CHEMISTRY LETTERS, pp. 45-48, 1982.

SYNTHESIS OF PEPTIDES CONTAINING HYDROXYAMINO ACIDS BY THE MIXED ANHYDRIDE METHOD WITHOUT PROTECTING THE HYDROXYL FUNCTIONS

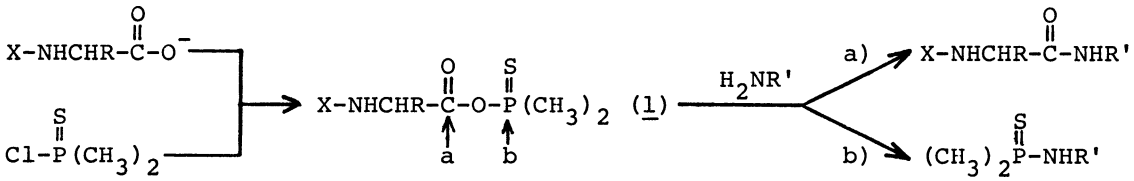
Masaaki UEKI* and Toshiyuki INAZU
Department of Applied Chemistry, Science University of Tokyo
1-3 Kagurazaka, Shinjuku-ku, Tokyo 162

Dimethylphosphinothioyl(Mpt) mixed anhydrides of *N*-protected amino acids were found to be isolatably stable and useful for the synthesis of hydroxyamino acid containing peptides without protecting the side-chain hydroxyl functions. They were applied for the solid phase synthesis of an enkephalin analog extended at C-terminal.

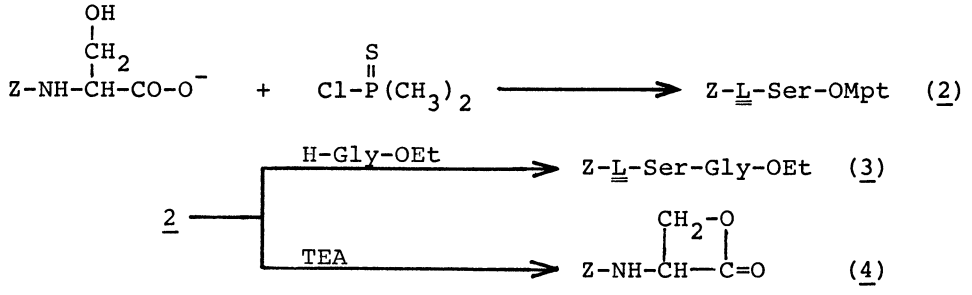
The mixed anhydride method is one of the most useful methods of peptide bond formation.[1] In order to achieve the selective acylation by this method, selection of a proper second acid component is very important. Carbonic monoesters are most widely used, but the use of phosphoric acid derivatives is not a few.[2] Recently it was reported that mixed anhydrides obtained by the reaction of protected amino acids with diphenylphosphinyl chloride(Dpp-Cl) were extremely useful for peptide synthesis because they were highly stable and showed very little tendency to disproportionate.[3,4] We also studied the use of diphenylphosphinothioyl chloride (Ppt-Cl) since this chloride was much more stable to hydrolysis than Dpp-Cl.[5] But, the activity of Ppt-Cl was not sufficiently high for some amino acids with less hydrophobic side-chains. In this work this restriction was overcome by the use of dimethylphosphinothioyl chloride(Mpt-Cl) and further, synthesis of peptides containing hydroxyamino acids by the mixed anhydride method without protecting the side-chain hydroxyl functions was made possible.

The stability of the Dpp mixed anhydrides in solution has already been shown by means of NMR.[4] In the case of the Mpt mixed anhydrides their stability was clearly shown in the success of isolation of the pure compounds. For example, Mpt-Cl was added to a cooled solution of *N*-Mpt-L-tryptophan(Mpt-L-Trp-OH) and

triethylamine(TEA) in chloroform and the reaction mixture was stirred for 15 min at room temperature. The mixed anhydride(Mpt-L-Trp-OMpt)(1) was obtained by means of column chromatography as a chromatographically homogeneous viscous oily material in a 98% yield. To determine the selectivity in acylation, the mixed anhydride 1 was allowed to react with L-tryptophan methyl ester(H-L-Trp-OMe) in the presence of an equimolar amount of TEA to yield the desired compound, Mpt-L-Trp-L-Trp-OMe, in a 92 % yield. The yield of the undesirable side product, Mpt-L-Trp-OMe, was determined spectrophotometrically as 0.06%.



An extreme advantage of the Mpt mixed anhydride method exists in the ability of activation of hydroxyamino acids without protecting the side-chain hydroxyl functions. When benzyloxycarbonyl-L-serine(Z-L-Ser-OH) was treated with the Mpt-Cl in the presence of TEA, the corresponding Mpt mixed anhydride(Z-L-Ser-OMpt)(2) was obtained in a 17% yield according to the similar isolating procedures as those described above. The structure of 2 was ascertained by ^{1}H NMR($CDCl_3$) δ=2.06(6H, d, J=14Hz, P-CH_3) and IR(neat, $\nu_{C=O}$1763 cm^{-1}) measurements. This mixed anhydride was considerably stable at room temperature in the absence of a base and acylated selectively the amino function of glycine ethyl ester, for example, to give Z-L-Ser-Gly-OEt(3) in a 53% yield calculated from the mixed anhydride. When a base was added, 2 was rapidly converted to the lactone 4.[6] Isolation of the corresponding Dpp mixed anhydride was not successful. When the reaction was carried out without the isolation of the mixed anhydride 2, the peptide 3 was obtained in a 78% yield.



Next, the possibility of the *o*-acylation of side-chain hydroxyl functions in the amino component during the mixed anhydride coupling was examined. Usually all the hydroxyl functions in the side-chain should be protected in the peptide syn-

thesis by the mixed anhydride method. The active ester method has claimed to be allowed to couple with hydroxyamino acids without protection of the hydroxyl functions,[7] but recently Bodanszky revealed that the *O*-acylation could be a severe side reaction in the peptide synthesis by the active ester method.[8] It was also reported that complex product mixture was obtained in the synthesis of *t*-butoxycarbonyl-L-tryptophyl-L-serine methyl ester by the hydroxysuccinimide ester method.[9] When the Mpt mixed anhydride method was applied for the synthesis of such model compounds, high yields of the desired selectively *N*-acylated products were obtained as shown in Table 1.

Table 1. Peptides Synthesized by The Mpt Mixed Anhydride Method

Peptide	Yield(%)	Mp(°C)	$[\alpha]_D$
Z-L-Ser-Gly-OEt	78	99-103	-5°(*c* 1, EtOH)
		(lit, 98-100, -5.5°(*c* 1, EtOH))[10]	
Z-L-Val-L-Tyr-OMe	92	149-150	+5°(*c* 1, pyridine)
		(lit, 150, +12.5°(*c* 1, pyridine))[11]	
Boc-L-Trp-L-Ser-OMe	93	82-84(dec)	-4°(*c* 1, EtOH)

As an application of the Mpt mixed anhydride method, solid phase synthesis of an enkephalin analog extended at C-terminal(H-L-Tyr-D-Ser-Gly-L-Phe-L-Leu-L-Thr-OH)[12] was attempted. The Mpt-L-threonine resin(2 g, polystyrene-1%-divinylbenzene copolymer, Thr content; 0.12 meq./g), synthesized by the Gisin's method,[13] was placed in a reaction vessel of a Beckman Model 990 peptide synthesizer. The Mpt group was removed by 0.2 M HCl(0.2 M triphenylphosphine) in CH_2Cl_2. After neutralization by 10% TEA in $CHCl_3$, 2 eq. of the mixed anhydride solution prepared just before use by the reaction of dicyclohexylamine salt of an Mpt-amino acid with Mpt-Cl in $CHCl_3$ was added and mixing was performed for 30 min in the presence of an equimolar amount of TEA. The coupling was repeated. After the final removal of the *N*-terminal Mpt group, the hexapeptide hydrochloride resin was thoroughly washed and dried *in vacuo*. The crude peptide was cleaved from the resin support by means of HBr in trifluoroacetic acid in the presence of anisole. Purification by preparative silica-gel thin-layer chromatography, successive gel chromatography on Sephadex LH-20(methanol) and droplet counter current distribution gave chromatographically homogeneous white solid, 91.7 mg(55% yield calculated from the Mpt-L-threonine resin). Amino acid ratios in hydrolyzate obtained by the use of 6 M HCl at 110°C for 24 h: $Tyr_{0.98}$, $Ser_{0.82}$, $Gly_{1.02}$, $Phe_{0.98}$, $Leu_{1.03}$, $Thr_{0.86}$. Biologi-

cal activity of this peptide was identical with the reported value.[12]

Further applications of the Mpt mixed anhydride method for peptide synthesis are now in progress.

Acknowledgement

We are deeply indebted to Professor Tetsuo Oka, Tokai University, for his biological activity measurements.

References

1) J. Meienhofer, "The Peptides", Vol. 1, ed. by E. Gross and J. Meienhofer, Academic Press, New York(1979), p. 264.
2) M. Bodanszky, Y. S. Klausner, and M. A. Ondetti, "Peptide Synthesis", John Wiley & Sons, New York(1976), p. 90.
3) A. G. Jackson, G. W. Kenner, G. A. Moore, R. Ramage, and W. D. Thorpe, Tetrahedron Lett., 1976, 3627.
4) I. J. Galpin, A. E. Robinson, and R. G. Tyson, "Peptides 1980: Proceedings of the 16th European Peptide Symposium", ed. by K. Brunfeldt, Scriptor, Copenhagen (1981), p. 169.
5) F. Tonegawa, R. Kuronuma, and M. Ueki, "Peptide Chemistry 1978: Proceedings of the 16th Symposium on Peptide Chemistry", ed. by N. Izumiya, Protein Research Foundation, Osaka(1979), p. 45.
6) W. König and R. Geiger, Chem. Ber., 103, 788(1970).
7) M. Bodanszky, "The Peptides", Vol. 1, ed. by E. Gross and J. Meienhofer, Academic Press, New York(1979), p. 106.
8) J. Martinez, J. Tolle, and M. Bodanszky, Int. J. Peptide Protein Res., 13, 22 (1979).
9) G. R. Pettit, T. S. Krupa, and R. M. Reynolds, Int. J. Peptide Protein Res., 14, 193(1979).
10) T. Mukaiyama, R. Matsueda, and M. Suzuki, Tetrahedron Lett., 1970, 1901.
11) J. Ramachandran and C. H. Li, J. Org. Chem., 28, 173(1963).
12) G. Gacel, M. Fournie-zalusky, and B. P. Roques, FEBS Lett., 118, 245(1980).
13) B. F. Gisin, Helv. Chim. Acta, 56, 1476(1973).

(Received October 12, 1981)