Amide Protection and Amide Supports in Solid-phase Peptide Synthesis

By P. G. PIETTA† and GARLAND R. MARSHALL*

(*Department of Physiology and Biophysics and of Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri* 63110)

Summary A protecting group for side-chain amide functions, and polymeric amino-supports for use in the synthesis of *C*-terminal amide peptides, have been developed, and their use demonstrated in the syntheses of gastrin tetrapeptide by the solution and the solid-phase procedures.

GLUTAMINE and asparagine when used in peptide synthesis are subject to rearrangements to give the cyclic-imide and pyrollidone derivatives as well as dehydration to the nitrile when used with dicyclohexylcarbodi-imide (DCC) coupling procedures. Protection of the side-chain amide group as a means of avoiding these side reactions has been shown[1-3] with the bis(2,4-dimethoxybenzyl) group, 2,4-dimethoxybenzyl group and 2,4,6-trimethoxybenzyl group. These groups are relatively acid-sensitive, however, and are not sufficiently stable under the acidic conditions normally associated with deprotection in solid-phase synthesis[4] (1 N-HCl–HOAc, 4 N-HCl–dioxan, and 50%-CF_3CO_2H–CH_2Cl_2).

TABLE

Pmb amino-acid derivatives

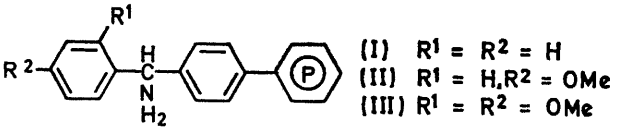
Compound	M.p.	R_F	System*
Boc–Asn(Pmb)–OBz	105—106	0·74	3
Boc–Asn(Pmb)–OH	132—133	0·71	4
Boc–Gln(Pmb)–OBz	108—109	0·80	3
Boc–Gln(Pmb)–OH	96—97	0·70	4
Boc–Gly–NH–Pmb	69—70	0·81	3
HCl·NH_2–Gly–NH–Pmb	162—163	0·77	2
Boc–Met–NH–Pmb	95—97	0·72	3
Boc–Phe–NH–Pmb	122	0·86	3
HCl·NH_2–Phe–NH–Pmb	177—178	0·85	2
Z–Phe–NH–Pmb	157—158	0·60	1
Boc–Val–NH–Pmb	122—123	0·65	3

* The t.l.c. solvent systems were prepared with the following proportions (v/v) and used with silica gel G plates in the ascending manner. 1 = Benzene–EtOAc–petroleum ether (50 : 25 : 25); 2 = Bu^nOH–AcOHH_2O (4 : 1 : 4); 3 = Benzene–EtOAc–petroleum ether (25 : 70 : 5); 4 = $CHCl_3$–MeOH–AcOH (18 : 2 : 1).

In contrast, the *p*-methoxybenzyl (Pmb) group is stable as an amide protecting group at room temperature in CF_3CO_2H, 1 N-HCl–HOAc, and 4 N-HCl–dioxan. Deprotection to give the free amide is accomplished by treatment with anhydrous HF. This group has been introduced by reacting a free carboxyl function with *p*-methoxybenzylamine by the use of either DCC or *N*-ethynylmethyldiethylamine.[5] The reaction of *N*-Boc-L-glutamic acid α-benzyl ester (Boc = t-butoxycarbonyl) and *N*-Boc-L-aspartic acid α-benzyl ester with *p*-methoxybenzylamine gave *N*-Boc-L-glutamine(Pmb) α-benzyl ester and *N*-Boc-L-asparagine(Pmb) α-benzyl ester, respectively. Hydrogenolysis gave the desired crystalline *N*-Boc-L-glutamine-(Pmb)–OH and *N*-Boc-L-asparagine(Pmb)–OH. An analogous procedure has given other Pmb-protected amino-acid amides, some of which are shown in the Table. Starting with crystalline H–Phe–NH–Pmb, the crystalline Boc–Phe–Ala–Phe–NH–Pmb as well as the crystalline protected *C*-terminal tetrapeptide of gastrin, Boc–Trp–Met–Asp-(CH_2Ph)–Phe–NH–Pmb (194—195°), have been synthesized by the stepwise nitrophenyl-ester procedure. The tripeptide amide, H–Phe–Ala–Phe–NH_2, and the free tetrapeptide amide, H–Trp–Met–Asp–Phe–NH_2, were obtained by treatment of the protected peptides with anhydrous HF to which anisole was added.

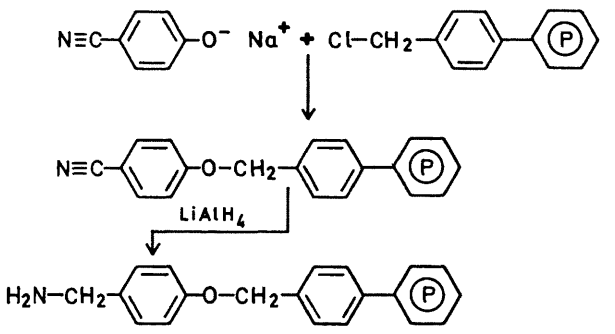
Gastrin tetrapeptide is only one of the many biologically-active peptides with a *C*-terminal amide. The use of a polymeric support which allowed the attachment directly through the amide bond seemed advantageous for the synthesis of such peptides. Although the cleavage of the ester link to solid-phase supports by ammonolysis has been successful in the synthesis of oxytocin[6] and [Lys^8]-vasopressin,[7] transesterification has been reported as a major side reaction in some cases,[8] and the presence of side-chain carboxyl groups presents additional difficulties. Gastrin tetrapeptide has been synthesized by Shchukina *et al.*[9] by an elaborate method. The simplicity offered by covalently linking the *C*-terminal residue directly through an amide bond appeared desirable. A polymeric *p*-methoxybenzylamine support was prepared by the reaction of sodium *p*-cyanophenoxide with chloromethylated polystyrene–2%-divinylbenzene. The reduction of the cyano-group with $LiAlH_4$ in diglyme gave the required polymeric amine as shown in the Scheme.

As well as supports based on the *p*-methoxybenzyl group, modified diphenylmethylamine supports are also under investigation; compounds (I), (II), and (III) have been prepared. As an example, support (I) was prepared by the treatment of the diphenylmethyl bromide or chloride



support used by Southard *et al.*[10] with a saturated solution of ammonia in methylene chloride at 0°. Boc–Phe–OH was treated with DCC and support (I) in CH_2Cl_2. Amide peaks were present in the i.r. spectra of the resulting polymer and any remaining amine was acetylated with acetic anhydride. Treatment of the polymer with anhydrous HF cleaved the amide from the support and gave crystalline H–Phe–NH_2 when the filtrate was evaporated. The amide bands were no longer present when the polymer was examined by i.r. Treatment of the Boc–Phe–NH-support with HCl-HOAc did not remove H–Phe–NH_2 from the polymer, indicating that the peptide chain can be elongated by normal solid-phase procedures. Similar experimental results were obtained with support (II). Synthesis of

† Guest investigator (N.A.T.O.) from Institute of Organic Chemistry, Milan, Italy.



SCHEME. *Synthesis of* p-*methoxybenzylamine support.*

peptides with support (I) was demonstrated by the preparation of H–Phe–Ala–Phe–NH_2 and H–Trp–Met–Asp–Phe–NH_2 using conventional solid-phase procedures with the amide support, followed by cleavage with HF. The peptides obtained were identical with those obtained previously by the traditional solution procedures. In addition, the gastrin tetrapeptide obtained in both cases behaved identically compared with an authentic sample of gastrin tetrapeptide in t.l.c. in three systems, in immuno-assay, and in bio-assay.

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