NEW HYDROXYL PROTECTING GROUPS OF A SAFETY-CATCH TYPE REMOVABLE BY REDUCTIVE ACIDOLYSIS[1)]

Yoshiaki KISO,* Shigeki TANAKA, Tooru KIMURA, Hisatomi ITOH and Kenichi AKAJI

Department of Medicinal Chemistry, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607, Japan

New hydroxyl protecting groups of a safety-catch type, *i.e.*, 4-methylsulfinylbenzyloxycarbonyl (Msz) group for Tyr and 4-methylsulfinylbenzyl (Msob) ether for Thr, have been developed. O-Msz and O-Msob groups are stable under both acidic and basic conditions and can be removed by a one-pot reaction involving reductive acidolysis using tetrachlorosilane-trifluoroacetic acid (TFA)-scavengers. Using these new protecting groups, a 17 residue-peptide, γ-endorphin, was successfully synthesized by the efficient solid phase method.

KEYWORDS solid phase peptide synthesis; 4-methylsulfinylbenzyloxycarbonyl (Msz); 4-methylsulfinylbenzyl (Msob); safety-catch type protecting group; reductive acidolysis; fluoride ion deprotection

Recently, we have developed a safety-catch type of protecting group: 4-methylsulfinylbenzyl (Msob) ester[2-4)] for carboxyl and 4-methylsulfinylbenzyloxycarbonyl (Msz)[3)] for amino groups. The safety-catch type of protecting group can be smoothly removed by a one-pot reaction involving reductive acidolysis using tetrachlorosilane-trifluoroacetic acid (TFA)-scavengers,[3,4)] which is a mild acidolysis system having strong reductivity. In this paper, we report new hydroxyl protecting groups of the safety-catch type removable by reductive acidolysis, *i.e.*, Tyr(Msz) and Thr(Msob).

The preparation of Boc-Tyr(Msz)-OH (Boc=*t*-butoxycarbonyl) is illustrated in Fig. 1. 4-Methylthiobenzyl 4'-nitrophenyl carbonate (Mtz-ONp, Mtz=4-methylthiobenzyloxycarbonyl) (**2**) (mp 78-79°C, *Anal.* Calcd for $C_{15}H_{13}NO_5S$: C, 56.42; H, 4.10; N, 4.39. Found; C, 56.38; H, 4.18; N, 4.29.) was

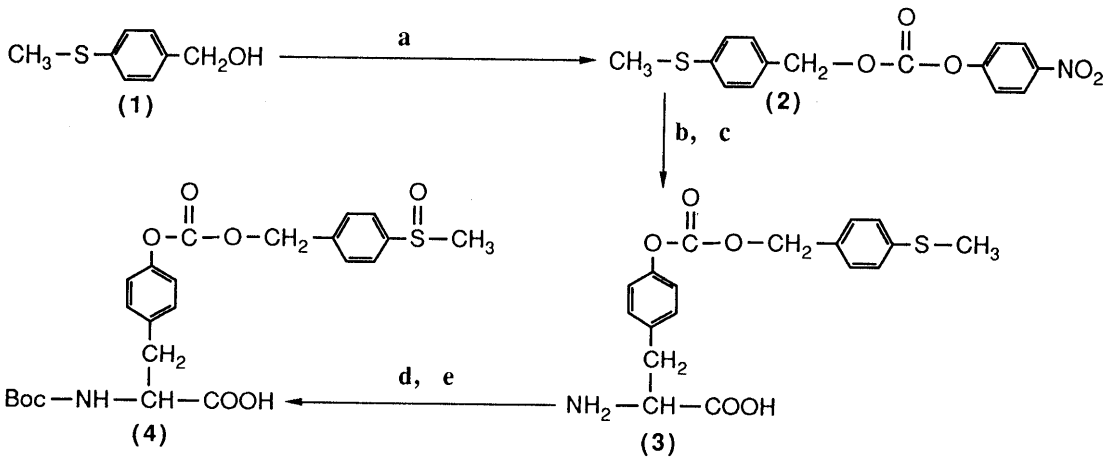

Fig. 1. Preparation of Boc-Tyr(Msz)-OH
Reagents: **a**, 4-nitrophenyl chloroformate / pyridine; **b**, Tyr•1/2Cu, $NaHCO_3$ / DMF-H_2O; **c**, EDTA•2Na / H_2O; **d**, Boc_2O, Et_3N / tetrahydrofurane-H_2O; **e**, $NaBrO_2$•$3H_2O$ / AcOEt-H_2O.

prepared in 70% yield by the reaction of 4-methylthiobenzyl alcohol (**1**) and 4-nitrophenyl chloroformate in pyridine at 25°C for 4h. Mtz-group was introduced to phenolic moiety of tyrosine by the reaction of copper complex of tyrosine with Mtz-ONp (**2**) in 70% aqueous DMF (25°C, 24h) followed by treatment with aqueous ethylenediaminetetraacetic acid (EDTA) disodium salt to give H-Tyr(Mtz)-OH (**3**) [mp 213-216°C, $[\alpha]_D^{26}$ -16.07° (c=0.56, 50% AcOH)] in 68% yield. Boc-Tyr(Msz)-OH (**4**) [mp 61-64°C, $[\alpha]_D^{26}$ -167.82 (c=0.44, MeOH), *Anal.* Calcd for $C_{23}H_{27}NO_8S \cdot 3/4H_2O$: C, 56.26; H, 5.54; N, 2.85. Found: C, 56.45; H, 5.79; N, 2.88.] was obtained in 50% yield from H-Tyr(Mtz)-OH (**3**) by the conventional *t*-butoxycarbonylation with $(Boc)_2O$ and following oxidation using sodium bromite trihydrate[5] in AcOEt-H_2O at 25°C for 30min. Boc-Thr(Msob)-OH [mp 114-116°C, $[\alpha]_D^{26}$ -176.47° (c=0.60, MeOH), *Anal.* Calcd for $C_{17}H_{25}NO_6S \cdot H_2O$: C, 52.42; H, 6.47; N, 3.60. Found: C, 52.48; H, 6.78; N, 3.50.] and Boc-Ser(Msob)-OH [mp 69-70°C, $[\alpha]_D^{26}$+34.15° (c=0.41, MeOH) *Anal.* Calcd for $C_{16}H_{23}NO_6S \cdot 1/2H_2O$: C, 52.44; H, 6.33; N, 3.82. Found: C, 52.44; H, 6.50; N, 3.82.] were prepared using Msob-Br and NaI in essentially the same manner as described by Futaki et al.[6]

The stabilities of Tyr(Msz), Thr(Msob) and Ser(Msob) under various conditions were examined by TLC. These protecting groups were sufficiently stable under the acidic condition [TFA-anisole (25°C, 24h), or 0.5M methanesulfonic acid / dichloromethane : dioxane (9:1) containing 2% anisole (25°C, 24h)][7] and the basic condition [5% Et_3N / dimethylformamide (DMF) (25°C, 24h), or 5% diisopropylethylamine / DCM (25°C, 24h)]. O-Msz or O-Msob group was rapidly cleaved with reductive acidolysis system [1M $SiCl_4$ (10eq) / TFA-anisole (10eq), or 1M $SiCl_4$ (10eq) / TFA-anisole, thioanisole, ethanedithiol (EDT) (10eq each) (25°C, 10min)] to give a sole product on TLC. The O-Msz group of Tyr was also cleaved by saponification [1N NaOH / MeOH (25°C, 24h)], while the Msob ether of Thr or Ser was kept completely intact under this condition.

In Merrifield type solid phase peptide synthesis, it is inevitable that slight cleavage of semi-permanent protecting groups occurs during repetitive TFA treatment. Thus, we have compared the stabilities of Msz and Msob groups to TFA with those of conventional benzyl alcohol type protecting groups in detail using an amino acid analyzer. As shown in Fig. 2, Msz group of Tyr was most stable among the phenolic protecting groups tested, *i.e.*, benzyl (Bzl), *o*-bromobenzyloxycarbonyl (Br-Z), 2,6-dichlorobenzyl (Cl_2Bzl) and Msob[8] groups. The Msob ethers of Ser and Thr were about 10 times more stable than the corresponding Bzl ethers. The high stability of Msz and Msob groups seemed to be advantageous, especially for the solid phase synthesis of long chain peptides.

In order to demonstrate the usefulness of the new hydroxyl protecting groups of the safety-catch type, we synthesized γ-endorphin consisting of 17 amino acid residues (Fig. 3). The peptide backbone was constructed on the phenacyl (Pac) resin[9] by the efficient method[10] of solid phase peptide synthesis, which consists of selective N^{α}-deprotection by dilute methanesulfonic acid, *in situ* neutrali-

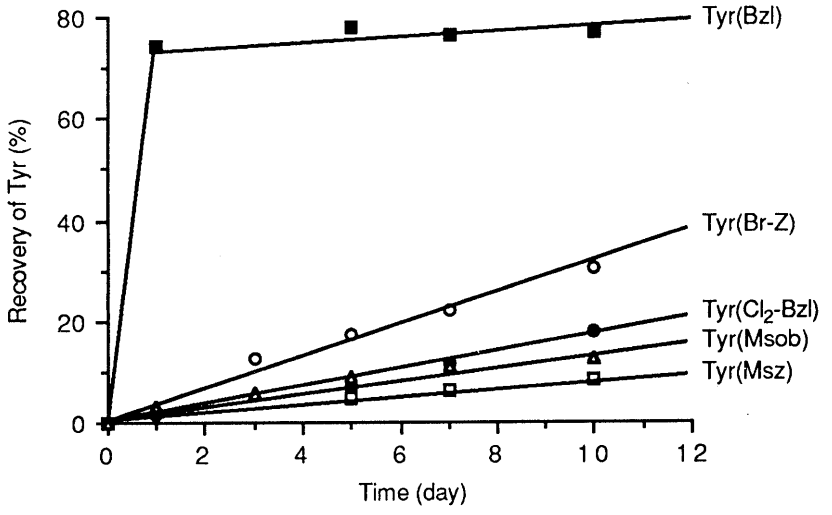

Fig. 2. Stability of Msz and Msob Group in TFA-Anisole (25°C)

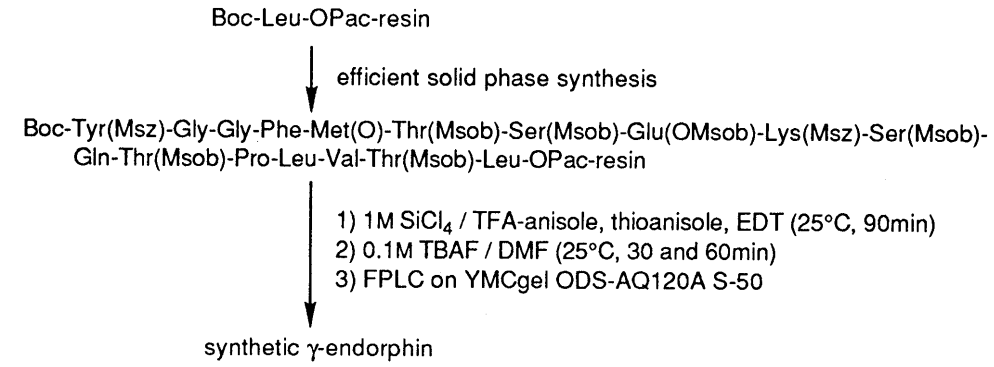

Fig. 3. Synthesis of γ-Endorphin

zation, and rapid coupling reaction using benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate. Together with Boc-Tyr(Msz), Boc-Thr(Msob) and Boc-Ser(Msob), Boc-amino acid derivatives bearing safety-catch type protecting groups were employed, *i.e.*, Boc-Glu(OMsob)[11] and Boc-Lys(Msz).[3] After the completion of the peptide chain elongation, the protected peptide-resin was treated with 1M $SiCl_4$ / TFA-anisole, thioanisole, EDT at 25°C for 90min to remove all of the side chain protecting groups. After washing the resin, the deprotected peptide was cleaved from the Pac resin by treating twice (30min and the 60min) with 0.1M tetra-*n*-butylammonium fluoride trihydrate (TBAF) / DMF at 25°C.[7,10,11] The product was purified by fast protein liquid chromatography (FPLC, Pharmacia) on a column packed with YMC-gel ODS-AQ 300A (S-50) (20x500mm). The homogeneous peptide was obtained in 49% yield from the starting resin. The purity of synthetic γ-endorphin was confirmed by analytical HPLC on YMC AM302 column (4.6x150mm) [retention time; 19.53min, on a gradient elution with MeCN (10-60% in 30min) in 0.1% aqueous TFA, 0.7ml/min] and by amino acid analysis after acid hydrolysis with 6N HCl-phenol [amino acid ratios (theoretical numbers in parentheses): Leu 2.00 (2), Thr 2.86 (3), Ser 1.87 (2), Glu 2.04 (2), Gly 2.02 (2), Val 0.99 (1), Met 0.94 (1), Tyr 0.97 (1), Phe 0.98 (1), Lys 0.99 (1), Pro 0.98 (1), Rec. of Leu 61%].

These excellent results demonstrate the usefulness of the new hydroxy protecting groups of the safety-catch type removable by reductive acidolysis in practical solid phase peptide synthesis.

REFERENCES AND NOTES

1) Presented in part at the 28th Symposium on Peptide Chemistry, Oct. 25-27, 1990, Osaka, Japan.
2) J. M. Samanen and E. Brandeis, "Peptides: Structure and Function," eds. C. Deber, K. Kopple, Pierce Chemical, Rockford, IL, 1985, p. 225; J. M. Samanen and E. Brandeis, *J. Org. Chem.*, **53**, 561 (1988); Y. Kiso, M. Yoshida, T. Kimura, M. Shimokura and T. Fujisaki, "Peptides: Chemistry and Biology," ed. G. R. Marshall, ESCOM Sci. Publishers B. V., Leiden, Netherlands, 1988, p. 229.
3) Y. Kiso, T. Kimura, M. Yoshida, M. Shimokura, K. Akaji and T. Mimoto, *J. Chem. Soc., Chem. Commun.*, **1989**, 1511.
4) Y. Kiso, M. Shimokura, T. Kimura, T. Mimoto and T. Fujisaki, "Peptide Chemistry 1986", ed. T. Miyazawa, Protein Res. Found., Osaka, Japan, 1987, p. 211.
5) T. Kageyama, Y. Ueno and M. Okawara, *Synthesis*, **1983**, 815.
6) S. Futaki, T. Taike, T. Akita and K. Kitagawa, *J. Chem. Soc., Chem. Commun.*, **1990**, 523.
7) Y. Kiso, T. Kimura, Y. Fujiwara, M. Shimokura and A. Nishitani, *Chem. Pharm. Bull.*, **36**, 5024 (1988).
8) S. Futaki, T. Yagami, T. Taike, T. Akita and K. Kitagawa, *J. Chem. Soc., Perkin Trans. 1*, **1991**, 653.
9) F. Weygand and R. Obermeier, *Z. Naturforsch.*, Teil B, **23**, 1390 (1968); T. Mizoguchi, K. Shigezane and N. Takamura, *Chem. Pharm. Bull.*, **18**, 1465 (1970).
10) Y. Kiso, T. Kimura, Y. Fujiwara, H. Sakikawa and K. Akaji, *Chem. Pharm. Bull.*, **38**, 270 (1990).
11) Y. Kiso, Y. Fujiwara, T. Kimura, H. Itoh and K. Akaji, "Peptide Chemistry 1989", ed. N. Yanaihara, Protein Res. Found., Osaka, Japan, 1990, p. 159.

(Received September 27, 1991)