4-Methoxy-2,6-dimethylbenzenesulphonyl (Mds): a New Protecting Group of the Guanidino Function in Peptide Synthesis

By MASAHIKO FUJINO,* OSAMU NISHIMURA, MITSUHIRO WAKIMASU, and CHIEKO KITADA
(*Central Research Division, Takeda Chemical Industries, Ltd., Yodogawa-ku, Osaka* 532, *Japan*)

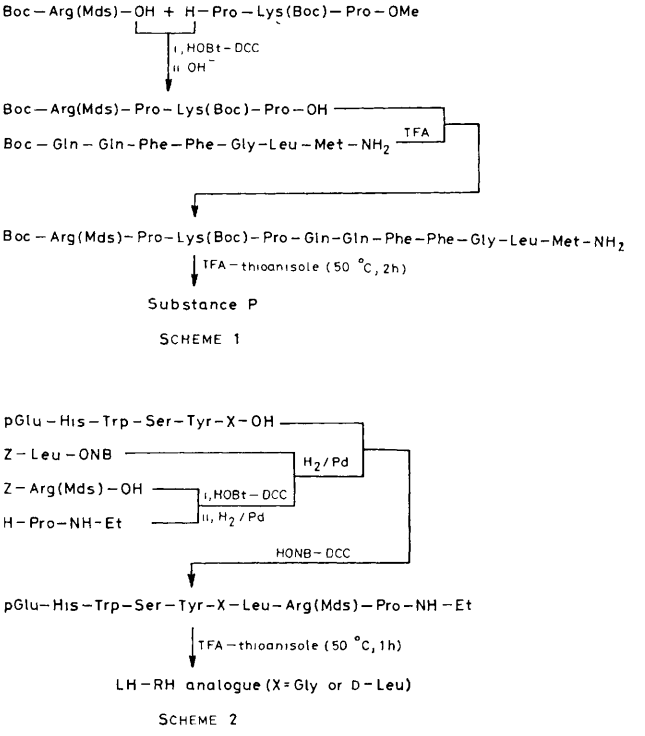
Summary The 4-methoxy-2,6-dimethylbenzenesulphonyl (Mds) group for the protection of the guanidino function, which is readily removed with trifluoroacetic acid–thioanisole but is resistant to hydrogenolysis or treatment with dilute hydrogen chloride, can be used in the solution synthesis of arginine-containing peptides; this protecting group was effectively used in the synthesis of substance P and two LH–RH analogues.

INTRODUCTION of the *p*-methoxybenzenesulphonyl (Mbs)[1] group and the mesitylene-2-sulphonyl (Mts)[2] group has made methanesulphonic acid (MSA)[3] of wide use as a

deprotecting reagent in the final step of peptide synthesis.[4] However, an unwanted side reaction, the formation of succinimide at the Asp and Asn residues of peptides, has been observed during the deblocking step with MSA.[5]

We now report the use of the 4-methoxy-2,6-dimethylbenzenesulphonyl (Mds) group as a new N^G-protecting group of Arg which has excellent stability against hydrogenolysis or treatment with dilute hydrogen chloride. Z-Arg(Mds)-OH was prepared from Z-Arg-OH and 4-methoxy-2,6-dimethylbenzenesulphonyl chloride (Mds-Cl)[6] by a procedure similar to that described for Z-Arg(Mbs)-OH,[1] and characterized as its cyclohexylamine salt, m.p. 140—141 °C, $[\alpha]_D^{23}$ + 5·7° (*c* 0·5, MeOH). Hydrogenation of Z-Arg(Mds)-OH over palladium black afforded H-Arg(Mds)-OH, m.p. 120—122 °C (decomp.), $[\alpha]_D^{23}$ −7·8° (*c* 0·7, MeOH), which was easily crystallized from water. Boc-Arg(Mds)-OH was prepared by t-butoxycarbonylation of H-Arg(Mds)-OH with 4,6-dimethyl-2-(t-butoxycarbonylthio)pyrimidine[7] and obtained in crystalline form, m.p. 175—176 °C (decomp.), $[\alpha]_D^{26}$ + 3·5° (*c* 0·5, MeOH). In spite of the complete stability of the Mds group to 1N-HCl in dioxan at 20 °C for 2 h, the group could be removed cleanly by treatment with trifluoroacetic acid (TFA) in the presence of thioanisole (5%) at 50 °C for 1—2 h (or 21 °C for 5—8 h). Under these conditions of deblocking, no serious side reaction was observed in studies on model peptides† and thus, the Mds group may be used in peptide synthesis.

A biologically active peptide, substance P,[8] and two LH-RH agonists, des-Gly^9-LH-RH-ethylamide[10] and des-Gly^9-[D-Leu]^6-LH-RH-ethylamide,[9] were synthesized in solution to demonstrate the usefulness of the Mds-protecting group. Boc-Arg(Mds)-Pro-Lys(Boc)-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2, m.p. 245—247 °C (decomp.), $[\alpha]_D^{26}$ −34·0° (*c* 0·5, DMF) and pGlu-His-Trp-Ser-Tyr-X-Leu-Arg(Mds)-Pro-NH-Et {X = Gly; m.p. 135—140 °C (decomp.), $[\alpha]_D^{23}$ −26·7° (*c* 0·5, dimethylformamide), and X = D-Leu; m.p. 105—106 °C (decomp.), $[\alpha]_D^{23}$ −28·2° (*c* 0·6, dimethylformamide)} were prepared by fragment assembly (Schemes 1 and 2) and then treated with TFA–thioanisole (95:5) at 50 °C for 2 h. Pure substance P and LH-RH agonists (checked by amino acid analysis, t.l.c., paper electrophoresis, and h.p.l.c.) were obtained in good yield (overall yield, *ca.* 60%, in the deprotection and purification steps). These results indicate that the use of this protecting group for the guanidino function together with the deprotection procedure of the 'push-pull' mechanism reported by Kiso *et al.*[11] should be useful in the synthesis of complicated peptides.



HOBt = *N*-hydroxybenzotriazole; HONB = *N*-hydroxy-norborn-5-ene-2,3-dicarboximide; –ONB = HONB ester; –NH–Et = ethylamide; DCC = dicyclohexyl carbodi-imide.

We thank Drs. E. Ohmura and M. Nishikawa for their interest and Prof. H. Yajima, Kyoto University, for helpful discussions.

(*Received, 15th April* 1980; *Com.* 391.)

† When a model peptide, H-Phe-Asp-Asn-Ala-OH, was treated with TFA–thioanisole (50 °C, 2 h), only the intact peptide was recovered (checked by h.p.l.c.), whereas complete transformation into the succinimide derivative of the peptide was observed on treatment with MSA (20 °C, 1 h) and partial formation of the succinimide (*ca.* 40%) was also found on treatment with HF (0 °C, 1 h).

[1] O. Nishimura and M. Fujino, *Chem. Pharm. Bull.*, 1976, **24**, 1568.
[2] H. Yajima, M. Takeyama, J. Kanaki, and K. Mitani, *J. Chem. Soc., Chem. Commun.*, 1978, 482.
[3] H. Yajima, Y. Kiso, H. Ogawa, N. Fujii, and H. Irie, *Chem. Pharm. Bull.*, 1975, **23**, 1164.
[4] M. Fujino, S. Shinagawa, M. Wakimasu, C. Kitada, and H. Yajima, *Chem. Pharm. Bull.*, 1978, **26**, 101; M. Fujino, M. Wakimasu, S. Shinagawa, C. Kitada, and H. Yajima, *ibid.*, p. 539; H. Yajima and N. Fujii, *J. Chem. Soc., Chem. Commun.*, 1980, 115.
[5] K. Koyama, M. Takeyama, and H. Yajima, 'Peptide Chemistry, 1979,' ed. H. Yonehara, Protein Research Foundation, Osaka, p. 119.
[6] G. W. Buchanan, C. Reyes-Zamora, and C. Cheung, *J. Org. Chem.*, 1975, **40**, 2537.
[7] T. Nagasawa, K. Kuroiwa, K. Narita, and Y. Isowa, *Bull. Chem. Soc. Jpn*, 1973, **46**, 1269.
[8] M. M. Chang, S. E. Leeman, and H. D. Niall, *Nature (London), New Biol.*, 1971, **232**, 86.
[9] M. Fujino, T. Fukuda, S. Shinagawa, S. Kobayashi, I. Yamazaki, R. Nakayama, J. H. Seeley, W. F. White, and R. H. Rippel, *Biochem. Biophys. Res. Commun.*, 1974, **60**, 406.
[10] M. Fujino, S. Shinagawa, I. Yamazaki, S. Kobayashi, M. Obayashi, T. Fukuda, R. Nakayama, W. H. White, and R. H. Rippel, *Arch. Biochem. Biophys.*, 1973, **154**, 488.
[11] Y. Kiso, K. Ukawa, and T. Akita, *J. Chem. Soc., Chem. Commun.*, 1980, 101; Y. Kiso, K. Ukawa, S. Nakamura, K. Ito, and T. Akita, *Chem. Pharm. Bull.*, 1980, **28**, 673.