4-Methoxy-2,3,6-trimethylbenzenesulphonyl (Mtr): a New Amino-protecting Group in Peptide Synthesis

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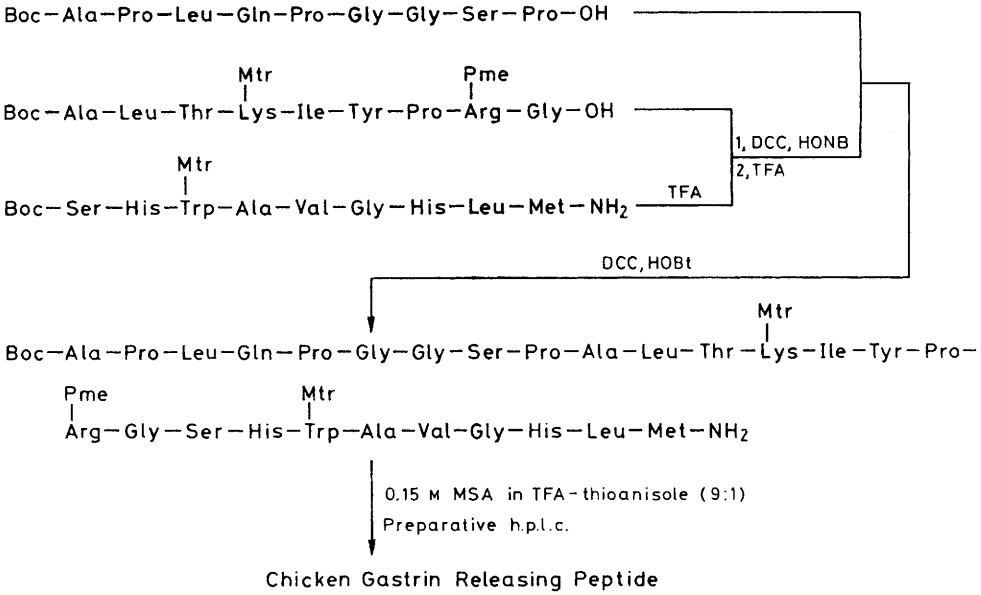
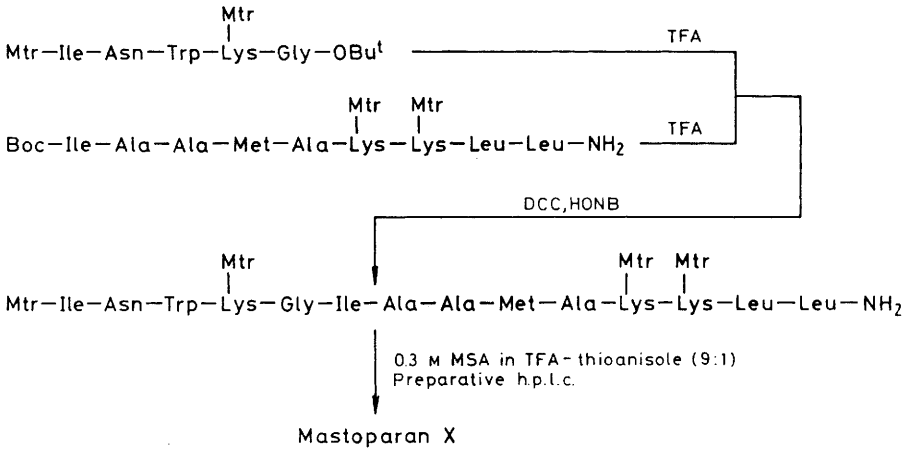
The 4-methoxy-2,3,6-trimethylbenzenesulphonyl (Mtr) group used to protect the ε-amino function of lysine can be readily removed with 0.15—0.3 M methanesulphonic acid in trifluoroacetic acid–thioanisole (9:1) but is completely resistant to hydrogenolysis or treatment with neat trifluoroacetic acid and hydrogen chloride, and thus can be used in the solution synthesis of methionine-containing peptides, such as mastoparan X and chicken gastrin releasing peptide (c-GRP).

The most popular procedures for deprotection in peptide synthesis are acidolysis and hydrogenolysis, even for the cleavage of temporary or semi-permanent protecting groups.[1] Syntheses of complicated or long peptides seem to require absolute selectivity of the cleavage of temporary protecting groups together with the ready availability of the starting materials, protected amino acids. This paper describes the use of a new ε-amino-protecting group, 4-methoxy-2,3,6-trimethylbenzenesulphonyl (Mtr), of lysine which has excellent stability against hydrogenolysis or treatment with neat trifluoroacetic acid (TFA) and dilute hydrogen chloride. H-Lys(Mtr)-OH, m.p. 228—230 °C, $[\alpha]_D^{23}$ +1.1° (*c*, 0.9 in MeOH), was prepared from the copper(II) complex of lysine[2] and 4-methoxy-2,3,6-trimethylbenzenesulphonyl chloride (Mtr-Cl), m.p. 56—58 °C. The latter was easily prepared from 2,3,5-trimethylanisole and chlorosulphonic acid[3] without formation of any



Scheme 1. HONB = N-hydroxynorborn-5-ene-2,3-dicarboximide, HOBt = N-hydroxybenzotriazole, DCC = dicyclohexylcarbodi-imide, Pme = pentamethylbenzenesulphonyl.



Scheme 2. HONB = *N*-hydroxynorborn-5-ene-2,3-dicarboximide, DCC = dicyclohexylcarbodi-imide.

isomeric chlorosulphonate, in contrast with the preparation of 4-methoxy-2,6-dimethylbenzenesulphonyl chloride (Mds-Cl).† Acylation of H-Lys(Mtr)-OH in the usual manner gave the Z and Boc derivatives. These derivatives were characterized as the corresponding dicyclohexylamine (DCHA) salts: Z-Lys-(Mtr)-OH·DCHA, m.p. 164—165 °C, $[\alpha]_D^{23}$ +5.8° (*c*, 0.9 in MeOH); Boc-Lys(Mtr)-OH·DCHA, m.p. 169—170 °C, $[\alpha]_D^{22}$ +8.6° (*c*, 0.9 in MeOH). Mtr-Ile-OH was also prepared by direct acylation of isoleucine with Mtr-Cl and characterized as the cyclohexylamine (CHA) salt: m.p. 189—190 °C, $[\alpha]_D^{23}$ +13.6° (*c*, 1.1 in MeOH).

In contrast with the complete stability of the Mtr group to neat TFA at 20 °C for 60 min and to catalytic hydrogenation, the group could be removed cleanly and smoothly by treatment with 0.15—0.3 M methanesulphonic acid (MSA) in TFA–thioanisole (9:1) at 20 °C for 1–2 h. Under these conditions of deblocking, serious side reactions, like aspartimide formation, were reduced to 10—20% of the total product in studies on model peptides, H-Trp-Asp(OBu^t)-Asn-Gln-OBu^t and dynorphin.[4] Thus the Mtr group may be used in solution synthesis of complicated peptides. Two biologically active peptides, mastoparan X[5] and chicken gastrin releasing peptide (c-GRP),[6] were synthesized in solution to demonstrate the applicability of the Mtr group. As shown in Schemes 1 and 2, fully protected mastoparan X and c-GRP were prepared by fragment assembly. The fragments were mostly prepared by a stepwise chain elongation using N^{α}-Z-or Boc-amino acids. In the case of c-GRP, the guanidino function of arginine was blocked by the previously reported pentamethylbenzenesulphonyl (Pme) group[3] and the indole nitrogen was blocked with the Mtr group to avoid unwanted oxidation and alkylation of the indole moiety.[7] These protecting groups could be cleanly removed by the MSA–TFA–thioanisole treatment. As the final step of synthesis, these fully protected peptides were treated with 0.15 or 0.3 M MSA in TFA–thioanisole (9:1) at 20 °C for 1–2 h, and pure mastoparan X‡ and c-GRP§ were obtained in good yields after preparative reverse-phase h.p.l.c.

These results indicate that the use of this protecting group for the ε-amino function together with the deprotection procedure using dilute MSA in TFA–thioanisole should be useful in the synthesis of complicated peptides, owing to great flexibility in the choice of temporary protecting groups.

We thank Professor H. Yajima (Kyoto University) for a reference sample of c-GRP and Professor T. Nakajima (Tokyo Medical and Dental University) for the isolated natural mastoparan X. We also thank Drs. E. Ohmura, K. Morita, and M. Nishikawa for their interest and helpful discussions.

Received, 18th January 1982; Com. 047

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† Smith *et al.* (C. W. Smith, G. Skala, and J. R. Boal, *J. Chem. Soc., Chem. Commun.*, 1981, 1243) have recently reported the multiplicity of products obtained using Mds-Cl; however, we reported previously the formation of the isomeric product (mainly 2-methoxy-4,6-dimethylbenzenesulphonyl chloride, *ca.* 27% by n.m.r. analysis) by the usual chlorosulphonation of 3,5-dimethylanisole and stated that pure crystalline Mds-Cl, m.p. 27—28 °C, could be obtained by a simple filtration on a silica gel column (hexane–$CHCl_3$, 9:1) (M. Fujino, M. Wakimasu, O. Nishimura, and C. Kitada, 'Peptide Chemistry, 1980,' p. 21, ed. K. Okawa, Protein Research Foundation, Osaka, 1981; M. Wakimasu, C. Kitada, and M. Fujino, *Chem. Pharm. Bull.*, 1981, **29**, 2582).

‡ The synthetic product was identical with natural mastoparan X according to h.p.l.c. [column, Toyosoda LS-410 (0.4 × 20 cm); solvent, 0.1% TFA in MeCN–H_2O (37:63); flow rate, 1 ml/min; elution time = 10.2 min] and t.l.c. [cellulose plate, R_f^1 (Bu^{n}OH–pyridine–AcOH–H_2O, 15:10:3:12) = 0.66; R_f^2(AcOEt–Bu^{n}OH–AcOH–H_2O, 1:1:1:1) = 0.64]; $[\alpha]_D^{22}$ −63.5° (*c*, 0.3 in 3% AcOH).

§ The synthetic product was identical with a reference sample of c-GRP according to h.p.l.c. [column, Toyosoda LS-410 (0.4 × 20 cm); solvent, 0.1% TFA in MeCN–H_2O (1:3); flow rate, 1 ml/min; elution time = 10.1 min] and t.l.c. [cellulose plate, R_f^1 = 0.69; R_f^2 = 0.64]; $[\alpha]_D^{22}$ −102.2°(*c*, 0.3 in 1% AcOH).