N-Carbamoyl Derivatives and Their Nitrosation by Gaseous NO_x − A New, Promising Tool in Stepwise Peptide Synthesis

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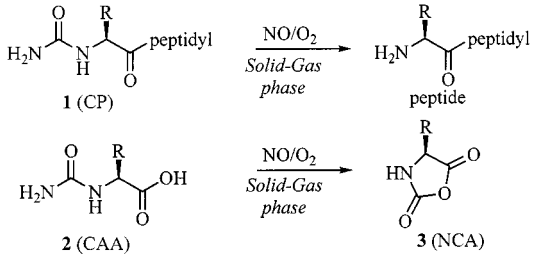
New uses of the *N*-carbamoyl group in peptide synthesis – as an N^{α}-protecting group in classical peptide coupling methods, and as a preactivating group for stepwise coupling by NCA formation – are presented. In the first application, the *N*-carbamoyldipeptide esters C-Val-Gly-OEt, C-Leu-Gly-OEt, C-Ala-Gly-OEt, and C-Ala-Phe-OEt were obtained in good yields by treatment of the corresponding *N*-carbamoyl-amino acids (CAA) with amino acid esters. Quantitative *N*-deprotection without racemisation was then achieved in the solid through nitrosation by gaseous NO_x. The extent of racemisation occurring in the coupling step is discussed. In the second application, an easy route to amino acid *N*-carboxy anhydrides (NCAs) through nitrosation of CAA under the same conditions as above allowed straightforward "one-pot" peptide stepwise coupling, as demonstrated by the formation of Leu-Gly and Val-Gly in good yields and enantiomeric excess.

Introduction

Despite the ease of *N*-carbamoylation of amino acids and peptides, the carbamoyl group is hardly or never used as an N^{α}-protecting group in stepwise peptide synthesis, the removal of an *N*-carbamoyl moiety requiring drastic basic conditions or the use of enzymes, conditions not convenient in peptide chemistry. Recent studies in our laboratory, however, have shown that nitrosation of *N*-carbamoylpeptides (CPs) **1**, carried out in the solid phase with gaseous NO_x as the nitrosating agent, results in fast, smooth and quantitative decarbamoylation of the *N*-carbamoyl group, with neither peptide bond cleavage nor deamination side reactions.[1] Under the same conditions, *N*-carbamoylamino acids (CAAs) **2** afford the corresponding *N*-carboxy anhydrides (NCAs) **3**, which can be isolated quantitatively prior to hydrolysis into the free amino acids (Scheme 1).[2] Both reactions operate without racemisation, and yield nitrogen and water as the only waste materials, thus also offering interesting perspectives as an eco-friendly reaction.

In this context, we examined two possible uses of the carbamoyl group in peptide synthesis, scrutinising the enantioselectivity of the reactions. The first was as an N^{α}-protecting group for classical peptide coupling methods, the second was as a preactivating group for peptide stepwise coupling by NCA formation. Both strategies rely on the nitrosation of the *N*-alkylurea group in the solid phase by gaseous NO_x.



Scheme 1

Results and Discussion

N-Carbamoylation Reaction

Both strategies required efficient *N*-carbamoylation of amino acids (and of peptides). Although several synthetic routes exist, such as the hydrolysis of hydantoins by strong bases[3] or enzymatic systems,[4] or *trans*-*N*-carbamoylation from urea,[5] the simplest and cheapest method for amino group *N*-carbamoylation was treatment with aqueous mineral cyanate.[6,7] This reaction appeared to be highly dependent on the pH; a kinetic study of cyanate reactivity allowed optimised conditions to be identified: pH = 8−8.5 at 40−50 °C for a few hours.[8] This reaction was reasonably robust, however, and could be achieved over a wider pH range (6.5−10). The CAA or CP was usually isolated in good yields by precipitation from the chilled, acidified reac-

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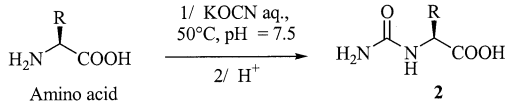
tion mixture (Scheme 2). No racemisation was found to occur under these conditions.

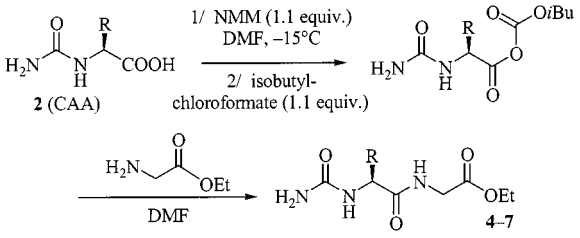


Scheme 2

The Carbamoyl Moiety as an N^{α}-Protecting Group

Peptide Coupling Reaction

We first investigated the possibility of using the carbamoyl group as an N^{α}-protecting group for peptide coupling, focusing on the mixed-anhydride method, well known in peptide chemistry for its efficiency in terms of yield and absence of racemisation.[9,10] The carboxyl group of the *N*-protected amino acid is activated to a mixed anhydride by treatment with isobutyl chloroformate (IBCF) in the presence of *N*-methylmorpholine (NMM, Scheme 3). The mixed anhydride is usually formed prior to the addition of the *O*-protected amino acid (a procedure established by Anderson et al.[10]).



Scheme 3

This method was applied to produce *N*-carbamoyldipeptide esters **4**−**7** (Table 1). Coupling of C-Val or C-Leu to Gly-OEt afforded the respective dipeptides **4**−**5** in good yields, but this proved poorly efficient when C-Ala was the starting material. A modification of the reagent addition sequence (amino ester addition prior to IBCF), in order to form the mixed anhydride of C-Ala in the presence of the amino ester, significantly improved the yield of isolated C-Ala-Gly-OEt (**6**). Therefore this *alternative* procedure (named in contrast with Anderson's *classical* procedure) was directly used for the preparation of C-Ala-Phe-OEt (**7**), isolated in good yield. No significant change was observed for the preparation of C-Leu-Gly-OEt (**5**) either with the classical or with the alternative procedure, while the results for the preparation of **6** suggested a high instability of the mixed anhydride derived from C-Ala, which would then readily decompose prior to aminolysis in the classical procedure.

Epimerisation

To evaluate the extent of epimerisation during the coupling step, the enantiomeric excesses of peptide residues were measured after *N*-decarbamoylation (see next section) and then after peptide bond hydrolysis with boiling 6 N HCl.[11] The enantiomeric excesses (*ee*s) of the resulting amino acids were measured by GC analysis after derivatisation with (−)-menthyl chloroformate in 2-propanol.[12] To determine the extent of racemisation occurring during the hydrolysis step, a phenomenon described in the literature,[13,14] commercial dipeptide ester samples were submitted to the same treatment, and showed ca. 5% racemisation (Table 1).

Though Val-Gly or Leu-Gly coupling occurred with (almost) no epimerisation (the measured *ee*s remaining very close to those of commercial dipeptides after the hydrolysis step), almost complete epimerisation (together with lower yields) occurred during both Ala-Gly and Ala-Phe coupling, whether the classical or the alternative coupling procedure was applied. Attempts to use *N*,*N*-dimethylacetamide (DMAc, known as a low-racemisation medium) instead of DMF as the reaction solvent did not significantly improve either the yield or the *ee* for the synthesis of C-Ala-Gly-OEt. No change/difference was observed with a change in solvent for the preparation of C-Val-Gly-OEt (**4**).

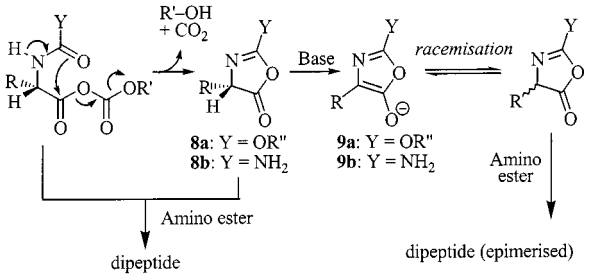
Epimerisation during the peptide coupling step may be explained in terms of the formation and racemisation of a cyclic intermediate, as previously observed in the activation of amino acids N^{α}-protected with groups of the urethane type by the mixed-anhydride method.[10,15,16] The formation of the oxazolone **8a** from the mixed anhydride (Scheme 4) was found to depend on various factors such as the nature of the *N*-protecting group, the amino acid side chain and the activating base. Although the nucleophilic attack on **8a** by the free amino ester also results in the peptide coupling, this last reaction is in competition with the reversible depro-

Table 1. Results of coupling amino acid esters to CAA by the mixed-anhydride method (yields are not optimised)

Compound	Yield (%)		*ee* (%) at the N residue[a]		
	classical[b]	alternative[b]	classical[b]	alternative[b]	(blank)[c]
4 (C-Val-Gly-OEt)	87	–	89	–	89.6
5 (C-Leu-Gly-OEt)	80	82	89.6	89.6	90.6
6 (C-Ala-Gly-OEt)	52	84	10	10	90
7 (C-Ala-Phe-OEt)	–	91	–	10	–

[a] Measured by GC after peptide *N*-deprotection followed by hydrolysis. [b] Peptide coupling procedure used (see text). [c] *ee* measured after hydrolysis (same method) of the corresponding commercial dipeptide esters.

tonation of **8a** at the α position to give the planar cyclic anion **9a**. Racemisation of **8a** is then possible through **9a**.

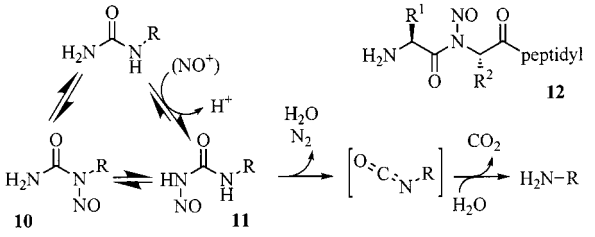


Scheme 4

By structural analogy, such a mechanism was also likely to occur in our case, and would involve the formation and racemisation of **8b**. However, such 2-amino-5-(4*H*)-oxazolones (e.g. **8b**) have never been reported in the literature. If such a mechanism were involved, the racemisation rate should be very powerfully affected by the steric hindrance of the side chain, explaining why almost complete racemisation was observed upon treatment of C-Ala, while bulky side chain compounds such as C-Val or C-Leu were not affected. This explanation, however, is insufficient to account for epimerisation with C-Ala, in comparison with such notoriously nonracemising urethane derivatives as Z-Ala or Boc-Ala, in which the bulky OR′′ substituent probably efficiently prevents α-proton abstraction from **8a** despite its expected (from the comparative electronegativities of OR′′ and NH_2) higher acidity in comparison with **8b**. It is also possible, however, that the (unhindered) mixed anhydride would itself be subject to α-proton abstraction, and then to racemisation.

Deprotection Reaction

The *N*-deprotection of *N*-carbamoylpeptides was carried out by nitrosation in solid/gas phase:[1] the solid *N*-carbamoyldipeptide was exposed (under nitrogen) to a mixture of nitric oxide and oxygen (in a 2.5:1 ratio). A small amount of water was necessary to initiate the reaction (the progress of which was visible by the disappearance of the red-brown colour of the NO_2) and to obtain quantitative conversion in a reasonable time. The *N*-deprotected dipeptides were obtained as their corresponding salts with nitric acid. The nitrosation probably proceeds through species such as N_2O_3 or N_2O_4, although the ultimate intermediate is probably



Scheme 5

NO^+. These solid/gas conditions produced a significant acidification of the medium due to release of HNO_2/HNO_3, thus ensuring a fast equilibrium between the two nitroso urea adducts **10** and **11** (detailed investigations to be published elsewhere). While base-catalysed decomposition of **10** (into diazo compound and deamination products) did not occur, due to the absence of HO^-, the unstable **11** spontaneously decomposed into an isocyanate that finally underwent hydrolysis (Scheme 5).

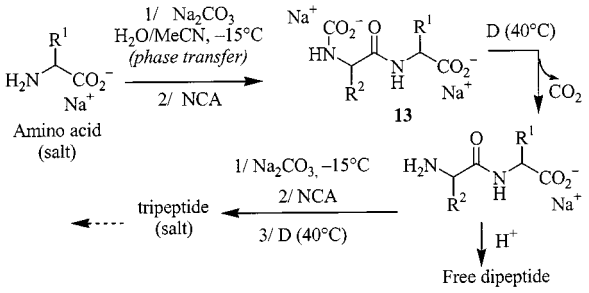
Only traces of nitroso adducts at the peptide bond nitrogen atom (compounds **12**) were observed in the crude reaction mixtures. Although peptidic nitrogen atoms are probably less reactive than ureidic nitrogen atoms towards nitrosation, the acid-catalysed denitrosation was probably also involved in this case. That might explain why neither peptide bond cleavage nor deamination were observed, in contrast with, for instance, the results of Garcia et al.[17] in a model study of lung tissue damage caused by polluted urban air, in which the nitrosating agent was obtained from an NO_2 stream through a sodium acetate suspension in dichloromethane.

In view of future applications, however, the presence of nitroso adducts in the final products had to be strictly avoided, due to their toxicity: numerous studies have examined the carcinogenic, mutagenic or teratogenic character of nitroso ureas.[18−21] Our nitrosation method offers the advantage that only trace amounts of these undesirable adducts will have to be removed, but a further denitrosation procedure will have to be applied anyway. In a preliminary study, a solution of N^{ε}-nitroso-N^{ε}-carbamoyllysine NCA in acetonitrile was efficiently and quantitatively denitrosated by bubbling of dry HCl through the solution. The use of a suitable scavenger (such as hydrazine, urea, etc.) may be necessary to remove final traces of nitroso ureas and nitroso amides, the presence of which being a serious limitation to this protecting group strategy otherwise. Details regarding this concern will be discussed in a future paper.

The N-Carbamoyl Group as a Preactivating Group for Peptide Coupling via NCAs

An alternative use of ureido derivatives in peptide synthesis is as starting materials for NCA synthesis through nitrosation:[2] this novel reaction offers much easier access to NCAs than the classical phosgene route does.[22,23] NCAs are well-known monomers for polypeptide synthesis; control of their polymerisation is the object of important investigations into the obtainment of well-defined homopolymers and statistical[24] or − recently − block copolymers.[25] NCA reactivity has also been investigated in the context of controlled oligomerisation and stepwise peptide coupling: Bailey[26] reported oligopeptide synthesis by treatment of NCAs with amino acid esters at low temperature in anhydrous organic media, without being able to avoid the formation of dioxopiperazine. On the basis of a kinetic study of NCA hydrolysis and reaction with amino groups in aqueous solution,[27] Denkewalter et al.[28,29] succeeded in controlling stepwise NCA oligomerisation in aqueous media.

In a reactivity study, Iwakura et al.[30] showed that the propagation reaction in acetonitrile proceeded only through a nucleophilic attack on the NCA at C-5 by the amino end-group of the growing chain. He proposed a phase-transfer method for the preparation of sequential oligopeptides (up to 4 residues) in good yields without isolation of the intermediates: the coupling occurred at −15 °C at the interface between an acetonitrile solution of the NCA and an aqueous solution of the free amino acid (or peptide). Saturation of the aqueous phase with sodium carbonate stabilised the carbamate intermediate **13**, thus preventing further NCA coupling (Scheme 6). After separation of the two layers and *N*-decarboxylation of **13** by simple warming to 40 °C, the elongated peptide could be treated with a further batch of NCA, or recovered after acidification as the free peptide. The phase-transfer system efficiently prevented side reactions such as hydrolysis of the NCA, which could therefore be used in excess to obtain complete conversion.



Scheme 6

Our NCA synthetic route offered the opportunity to adapt the method of Iwakura through a straightforward "one-pot" procedure. The NCA was prepared by nitrosation of the CAA by a mixture of NO and O_2 (4:1) at room temperature and under atmospheric pressure, in the same way as the decarbamoylation of C-peptides **4**−**7**;[2] the crude NCA was then directly used in the peptide coupling reaction, by Iwakura's procedure.

The dipeptides Val-Gly and Leu-Gly were thus obtained in good yields (Table 2). On the basis of preliminary experiments on the nitrosation step, which showed about 90% conversion, the initially reacting CAA amounted to ca. 1.2−1.3 equiv. of the glycine involved in the coupling step, hence ensuring a sufficient NCA/amino acid stoichiometric excess. Optical rotation measurement, with comparison with commercial authentic samples, showed that almost no racemisation occurred (ca. 97% *ee* in final products).

Table 2. Dipeptide coupling by NCA formation

Dipeptide	Yield (%)[a]	*ee* (%)	$[\alpha]_D$	$[\alpha]_D$ ref.[b]
Val-Gly	86 (90)[c]	96.8	+42.9	+44.3
Leu-Gly	82 (87)[c]	97.0	+39.0	+40.2

[a] Overall yield. [b] Of commercial authentic samples. $[\alpha]_D$ measured at 20 °C (c = 2.5, 2 N HCl). [c] Values in parentheses: yield after first step.

Conclusion

The solid-phase nitrosation of *N*-carbamoylpeptides or of CAAs by gaseous NO_x, affording *N*-deprotected peptides or NCAs, respectively, offers interesting possibilities in stepwise peptide synthesis. We have shown that the *N*-carbamoyl group may be used as an *N*-protecting group in peptide coupling by the mixed-anhydride method. Furthermore, complementary studies to evaluate this protecting group strategy with other peptide-coupling methods and to overcome its major limitations, namely the presence of trace nitroso adducts in coupling products and α-C epimerisation, remain to be done. This last concern might be favourably addressed by use of improved IBCF/NMM operating procedures.[31]

On the other hand, the easy route to NCAs offered by CAA nitrosation allows stepwise peptide elongation to be carried out without protecting groups in a simple, one-pot procedure. Both strategies share the important advantage of cheapness of starting *N*-carbamoylamino acids, in comparison with the usual *N*-protected amino acids.

Experimental Section

Hazardous Compounds: Nitric oxide is skin- and mucous-tissue-irritant. Nitroso ureas are potent carcinogens. All operations involving the handling of these compounds or their solutions were carried out in a fume hood, while wearing gloves. Effluents were destroyed by treatment with concentrated sodium hydroxide.

Materials and Methods: Methanol, acetonitrile, chloroform, and ethyl acetate were obtained from Baeckeroot (France). Alanine, valine, leucine, dimethylformamide (DMF), (−)-menthyl chloroformate, isobutyl chloroformate (IBCF), pyridine, and sodium borate were obtained from Aldrich. Glycine ethyl ester hydrochloride and phenylalanine ethyl ester hydrochloride were obtained from Acros Chemicals. *N*-Methylmorpholine was obtained from Fluka. Sodium glycinate was obtained from Sigma. Sodium carbonate and potassium cyanate were obtained from Prolabo. Hydrochloric acid (36%) was obtained from Carlo Erba. Nitric oxide and oxygen were obtained from L'Air Liquide (France). Acetonitrile, DMF, and *N*,*N*-dimethylacetamide (DMAc) were dried with molecular sieves prior to use. Potassium cyanate was washed with methanol and dried in vacuo prior to use. All other reagents and solvents were used as received. Melting points were taken with a Büchi 520 apparatus. ^{1}H NMR spectra were recorded with Bruker AC 200 or AC 250 spectrometers (200 or 250 MHz) in [D_6]DMSO solution. The pH regulation was performed with a Methrom Titrino 719S autotitrator. HPLC analyses were carried out with a Varian apparatus set up with a Varian 2510 pump, a Varian 2550 UV detector and a Varian 4290 integrator. Nucleosil RP-C18 (5 μm, 250 × 4.1 mm i.d.) analytical columns (obtained from Shandon, France) were used at room temperature under isocratic conditions (detailed conditions are provided below). Gas Chromatography (GC) analyses were carried out with a Shimadzu GC-14A apparatus equipped with a chromatopac C-9R 6A integrator, with OV-1701 silica (30 m × 0.25 mm i.d. film thickness 0.25 μm) capillary columns (obtained from The Quadrexp Corp., New Haven, CT). Optical rotation measurements were carried out with a Perkin−Elmer 241 polarimeter at the sodium emission wavelength (589 nm) in a 10-cm glass cell. The preparation of the *N*-carbamoylamino acids

C-Val, C-Ala, and C-Leu was carried out according to our previously published procedure.[8]

Peptide Coupling by the Mixed-Anhydride Method[9]

C-Val-Gly-OEt (4). − Classical Procedure: *N*-Methylmorpholine (0.6 mL, 5.5 mmol, 1.1 equiv.) was added to a chilled (−15 °C, liquid nitrogen/ethanol bath), stirred solution of *N*-carbamoylvaline (0.80 g, 5 mmol) in DMF (30 mL) under an inert gas; after 3−5 min of stirring, IBCF (0.7 mL, 5.5 mmol) was added to the mixture, whereupon a precipitate appeared. After another 1−2 min of stirring, a solution of glycine ethyl ester hydrochloride (0.70 g, 5 mmol) and *N*-methylmorpholine (0.6 mL, 5 mmol) in DMF [10 mL, this solution was prepared by dissolving the glycine ester hydrochloride in warm DMF, and then adding the *N*-methylmorpholine to the cooled (room temp.) solution and swirling] was added. After 20 min of stirring at −15 °C, the mixture was allowed to warm up to room temperature, and then stirred again for another 60 min at room temp. The solvent was then evaporated in vacuo, and the residue was recrystallised from methanol to afford 1.07 g (4.35 mmol, 87%) of pure material. M.p. 227−229 °C. NMR: δ_H = 0.84 (d, 3 H, H_γ^{Val}, $J_{\gamma\delta}$ = 7.0 Hz), 0.88 (d, 3 H, H_γ^{Val}, $J_{\gamma\delta}$ = 7.0 Hz), 1.20 (t, 3 H, O−CH_2−*CH_3*, J_1 = 7.0 Hz), 1.93 (m, 1 H, H_β^{Val}), 3.82 (2 H, ABX system, H_α^{Gly}, $J_{\alpha\text{-}NH}^{Gly} = J_{\alpha'\text{-}NH}^{Gly}$ = 6.0 Hz, $J_{\alpha\alpha'}^{Gly}$ = 17.5 Hz), 4.08 (dd, 1 H, H_α^{Val}, $J_{\alpha\text{-}NH}^{Gly}$ = 9.0 Hz, $J_{\alpha\text{-}\beta}^{Gly}$ = 7 Hz), 4.09 (q, 2 H, O−*CH_2*−CH_3, J_1 = 7.0 Hz), 5.60 (s, 2 H, −NH_2), 6.11 (d, 1 H, −CH_α^{Val}−*NH*−, J_1 = 9.0 Hz), 8.36 (t, 1 H, −CH_α^{Gly}−*NH*−, $J_{\alpha\text{-}NH}^{Gly}$ = 5.5 Hz). δ_C = 14.9 (O−CH_2−*CH_3*), 18.5 (C_γ^{Val}), 20.0 ($C_{\gamma'}^{Val}$), 32.0 (C_β^{Val}), 41.47 (C_α^{Gly}), 58.3 (C_α^{Val}), 61.2 (O−*C*H_2−CH_3), 159.2 (−NH−*C*O−NH_2), 170.6 (C_α^{Val}−*C*O−NH-), 173.5 (−*C*O−O−).

C-Leu-Gly-OEt (5). − Classical Procedure: The reaction was carried out with C-Leu (0.87 g, 5 mmol) and glycine ethyl ester hydrochloride (0.70 g, 5 mmol). The crude product was purified by silica gel chromatography (eluent ethyl acetate/methanol, 9:1) and then recrystallised from methyl acetate to afford 1.04 g (4 mmol, 80%) of pure material. M.p. 150−152 °C. NMR: δ_H = 0.88 (d, 3 H, H_δ^{Leu}, $J_{\gamma\delta}$ = 6.5 Hz), 0.92 (d, 3 H, H_δ^{Leu}, $J_{\gamma\delta}$ = 6.5 Hz), 1.20 (t, 3 H, O−CH_2−*CH_3*, J_1 =7.0 Hz), 1.40 (m, 2 H, H_β^{Leu}), 1.63 (m, 1 H, H_γ), 3.80 (2 H, ABX system, H_α^{Gly}, $J_{\alpha\text{-}NH}^{Gly} = J_{\alpha'\text{-}NH}^{Gly}$ = 6.0 Hz, $J_{\alpha\alpha'}^{Gly}$ = 17.0 Hz), 4.08 (q, 2 H, O−*CH_2*−CH_3, J_1 = 7.0 Hz), 4.18 (1 H, ABX system, H_α^{Leu}), 5.55 (s, 2 H, −NH_2), 6.13 (d, 1 H, CH_β^{Leu}−*NH*−, $J_{\alpha\text{-}NH}$ = 8.5 Hz), 8.38 (t, 1 H, −CH_α^{Gly}−*NH*−, $J_{\alpha\text{-}NH}^{Gly}$ = 6.0 Hz); δ_C = 14.9 (O−CH_2−*CH_3*), 22.8 (C_δ^{Leu}), 23.8 ($C_{\delta'}^{Leu}$), 24.9 (C_γ^{Leu}), 41.4 (C_β^{Leu}), 43.1 (C_α^{Gly}), 51.9 (C_α^{Leu}), 61.2 (O−*C*H_2−CH_3), 158.9 (−NH−*C*O−NH_2), 170.6 (C_α^{Leu}−*C*O−NH−), 174.5 (−*C*O−O−). − **Alternative Procedure:** *N*-Methylmorpholine (0.6 mL, 5.5 mmol, 1.1 equiv.) was added under an inert gas to a chilled (−15 °C), stirred solution of C-Leu (0.87 g, 5 mmol) in DMF (30 mL). After 3−5 min of stirring, a solution of glycine ethyl ester hydrochloride (0.70 g, 5 mmol) and *N*-methylmorpholine (0.6 mL, 5 mmol) in 10 mL of DMF (prepared as above) was added, followed (after another 3−5 min) by IBCF (0.7 mL, 5.5 mmol). After 20 min of stirring at −15 °C, the mixture was allowed to warm up to room temperature, and then stirred for another 60 min at room temp. After in vacuo evaporation of the solvent, the residue was purified as above to afford 1.06 g (0.41 mmol, 82%) of pure material, exhibiting the same physical characteristics as above.

C-Ala-Gly-OEt (6). − Classical Procedure: The reaction was carried out with *N*-carbamoylalanine (0.66 g, 5 mmol) and glycine ethyl ester hydrochloride (0.70 g, 5 mmol). The crude product was purified by silica gel chromatography (eluent ethyl acetate/methanol, 9:1) and then recrystallised from methyl acetate/methanol to afford 0.56 g (0.26 mmol, 52%) of pure material. M.p. 145−147 °C. NMR: δ_H = 1.17 (d, 3 H, H_β^{Ala}, $J_{\alpha\beta}^{Ala}$ = 7.0 Hz), 1.20 (t, 3 H, O−CH_2−*CH_3*, J_1 =7.0 Hz), 3.82 (2 H, ABX system, H_α^{Gly}, $J_{\alpha\alpha'}^{Gly}$ = 17.5 and $J_{\alpha\text{-}NH}^{Gly} = J_{\alpha'\text{-}NH}^{Gly}$ = 6.0 Hz), 4.09 (q, 2 H, O−*CH_2*−CH_3, J_1 =7.0 Hz), 4.16 (m, 1 H, H_α^{Ala}, $J_{\alpha\text{-}NH}^{ALa}$ = 7.5 Hz), 5.59 (s, 2 H, CO−*NH_2*), 6.18 (d, 1 H, CH^{Ala}−*NH*, $J_{\alpha\text{-}NH}^{ALa}$ = 7.5 Hz), 8.34 (t, 1 H, −CH_2^{Gly}−NH−, $J_{\alpha\text{-}NH}^{Gly}$ = 6.0 Hz); δ_C = 14.9 (O−CH_2−*CH_3*), 20.4 (C_β^{Ala}), 41.44 (C_α^{Gly}), 49.1 (C_α^{Ala}), 61.2 (O−*C*H_2−CH_3), 158.8 (−NH−*C*O−NH_2), 170.6 (C_α^{Ala}−*C*O−NH−), 174.7 (−*C*O−O−). − **Alternative Procedure:** The reaction was carried out with *N*-carbamoylalanine (0.66 g, 5 mmol), the crude product was purified as above to afford 0.91 g (4.2 mmol, 84%) of pure material, exhibiting the same physical characteristics as above.

C-Ala-Phe-OEt (7). − Alternative Procedure: The reaction was carried out with *N*-carbamoylalanine (0.66 g, 5 mmol) and L-phenylalanine ethyl ester hydrochloride (1.15 g, 0.5 mmol). The crude product was purified by silica gel chromatography (eluent ethyl acetate/methanol, 9:1) and recrystallisation from methyl acetate/methanol to afford 1.40 g (4.55 mmol, 91%) of pure material. M.p. 184−186 °C. NMR: δ_H = 0.96 (d, 3 H, H_β^{Ala}, $J_{\alpha\beta}$ = 7.0 Hz), 1.13 (dd, 3 H, O−CH_2−*CH_3*, J_1 = 7.0 Hz), 2.93 (m, 2 H, H_β^{Phe}), 4.04 (q, 2 H, O−*CH_2*−CH_3, J_1 = 7.0 Hz), 4.13 (m, 1 H, H_α^{ALa}), 4.45 (m, 1 H, H_α^{Phe}), 5.57 (s, 2 H, NH_2), 6.13 (d, 1 H, CH^{Ala}−*NH*, $J_{\alpha\text{-}NH}^{Ala}$ = 8.0 Hz), 7.25 (m, 5 H, H_{Ar}), 8.38 (d, 1 H, −CH^{Phe}−NH−, $J_{\alpha\text{-}NH}^{Phe}$ = 8.0 Hz); δ_C = 14.8 (O−CH_2−*CH_3*), 20.6 (C_β^{Ala}), 37.5 (C_β^{Phe}), 49.0 (C_α^{Ala}), 54.1 (C_α^{Phe}), 61.4 (O−*C*H_2−CH_3), 127.4 (C_δ^{Ar}), 129.1 (C_γ^{Ar}), 130.1 (C_β^{Ar}), 138.0 (C_α^{Ar}), 158.8 (−NH−*C*O−NH_2), 172.3 (C_α^{Ala}−*C*O−NH−), 174.2 (*C*O−O−).

***N*-Decarbamoylation of *N*-Carbamoyldipeptide Esters**

H-Val-Gly-OEt. − General Procedure: A 100-mL flask was fitted with a magnetic stirrer and capped with a silicon rubber septum, and finely powdered C-Val-Gly-OEt (100 mg, 0.41 mmol) was added, spread over 2-mm glass beads and flushed with nitrogen. Nitric oxide (27.5 mL, 1.23 mmol, 3 equiv.), oxygen (11 mL, 0.49 mmol, 1.2 equiv.), and then pure water (22 μL, 1.22 mmol, 3 equiv.) were introduced through separate glass syringes. After 30 min of stirring, the flask was rapidly flushed with nitrogen to remove the unchanged NO_x, and the crude product was then dried in vacuo in a heating desiccator (30 °C) to afford 108 mg (0.41 mmol, 100%) of pure H-Val-Gly-OEt (salt with nitric acid). NMR: δ_H = 0.98 (d, 3 H, H_γ^{Val}, $J_{\gamma\delta}$ = 7.0 Hz), 1.00 (d, 3 H, H_γ^{Val}, $J_{\gamma\delta}$ = 7.0 Hz), 1.21 (t, 3 H, O−CH_2−*CH_3*, J_1 = 7.0 Hz), 2.10 (m, 1 H, H_β^{Val}), 3.82 (d, 1 H, C_α^{Val}, $J_{\alpha\beta}^{Val}$ = 5.5 Hz), 3.97 (2 H, ABX system, H_α^{Gly}, $J_{\alpha\text{-}NH}^{Gly} = J_{\alpha'\text{-}NH}^{Gly}$ = 6.0 Hz, $J_{\alpha\alpha'}^{Gly}$ = 16.0 Hz), 4.13 (q, 2 H, O−*CH_2*−CH_3, J_1 = 7.0 Hz), 8.13 (s, 3 H, −NH_3), 8.86 (t, 1 H, −CH_α^{Gly}−*NH*−, $J_{\alpha\text{-}NH}^{Gly}$ = 6.0 Hz). δ_C = 14.8 (O−CH_2−*CH_3*), 18.4 (C_γ^{Val}), 18.94 ($C_{\gamma'}^{Val}$), 30.7 (C_β^{Val}), 41.6 (C_α^{Gly}), 58.2 (C_α^{Val}), 61.5 (O−*C*H_2−CH_3), 169.2 (C_α^{Val}−*C*O−NH−), 170.1 (−*C*O−O−).

H-Leu-Gly-OEt: The reaction was carried out with (finely powdered) C-Leu-Gly-OEt (100 mg, 0.39 mmol), nitric oxide (26 mL, 1.16 mmol, 3 equiv.), oxygen (10.5 mL, 0.47 mmol, 1.22 equiv.), and pure water (21 μL, 1.17 mmol, 3 equiv.) to afford 107 mg (0.39 mmol, 100%) of pure H-Leu-Gly-OEt. NMR: δ_H = 0.97 (d, 3 H, H_δ^{Leu}, $J_{\gamma\delta}$ = 6.0 Hz), 0.99 (d, 3 H, H_δ^{Leu}, $J_{\gamma\delta}$ = 6.0 Hz), 1.20 (t, 3 H, O−CH_2−*CH_3*, J_1 = 7.0 Hz), 1.58 (m, 2 H, H_β^{Leu}), 1.71 (m, 1 H, H_γ^{Val}), 3.81 (1 H, ABX system, H_α^{Leu}), 3.95 (2 H, ABX system, H_α^{Gly}, $J_{\alpha\text{-}NH}^{Gly} = J_{\alpha'\text{-}NH}^{Gly}$ = 6.0 Hz, $J_{\alpha\alpha'}^{Gly}$ = 10.5 Hz), 4.13 (q, 2 H, O−*CH_2*−CH_3, J_1 = 7.0 Hz), 8.26 (s, 3 H, −NH_3), 8.95 (t, 1

H, $-CH_{\alpha}^{Gly}-NH-$, $J_{\alpha\text{-}NH}^{Gly}$ = 6.0 Hz); δ_C = 14.9 ($O-CH_2-CH_3$), 22.6 (C_{δ}^{Leu}), 23.4 ($C_{\delta'}^{Leu}$), 24.9 (C_{γ}^{Leu}), 40.8 (C_{β}^{Leu}), 41.0 (C_{α}^{Gly}), 51.6 (C_{α}^{Leu}), 61.5 ($O-CH_2-CH_3$), 170.1 ($C_{\alpha}^{Leu}-CO-NH-$), 170.4 ($-CO-O-$).

H-Ala-Gly-OEt: The reaction was carried out with finely powdered C-Ala-Gly-OEt (100 mg, 0.46 mmol), nitric oxide (31 mL, 1.38 mmol, 3 equiv.), oxygen (12.5 mL, 0.55 mmol, 1.19 equiv.) and pure water (25 µL, 1.38 mmol, 3 equiv.) to afford 109 mg (0.46 mmol, 100%) of pure H-Ala-Gly-OEt. NMR: δ_H = 1.21 (t, 3 H, $O-CH_2-CH_3$, J_1 = 7.0 Hz), 1.38 (d, 3 H, H_{β}^{Ala}, $J_{\alpha\beta}^{Ala}$ = 7.0 Hz), 3.85 (m, 1 H, H_{α}^{Ala}), 3.95 (2 H, ABX system, H_{α}^{Gly}), 4.10 (q, 2 H, $O-CH_2-CH_3$, J_1 = 7.0 Hz), 8.12 (s, 3 H, $-NH_3$), 8.83 (t, 1 H, $-CH_2^{Gly}-NH-$, $J_{\alpha\text{-}NH}^{Gly}$ = 6.0 Hz); δ_C = 14.6 ($O-CH_2-CH_3$), 17.7 (C_{β}^{Ala}), 41.3 (C_{α}^{Gly}), 48.6 (C_{α}^{Ala}), 61.2 ($O-CH_2-CH_3$), 169.9 ($C_{\alpha}^{Ala}-CO-NH-$), 170.6 ($-CO-O-$).

H-Ala-Phe-OEt: The reaction was carried out with finely powdered C-Ala-Phe-OEt (100 mg, 0.33 mmol), nitrogen dioxide (22 mL, 0.98 mmol, 3 equiv.), oxygen (9 mL, 0.39 mmol, 1.2 equiv.), and pure water (18 µL, 1 mmol, 3 equiv.) to afford 106 mg (0.33 mmol, 100%) of pure H-Ala-Phe-OEt. NMR: δ_H = 1.12 (t, 3 H, $O-CH_2-CH_3$, J_1 = 7.0 Hz), 1.35 (d, 3 H, H_{β}^{Ala}, $J_{\alpha\beta}$ = 7.0 Hz), 3.82 (m, 1 H, H_{α}^{ALa}), 2.98 (m, 2 H, H_{β}^{Phe}), 4.11 (q, 2 H, $O-CH_2-CH_3$, J_1 = 7.0 Hz), 4.59 (m, 1 H, H_{α}^{Phe}), 7.31 (m, 5 H, H_{Ar}), 8.05 (s, 3 H, $-NH_3$), 8.85 (t, 1 H, $-CH^{Phe}-NH-$, $J_{\alpha\text{-}NH}^{Phe}$= 9.0 Hz); δ_C = 14.8 ($O-CH_2-CH_3$), 18.0 (C_{β}^{Ala}), 37.2 (C_{β}^{Phe}), 48.9 (C_{α}^{Ala}), 54.2 (C_{α}^{Phe}), 61.6 ($O-CH_2-CH_3$), 127.6 (C_4^{Ar}), 129.1 (C_3^{Ar}), 130.0 (C_2^{Ar}), 137.6 (C_1^{Ar}), 170.2 ($C_{\alpha}^{Ala}-CO-NH-$), 171.9 ($CO-O-$).

Peptide Hydrolysis[11] and Derivatisation[12] for GC Analysis: A solution of (*N*-deprotected) dipeptide ethyl ester (100 mg) in 6 N HCl (10 mL) was heated to 110−120 °C (oven) in a sealed Pyrex tube. After cooling and concentration to dryness in a rotary evaporator, the residue was dissolved in aqueous sodium borate (50 mM, 2 mL, pH = 9.0), and then diluted with sodium borate (0.2 M, 20 mL, pH = 7.7). The pH of the mixture was checked and adjusted if necessary to 7.5−8 with HCl. To this solution (50 µL) in a 1-mL vial tube was added a pyridine/methanol mixture (40 µL, 20:80, v/v), and then (−)-menthyl chloroformate (10 µL). After occasional shaking for 10 min at room temp., the mixture was extracted once with chloroform (100 µL). GC analysis of the chloroform layer was performed with nitrogen as the carrier gas (10 mL·min^{-1}), 300 °C at inlet and outlet, and a temperature program (190 °C to 230 °C at 4 °C·min^{-1}, and then 30 min at 230 °C). t_R [min] = 12.8 (L-Leu), 12.9 (D-Leu), 6.3 (L-Ala), 6.4 (D-Ala), 9.6 (L-Val), and 9.9 (D-Val).

Peptide Coupling by NCA Formation: The same nitrosation procedure as for *N*-carbamoylpeptide decarbamoylation was used, except that no water was injected into the reaction flask.

H-Leu-Gly-OH: C-Leu (400 mg, 2.3 mmol) was nitrosated with nitric oxide (154 mL) and oxygen (39 mL) in a 500-mL flask. The crude mixture was dissolved in dry acetonitrile (10−15 mL), filtered, and concentrated in vacuo to 5−7 mL (the solution was filtered again if necessary), and then added to a chilled (−10 °C), vigorously stirred mixture of sodium glycinate (158.5 mg, 1.63 mmol) and sodium carbonate (173 mg, 1.63 mmol) in 0.2 N NaOH (10 mL) and acetonitrile (7 mL). After 2 h of stirring at −10 °C, the two layers were separated and the aqueous layer was washed twice with 5 mL of cold (−10 °C, to ensure correct layer separation) acetonitrile. The aqueous layer was then concentrated to 5 mL at 40 °C under reduced pressure, and then neutralised with dilute sulfuric acid. After addition of 7−8 mL of ethanol and filtering off of the precipitated salts, the dipeptide was precipitated by ether addition and collected by filtration. The crude product was recrystallised from ethanol/ether to afford 251 mg (1.34 mmol, 82%) of pure material. NMR: δ_H = 0.92 (dd, 6 H, H_{δ}^{Leu}, $J_{\gamma\delta}$ = 6.0 Hz), 1.60 (m, 2 H, H_{β}^{Leu}), 1.70 (m, 1 H, H_{γ}^{Val}), 3.88 (2 H, ABX system, H_{α}^{Gly} and 1 H, ABX system, H_{α}^{Leu}), 8.15 (s, 3 H, $-NH_3$), 8.85 (t, 1 H, $-CH_{\alpha}^{Gly}-NH-$, $J_{\alpha\text{-}NH}^{Gly}$ = 5.5 Hz); δ_C = 22.7 (C_{δ}^{Leu}), 23.4 ($C_{\delta'}^{Leu}$), 24.3 (C_{γ}^{Leu}), 40.9 (C_{β}^{Leu}), 41.15 (C_{α}^{Leu}), 51.6 (C_{α}^{Leu}), 170.3 ($C_{\alpha}^{Leu}-CO-NH-$), 171.5 ($-COOH$).

H-Val-Gly-OH: The procedure was the same as above, with C-Val (370 mg, 2.3 mmol) and the same quantities of other reagents. The crude product was recrystallised from ethanol/ether to afford 244 mg (1.40 mmol, 86%) of pure material. NMR: δ_H = 0.98 (dd, 6 H, H_{γ}^{Val}, $J_{\gamma\delta}$ = 7.0 Hz), 2.10 (m, 1 H, H_{β}^{Val}), 3.65 (m, 1 H, C_{α}^{Val}), 3.89 (2 H, ABX system, H_{α}^{Gly}, $J_{\alpha\text{-}NH}^{Gly} = J_{\alpha'\text{-}NH}^{Gly}$ = 5.5 Hz, $J_{\alpha\alpha'}^{Gly}$ = 17.5 Hz), 8.11 (s, 3 H, $-NH_3$), 8.75 (t, 1 H, $-CH_{\alpha}^{Gly}-NH-$, $J_{\alpha\text{-}NH}^{Gly}$ = 5.5 Hz); δ_C = 17.2 (C_{γ}^{Val}), 17.8 ($C_{\gamma'}^{Val}$), 29.4 (C_{β}^{Val}), 40.3 (C_{α}^{Gly}), 56.9 (C_{α}^{Val}), 167.9 ($C_{\alpha}^{Val}-CO-NH-$), 171.3 ($-COOH$).

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[1] H. Collet, L. Boiteau, J. Taillades, A. Commeyras, *Tetrahedron Lett.* **1999**, *40*, 3355−3358.
[2] H. Collet, C. Bied, L. Mion, J. Taillades, A. Commeyras, *Tetrahedron Lett.* **1996**, *37*, 9043−9046.
[3] A. Rousset, M. Lasperas, J. Taillades, A. Commeyras, *Tetrahedron* **1980**, *36*, 2649−2661.
[4] A. Yamashiro, K. Kobuta, K. Yokozeki, *Agric. Biol. Chem.* **1988**, *52*, 2857−2863.
[5] V. F. Gonko, N. D. Shustova, G. M. Anoshina, T. E. Zubova, L. B. Radina, *Pharm. Chem. J.* **1978**, *12*, 601−606.
[6] D. G. Smyth, *J. Biol. Chem.* **1967**, *242*, 1579.
[7] A. Williams, W. P. Jencks, *J. Chem. Soc., Perkin Trans. 2* **1974**, 1753−1759.
[8] J. Taillades, L. Boiteau, I. Beuzelin, O. Lagrille, J. P. Biron, W. Vayaboury, O. Vandenabeele-Trambouze, O. Giani, A. Commeyras, *J. Chem. Soc., Perkin Trans. 2* **2001**, 1247−1254.
[9] J. Meienhofer, in: *The peptides*, vol. 1 (Ed.: E. Gross, J. Meinhofer), Academic Press, Orlando, FL, **1979**, chapter 6.
[10] G. W. Anderson, J. E. Zimmerman, F. M. Callahan, *J. Am. Chem. Soc.* **1967**, *89*, 5012−5017.
[11] C. H. Clapp, J. S. Swan, J. L. Poechmann, *J. Chem. Educ.* **1992**, *69*, 122−126.
[12] N. Domergue, M. Pugniere, A. Previero, *Anal. Biochem.* **1993**, *214*, 420−425.
[13] J. M. Manning, *J. Am. Chem. Soc.* **1970**, *92*, 7449−7454.
[14] N. E. Blair, W. A. Bonner, *Orig. Life* **1980**, *10*, 255−263.
[15] N. L. Benoiton, *Biopolymers (Peptide Science)* **1996**, *40*, 245−254.
[16] M. Crisma, G. Valle, F. Formaggio, C. Toniolo, A. Bagno, *J. Am. Chem. Soc.* **1997**, *119*, 4136−4142.
[17] J. Garcia, J. Gonzalez, R. Segura, J. Vilassara, *Tetrahedron* **1984**, *40*, 3121−3127.
[18] P. N. Magee, P. F. Swann, *Br. Med. Bull.* **1969**, *25*, 240−244.
[19] P. N. Magee, J. M. Barnes, *Adv. Cancer Res.* **1967**, *10*, 163−167.
[20] F. K. Zimmermann, *Biochem. Pharmacol.* **1971**, *20*, 985−995.
[21] H. Druckrey, *Xenobiotica* **1973**, *3*, 271−303.
[22] T. Curtius, K. Hochschwender, H. Meier, W. Lehmann, A.

Benckiser, M. Schenk, W. Wirbatz, J. Gaier, W. Mühlhaüsser, *J. Prakt. Chem.* **1930**, *125*, 211−302.
[23] A. C. Farthing, R. J. W. Reynolds, *Nature (London)* **1950**, *165*, 647.
[24] H. R. Kricheldorf, *Amino Acid N-Carboxy Anhydrides and Related Heterocycles*, Springer-Verlag, Berlin, **1987**.
[25] T. J. Deming, *Nature* **1997**, *390*, 386−387.
[26] J. L. Bailey, *J. Chem. Soc.* **1950**, 3461−3466.
[27] P. D. Bartlett, R. H. Jones, *J. Am. Chem. Soc.* **1957**, *79*, 2153−2160.
[28] G. Denkewalter, H. Schwam, R. G. Strachan, T. E. Beesley, D. F. Veber, E. F. Schoenewaldt, H. Barkemeyer, W. J. Paleveda, Jr., T. A. Jacob, R. Hirschmann, *J. Am. Chem. Soc.* **1966**, *88*, 3163−3164.
[29] R. Hirschmann, H. Schwam, R. G. Strachan, E. F. Schoenewaldt, H. Barkemeyer, S. M. Miller, J. B. Conn, V. Garsky, D. F. Veber, R. G. Denkewalter, *J. Am. Chem. Soc.* **1971**, *93*, 2746−2754.
[30] Y. Iwakura, K. Uno, M. Oya, R. Katakai, *Biopolymers* **1970**, *9*, 1419−1427.
[31] W.-C. Shieh, J. A. Carlson, M. E. Shore, *Tetrahedron Lett.* **1999**, *40*, 7167−7170.

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