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Studies on Peptides. LXXXV.^{1,2)} A New Deprotecting Procedure for
p-Toluenesulfonyl and *p*-Methoxybenzenesulfonyl Groups
from the N^{im}-Function of Histidine

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The chemical behavior of N^{im}-*p*-methoxybenzenesulfonylhistidine, His (MBS), was examined. This new N^{im}-protecting group was removable by HF or N-hydroxybenzotriazole, like the N^{im}-Tos group, but was more acid-stable than the Tos group with methanesulfonic acid or HBr. The N^{im}-MBS group was stable to treatment with trifluoroacetic acid in the presence of anisole, but was found to be cleaved smoothly by the same acid in the presence of dimethylsulfide at room temperature within an hour. The N^{im}-Tos group was also removable under similar conditions, but at a somewhat lower rate.

Keywords—N^{im}-*p*-methoxybenzenesulfonyl (MBS)-histidine; N^{im}-toluenesulfonyl (Tos)-histidine; trifluoroacetic acid-dimethylsulfide system for deprotection of His (MBS); trifluoroacetic acid-dimethylsulfide system for deprotection of His(Tos); N-hydroxybenzotriazole for deprotection of His(MBS); HF for deprotection of His(MBS)

Boc-His(Tos)-OH⁴⁾ is a useful derivative, like Boc-Arg(Tos)-OH,⁵⁾ for peptide synthesis. The Tos group is known to be cleaved from these two derivatives by treatment with sodium in liquid ammonia⁶⁾ or hydrogen fluoride.^{7,8)} In spite of this similarity, the Tos group attached at the N^{im}-function of histidine behaves quite differently from the same group attached at the guanidino function of arginine. The N^{im}-Tos group is removed by treatment with HOBT^{4,9)} or 1 N NaOH, while the N^g-Tos group remains intact under these conditions. Recently, the N^g-MBS and the Mts groups were introduced by Nishimura and Fujino¹⁰⁾ and by us,¹¹⁾ respectively, as acidolytically removable protecting groups for arginine. In view of the characteristic chemical features of the N^{im}-sulfonyl-type protecting groups for histidine mentioned above, we first examined the chemical behavior of the N^{im}-MBS group of histidine and found that this group was quantitatively removable by treatment with TFA in the presence

- 1) Part LXXXIV: H. Yajima, J. Kanaki, S. Funakoshi, Y. Hirai, and T. Nakajima, *Chem. Pharm. Bull.*, **27**, 882 (1979).
- 2) Amino acids, peptides and their derivatives mentioned in this paper are of the L-configuration. The following abbreviations are used: Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Boc=*t*-butoxycarbonyl, Bzl=benzyl, NP=*p*-nitrophenyl, Tos=toluenesulfonyl, MBS=*p*-methoxybenzenesulfonyl, Mts=mesitylene-2-sulfonyl, DCC=dicyclohexylcarbodiimide, HOBT=N-hydroxybenzotriazole, DCHA=dicyclohexylamine, TFA=trifluoroacetic acid, MSA=methanesulfonic acid, DMF=dimethylformamide.
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- 4) T. Fujii and S. Sakakibara, *Bull. Chem. Soc. Jpn.*, **47**, 3146 (1974).
- 5) R. Schwyzler and C.H. Li, *Nature* (London), **182**, 1669 (1958); E. Schnabel and C.H. Li, *J. Am. Chem. Soc.*, **82**, 4576 (1960); J. Ramachandran and C.H. Li, *J. Org. Chem.*, **27**, 4006 (1962).
- 6) R.H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).
- 7) S. Sakakibara, Y. Shimonishi, Y. Kishida, H. Okada, and H. Sugihara, *Bull. Chem. Soc. Jpn.*, **40**, 2164 (1967).
- 8) R.H. Mazur, *Exp.*, **24**, 661 (1968).
- 9) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
- 10) O. Nishimura and M. Fujino, *Chem. Pharm. Bull.*, **24**, 1568 (1976).
- 11) H. Yajima, M. Takeyama, J. Kanaki, and K. Mitani, *J.C.S. Chem. Comm.*, **1978**, 482; H. Yajima, M. Takeyama, J. Kanaki, O. Nishimura, and M. Fujino, *Chem. Pharm. Bull.*, **26**, 3752 (1978).

of dimethylsulfide at room temperature within an hour. The N^{im} -Tos group was also cleaved under similar conditions, but at a somewhat slower rate. Preparation of His(MBS) derivatives and the results of some model experiments are reported in this paper.

Z(OMe)-His(MBS)-OH was prepared from Z(OMe)-His-OH^{4,12)} and *p*-methoxybenzenesulfonyl chloride by the procedure used for the preparation of Boc-His(Tos)-OH⁴⁾ and was obtained, after purification through its DCHA salt, as a crystalline compound. The corresponding derivatives, Boc-His(MBS)-OH and Z-His(MBS)-OH, were similarly prepared and characterized as crystalline compounds. H-His(MBS)-OH was also obtained as a crystalline compound from aqueous methanol, by treatment of Z(OMe)-His(MBS)-OH with TFA in the presence of anisole.¹³⁾ Thus, various N^{im} -MBS derivatives of histidine were easily prepared.

The chemical behavior of the N^{im} -substituent was next examined. Like the N^{im} -Tos group, the N^{im} -MBS group could be removed by HOBT or under basic conditions, *i.e.*, completely in 1 *N* NaOH within an hour and partially in 80% hydrazine hydrate in methanol for 24 hours. This group survived intact under the catalytic hydrogenolysis conditions required for the removal of the Z group and under the acidic conditions required for the N^{α} -deprotection of Boc and Z(OMe) groups, such as treatment with TFA-anisole or 25% HBr-acetic acid.¹⁴⁾ Acidolytic removal of this group was achievable by treatment with hydrogen fluoride in the presence of anisole, but was not complete using the MSA-anisole system.¹⁵⁾ The N^{G} -MBS group is known to be cleaved completely by the latter treatment. Thus, compared to the N^{G} -MBS group, the N^{im} -MBS group showed greater stability to acid.

Despite the acid stability of the N^{im} -MBS group mentioned above we found that this group was removable by TFA in the presence of various sulfur compounds, such as thioanisole, dimethylsulfide, mercaptoethanol or ethanedithiol. Among the compounds tested, dimethylsulfide was judged to be the most effective reagent for this purpose. When H-His(MBS)-OH was exposed to TFA in the presence of dimethylsulfide (5 equiv.) at room temperature, histidine was regenerated quantitatively within 40 to 60 minutes. This result was confirmed using a dual-wavelength TLC scanner, as well as an amino acid analyzer.

This new finding prompted us to examine the TFA deprotection of the N^{im} -Tos group in the presence of various sulfur compounds. Again, dimethylsulfide was found to be the most effective reagent tested. When the progress of the deprotection by TFA-dimethylsulfide was monitored with a TLC scanner, it was found that the N^{im} -MBS group was removed at a slightly faster rate than the N^{im} -Tos group. Even in the latter instance, nearly quantitative recovery of histidine was obtained within 60 to 90 minutes. Though thiol compounds, such as ethanedithiol or mercaptoethanol, seemed to have little effect, thioanisole showed moderate effectiveness. The N^{im} -Tos and the N^{im} -MBS groups were removed by TFA-thioanisole to the extent of 17% and 30% respectively, within 60 minutes and completely when the solutions were stored at room temperature overnight.

It seems noteworthy that the MSA-dimethylsulfide system, a rather acidic system, was not as effective as the TFA-dimethylsulfide system mentioned above, and in addition Arg(Tos) and Arg(MBS) remained intact in the TFA-dimethylsulfide system for 60 to 90 minutes. Further information is required to account for the above phenomena. At present, as far as the N^{im} -sulfonyl type protecting group is concerned, it seems certain that the electron-rich sulfur atom of dimethylsulfide plays an important role as a potent cation acceptor, particularly in TFA, but not in MSA, since anisole-TFA or dimethylsulfide-acetic acid or methanol did not show such clear tendencies.

12) H. Yajima, F. Tamura, Y. Kiso, and M. Kurobe, *Chem. Pharm. Bull.*, **21**, 1380 (1973); E. Schaich, A.M. Fretzdorff, and F. Schneider, *Z. Physiol. Chem.*, **354**, 897 (1973).

13) F. Weygand and K. Hunger, *Chem. Ber.*, **98**, 1 (1962).

14) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952); D. Ben-Ishai, *ibid.*, **19**, 62 (1954); G.W. Anderson, J. Blodinger, and A.D. Welcher, *J. Am. Chem. Soc.*, **74**, 5309 (1952).

15) H. Yajima, Y. Kiso, H. Ogawa, N. Fujii, and H. Irie, *Chem. Pharm. Bull.*, **23**, 1164 (1975).

Z(OMe)-His(MBS)-OH·DCHA Salt—MBS-Cl (7.0 g, 35 mmol) in dioxane (150 ml) was added dropwise to an ice-chilled solution of Z(OMe)-His-OH^{4,12)} (5.43 g, 17 mmol) in 2 N NaOH (50 ml). After stirring for 5 hr, the solution was concentrated *in vacuo* and the residue was dissolved in H₂O (100 ml). The aqueous phase was washed with AcOEt, then acidified with citric acid and the resulting precipitate was extracted with AcOEt. The organic phase was washed with H₂O-NaCl, dried over Na₂SO₄ and concentrated. The oily residue was dissolved in a small amount of AcOEt and DCHA (3.6 ml, 20 mmol) was added. The resulting solid was recrystallized from AcOEt; yield 6.85 g (60%), mp 155–158°, $[\alpha]_D^{25} + 20.9^\circ$ ($c=0.6$, MeOH). *Anal.* Calcd for C₂₂H₂₃N₃O₈S·C₁₂H₂₃N: C, 60.87; H, 6.91; N, 8.35. Found: C, 60.77; H, 7.05; N, 8.32.

Boc-His(MBS)-OH·DCHA Salt—Starting with Boc-His-OH²¹⁾ (4.32 g, 17 mmol), the salt was prepared in essentially the manner described above; yield 7.15 g (69%), mp 155–158°, $[\alpha]_D^{25} + 22.7^\circ$ ($c=0.7$, MeOH). *Anal.* Calcd for C₁₈H₂₃N₃O₇S·C₁₂H₂₃N·H₂O: C, 57.67; H, 7.74; N, 8.97. Found: C, 57.08; H, 7.38; N, 8.91.

Z-His(MBS)-OH·DCHA Salt—Starting with Z-His-OH²²⁾ (4.92 g, 17 mmol), the salt was prepared as described above; yield 5.21 g (48%), mp 147–150°, $[\alpha]_D^{25} + 29.9^\circ$ ($c=2.5$, MeOH). *Anal.* Calcd for C₂₁H₂₁N₃O₇S·C₁₂H₂₃N·1/2H₂O: C, 60.99; H, 6.98; N, 8.62. Found: C, 60.83; H, 6.87; N, 8.70.

Z(OMe)-His(MBS)-OH—Z(OMe)-His(MBS)-OH·DCHA salt (1.35 g, 2 mmol) was dissolved in MeOH (10 ml) and 1 N HCl (3 ml) was added. The solvent was evaporated off and AcOEt was added. The DCHA hydrochloride was removed by filtration, then the filtrate was washed with H₂O-NaCl, dried over Na₂SO₄ and concentrated. Trituration of the residue with ether afforded a crystalline compound; yield 0.97 g (99%), mp 79–81°, $[\alpha]_D^{25} + 9.4^\circ$ ($c=1.3$, MeOH), R_f 0.50. *Anal.* Calcd for C₂₂H₂₃N₃O₈S: C, 53.98; H, 4.74; N, 8.59. Found: C, 53.72; H, 4.81; N, 8.37.

Boc-His(MBS)-OH—This compound was obtained from the DCHA salt as described above. mp 128–131°, $[\alpha]_D^{25} + 14.2^\circ$ ($c=1.1$, MeOH), R_f 0.45. *Anal.* Calcd for C₁₈H₂₃N₃O₇S: C, 50.81; H, 5.45; N, 9.88. Found: C, 50.67; H, 5.48; N, 9.67.

Z-His(MBS)-OH—This compound was obtained from the DCHA salt as described above. mp 64–65°, $[\alpha]_D^{25} + 7.2^\circ$ ($c=0.1$, MeOH), R_f 0.51. *Anal.* Calcd for C₂₁H₂₁N₃O₇S·1/2H₂O: C, 53.84; H, 4.73; N, 8.97. Found: C, 53.81; H, 4.80; N, 8.99.

H-His(MBS)-OH—Z(OMe)-His(MBS)-OH (derived from 1.35 g, 2 mmol, of the DCHA salt) was treated with TFA-anisole (3.0 ml–1 ml) in an ice-bath for 30 min, then dry ether was added. The resulting powder was collected by filtration, washed with ether and dissolved in a small amount of MeOH. The solution was neutralized with 5% NH₄OH and concentrated. The resulting powder was collected by filtration and recrystallized from 50% aqueous MeOH; yield 0.57 g (88%), mp 155–158°, $[\alpha]_D^{25} + 10.9^\circ$ ($c=2.4$, 10% AcOH), R_f 0.30. *Anal.* Calcd for C₁₃H₁₅N₃O₅S: C, 47.99; H, 4.65; N, 12.92. Found: C, 47.21; H, 4.60; N, 12.52.

Properties of His(MBS)—H-His(MBS)-OH (10 mg each) was exposed to various reagents and the treated samples were examined by TLC in CHCl₃-MeOH-H₂O (8:3:1). The ninhydrin color intensity of histidine was determined with a Shimadzu dual-wavelength TLC scanner, and the results are listed in Table I.

Effects of Various Sulfur Compounds on the Removal of the MBS and Tos Groups in TFA or in MSA—H-His(MBS)-OH and Boc-His(Tos)-OH (10 mg each) were treated with TFA (2 ml) or MSA (2 ml) in the

TABLE I. Properties of His(MBS)

Reagent		Temp. (°C)	Time	His regenerated (%)
1 N NaOH	2 equiv.	23	60 min	100
80% NH ₂ NH ₂	10 equiv.	23	24 hr	50
HOBt	2 equiv.	23	90 min	100
Pyridine-HCl	5 equiv.	23	60 min	34
Na-liquid NH ₃		–33	3 sec	100
H ₂ -Pd		23	3 hr	0
25% HBr-AcOH	6 equiv.	0	60 min	0
TFA-anisole		23	60 min	0
MSA-anisole		23	60 min	31
HF-anisole		0	60 min	100

21) B.O. Handford, T.A. Hylton, K.T. Wand, and B. Weinstein, *J. Org. Chem.*, **33**, 4251 (1968); G. Losse and U. Krychowski, *J. Prak. Chem.*, **312**, 1097 (1970); O.D. van Batenburg and K.E.T. Kerling, *Int. J. Peptide Protein Res.*, **8**, 1 (1976).

22) S. Akabori, K. Okawa, and F. Sakiyama, *Nature*(London), **181**, 772 (1958); F. Sakiyama, K. Okawa, T. Yamakawa, and S. Akabori, *Bull. Chem. Soc. Japan*, **31**, 926 (1958).

TABLE II. Effects of Various Sulfur Compounds on the Removal of the N^{im}-MBS and N^{im}-Tos Groups

Reagent	His regenerated from H-His(MBS)-OH %		His regenerated from Boc-His(Tos)-OH %	
	40 min	60 min	40 min	60 min
TFA-thioanisole		29.0		16.7
TFA-dimethylsulfide	94.4	100.0	87.0	99.8
TFA-ethanedithiol		14.3		13.3
TFA-mercaptoethanol		14.9		13.5
MSA-thioanisole		44.0		63.0
MSA-dimethylsulfide		66.9		48.3
MSA-ethanedithiol		40.1		52.3
MeOH-dimethylsulfide		0		0
AcOH-dimethylsulfide		6		0

presence of various sulfur compounds (5 equiv.) for a certain period, and the solutions were examined as described above. The results are listed in Table II.

Z-(OMe)-His(MBS)-ONP—DCC (1.03 g, 5 mmol) was added to a mixture of Z(OMe)-His(MBS)-OH (prepared from 3.35 g, 5 mmol, of the DCHA salt) and NP-OH (0.70 g, 5 mmol) in AcOEt (30 ml). After stirring at room temperature for 5 hr, the solution was filtered, then the filtrate was washed with 5% citric acid, 5% Na₂CO₃ and H₂O-NaCl, dried over Na₂SO₄ and concentrated. Trituration of the residue with ether afforded a powder, which was recrystallized from AcOEt and ether; yield 2.93 g (96%), mp 82–84°, [α]_D²⁵ +2.0° (c =2.3, DMF). R_f 0.83. *Anal.* Calcd for C₂₈H₂₆N₄O₁₀S: C, 55.08; H, 4.29; N, 9.18. Found: C, 54.93; H, 4.26; N, 9.04.

Boc-His(MBS)-ONP—Starting with Boc-His(MBS)-OH·DCHA (1.86 g, 3 mmol), the active ester was prepared as described above; yield 1.47 g (90%), mp 138–143°, [α]_D²⁵ –2.0° (c =2.3, DMF), R_f 0.92. *Anal.* Calcd for C₂₄H₂₆N₄O₉S: C, 52.74; H, 4.80; N, 10.25. Found: C, 52.18; H, 4.99; N, 9.96.

Z-His(MBS)-ONP—Starting with Z-His(MBS)-OH (1.28 g, 2 mmol), the active ester was prepared as described above; yield 0.96 g (83%), mp 65–68°, [α]_D²⁵ –4.5° (c =2.0, DMF), R_f 0.95. *Anal.* Calcd for C₂₇H₂₄N₄O₉S: C, 55.85; H, 4.17; N, 9.65. Found: C, 55.70; H, 4.02; N, 9.51.

Z(OMe)-His(MBS)-Gly-OBzl—Z(OMe)-His(MBS)-ONP (1.83 g, 3 mmol) was added to a stirred solution of H-Gly-OBzl (prepared from 1.68 g, 5 mmol, of the tosylate with 0.7 ml, 5 mmol, of Et₃N) in DMF (30 ml). After 24 hr, the solution was concentrated and the residue was dissolved in AcOEt. The organic phase was washed with 10% citric acid, 5% Na₂CO₃ and H₂O-NaCl, dried over Na₂SO₄ and concentrated. Trituration of the residue with *n*-hexane followed by recrystallization from MeOH and AcOEt afforded a powder; yield 1.75 g (92%), mp 122–124°, [α]_D²⁵ +9.1° (c =1.5, DMF), R_f 0.70. *Anal.* Calcd for C₃₁H₃₂N₄O₉S: C, 58.48; H, 5.07; N, 8.80. Found: C, 58.22; H, 5.20; N, 8.77.

Boc-His(MBS)-Gly-OBzl—Starting with Boc-His(MBS)-ONP (0.40 g, 0.73 mmol), this dipeptide ester was prepared as described above; yield 0.35 g (83%), mp 41–42°, [α]_D²⁵ +1.1° (c =1.0, DMF), R_f 0.72. *Anal.* Calcd for C₂₇H₃₂N₄O₈S·1/2H₂O: C, 55.75; H, 5.72; N, 9.63. Found: C, 55.61; H, 5.74; N, 9.19.

Z(OMe)-His-Gly-OBzl—A mixture of Z(OMe)-His(MBS)-Gly-OBzl (637 mg, 1 mmol) and HOBT (270 mg, 2 mmol) in THF (20 ml) was stirred at room temperature for 1.5 hr, while the starting material disappeared on TLC. The solvent was evaporated off and the residue was dissolved in 1 N H₂SO₄. The aqueous phase was washed with AcOEt, then neutralized with Na₂CO₃ in an ice-bath. The resulting precipitate was extracted with AcOEt. The extract was washed with H₂O-NaCl, dried over Na₂SO₄ and concentrated. Trituration with ether followed by recrystallization from AcOEt afforded the protected dipeptide; yield 410 mg (88%), mp 105–108°, [α]_D²⁵ –15.7° (c =1.2, DMF), R_f 0.61. *Anal.* Calcd for C₂₄H₂₅N₄O₆·1/2H₂O: C, 60.75; H, 5.52; N, 11.81. Found: C, 60.81; H, 5.72; N, 12.17.

***n*-p-Methoxybenzenesulfonyloxy-benzotriazole**—The AcOEt washing in the above experiment was washed with H₂O-NaCl, dried over Na₂SO₄ and concentrated. The resulting solid was recrystallized from AcOEt to give a crystalline compound; yield 238 mg (78%), mp 80–82°. *Anal.* Calcd for C₁₃H₁₁N₃O₄S: C, 51.14; H, 3.63; N, 13.76. Found: C, 50.84; H, 3.57; N, 13.80.

H-His(MBS)-Gly-OBzl—Z(OMe)-His(MBS)-Gly-OBzl (637 mg, 1 mmol) was treated with TFA-anisole (1.5 ml–0.4 ml) in an ice-bath for 30 min, then excess TFA was removed by evaporation and the residue was dissolved in a small amount of H₂O. The aqueous phase was washed with AcOEt, then made basic with 10% Na₂CO₃ and the resulting precipitate was extracted with AcOEt. The extract was washed with H₂O-NaCl, dried over Na₂SO₄ and concentrated. The oily residue turned to a powder on standing with

ether, and this was recrystallized from AcOEt and ether; yield 420 mg (89%), mp 108–110°, $[\alpha]_D^{25} -3.4^\circ$ ($c=2.0$, DMF), Rf_1 0.58. *Anal.* Calcd for $C_{22}H_{24}N_4O_6S$: C, 55.92; H, 5.12; N, 11.86. Found: C, 55.37; H, 5.06; N, 11.45.

Z(OMe)-His-Gly-OH—Z(OMe)-His(MBS)-Gly-OBzl (637 mg, 1 mmol) dissolved in MeOH (30 ml) was treated with 1 N NaOH (2 ml) at room temperature for 1 hr; loss of the starting material was monitored by TLC. After neutralization with AcOH, the solution was concentrated and the residue was dissolved in H_2O . The aqueous phase was washed with AcOEt and concentrated. The oily residue was treated with EtOH in a refrigerator overnight and the resulting solid was recrystallized from EtOH; yield 280 mg (74%), mp 175–178°, $[\alpha]_D^{25} +3.9^\circ$ ($c=1.5$, DMF), Rf_1 0.13. *Anal.* Calcd for $C_{17}H_{20}N_4O_6 \cdot H_2O$: C, 51.77; H, 5.62; N, 14.21. Found: C, 51.35; H, 5.54; N, 13.67.

Treatment of Z(OMe)-His(MBS)-Gly-OBzl by TFA-dimethylsulfide—Z(OMe)-His(MBS)-Gly-OBzl (100 mg) was treated with TFA (1 ml) in the presence of dimethylsulfide (50 equiv.) at room temperature for 60 min. Examination by TLC revealed the presence of two ninhydrin-positive spots: the spot with Rf_2 0.62 (main spot positive to the Pauly test) matched that of the TFA-treated sample of Z(OMe)-His-Gly-OBzl and the spot with Rf_2 0.21 (minor spot positive to the Pauly test) matched that of the TFA-treated sample of Z(OMe)-His-Gly-OH.

Z(OMe)-His(MBS)-Pro-NH₂—Z(OMe)-Pro-NH₂ (2.78 g, 10 mmol) was treated with TFA-anisole (5.0 ml–1.9 ml) as usual, then dry ether was added. The resulting powder was washed with ether and dissolved in DMF (30 ml) together with Et_3N (2.8 ml, 20 mmol) and Z(OMe)-His(MBS)-ONP (6.10 g, 10 mmol). After stirring for 48 hr, the solution was concentrated and the residue was dissolved in AcOEt. The organic phase was washed with 10% citric acid, 5% Na_2CO_3 and H_2O -NaCl, dried over Na_2SO_4 and concentrated. Trituration of the residue with ether afforded a powder, which was recrystallized from AcOEt and ether; yield 3.15 g (54%), mp 139–142°, $[\alpha]_D^{27} -17.7^\circ$ ($c=0.9$, MeOH), Rf_1 0.67. *Anal.* Calcd for $C_{27}H_{31}N_5O_8S \cdot 1/2 \cdot H_2O$: C, 54.53; H, 5.42; N, 11.78. Found: C, 54.65; H, 5.37; N, 11.65.

Z-Pyr-His-Pro-NH₂—Z(OMe)-His(MBS)-Pro-NH₂ (0.40 g, 0.67 mmol) was treated with TFA (4 ml) in the presence of anisole (0.36 ml, 5 equiv.) and dimethylsulfide (0.49 ml, 10 equiv.) at room temperature for 60 min, then *n*-hexane was added. Trituration of the oily precipitate with ether afforded a powder, which was dried over KOH pellets *in vacuo* for 3 hr, then dissolved in DMF (4 ml) together with Et_3N (0.27 ml, 3 equiv.) and Z-Pyr-ONP (0.26 g, 0.67 mmol). After stirring at room temperature for 48 hr, the solution was concentrated and the residue was purified by column chromatography on CM-cellulose (2.2×5 cm), eluting with 0.1 M $AcONH_4$ buffer (pH 5.0) through a mixing flask containing H_2O (200 ml). Individual fractions (3 ml each) were collected and the absorption at 245 nm was determined. Two peaks were detected; the front peak was due to the contaminating scavengers. The fractions corresponding to the main peak (tube No. 29–34) were combined and the solvent was removed by evaporation. Repeated lyophilization of the residue afforded a powder; yield 0.22 g (68%), $[\alpha]_D^{27} -41.3^\circ$ ($c=0.8$, DMF), (lit.¹⁷) -43° in DMF, Rf_1 0.58. Amino acid ratios in the 6 N HCl hydrolysate: Glu 1.01, His 1.06, Pro 1.00 (average recovery 88%). *Anal.* Calcd for $C_{24}H_{28}N_6O_6 \cdot 3H_2O$: C, 52.35; H, 6.22; N, 15.27. Found: C, 52.08; H, 5.78; N, 15.32.

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