Efficient Removal of *N*-Benzyloxycarbonyl Group by a 'Push–Pull' Mechanism using Thioanisole–Trifluoroacetic Acid, Exemplified by a Synthesis of Met-Enkephalin†

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Summary The *N*-benzyloxycarbonyl group can be smoothly cleaved under mild conditions, using thioanisole–trifluoroacetic acid, which can deprotect *O*-benzyltyrosine without the formation of *O*-to-*C* rearrangement products, this deblocking method was successfully applied to the synthesis of Met-enkephalin

THE benzyloxycarbonyl (Z) group,[1] which can be removed by hydrogenolysis, is an important amino-protecting group in peptide chemistry In the syntheses of the sulphur-containing peptides, removal of the *N*-benzyloxycarbonyl group is accomplished only under drastic conditions such as HF,[2] HBr–AcOH,[3] trifluoromethanesulphonic acid (TFMSA),[4] methanesulphonic acid,[5] sodium in liquid

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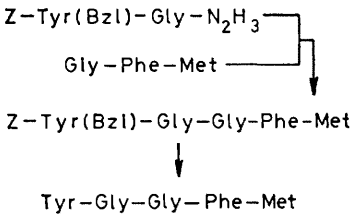
ammonia,[6] boiling trifluoroacetic acid (TFA),[7] and unusually prolonged (for 2·5 days) treatment with TFA.[8] We therefore sought and can now report a mild method for removal of the *N*-benzyloxycarbonyl group using thioanisole–TFA, which can deprotect *O*-benzyltyrosine without the formation of *O*-to-*C* rearrangement products and does not completely deprotect *O*-benzylserine and *O*-benzylthreonine.[9]

The mechanism for this cleavage reaction is analogous to the one for the cleavage reaction of *O*-benzyltyrosine with thioanisole–TFA[9] and *O*-methyltyrosine with thioanisole–TFMSA.[10] This reaction occurs by addition of H^+ (a hard acid)[11] to the oxygen atom (a hard base) of the carbonyl and nucleophilic attack of sulphur (a soft base) on the electron-deficient benzyl carbon atom (a soft acid). This reaction thus involves the co-operative action of a soft nucleophile and a hard electrophile on a substrate (push–pull mechanism) and proceeds in a favourable manner. The reaction rate depended on the nature of the attacking soft nucleophiles. The promoting effect on this cleavage reaction of the nucleophiles examined was in the order thioanisole > dimethyl sulphide > ethanedithiol ~ phenol > anisole. The complete cleavage of N^{ε}-benzyloxycarbonyl-lysine (0·1 mmol)[12] was achieved by thioanisole (5 mmol) in TFA (27 mmol) at 25 °C for 3 h, while in anisole–TFA, the cleavage of N^{ε}-benzyloxycarbonyl-lysine was incomplete even after a period of 27 h at 25 °C.

^{1}H-N.m.r. evidence for the formation of the sulphonium ion was obtained with the reaction mixture showing a methyl signal at δ 3·20 (s, Me in benzylmethylphenylsulphonium ion) and a methylene signal at δ 4·66 and 4·82 (AB-type q, J_{gem} 12 Hz, CH_2 in benzylmethylphenylsulphonium ion).

The benzyl carbamate was more reactive toward thioanisole–TFA than the benzyl esters (reaction time at 25 °C: glycine benzyl ester tosylate, 40 h, phenylalanine benzyl ester tosylate, incomplete at 75 h). The easier cleavage of benzyl carbamate than benzyl ester can be explained by enhancement of the initial protonation owing to the presence of the lone pair on nitrogen.



SCHEME Synthesis of Met-enkephalin

In order to evaluate the usefulness of this mild method for deprotection of *N*-benzyloxycarbonyl group and *O*-benzyltyrosine by thioanisole–TFA, we have applied it to the conversion of Z-Tyr(Bzl)–Gly–Gly–Phe–Met into a biologically active peptide, Met-enkephalin.[13] Z-Tyr(Bzl)[14] was condensed with Gly–OEt by dicyclohexylcarbodi-imide and the resulting dipeptide ethyl ester[14] was converted into the corresponding hydrazide, Z-Tyr(Bzl)–Gly–N_2H_3. This hydrazide was condensed by the azide method with Gly–Phe–Met, prepared by conventional methods (Scheme). The resulting protected pentapeptide, Z-Tyr(Bzl)–Gly–Gly–Phe–Met was deblocked with thioanisole–TFA at 25 °C for 4 h. The deprotected material was purified by ion-exchange chromatography on DEAE-Sephadex A-25 (acetate form) using 1% pyridine–0·04% acetic acid aqueous buffer[15] and reprecipitation from EtOH–AcOEt. The homogeneous peptide‡ thus obtained (overall yield 57% in the deprotection and purification steps) possessed properties identical with those of the Met-enkephalin obtained by thioanisole–TFMSA method.[16]

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‡ M.p. 195—196 °C, $[\alpha]_D^{25}$ + 24·0° (*c* 0·5, 3% AcOH), t.l.c. (silica, Bu^nOH–AcOH–pyridine–H_2O = 4·1:1:2) R_f = 0·70. Satisfactory elemental analyses were obtained for C, H, and N. Amino acid ratios (acid hydrolysate): Tyr 0·95, Gly 2·07, Phe 0·97, and Met 0·96. The biological activity was determined by inhibition of the electrically evoked contraction of the guinea pig ileum to give an ED_{50} of 33 ± 11 nM (n = 22).

[1] M. Bergmann and L. Zervas, *Ber.*, 1932, **65**, 1192.
[2] S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Japan*, 1967, **40**, 2164.
[3] D. Ben-Ishai and A. Berger, *J. Org. Chem.*, 1952, **17**, 1564.
[4] H. Yajima, N. Fujii, H. Ogawa, and H. Kawatani, *J.C.S. Chem. Comm.*, 1974, 107.
[5] H. Yajima, Y. Kiso, H. Ogawa, N. Fujii, and H. Irie, *Chem. and Pharm. Bull. (Japan)* 1975, **23**, 1164.
[6] R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, 1935, **108**, 753.
[7] F. Weygand and W. Steglich, *Z. Naturforsch.*, 1959, **14b**, 472.
[8] M. Bodanszky, Y. S. Klausner, C. Y. Lin, V. Mutt, and S. I. Said, *J. Amer. Chem. Soc.*, 1974, **96**, 4973.
[9] Y. Kiso, K. Ukawa, S. Nakamura, K. Ito, and T. Akita, *Chem. and Pharm. Bull. (Japan)*, in the press.
[10] Y. Kiso, S. Nakamura, K. Ito, K. Kitagawa, T. Akita, and H. Moritoki, Abstracts of papers of the ACS/CSJ Chemical Congress, April, 1979, Honolulu, Hawaii, ORGN 366, Y. Kiso, S. Nakamura, K. Ito, K. Ukawa, K. Kitagawa, T. Akita, and H. Moritoki, *J.C.S. Chem. Comm.*, 1979, 971.
[11] R. G. Pearson, *J. Amer. Chem. Soc.*, 1963, **85**, 3533, B. Saville, *Angew. Chem. Internat. Edn.*, 1967, **6**, 928, T. Ho, *Chem. Rev.*, 1975, **75**, 1, 'Hard and Soft Acids and Bases Principle in Organic Chemistry,' Academic Press, New York, 1977.
[12] M. Bergmann, L. Zervas, and W. F. Ross, *J. Biol. Chem.*, 1935, **111**, 245.
[13] J. Hughes, T. W. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgan, and H. R. Morris, *Nature*, 1975, **258**, 577.
[14] E. Wunsch, G. Fries, and A. Zwick, *Chem. Ber.*, 1958, **91**, 542.
[15] J. D. Bower, K. P. Guest, and B. A. Morgan, *J.C.S. Perkin I*, 1976, 2488.
[16] 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.