Deprotection of *O*-Methyltyrosine by a 'Push–Pull' Mechanism Using the Thioanisole–Trifluoromethanesulphonic Acid System. Application to the Convenient Synthesis of a Potent *N*-Methylenkephalin Derivative†

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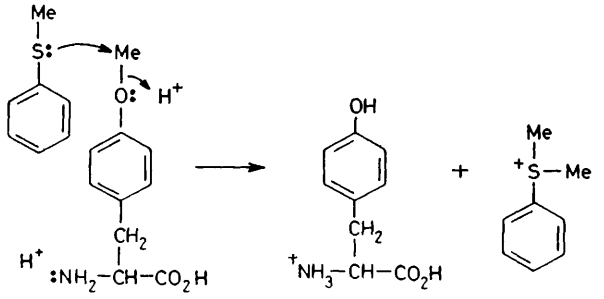
Summary The methyl group attached at the phenolic oxygen of tyrosine can be smoothly cleaved by a thioanisole–trifluoromethanesulphonic acid system; this deblocking method was successfully applied to the synthesis of a new potent enkephalin derivative, MeTyr-Gly-Gly-Phe-Metol (Metol = L-methioninol residue).

THE methyl group in *O*-methyltyrosine[1] has represented an irreversible protection of the hydroxy-group since its removal is accomplished only under very drastic conditions.[2] We now report a mild method for cleavage of the methyl group at the phenolic oxygen of tyrosine using a thioanisole–trifluoromethanesulphonic acid (TFMSA) system.[3–5]



SCHEME 1

This cleavage reaction occurs by addition of H^+ (a hard acid)[6] to the oxygen atom (a hard base) of the ether bond and nucleophilic attack of sulphur (a soft base) on the electron-deficient methyl carbon atom (a soft acid) (Scheme 1). This reaction thus involves the co-operative action of a soft nucleophile and a hard electrophile on a substrate (push–pull mechanism) and proceeds favourably. The reaction rate depended on the nature of the attacking sulphur nucleophiles; thioanisole was more effective in

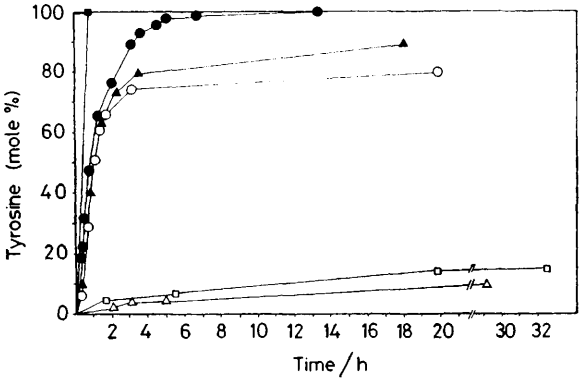


FIGURE. Reaction of *O*-methyltyrosine (0·1 mmol) with soft nucleophile–TFMSA–TFA (2 ml) at 25 °C: (○) thioanisole (5 mmol)–TFMSA (0·5 mmol); (□) dimethyl sulphide (5 mmol)–TFMSA (0·5 mmol); (△) ethanedithiol (5 mmol)–TFMSA (0·5 mmol); (▲) thioanisole (10 mmol)–TFMSA (0·5 mmol); (●) thioanisole (5 mmol)–TFMSA (1 mmol); (■) thioanisole (5 mmol)–TFMSA (10 mmol).

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TABLE. Activities of enkephalin analogues in the guinea pig ileum

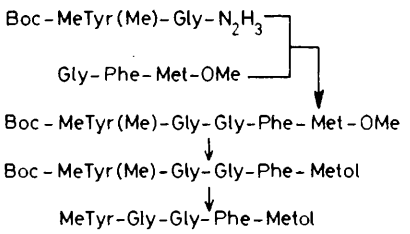
Compound	ED_{50} ($\times 10^{-10}$ M) (mean ± S.E.M.)			Relative potency[a]
Morphine	651	± 127	(n = 17)[b]	100
Tyr-Gly-Gly-Phe-Met[c]	335	± 117	(n = 22)	194
MeTyr-Gly-Gly-Phe-Met[d]	327	± 116	(n = 21)	199
Tyr-Gly-Gly-Phe-Metol[e]	654	± 192	(n = 12)	100
MeTyr-Gly-Gly-Phe-Metol (**1**)	7·0	± 1·1	(n = 13)	9300
MeTyr(Me)-Gly-Gly-Phe-Metol	7120	± 490	(n = 7)	9

[a] Relative potency expressed as percentage of morphine. [b] n = number of experiments. [c] Met-enkephalin; J. Hughes, T. W. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgan, and H. R. Morris, *Nature*, 1975, **258**, 577. [d] A. F. Bradbury, D. G. Smyth, C. R. Snell, J. F. W. Deakin, and S. Wendlandt, *Biochem. Biophys. Res. Comm.*, 1977, **74**, 748; B. A. Morgan, J. D. Bower, K. P. Guest, B. K. Handa, G. Metcalf, and C. F. C. Smith, Proceedings of the Fifth American Peptide Symposium, eds. M. Goodman and J. Meienhofer, Wiley, New York, 1977, p. 111. [e] J. Pless, W. Bauer, F. Cardinaux, A. Closse, D. Hauser, R. Huguenin, D. Roemer, H. Buescher, and R. C. Hill, *Helv. Chim. Acta*, 1979, **62**, 398.

promoting demethylation than was dimethyl sulphide (Figure), owing to the stabilization of the resulting sulphonium ion by conjugation with the benzene ring. The reaction rate also depended on the concentration of both H^+ and thioanisole, with complete cleavage of 0·1 mmol of *O*-methyltyrosine by 5 mmol of thioanisole plus 10 mmol of TFMSA in 2 ml of trifluoroacetic acid (TFA) at 25 °C for 50 min.

N.m.r. evidence for the formation of the sulphonium ion was obtained when anisole and thioanisole were mixed with TFMSA–TFA. The mixture showed a methyl signal at δ 3·02 ($Me_2\overset{+}{S}Ph$) and complete disappearance of signals at δ 3·77 (anisole Me) and δ 2·26 (thioanisole Me). No evidence of O → C rearrangements was obtained.

In order to evaluate the usefulness of this deblocking method, we have applied it to the synthesis of an *N*-methyltyrosine-containing peptide, [MeTyr[1], Metol[5]]enkephalin.‡ We had already demonstrated[5] that it was possible to synthesize a methionine-containing peptide using the thioanisole–TFMSA system. Boc-MeTyr(Me), prepared in one step from Boc-Tyr with MeI and NaH,[7] was condensed with Gly-OEt using dicyclohexylcarbodi-imide, and the resulting dipeptide ethyl ester was converted into the corresponding hydrazide, Boc-MeTyr(Me)-Gly-N_2H_3. This hydrazide was condensed by the azide method with Gly-Phe-Met-OMe, prepared by conventional methods, and the resulting pentapeptide methyl ester, Boc-MeTyr(Me)-Gly-Gly-Phe-Met-OMe, was converted into the alcohol derivative by reduction using $NaBH_4$[8] in MeOH (Scheme 2). The protected pentapeptidyl alcohol, Boc-MeTyr(Me)-Gly-Gly-Phe-Metol thus obtained was deblocked with thioanisole–TFMSA–TFA at 0 °C for 30 min, and at 25 °C for 2 h. Purification of the deprotected material by partition



(1)

SCHEME 2

chromatography on Sephadex G-25,[9] using the solvent system n-butanol–acetic acid–water (4 : 1 : 5), gave the pure [MeTyr[1], Metol[5]]enkephalin§ (**1**) (overall yield 43% in the deprotection and purification steps). In addition, several other enkephalin derivatives (Table) were synthesized either with this new thioanisole–TFMSA deblocking method or the usual TFA deblocking method to provide material for studying structure–function relationships of the enkephalins.

The biological activity of these peptides was determined by inhibition of electrically evoked contraction of the isolated guinea pig ileum.[10] The results, summarized in the Table, show that the combination of amino-terminal (MeTyr) and carboxy-terminal (Metol) modification produces a surprisingly potent enkephalin analogue (**1**), and the hydroxy-group of tyrosine plays an important role in activity. Details of the pharmacological properties of these analogues will be published elsewhere.

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‡ Metol = L-methioninol residue.

§ Satisfactory elemental analyses were obtained for $C_{28}H_{39}O_6N_5S\cdot MeCO_2H\cdot H_2O$: t.l.c. (silica), R_f ($CHCl_3$–MeOH–H_2O, 8 : 3 : 1, lower layer) 0·40, (Bu^nOH–AcOH–H_2O, 3 : 1 : 1) 0·56.

[1] N. Izumiya and A. Nagamatsu, *Bull. Chem. Soc. Japan*, 1952, **25**, 265.
[2] E. Schröder and K. Lübke, 'The Peptides,' Vol. I, Academic Press, New York, 1965, p. 225.
[3] H. Irie, N. Fujii, H. Ogawa, H. Yajima, M. Fujino, and S. Shinagawa, *J.C.S. Chem. Comm.*, 1976, 922; N. Fujii, S. Funakoshi, T. Sasaki, and H. Yajima, *Chem. and Pharm. Bull. (Japan)*, 1977, **25**, 3096.
[4] Y. Kiso, H. Isawa, K. Kitagawa, and T. Akita, *Chem. and Pharm. Bull. (Japan)*, 1978, **26**, 2562.
[5] Y. Kiso, K. Ito, S. Nakamura, K. Kitagawa, T. Akita, and H. Moritoki, 'Peptide Chemistry 1978,' ed. N. Izumiya, Protein Research Foundation, Osaka, 1979, p. 25; *Chem. and Pharm. Bull. (Japan)*, 1979, **27**, 1472.
[6] R. G. Pearson, *J. Amer. Chem. Soc.*, 1963, **85**, 3533; B. Saville, *Angew. Chem. Internat. Edn.*, 1967, **6**, 928; T. Ho, 'Hard and Soft Acids and Bases Principle in Organic Chemistry,' Academic Press, New York, 1977.
[7] S. D. Jolad, J. J. Hoffmann, S. J. Torrance, R. M. Wiedhopf, J. R. Cole, S. K. Arora, R. B. Bates, R. L. Gargiulo, and G. R. Kriek, *J. Amer. Chem. Soc.*, 1977, **99**, 8040.
[8] O. Yonemitsu, T. Hamada, and Y. Kanaoka, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 2075.
[9] D. Yamashiro, *Nature*, 1964, **201**, 76.
[10] H. W. Kosterlitz and A. J. Watt, *Brit. J. Pharmacol.*, 1968, **33**, 266.