Efficient Deprotection of N^G-Tosylarginine with a Thioanisole–Trifluoromethanesulphonic Acid System†

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Summary The tosyl group attached at the guanidino function of arginine can be efficiently cleaved by a thioanisole–trifluoromethanesulphonic acid system, which can deprotect *O*-2,6-dichlorobenzyltyrosine without the formation of *O*-to-*C* rearrangement products; the N^G-mesitylene-2-sulphonyl group was also cleaved by a thioanisole–trifluoroacetic acid system.

THE tosyl (*p*-tolylsulphonyl) group in N^G-tosylarginine,[1] which is one of the most important protecting groups in peptide synthesis, can be removed by HF–anisole,[2] but special apparatus is needed.[3] Deprotection of Arg(Tos)‡ by trifluoromethanesulphonic acid (TFMSA)–anisole is accomplished only under very drastic conditions such as use of a large excess of TFMSA (neat) and a high temperature.[4] We now report a mild and simple method for removal of the tosyl group attached at the guanidino function of arginine using a thioanisole–TFMSA system,[5–8] which can deprotect *O*-2,6-dichlorobenzyltyrosine and *O*-methyltyrosine without the formation of *O*-to-*C* rearrangement products.[6,8]

The rate of this cleavage reaction depended on the nature of the nucleophiles used. The promoting effect on the reaction of the nucleophiles examined was in the order: thioanisole > anisole > dimethyl sulphide > phenol ~*o*-

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‡ Abbreviations used are those recommended by the I.U.P.A.C.–I.U.B. Commission on Biochemical Nomenclature: *J. Biol. Chem.*, 1972, **247**, 977. Tos = tosyl; Boc = t-butoxycarbonyl; 2,6-Cl_2Bzl = 2,6-dichlorobenzyl; ONp = *p*-nitrophenyl ester.

cresol ~ ethanedithiol (Figure) The complete deprotection of Arg(Tos) (0·1 mmol) was achieved by thioanisole (5 mmol) and TFMSA (0·5 mmol) in trifluoroacetic acid (TFA) (2 ml) at 25 °C for 90 min, whereas in anisole–TFMSA–TFA, the deprotection of Arg(Tos) was incomplete even after 23 h at 25 °C

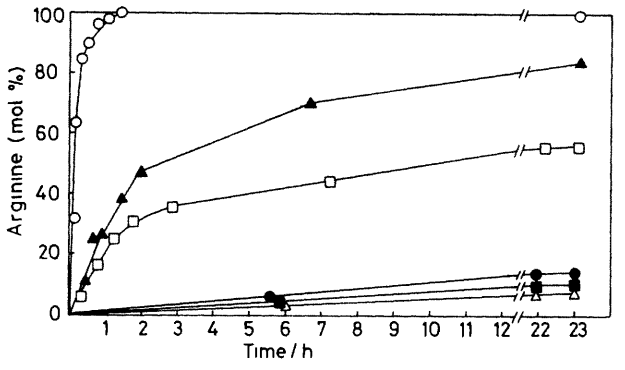

FIGURE Reaction of [N^G-tosylarginine (0 1 mmol) with nucleophile (5 mmol)–TFMSA (0 5 mmol)–TFA (2 ml) at 25° C Nucleophile ○, thioanisole, ▲, anisole, □, dimethyl sulphide, ●, phenol, ■, *o*-cresol, △, ethanedithiol

In order to evaluate the usefulness of this efficient method for deprotection of N^G-tosylarginine and *O*-2,6-dichlorobenzyltyrosine by a thioanisole–TFMSA system, we applied it to the conversion of Boc-Tyr(2,6-Cl_2Bzl)-Arg(Tos) into kyotorphin (Tyr-Arg),[9] since we had already demonstrated that it was possible to deprotect *O*-2,6-dichlorobenzyltyrosine without the formation of *O*-to-*C* rearrangement products,[6] and to synthesize biologically active peptides[7,8] using the thioanisole–TFMSA system

Boc-Tyr(2,6-Cl_2Bzl)-ONp was prepared from Boc-Tyr (2,6-Cl_2Bzl)[10] and *p*-nitrophenol using dicyclohexylcarbodiimide This active ester was treated with Arg(Tos) in dimethylformamide (DMF) containing triethylamine (non-aqueous media) Arg(Tos) is a useful derivative compared with Arg(NO_2), which is insoluble in DMF and requires H_2O as solvent Boc-Tyr(2,6-Cl_2Bzl)-Arg(Tos) thus obtained was deblocked with thioanisole–TFMSA–TFA at 0 °C for 30 min and then at 25 °C for 3 h Purification of the deprotected material by partition chromatography on Sephadex G-25, using the solvent system n-butanol–acetic acid–water (4 : 1 : 5), gave pure L-tyrosyl-L-arginine (kyotorphin)§ (overall yield 57% in the deprotection and purification steps)

It is noteworthy that the complete deprotection of N^G-2-mesitylsulphonylarginine[11] (0 05 mmol) was also achieved using a thioanisole (2·5 mmol)–TFA (1 ml) system[12,13] at 25 °C for 72 h, which could remove both *N*-benzyloxycarbonyl and *O*-benzyl groups On the other hand, in anisole–TFA, the cleavage of N^G-2-mesitylsulphonylarginine was incomplete even after 3 weeks at 25 °C

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§ The synthetic product was identical to a reference sample purchased from Kokusan Kagaku Inc (Tokyo), t l c (silica, Bu^nOH–AcOH–H_2O = 3 : 1 : 1) R_f = 0 31 Satisfactory elemental analyses were obtained for C, H, and N

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