of *N*-carboxamidomethyl-α-amino acids. The reactions of L-alanine benzylamide and L-phenylalanine benzylamide hydrochlorides (**2** and **3**) with paraformaldehyde in the presence of amides of aromatic and heteroaromatic carboxylic acids afforded *N*-carboxamidomethyl-L-alanine and *N*-carboxamidomethyl-L-phenylalanine benzylamine hydrochlorides, which are very poorly dissolved in organic solvents. To identify these compounds, they were transformed to the corresponding bases **13**—**18** by treatment with an equimolar amount of aqueous alkali. The highest yields of adducts **13**—**18** were obtained when the reaction was carried out in DMF in the presence of TsOH (Scheme 4).

We also succeeded in carrying out the three-component condensation of amide **4** with formaldehyde and phthalimide (compound **4** was generated *in situ* from hydrochloride **3** in propan-2-ol under the action of AcONa), which afforded *N*-phthalimidomethyl-L-phenylalanine benzylamide (**19**) in 39 % yield (Scheme 5).

The transformations described indicate the possibility of the direct synthesis of peptides modified with amido- and imidomethyl substituents at the terminal amino group of the peptide chain. It was sensible to assume that both mineral acid and the carboxy group of a peptide can perform the function of the acid that protects the peptide amino group from the side intramolecular condensation. In fact, the reaction of the hydrochloride of ethyl *N*-(L-phenylalanine)glycinate **5** with

Scheme 4

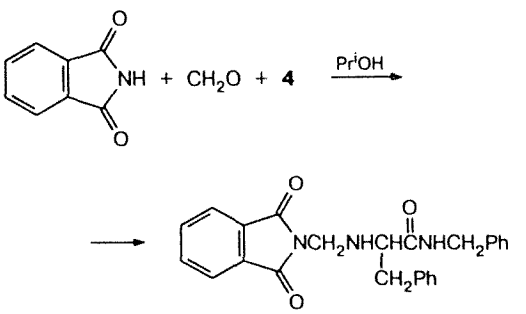


2, 3

13—18

2, 13, 14: R = Me
3, 15—18: R = $PhCH_2$
14, 15: Ar = Ph
13, 16: Ar = p-$NO_2C_6H_4$
17: Ar = pyridyl-3
18: Ar = 3,5-dichloroisothiazolyl-4

Scheme 5



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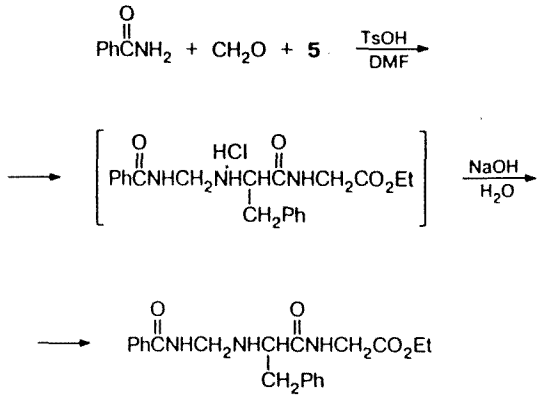
formaldehyde and benzamide in DMF in the presence of TsOH followed by workup with NaOH afforded peptide **20** in 20 % yield (Scheme 6).

The condensation of glycylglycine **6** with formaldehyde and amides (imides) under similar conditions proceeds also as an intermolecular process, and both hydrogen atoms of the peptide amino group enter into the reaction irrespective of the ratio of the components. The reaction products are *N,N*-bis(carboxamidomethyl)- and *N,N*-bis(phthalimidomethyl)glycylglycines (**21—28**). The highest yields (50—78 %) were achieved with benzamide, nicotinamide, the amide of 1-adamantanecarboxylic acid, and phthalimide (Scheme 7).

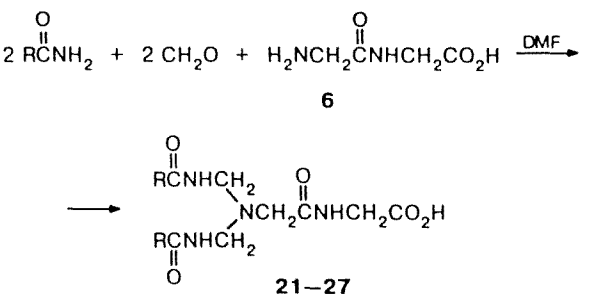
The derivatives of the *N*-carboxamidomethyl- and *N*-phthalimidomethyl-α-amino acids and peptides synthesized react at amino and carboxy groups with the

Scheme 6



20

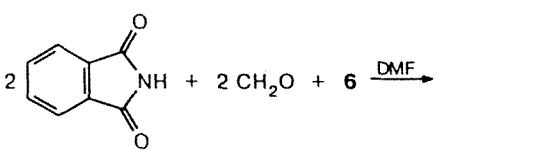
Scheme 7

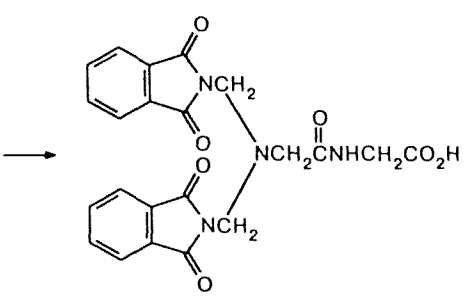


6

21—27

21: R = Me
22: R = 1-adamantyl
23: R = $PhCH_2$
24: R = Ph
25: R = o-FC_6H_4
26: R = p-$NO_2C_6H_4$
27: R = pyridyl-3





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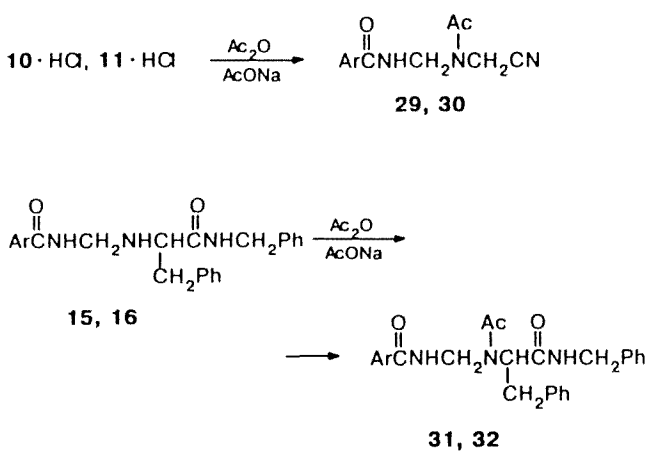
retention of the $N—CH_2—N$ group. For example, the reactions of nitriles and amides of *N*-carboxamidomethyl-α-amino acids **10**, **11**, **15**, and **16** with acetic anhydride

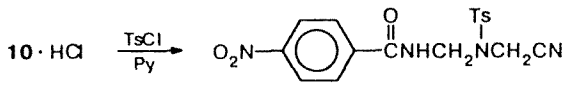
in the presence of sodium acetate yielded the corresponding *N*-acyl derivatives **29—32** (Scheme 8).

Scheme 8



10, 16, 29, 32: Ar = *p*-$NO_2C_6H_4$
11, 30: Ar = *o*-FC_6H_4
15, 31: Ar = Ph

The nitrile of *N*-(4-nitrobenzamidomethyl)-*N*-tosylglycine (**33**) was obtained by the reaction of nitrile hydrochloride **10** with tosyl chloride in pyridine.



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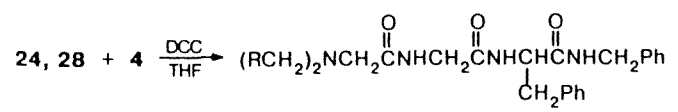
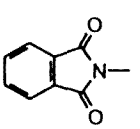
The reaction of glycylglycine derivatives **24** and **28** with amide **4** in the presence of DCC afforded tripeptides **34** and **35**. This reaction is of principle value since it opens the way to the synthesis of higher peptides modified with amido- and imidomethyl groups (Scheme 9).

Scheme 9



34, 35

24, 34: R = PhCONH; **28, 35:**



The functional derivatives of *N*-carboxamidomethyl- and *N*-phthalimidomethyl-α-amino acids and peptides synthesized are, as a rule, stable crystalline substances (products **20, 21,** and **32** were isolated as viscous noncrystallizable oils), the structure of which was confirmed by the IR, ^1H NMR (in some cases, ^{13}C NMR), and the elementary analysis data.

Experimental

IR spectra were recorded on a Specord-IR-75 spectrometer in KBr pellets for solid compounds and in thin layers for liquid samples. ^1H NMR spectra were obtained on a Bruker AM-300 spectrometer (300.13 (^1H) MHz) in DMSO-d_6, acetone-d_6, and $CDCl_3$. TLC was carried out on a Silpearl UV-250 silica gel. Compound **1** was synthesized by the known procedure.[13]

Benzylamides of *N*-*tert*-butoxycarbonyl-L-alanine (7) and -L-phenylalanine (8). A solution of dicyclohexylcarbodiimide (27.6 mmol) in abs. THF (1—2 mL) was added to a mixture of *N*-*tert*-butoxycarbonyl-L-alanine or -L-phenylalanine (26.4 mmol) and benzylamine (25.1 mmol) in abs. THF (5—7 mL) with stirring. The reaction mixture was kept for 12 h at ~20 °C. The solvent was distilled off *in vacuo*, DMF (3—5 mL) was added to the residue, and the undissolved precipitate was filtered off. The filtrate was diluted with water (30—40 mL) and kept for 12 h at 5 °C. The precipitate formed was filtered off, washed with water, dried in an air stream, and crystallized from an ethyl acetate—petroleum ether mixture to afford amide **7** in 56 % yield, m.p. 97—100 °C or amide **8** in 64 % yield, m.p. 130.5—132.5 °C. ^1H NMR of **7** (DMSO-d_6), δ: 1.29 (s, 9 H, CH_3); 1.20 (d, 3 H, CH_3, J = 6.5 Hz); 3.30 (m, 1 H, CH); 4.00 (d, 2 H, CH_2Ph); 7.0 (d, 1 H, NHCH, J = 6.7 Hz); 7.20—7.45 (m, 5 H, Ph); 8.35 (t, 1 H, $NHCH_2$). ^1H NMR of **8** (DMSO-d_6), δ: 1.32 (s, 9 H, CH_3); 2.60 (dd, 1 H, $\underline{CH_2}Ph$, ^2J = 13.0, ^3J = 8.0 Hz); 2.97 (dd, 1 H, $\underline{CH_2}Ph$, ^2J = 13.0, ^3J = 5.0 Hz); 4.13—4.22 (m, 1 H, CH); 4.29 (d, 2 H, $\underline{CH_2}Ph$, J = 6.6 Hz); 7.01 (d, 1 H, NH, J = 7.4 Hz); 7.15—7.36 (m, 10 H, 2Ph); 8.05 (t, 1 H, NHCO, J = 5.0 Hz).

Ethyl *N*-[(*N*-*tert*-butoxycarbonyl)-L-phenylalanyl]glycinate (9) was obtained similarly to compounds **7** and **8** starting from *N*-*tert*-butoxycarbonyl-L-phenylalanine (4.17 g, 1.57 mmol), ethyl glycinate (1.56 g, 1.50 mmol), and dicyclohexylcarbodiimide (3.39 g, 1.65 mmol) in abs. THF (8—10 mL). The yield of **9** was 1.81 g (30 %), m.p. 85—87 °C (ethyl acetate—petroleum ether) (*cf.* Ref. 14: m.p. 89.5—90 °C).

Hydrochlorides of benzylamides of L-alanine (2) and L-phenylalanine (3). Amide **8** (0.7 mmol) was added to a saturated solution of dry HCl in acetic acid (1 mL) and the reaction mixture was kept at 0—5 °C until liberation of CO_2 ceased. After 20 min, ether (30—40 mL) was added to the solution, and the precipitate formed was filtered off, washed with ether, and air dried to afford compound **3** in 84 % yield, m.p. 173.5—176 °C. ^1H NMR (DMSO-d_6), δ: 3.00—3.23 (m, 2 H, $CH\underline{CH_2}Ph$, J = 4.8 Hz); 4.18 (dd, 1 H, $NH\underline{CH_2}Ph$, ^2J = 13.0, ^3J = 5.0 Hz); 4.35 (dd, 1 H, $NH\underline{CH_2}Ph$, ^2J = 13.0, ^3J = 5.0 Hz); 7.07 (d, 2 H, Ph, J = 7.0 Hz); 7.15—7.35 (m, 8 H, Ph); 8.60 (br.s, 3 H, NH_3^+); 9.22 (br.s, 1 H, NHCO).

Compound **2** was obtained by a similar procedure and isolated in 62 % yield as an oil, ^1H NMR (DMSO-d_6), δ: 1.4 (d, 3 H, CH_3, J = 8.57 Hz); 3.82—3.98 (m, 1 H, CH); 4.30

Table 1. Yields and physico-chemical characteristics of *N*-carboxamidomethyl derivatives of glycine nitrile **10**, **11**, **29**, **30**, and **33**

Compound	Yield (%)	M.p. /°C	IR, ν/cm^{-1}			^{1}H NMR, δ (*J*/Hz)				
			C=O	NO_2	NH	ArCO	NCH_2N	NCH_2C	CONH	Other signals
10	30	115—117	1635 1665 1740	1520 1540	3290—3350	8.13 (d, 2 H, *J* = 9.0); 8.31 (d, 2 H, *J* = 9.0)	4.44 (d, *J* = 7.3)	3.77 (s)	8.66 (br.s, 1 H)	—
11	13	60—62	1650 1670 1750	—	3310	7.05—7.19 (m, 1 H); 7.20—7.30 (m, 1 H); 7.32—7.42 (m, 1 H); 7.42—7.55 (m, 1 H)	4.48 (d, *J* = 6.9)	3.67 (s)	8.05 (t, 1 H, *J* = 6.9)	—
29	41	188—189.5	1630 1660 1745	1520 1540	3280—3310	8.02 (d, 2 H, *J* = 9.0); 8.21 (d, 2 H, *J* = 9.0)	4.98 (d, *J* = 6.3)	4.35 (s, 1.68 H) 4.58 (s, 0.32 H)	9.25 (t, 0.16 H, *J* = 6.3) 9.35 (t, 0.84 H, *J* = 6.3)	2.15 (s, 0.5 H, Me) 2.35 (s, 2.5 H, Me)
30	62	106—109	1645 1670 1745	—	3380	7.08—7.36 (m, 2 H); 7.45—7.80 (m, 2 H)	5.01 (d, 0.88 H, *J* = 9.0); 5.11 (d, 1.12 H, *J* = 9.0)	4.47 (s) (1.12 H) 4.61 (s) (0.88 H)	8.07 (t, 1 H, *J* = 9.0)	2.23 (s, 1.32 H, Me) 2.40 (s, 1.68 H, Me)
33	24	174—178	1620 1650 1750	1515 1540	3310	8.08 (d, 2 H, *J* = 8.3); 8.31 (d, 2 H, *J* = 8.3)	5.07 (d, *J* = 6.3)	4.55 (s)	8.85 (br.s, 1 H)	2.35 (s, 3 H, Me); 7.40 (d, 2 H, *J* = 7.6, Ph); 7.85 (d, 2 H, *J* = 7.6, Ph)

(d, 2 H, $\underline{CH_2}Ph$, *J* = 5.35 Hz); 7.18—7.47 (m, 5 H, Ph); 8.35 (br.s, 3 H, NH_3^+); 9.18 (t, NHCO, *J* = 5.35 Hz).

The hydrochloride of ethyl L-phenylalanylglycinate (5) was obtained by the known procedure[14] from ester **9** in 94 % yield as an oil.

L-Phenylalanine benzylamide (4). A mixture of hydrochloride **3** (1.04 g, 3.58 mmol), NaOH (3.58 mmol), and water (3 mL) was stirred for 12 h at ~20 °C. The precipitate formed was filtered off, washed with water, and air dried to afford 0.80 g (90 %) of amide **4**, m.p. 64—65.5 °C. IR, ν/cm^{-1}: 1645 (C=O); 3290—3340 (NH). ^{1}H NMR ($CDCl_3$), δ: 2.65—2.90 (m, 1 H, CH—$\underline{CH_2}Ph$); 3.20—3.40 (m, 1 H, CH—$\underline{CH_2}Ph$); 3.58—3.78 (m, 1 H, CH); 4.33—4.59 (m, 2 H, NH$\underline{CH_2}Ph$); 7.10—7.50 (m, 10 H, 2 Ph); 7.55—7.73 (m, 1 H, NH).

Nitriles of *N*-carboxamidomethylglycines (10 and 11). A mixture of carboxamide (1.2 mmol), hydrochloride **1** (1.2 mmol), paraformaldehyde (1.32 mmol), and TsOH · H_2O (0.13 mmol) was dissolved in DMF (1 mL) at 60—80 °C. The reaction mixture was kept for 24 h at ~20 °C. The precipitate formed was filtered off, washed with acetone and ether, and air dried. A 45 % solution of NaOH (1.1 mmol per 1 mmol of hydrochloride) was added dropwise to a suspension of the precipitate in water (2—3 mL), and the reaction mixture was stirred for 24 h at ~20 °C. For compound **10**, the precipitate was filtered off, washed with water, air dried, and isolated by TLC on a SG silica gel (eluent ethyl acetate, R_f = 0.5). For compound **11**, the aqueous solution was extracted with ethyl acetate, and the organic phase was several times washed with water, dried with $MgSO_4$, and isolated by TLC on a SG silica gel (eluent ethyl acetate, R_f = 0.54). The yields, melting points, and spectral characteristics of compounds **10** and **11** are given in Table 1.

3,5-Dibenzylphthalimidazolidone-4 (12). A mixture of benzamide (0.095 g, 0.78 mmol), amide **4** (0.09 g, 0.78 mmol), and paraformaldehyde (0.025 g, 0.83 mmol) was dissolved in DMF (1 mL) at 60—80 °C. The reaction mixture was kept for 12 h at ~20 °C, diluted with ether (5—7 mL), and the precipitate formed was filtered off and air dried to afford 0.02 g (19 %) of compound **12**, m.p. 113—116 °C. ^{1}H NMR (acetone-d_6), δ: 2.85 (dd, 1 H, CH_2Ph, ^2J = 13.6, ^3J = 5.8 Hz); 3.03 (dd, 1 H, $\underline{CH_2}Ph$, ^2J = 13.6, ^3J = 7.8 Hz); 3.40 (t, 1 H, CH, *J* = 6.7 Hz); 3.80 (s, 2 H, $\underline{CH_2}Ph$); 4.12 (dd, 1 H, N—CH_2—N, ^2J = 12.9, ^3J = 5.8 Hz); 4.86 (dd, 1 H, N—CH_2—N, ^2J = 12.9, ^3J = 5.8 Hz); 7.04—7.32 (m, 10 H, 2 Ph); 7.80 (t, 1 H, NH, *J* = 4.9 Hz).

Benzylamides of *N*-carboxamidomethyl-L-α-amino acids (13—18). A mixture of carboxamide (0.83 mmol), the hydrochloride of the benzylamide of L-α-amino acid **2** or **3** (0.83 mmol), paraformaldehyde (1 mmol), and TsOH · H_2O (0.08 mmol) was dissolved in DMF (1—2 mL) at 60—80 °C. The reaction mixture was kept for 12 h at ~20 °C. The hydrochlorides of the benzylamides of *N*-carboxamidomethyl-L-α-amino acids were either precipitated at the end of keeping, or were crystallized upon dilution of the reaction mixture with an ether—acetone (4 : 1) mixture. A 45 % solution of NaOH (1.1 mmol per 1 mmol of hydrochloride) was added dropwise to a suspension of the hydrochloride in water (3—4 mL). The reaction mixture was stirred for 24 h at ~20 °C. The precipitate of compounds **13—18** was filtered off, washed with water, and air dried. The yields, melting points, and the IR and ^{1}H NMR spectral data are given in Table 2.

Table 2. Yields and physico-chemical characteristics of *N*-carboxamidomethyl derivatives of amides of alanine and phenylalanine **13—20, 31, and 32**

Compound	Yield (%)	M.p. /°C	IR, ν/cm^{-1}			^{1}H NMR, δ (*J*/Hz)						
			C=O	NO_2	NH	Ar	R	NCH_2N	NCHC	NCH_2Ph	CONH	Other signals
13	34	132—134	1610 1650	1525 1540	3310	8.05 (d, 2 H, *J* = 8.0); 8.30 (d, 2 H, *J* = 8.0)	1.19 (d, 3 H, *J* = 6.5)	4.25 (d, 2 H, *J* = 5.0)	3.35 (q, 1 H, *J* = 6.5)	4.10 (dd, 1 H, CH_2, ^2J = 12.0, ^3J = 5.0); 4.30 (dd, 1 H, CH_2, ^2J = 12.0, ^3J = 5.0); 7.10—7.35 (m, 5 H, Ph)	8.36 (t, 1 H, *J* = 5.0); 9.10 (t, 1 H, *J* = 5.0)	2.75 (br.s, 1 H)
14	12	83—86	1650	—	3300	7.40—7.65 (m, 3 H); 7.68 (d, 2 H, *J* = 7.5)	1.20 (d, 3 H, *J* = 5.8)	4.19—4.40 (m, 2 H)	3.32 (q, *J* = 5.8)	4.00—4.18 (m, 1 H); 4.19—4.40 (m, 1 H); 7.10—7.38 (m, 5 H, Ph)	8.40 (br.s, 1 H); 8.82 (br.s, 1 H)	—
15	76	128—130	1650	—	3290	7.40—7.60 (m, 3 H); 7.90 (d, 2 H, *J* = 7.5)	3.00 (d, 2 H, CH_2, *J* = 6.6); *a*	4.20 (d, 1 H, *J* = 5.0); 4.28 (d, 1 H, *J* = 5.0)	3.88 (t, *J* = 6.6)	4.10—4.25 (m, 1 H, CH_2); 4.32—4.43 (m, 1 H, CH_2); *a*	8.59 (t, 1 H, *J* = 5.0); 9.04 (t, 1 H, *J* = 5.0)	—
16	29	142—145	1605 1650	1535	3320	8.50 (d, 2 H, *J* = 8.0); 8.75 (d, 2 H, *J* = 8.0)	3.25 (dd, 1 H, CH_2, ^2J = 14.0, ^3J = 8.0); 3.53 (dd, 1 H, CH_2, ^2J = 14.0, ^3J = 5.0); *b*	4.80 (d, 2 H, *J* = 5.0)	4.15 (dd, 1 H, ^3J = 8.0, 5.0)	4.50 (dd, 1 H, CH_2, ^2J = 13.0, ^3J = 5.5); 4.85 (dd, 1 H, CH_2, ^2J = 13.0, ^3J = 5.5); *b*	8.71 (br.s, 1 H); 9.45 (t, 1 H, *J* = 5.0)	—
17	28	129—132	1655	—	3330	8.60 (d, 1 H, *J* = 7.5); 9.18 (d, 1 H, *J* = 3.7); 9.43 (br.s, 2H)	3.22 (dd, 1 H, CH_2, ^2J = 13.6, ^3J = 8.0); 3.47 (dd, 1 H, CH_2, ^2J = 13.6, ^3J = 5.0); *c*	4.75 (d, 2 H, *J* = 5.0)	4.10 (t, *J* = 6.5)	4.49 (dd, 1 H, CH_2, ^2J = 13.0, ^3J = 5.0); 4.61—4.90 (m, 1 H); *c*	7.95 (t, 1 H, *J* = 5.0); 8.82 (t, 1 H, *J* = 5.0)	—
18	24	110—111.5	1650 1660	—	3360	—	2.95 (dd, 1 H, CH_2, ^2J = 13.6, ^3J = 7.8); 3.12 (dd, 1 H, CH_2, ^2J = 13.6, ^3J = 5.0); *d*	4.54 (dd, 2 H, ^2J = 5.0, ^3J = 1.2)	3.73 (t, *J* = 6.5)	4.20 (dd, 1 H, CH_2, ^2J = 13.0, ^3J = 5.5); 4.41 (dd, 1 H, CH_2, ^2J = 13.0, ^3J = 5.5); *d*	7.95 (t, 1 H, *J* = 5.5); 8.20 (t, 1 H, *J* = 5.0)	—

to be continued

Table 2. (*continued*)

Compound	Yield (%)	M.p. /°C	IR, ν/cm^{-1}			^{1}H NMR, δ (*J*/Hz)						
			C=O	NO_2	NH	Ar	R	NCH_2N	NCHC	NCH_2Ph	CONH	Other signals
19	39	124—128	1660 1720 1740 1750	—	3320	7.97—8.12 (m, 4 H)	2.95 (dd, 1 H, CH_2, ^2J = 14.0, ^3J = 9.6); 3.51 (dd, 1 H, CH_2, ^2J = 14.0, ^3J = 4.2); 7.25—7.42 (m, 5 H, Ph)	4.82 (s, 2 H)	3.85 (dd, ^3J = 9.6, 4.2)	4.73 (d, 2 H, CH_2, *J* = 5.5); 7.48—7.72 (m, 5 H, Ph)	7.45 (t, *J* = 5.5)	—
20	20	Oil		—		7.40—7.58 (m, 3 H); 7.82 (d, 2 H, *J* = 8.0)	2.75 (dd, 1 H, CH_2, ^2J = 13.0, ^3J = 7.0); 3.03 (dd, 1 H, CH_2, ^2J = 13.0, ^3J = 4.0); 7.08—7.35 (m, 5 H, Ph)	4.25 (d, 2 H, *J* = 6.0)	3.61—3.75 (m)	—	8.40 (br.s, 1 H); 8.82 (br.s, 1 H)	1.20 (t, 3 H, Et, *J* = 7.0); 4.10 (q, 2 H, Et, *J* = 7.0); 4.27—4.40 (m, CH_2CO_2)
31	49	160—163	1640 1660	—	3290	7.40—7.60 (m, 3 H); 7.82 (d, 2 H, *J* = 7.7)	3.12 (dd, 1 H, CH_2, ^2J = 13.8, ^3J = 6.9); 3.35 (dd, 1 H, CH_2, ^2J = 13.8, ^3J = 7.7); [e]	4.72 (d, 1 H, *J* = 14.6); 5.49 (dd, 1 H, ^2J = 14.6, ^3J = 7.0)	5.35 (t, *J* = 7.3)	4.24 (dd, 1 H, ^2J = 13.8, ^3J = 3.8); 4.43 (dd, 1 H, ^2J = 13.8, ^3J = 5.0); [e]	8.42 (d, *J* = 7.0)	2.18 (s, 3 H, Me)
32	18	Oil	1650 1665	1540 1555	3290	8.00 (d, 2 H, *J* = 8.6); 8.25 (d, 2 H, *J* = 8.6	3.32 (dd, 1 H, CH_2, ^2J = 23.0, ^3J = 12.0); 3.42 (dd, 1 H, CH_2, ^2J = 23.0, ^3J = 6.0); 7.15 or 7.26 (both br.s, 5 H, Ph)	4.83 (d, 1 H, *J* = 14.6); 5.05 (dd, 1 H, ^2J = 14.6, ^3J = 6.9)	5.18 (dd, *J* = 6.0, 12.0)	4.30 (dd, 1 H, CH_2, ^2J = 12.9, ^3J = 4.5); 4.42 (dd, 1 H, CH_2, ^2J = 12.9, ^3J = 4.5); 7.15 or 7.26 (both br.s, 5 H, Ph)	7.85 (br.s, 1 H); 8.9 (br.s, 1 H)	2.18 (s, 3 H, Me)

[a] 7.07 (d, 2 H, Ph, *J* = 7.0); 7.15—7.30 (m, 8 H, Ph). [b] 7.50—7.75 (m, 10 H, Ph). [c] 7.50—7.82 (m, 10 H, Ph). [d] 7.15—7.38 (m, 10 H, Ph). [e] 7.07—7.41 (m, 10 H, Ph).

***N*-Phthalimidomethyl-L-phenylalanine benzylamide (19).** Sodium acetate (0.03 g, 0.34 mmol) and a 29 % aqueous solution of formalin (0.04 mL, 0.34 mmol) were added to a boiled solution of phthalimide (0.05 g, 0.34 mmol) and hydrochloride **3** (0.10 g, 0.34 mmol) in Pr^{i}OH (5 mL) with stirring. The reaction mixture was cooled and kept for 24 h at ~20 °C. The precipitate of NaCl was filtered off, the solvent from the filtrate was removed *in vacuo*, and the compound was isolated by TLC on a SG (eluent benzene—ether, 1 : 1, R_f = 0.4) to afford 0.06 g (39 %) of compound **19**. Its spectral characteristics are given in Table 2.

Ethyl *N'*-[(*N*-benzamidomethyl)-L-phenylalanyl]glycinate (20). A mixture of benzamide (0.08 g, 0.66 mmol), the hydrochloride of ethyl ester **5** (0.22 g, 0.66 mmol), paraformaldehyde (0.025 g, 0.83 mmol), and $TsOH \cdot H_2O$ (0.01 g, 0.07 mmol) was dissolved in DMF at 60—80 °C. The reaction mixture was kept for 12 h at ~20 °C and then diluted with ether (3—5 mL). The solvent was decanted, the oil-like resi-

Table 3. Yields and physico-chemical characteristics of *N,N*-bis(carboxamidomethyl) and *N,N*-bis(phthalimidomethyl) derivatives of glycylglycine **21—28**

Compound	Yield (%)	M.p. /°C	IR, ν/cm^{-1}			^{1}H NMR, δ (*J*/Hz)				
			C=O	NO_2	NH	R	NCH_2N	NCH_2C	$NHCH_2$	NH
21	60	Oil	1630 1650 1665 1730	—	3290 3320	1.85 (s, 6 H, Me)	3.98 (d, 4 H, *J* = 5.5)	3.12 (s)	3.78 (d, 2 H, *J* = 5.5)	8.16 (t, 2 H, *J* = 5.5); 8.22 (t, 1 H, *J* = 5.5)
22	78	157.5—159.5	1650 1670 1730	—	2860 2910 3330 3370	1.63 (s, 12 H); 1.75 (s, 12 H); 1.95 (s, 6 H)	3.98 (d, 4 H, *J* = 5.0)	3.07 (s)	3.77 (d, 2 H, *J* = 5.5)	7.75 (t, 2 H, *J* = 5.0); 8.20 (t, 1 H, *J* = 5.5)
23	7	122—125	1620 1650 1670 1730	—	3220 3270	7.10—7.40 (m, 10 H Ph); 3.48 (s, 4 H, CH_2)	4.05 (br.s, 4 H)	3.15 (s)	3.89 (br.s, 2 H)	8.18 (t, 1 H, *J* = 5.0); 8.30 (t, 2 H, *J* = 5.0)
24	65	140—143	1640—1670 1750	—	3280 3370	7.41—7.63 (m, 6 H); 7.86 (d, 4 H, *J* = 7.0)	4.38 (d, 4 H, *J* = 5.0)	3.35 (s)	3.85 (d, 2 H, *J* = 5.0)	8.32 (t, 1 H, *J* = 5.0); 8.83 (t, 2 H, *J* = 5.0)
25	20	129—133	1650 1740	—	3210	7.20—7.42 (m, 4 H); 7.58 (q, 2 H, *J* = 7.5); 7.70 (t, 2 H, *J* = 7.5)	4.40 (d, 4 H, *J* = 5.5)	3.42 (s)	3.85 (d, 2 H, *J* = 5.5)	8.35 (t, 1 H, *J* = 5.5); 8.72 (t, 2 H, *J* = 5.5)
26	32	108.5—111.5	1650 1665 1740	1540 1555	3220	8.09 (d, 4 H, *J* = 7.5); 8.30 (d, 4 H, *J* = 7.5)	4.39 (d, 4 H, *J* = 5.0)	3.41 (s)	3.80 (d, 2 H, *J* = 5.5)	8.27 (t, 1 H, *J* = 5.5); 9.15 (t, 2 H, *J* = 5.0)
27	60	154—156.5	1650—1670 1740	—	2950 3080 3280 3360	7.52 (br.s, 2 H); 8.22 (d, 2 H, *J* = 7.5); 8.71 (d, 2 H, *J* = 7.5); 9.05 (s, 2 H)	4.37 (br.s, 4 H)	3.40 (s)	3.80 (br.s, 2 H)	8.22 (s, 1 H) 9.00 (br.s, 2 H)
28	50	167—171	1650 1700 1720—1775	—	2900—3100 3350	7.85 (s, 8 H)	4.77 (s, 4 H)	3.53 (s)	3.60 (d, 2 H, *J* = 6.0)	8.00 (t, 1 H, *J* = 6.0)

due of the hydrochloride was mixed with water, and NaOH (1.1 mmol per 1 mmol of hydrochloride) was added to the suspension formed. The reaction mixture was stirred for 12 h at ~20 °C. The aqueous solution was decanted, and the residue was dried *in vacuo* to afford 0.06 g (20 %) of compound **20**. Its physico-chemical characteristics are given in Table 2.

Nitriles of *N*-carboxamidomethyl-*N*-acetylglycine (29 and 30). Benzylamides of *N*-carboxamidomethyl-*N*-acetyl-L-phenylalanylglycine (31 and 32). Compounds **29—32** were synthesized by the known procedure[15] from hydrochlorides **10** or **11**, or benzylamides **15** or **16** (0.78 mmol), acetic anhydride (30.0 mmol), and NaOAc (2.34 mmol). The yield of **29** was 41 %, m.p. 188—189.5 °C; the yield of **30** was 62 %, m.p. 106—109 °C. The yield of compound **31** was 49 %, m.p. 160—163.5 °C, and the yield of compound **32** (oil) was 18 %. The spectral characteristics of **29** and **30** are given in Table 1 and **31** and **32** in Table 2.

Nitrile of *N*-(*p*-nitrobenzamidomethyl)-*N*-tosylglycine (33) was obtained by the known procedure[15] from hydrochloride **10** (0.28 g, 1.03 mmol) and tosyl chloride (0.4 g, 2.06 mmol) in pyridine (2 mL). The yield of **33** was 24 %, m.p. 174—178 °C. Its spectral characteristics are given in Table 1.

***N,N*-Bis(carboxamidomethyl)glycylglycines (21—27) and *N,N*-bis(phthalimidomethyl)glycylglycine (28).** A mixture of carboxamide (1.5 mmol) or phthalimide (0.22 g, 1.5 mmol), glycylglycine (0.1 g, 0.75 mmol), and paraformaldehyde (1.5 mmol) was dissolved in DMF (1—2 mL) at 60—80 °C. The reaction mixture was kept for 12 h at ~20 °C, diluted with an ether—acetone (4 : 1) mixture (5—8 mL), and allowed to stand for 30—60 min at 5 °C. The solvent was decanted, and

products **24**, **25**, and **28** were isolated by precipitation from the methanolic solution with ether; compound **23** crystallized upon treatment with acetone, compound **27** crystallized upon treatment with methanol, and compound **21** was isolated as an oil. For compound **26**, the precipitate formed upon dilution of the reaction mixture with an ether—acetone (4 : 1) mixture was washed with acetone, and the product from the acetone filtrate was precipitated with ether. Compound **22** was filtered off from the reaction mixture. The yields, melting points, and the IR and ^{1}H NMR spectral data are given in Table 3.

***N,N*-Bis(benzamidomethyl)glycylglycyl-L-phenylalanine benzylamide (34) and *N,N*-bis(phthalimidomethyl)glycylglycyl-L-phenylalanine benzylamide (35).** A solution of dicyclohexylcarbodiimide (0.15 mmol) in THF (1 mL) was added to a mixture of compound **24** or **28** (0.73 mmol) and benzylamide **4** (0.17 mmol) in abs. THF (5—7 mL) with stirring. The reaction mixture was kept for 12 h at ~20 °C. The solvent was removed *in vacuo*, DMF (3—5 mL) was added to the residue, and the undissolved *N,N'*-dicyclohexylcarbamide was filtered off. The filtrate was diluted with water (20—30 mL) and kept for 2—3 h at 5 °C. The precipitate formed was filtered off, washed with water, methanol, and ether, and air dried to afford 0.27 g (53 %) of amide **34** or **35**. Compound **34**, m.p. 192—196 °C, ^{1}H NMR (DMSO-d_6), δ: 2.87 (dd, 1 H, CH—$\underline{CH_2}$Ph, ^2J = 13.5, ^3J = 8.5 Hz); 3.09 (dd, 1 H, CH—$\underline{CH_2}$Ph, ^2J = 13.5, ^3J = 5.0 Hz); 3.38 (s, 2 H, N—CH_2CO); 3.70 (dd, 1 H, NH—$\underline{CH_2}$CO, ^2J = 16.0, ^3J = 6.0 Hz); 3.82 (dd, 1 H, NH—$\underline{CH_2}$CO, ^2J = 16.0, ^3J = 6.0 Hz); 4.00 (br.s, 1 H, CH); 4.25 (br.s, 2 H, N—CH_2—N); 4.37 (d, 2 H, N—CH_2—N, J = 5.5 Hz); 4.60 (br.s, 2 H, NH—$\underline{CH_2}$Ph); 7.10—7.35 (m, 10 H, 2 Ph); 7.40—7.64 (m, 6 H, Ph); 7.90 (d, 4 H, Ph, J = 7.0 Hz); 8.26 (d, 2 H, ArCONH, J = 5.5 Hz); 8.50 (t, NHCO, J = 5.5 Hz); 8.93 (t, 1 H, NHCO, J = 5.5 Hz). Compound **35**, yield 0.30 g (61 %), m.p. 184—188 °C, ^{1}H NMR (DMSO-d_6), δ: 2.69—2.92 (m, 1 H, CH—$\underline{CH_2}$Ph); 2.92—3.09 (m, 1 H, CH—$\underline{CH_2}$Ph); 3.45—3.67 (m, 4 H, N—CH_2—N, NH—$\underline{CH_2}$Ph); 4.24 (br.s, 2 H, N—$\underline{CH_2}$Ph); 4.50 (br.s, 1 H, NH—$\underline{CH}$CO); 4.75 (s, 4 H, N—CH_2—N); 7.00—7.39 (m, 10 H, 2 Ph); 7.80 (s, 8 H, C_6H_4); 7.90 (br.s, 1 H, NH); 8.05 (br.s, 1 H, NH); 8.42 (br.s, 1 H, NH).

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