Peptide Synthesis under High Pressure. Coupling Reactions of *N*-(Benzyloxycarbonyl)-amino Acid *N*-Hydroxysuccinimide Esters with *N*-(Carboxymethyl)amino Acid Diesters

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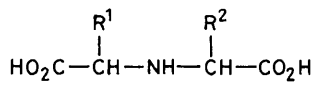
Coupling reactions of *N*-(benzyloxycarbonyl)amino acid *N*-hydroxysuccinimide esters with *N*-(carboxymethyl)amino acid diesters are significantly accelerated by using high pressure (10 kbar).

Iminodicarboxylic acids (**1**) are receiving increased attention because of their biological importance, having been found in crown gall tumours of plants and correlated with tumourigenesis,[1] and a series of synthetic dipeptides containing (**1**) have been demonstrated to be potently active as inhibitors of angiotensin-converting enzyme[2] and thermolysin.[3]

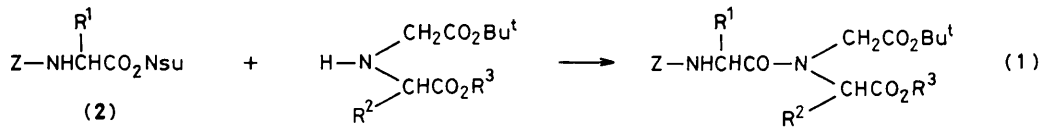
Recently *N*-carboxymethylamino acids (Cm-a.a.),† a simple type of (**1**) (R^1=H), have been investigated,[4] and it has been shown that the imino group of a Cm-a.a.† has remarkably poor reactivity, *i.e.*, the attempted couplings of *N*-(benzyloxycarbonyl)phenylalanine with the diester of *N*-(carboxymethyl)valine by various conventional methods of peptide synthesis have been unsuccessful, except when the acid chloride method is used.[4b] Presumably, this is because of the disadvantageous combination of a negative inductive effect and steric hindrance by the alkoxycarbonylmethyl group of Cm-amino acid diesters. The recent success of organic synthesis at high pressure, which offers a method of accelerating sluggish bond-forming reactions on sterically crowded substrates,[5] has prompted us to utilize high pressure to overcome this difficulty.

We report here that the coupling reactions of *N*-(benzyloxycarbonyl)amino acid *N*-hydroxysuccinimide esters (**2**) with Cm-amino acid diesters (**3**) are significantly accelerated by



(**1**)

† Abbreviations used: a. a. = amino acid, Cm = carboxymethyl, Nsu = succinimido, Z = benzyloxycarbonyl.



(2) (3) (4) (1)

a; $R^1 = CH_2Ph$ (Phe) **a**; $R^2 = Me$ (CmAla), $R^3 = Bu^t$
b; $R^1 = Me$ (Ala) **b**; $R^2 = Bu^i$ (CmLeu), $R^3 = Et$
c; $R^1 = Bu^i$ (Leu) **c**; $R^2 = Pr^i$ (CmVal), $R^3 = Et$
d; $R^1 = Pr^i$ (Val)

Table 1. Coupling reactions of *N*-(benzyloxycarbonyl)amino acid *N*-hydroxysuccinimide esters (**2**) with *N*-(carboxymethyl)amino acid diesters (**3**) [equation (1)][a]

Entry	(2)	(a.a.)	(3)[b]	(Cma.a.)	% Yield of (4)[c] 10 kbar	1 bar
1	(**2a**)	(Phe)	(**3a**)	(CmAla)	82.2	10.5
2	(**2b**)	(Ala)	(**3a**)	(CmAla)	70.1	8.7
3	(**2c**)	(Leu)	(**3a**)	(CmAla)	61.9	4.1[d]
4	(**2d**)	(Val)	(**3a**)	(CmAla)	27.9	1.6
5	(**2a**)	(Phe)	(**3b**)	(CmLeu)	32.4	3.8[d]
6	(**2b**)	(Ala)	(**3b**)	(CmLeu)	16.0	2.0[d]
7	(**2c**)	(Leu)	(**3b**)	(CmLeu)	15.9	0.3[d]
8	(**2d**)	(Val)	(**3b**)	(CmLeu)	2.2	nil[d]
9	(**2a**)	(Phe)	(**3c**)	(CmVal)	13.1	0.1[d]
10	(**2b**)	(Ala)	(**3c**)	(CmVal)	5.7	0.1[d]
11	(**2c**)	(Leu)	(**3c**)	(CmVal)	2.3	nil[d]
12	(**2d**)	(Val)	(**3c**)	(CmVal)	2.3	nil[d]

[a] Conditions are not optimized. [b] Prepared following a general procedure from amino acid ethyl or t-butyl ester, t-butyl bromoacetate, and triethylamine in dry tetrahydrofuran at room temperature (ref. 4): (**3a**), 53%, b.p. 78—80 °C/0.18 Torr, $[\alpha]_D^{25}$ −21.7° (*c* 1.19, MeOH); (**3b**), 67%, b.p. 96—103 °C/0.35 Torr, $[\alpha]_D^{25}$ −17.3° (*c* 1.16, MeOH); (**3c**), 47%, b.p. 91—94 °C/0.25 Torr, $[\alpha]_D^{25}$ −16.9° (*c* 0.95, MeOH). [c] Isolated yield, unless otherwise specified. All new compounds were characterized by ^1H n.m.r. and elemental analyses. [d] Determined by h.p.l.c.

using high pressures (10 kbar‡), affording the desired peptides in highly improved yields [equation (1)].§ The active ester method, not the acid chloride method, was chosen because of less equivocal reactions and the relative stability and moderate reactivity of the active esters.[6]

The reactions at high pressure were performed in a Teflon capsule (7.1 ml capacity) containing (**2**) (2 mmol), (**3**) (2 mmol), and dry dichloromethane (*ca.* 7 ml), compressed in a stainless steel apparatus[7] at ambient temperature for 7 days. After depressurisation, the reaction mixture was concentrated *in vacuo* to afford a crude product, which was chromatographically purified after treatment with 1-(2-aminoethyl)piperazine and, if necessary, with Amberlyst A-15. Control experiments at ambient pressure (1 bar) were also carried out by the reaction of (**2**) (2 mmol) with (**3**) (2 mmol) in dichloromethane (8 ml) at 20 °C for 7 days. The results are summarised in Table 1.

‡ 1 bar = 10^5 Pa

§ The a.a.s including the Cm-a.a. used here are all of the L-configuration.

The results of control experiments at 1 bar show the poor reactivity of Cm-amino acid diesters (**3**). The coupling of any active ester (**2**) with ethyl *N*-(t-butoxycarbonylmethyl)valinate (**3c**) afforded virtually no formation of (**4**) (entries 9—12).

However, when (**2**) was treated with (**3**) at high pressure, yields of (**4**) were significantly improved; coupling of (**2a—c**) with (**3a**) gave good yields (entries 1—3). Reactions of (**2**) and (**3**) containing more bulky substituents gave highly improved, but low yields; nevertheless, the desired product was easily isolated because of the few side reactions (entries 4—7, 9). The reaction of (**2b**) with (**3a**) at 1 bar for 16 days, however, afforded a 33.6% yield of (**4**) (cf. entry 2). Thus, it is the prolonged reaction time or the higher pressure that presumably affords increased yield.

This coupling reaction is susceptible to the bulkiness of the side-chains of the amino acids, especially of (**3**). The yield of (**4**) remained low even at 10 kbar in the reaction of the relatively bulky valine as the amino acid part of (**2**) or (**3**) (entries 8, 10—12). It is noteworthy that in this coupling reaction the steric requirement of phenylalanine in (**2**) seems to be smaller than that of alanine in (**2**) (Compare entry 1 with 2, 5 with 6, or 9 with 10).

In conclusion, high pressure conditions enable some of the title coupling reactions, which gave yields < 10% at atmospheric pressure, to be carried out in high yields. To our knowledge, this is the first application of high pressure to peptide bond formation.

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References

1 J. D. Kemp, *Biochem. Biophys. Res. Commun.*, 1976, **69**, 816; M. Drummond, *Nature*, 1979, **281**, 343; N. Murai and J. D. Kemp, *Proc. Natl. Acad. Sci. USA*, 1982, **79**, 86.

2 A. A. Patchett, E. Harris, E. W. Tristram, M. J. Wyvratt, M. T. Wu, D. Taub, E. R. Peterson, T. J. Ikeler, J. ten Broeke, L. G. Payne, D. L. Ondeyka, E. D. Thorsett, W. J. Greenlee, N. S. Lohr, R. D. Hoffsommer, H. Joshua, W. V. Ruyle, J. W. Rothrock, S. D. Aster, A. L. Maycock, F. M. Robinson, R. Hirschmann, C. S. Sweet, E. H. Ulm, D. M. Gross, T. C. Vassil, and C. A. Stone, *Nature*, 1980, **288**, 280; M. Vincent, G. Remond, B. Portevin, B. Serkiz, and M. Laubie, *Tetrahedron Lett.*, 1982, **23**, 1677.

3 A. L. Maycock, D. M. DeSousa, L. G. Payne, J. ten Broeke, M. T. Wu, and A. A. Patchett, *Biochem. Biophys. Res. Commun.*, 1981, **102**, 963.

4 (a) T. Miyazawa, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 2555; (b) *ibid.*, p. 3661.

5 (a) N. S. Isaacs, 'Liquid Phase High Pressure Chemistry,' Wiley Interscience, New York, 1981; (b) K. Matsumoto, A. Sera, and T. Uchida, *Synthesis*, in the press.

6 M. Bodanszky, 'The Peptides,' ed. E. Gross and J. Meienhofer, Academic Press, New York, 1979, vol. 1, chap. 3.

7 For a description of the high pressure equipment used see ref 5(b).