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VALIDITY OF METHOXYCARBONYL AS AN N-PROTECTING GROUP IN PEPTIDE SYNTHESIS: NEW SYNTHESIS OF MSH-RELEASE INHIBITING FACTOR

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N-Methoxycarbonyl (MOC)-amino acids regenerate the parent amino acids by treatment with dimethyl sulphide and methanesulphonic acid. Synthesis of MSH-Release Inhibiting Factor was accomplished using MOC-amino acids and applying the above mentioned deblocking method.

There have been many urethane type protecting groups in peptide synthesis. E. Fischer,[1] a pioneer of peptide synthesis, indicated that ethoxycarbonyl was so stable under the usual hydrolysis condition that it was not available to the purpose. Recently, Olah and Ho,[2] and Jung and Lyster[3] reported a new method for ester hydrolysis with trimethylsilyl iodide followed by water. We report here a new useful method to regenerate amino acids from N-methoxycarbonyl (MOC) amino acids (equivalent to Fischer's ethoxycarbonyl amino acids) as an extension of our work concerning cleavage reaction of aromatic ethers with methionine and methanesulphonic acid.[4]

Treatment of amino acids with methyl chloroformate in aqueous alkaline solution under cooling gave the MOC-amino acids in good yield. N^{α}-p-Methoxybenzyloxycarbonyl-N^{ε}-MOC-Lys-OH was prepared as follow: (i) lysine-Cu-complex[5] was treated with methyl chloroformate to give N^{ε}-MOC-lysine-Cu-complex, (ii) after treating it with ethylenediaminetetraacetic acid di-sodium salt (EDTA), α-amino group was acylated with p-methoxybenzyl S-4,6-dimethylpyrimid-2-yl-thiocarbonate[6] to give Z(OMe)-Lys-(MOC)-OH. Reaction of the MOC-amino acids (from MOC-Gly-OH to MOC-Val-OH in Table) with dimethyl sulphide in methanesulphonic acid (neat) at 5°C for several hours resulted in regeneration of the parent amino acids, the quantitative yields of which were revealed by amino acid analysis. Thioanisole[7] was also available as a methyl acceptor. On the other hand, acidolytical removal of Z(OMe)-group of Z(OMe)-Lys-(MOC)-OH with trifluoroacetic acid gave H-Lys-(MOC)-OH as a sole product (revealed by thin layer chro-

Table. Physical Data of MOC-Amino Acids

MOC-Amino acids[a)]	Isolated yields of MOC-amino acids from parent amino acids	M.p. (°C)	$[\alpha]_D$ (in methanol) (c=1.2)
MOC-Gly-OH	58%	97-98	-5.1°
MOC-Ala-OH	75%	118-119[b)]	-3.1°
MOC-Leu-OH	75%	155-157[b)]	-11.8°
MOC-Ile-OH	71%	71-73	-0.4°
MOC-Phe-OH	65%	153-154[b)]	+34.7°
MOC-Pro-OH	66%	54	-65.0°
MOC-Val-OH	76%	110-111	-8.4°
Z(OMe)-Lys-(MOC)-OH	60%	124-126[c)]	-2.1°

a) Satisfactory elemental analyses were obtained all the compounds cited here.
b) Cyclohexylamine salt. c) Dicyclohexylamine salt.

Chart

R-Leu-Gly-X

(2) R=MOC; X=OMe

(3) R=MOC; X=NH_2

(4) R=H; X=NH_2

(6) R=MOC; X=NH-NH_2

R-Pro-Leu-Gly-NH_2

(5) R=MOC

(1) R=H

matography). Further treatment of H-Lys-(MOC)-OH with dimethyl sulphide in methanesulphonic acid as mentioned above yielded H-Lys-OH in quantitative yield,[8)] suggesting that MOC group had usefulness as a protecting group to N^{ε}-amino group of lysine.

Based on the above result, synthesis of Melanocyte Stimulating Hormone-Release Inhibiting Factor (MIF)[9)] (1) was undertaken. Thus, reaction of MOC-Leu-OH with H-Gly-OMe in dimethylformamide and tetrahydrofuran using dicyclohexylcarbodiimide gave MOC-Leu-Gly-OMe (2) in 45% yield[10)]. The MOC-dipeptide was smoothly transformed to the amide (3) with ammonia in methanol for 5 h. Treatment of the amide (3) with dimethyl sulphide (5 times molar excess) in methanesulphonic acid at 5°C for 5 h

gave H-Leu-Gly-NH_2 (4) in 90% yield. Condensation of the amide (4) with MOC-Pro-Cl[10] in pyridine furnished MOC-Pro-Leu-Gly-NH_2 (5) in 85% yield (purified by a column chromatography on silica gel in chloroform-ethanol) which showed the molecular ion peak at m/e 342 in its mass spectrum. The MOC-tripeptide (5) was subjected to the deblocking reaction in the same manner as mentioned above to complete the synthesis of MIF in 30% isolated yield, which was identical with the authentic sample[11] in i.r. spectrum (KBr-disc) and behaviour on thin layer chromatography, indicating the validity of MOC group in peptide synthesis.

Furthermore, MOC-Leu-Gly-OMe (2) was easily converted to the hydrazide (6) in the usual manner without impairment on MOC group, suggesting that MOC was available to the azide coupling method in peptide synthesis.

References

1) E. Fischer and E. Fourneau, Chem. Ber., 34, 2868 (1901); E. Fischer, ibid, 39, 530 (1906).

2) T-L. Ho and G.A. Olah, Angew. Chem., Int. Ed., 15, 774 (1976).

3) M.E. Jung and M.A. Lyster, J. Am. Chem. Soc., 99, 968 (1977).

4) H. Irie, N. Fujii, H. Ogawa, and H. Yajima, J. C. S. Chem. Commun., 1976, 922; H. Irie, N. Fujii, H. Ogawa, H. Yajima, M. Fujino, and S. Shinagawa, Chem. Pharm. Bull., 25, 2929 (1977); N. Fujii, H. Irie, and H. Yajima, J. Chem. Soc., Perkin I, 1977, 2288.

5) A. Neuberger and F. Sanger, Biochem. J., 37, 515 (1943);J.P. Greenstein and M. Winitz, Chemistry of Amino Acids, Vol. II p. 1059 (1961), John Wiley & Sons, New York.

6) T. Nagasawa, K. Kuroiwa, K. Narita, and Y. Isowa, Bull. Chem. Soc. Jpn., 46, 1269 (1973).

7) Recently, Kiso and his co-workers reported a thioanisole-trifluoromethanesulphonic acid system for cleavage of methoxy group in O-methyltyrosine and application of this system to a synthesis of N-methylenkephalinol. Y. Kiso, S. Nakamura, K. Ito, K. Ukawa, K. Kitagawa, T. Akita, and H. Moritoki, J. C. S. Chem. Commun., 1979, 971.

8) In the case of H-Lys-(MOC)-OH, dimethyl sulphide showed much better result in this deblocking procedure than thioanisole different from Kiso's observation.

9) R.M.G. Nair, A.J. Kastin, and A.V. Schally, Biochem. Biophys. Res. Commun., 43, 1376 (1971); M.E. Celis, S. Taleisnik, and R. Walter, Proc. Natl. Acad. Sci. U.S.A., 68, 1428 (1971).

10) MOC-Leu-Gly-OMe was conveniently obtained by treatment of H-Gly-OMe with MOC-Leu-Cl in triethylamine. MOC-Leu-Cl and MOC-Pro-Cl were prepared from MOC-Leu-OH and MOC-Pro-OH with thionyl chloride in benzene, respectively, without racemisation.

11) The authentic sample of MIF was supplied from Protein Research Foundation (Japan).

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