PEPTIDE SYNTHESIS UNDER HIGH PRESSURE BY AMINOLYSIS OF METHYL AND PHENYL ESTERS

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Received February 2, 1990
Accepted March 28, 1990

High pressure may, depending on solvent, enhance the reactivity of methyl and phenyl ester of the N-protected dipeptide Ala-Ala with the amino component, valine tert-butyl ester, yielding the protected tripeptide. Results of this reaction in several solvents are discussed.

Methyl ester was the first functionality used by Curtius for the synthesis of peptide bond in the reaction of glycine methyl ester[1]. The limitations of the method due to low reactivity were soon recognized[2]. Nevertheless, the reaction was given considerable attention (for review see ref.[3]). More recently, methyl esters yielded protected peptides in a reaction catalyzed by imidazole[4,5] and by engaging the ester in a complex with transition metal[6-8]. In both methods some features prevented wider application. Besides, methyl esters find extensive application in peptide synthesis as protecting group. Phenyl esters were studied for use in peptide synthesis as activating group[9], but their low reactivity could not keep pace with substituted phenyl esters and other means of activation. In the present context of peptide synthesis phenyl esters could also be regarded a carboxyl protecting group.

It has been shown recently, that under high pressure of 0·8 GPa (8 kBar) methyl and ethyl esters of simple mostly aliphatic carboxylic acids react at slightly elevated temperatures with amines in acetonitrile to give amides in good yields[10]. Although there exist a priori restrictions regarding direct applicability to peptide synthesis which stem from the ester character of many protecting groups in use, the reaction deserves further investigation.

We have chosen as a model system the reaction between Nps-Ala-Ala-OX (X = = Me, Ph) and Val-OBu^t. Under high pressure both components react at ambient temperature in several solvents yielding the protected tripeptide as the main product.

Results of the underlying experiments are summarized in Table I. In general, best results we achieved so far in THF and in methanol (experiments 9, 10, 13, and 7).

Two side reactions were observed and found to be related to the protection groups used. We noticed a transfer of the amino protecting *o*-nitrophenylsulfenyl (Nps) group to the amino group of Val-OBu^t, giving rise to Nps-Val-OBu^t. It takes part in reactions performed in the two chlorinated solvents studied and in reactions of the phenyl ester. This side reaction is probably associated with the presence of anionic species: chloride anion which may be generated from chlorinated solvents in the presence of base, and phenolate ion from the reactions of the phenol ester. At 80°C in dichloromethane, it accounted for most of the material bearing chromophore absorbing at the wavelength of maximum absorbtion of the Nps group. DMFA seems to promote this side reaction (Table I, experiment 12), but it is not effective alone (experiment 11). Another side product identified in reactions performed in methanol is Nps-Ala-Ala--Val-OMe, apparently the result of reesterification of the product, Npa-Ala-Ala-Val--OBu^t. Along with this, dioxopiperazine derived from valine was isolated in experiment 7. The inhibiting effect of high concentration of amine (experiment 7) could be explained by its participation in a complex with the tert. butyl ester, which colapses rather in the direction of starting components, but interferes in the interaction with

TABLE I

Results of the model reaction performed at pressure 1·4 GPa (14 kBar)

Experiment	Val-OBu^t		Solvent	Time, h/°C	Composition of reaction mixture, %				
	c, mol l^{-1}	excess			*I*	*II*	*III*	*IV*	*V*
1	0·6	2·45	CH_2Cl_2	5/50	81	11	—	0	1·5
2	as above, + additional			5/80	2·6	tr.	—	0	88
3[a]	0·6	2·45	$(CH_2Cl)_2$	5/50	95	2	—	0	tr.
4	0·76	2·9	MeOH	5/50	28	33	2·6	34	0
5	0·6	3·2	MeOH	5/50	41	24	1·4	29	0
6	0·8	5·3	MeOH	24/20	8	52	0·6	38	0
7	2·75	15	MeOH	72/20	0·2	87	<0·1	6	tr.
8[b]	2·75	15	MeOH	22/50	87	2	—	0	2
9	0·66	1·5	THF	5/50	79	21	—	0	0
10	as above, +6·5 eq. MeOH +			5/50	48	51	<0·02	0	1
11[a]	0·75	2·2	DMFA	24/20	100	0	—	0	0
12[a]	0·8	4	DMFA	24/20	5[c]	41	30	0	27
13	0·44	1·46	THF	24/20	0[c]	94	<0·03	0	2

[a] Reactions below the freezing point of the solvent; [b] reaction at pressure 1 Bar; [c] Nps-L-Ala-L--Ala-OPh.

methanol. In the same experiment, the extent of racemization was lower than in the rest of experiments in methanol.

It is interesting to compare the results obtained in DMFA and THF in view of the known effectivness of DMFA as a catalyst in aminolysis of esters[11,12]. While in THF the reaction proceeded a clean way both from the aspect of side reactions and optical purity, in DMFA there was no reaction with the methyl ester while impure and highly racemized product resulted from the reaction of the phenyl ester. It points to the possibility of a difference in character of the respective activated complexes deserving further study. The striking absence of any reaction in DMFA (experiment 11) can hardly be explained by diffusion problems in the pressure solidified solution because of reactivity of the phenyl ester in the same solvent. Moreover some reaction proceeded also in dichloroethane, the choice of which was motivated by the solidification induced acceleration and increased selectivity of glycosylation as earlier observed[13].

I, Nps-L-Ala-L-Ala-OMe
II, Nps-L-Ala-L-Ala-L-Val-OBu^t
III, Nps-L-Ala-D-Ala-L-Val-OBu^t
IV, Nps-L-Ala-L-Ala-L-Val-OMe
V, Nps-L-Val-OBu^t

From the preparative point of view, the reaction of methyl ester in THF, though clean, is slow in spite of some acceleration which can be attained by the addition of small amount of methanol without significant deterioration of the result (experiment 10). A prerequisite for a trial to speed up the reaction by acid catalysis is a choice of another amino protecting group. On the other hand, phenyl ester is perhaps a candidate for further screening, as can be judged from Table I (experiment 13).

EXPERIMENTAL

The reactions were performed in thin-walled teflon vials in 0·5 ml volume. Samples were subjected to external hydrostatic pressure of 1·4 GPa (14 kBar) and analyzed by HPLC on C_8 and C_{18} columns in acetonitrile–phosphate buffer pH 6·5. The product was identified by comparison with the authentic sample synthetized in a conventional way and detected in the reaction mixture by ^{1}H NMR (Bruker 500 MHz, courtesy of Dr N. F. Sepetov, Institute of Experimental Cardiology). Side products were isolated in chromatographic experiments and analyzed by mass spectrometry (VG Analytical, ZAB-EQ). The extent of racemization was estimated by HPLC on Spectrosil column in ethyl acetate–petroleum ether by comparison with the authentic sample of the diastereomeric Nps-L-Ala-D-Ala-L-Val-OBu^t synthetized in a conventional way.

Nps-L-Ala-L-Ala-L-Val-OBu^t

Nps-L-Ala-L-Ala-OPh (58 mg, 0·15 mmol) was dissolved in a mixture of THF (0·41 g) and L Val-OBu^t (38 mg, 0·22 mmol) and subjected to pressure of 1·4 GPa for 24 h at 20°C. The reaction mix-

ture was analyzed by HPLC (Table I, experiment 13) and evaporated at 45°C. The sirupy residue was dissolved in ethyl acetate, washed with 1M solution of $KHSO_4$, three times with saturated solution of sodium sulfate, 1M-NaOH and twice with saturated sodium sulfate, dried with anhydrous sodium sulfate and evaporated to dryness. The residue (59 mg) was dissolved in ether and diluted with cyclohexane. Yield 13 mg of large yellow crystals, m.p. 110–111°C. For $C_{21}H_{32}N_4O_6S.1/2\ C_6H_{12}$ (510·7) calculated 56·45% C, 7·50% H, 10·97% N; found 56·85% C, 7·60% H, 10·94% N. Another portion was obtained from mother liquors, yield 43 mg, m.p. 80–100°C.

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Translated by the author (V.G.).