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Further Studies on the Use of Multi-substituted Benzenesulfonyl Groups for Protection of the Guanidino Function of Arginine[1)]

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Various multi-substituted benzenesulfonyl protecting groups for the guanidino function of arginine, removable under mild conditions such as with trifluoroacetic acid (TFA)-thioanisole, were studied. Among them, the 4-methoxy-2,6-dimethylbenzenesulfonyl (Mds) group, the 2,3,4,5,6-pentamethylbenzenesulfonyl (Pme) group, and the 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr) group were found to be useful. Both Mds and Pme show considerable resistance to TFA and, therefore, are suitable for procedures in which the intermediates are deprotected by TFA treatment, while Mtr is the most useful protecting group when TFA treatment is not necessary.

Keywords——4-methoxy-2,6-dimethylbenzenesulfonyl (Mds); 2,4,6-trimethoxybenzenesulfonyl (Mtb); 2,3,4,5,6-pentamethylbenzenesulfonyl (Pme); 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr); trifluoroacetic acid-thioanisole deprotection

Introduction of the *p*-methoxybenzenesulfonyl (Mbs)[2)] group and the mesitylene-2-sulfonyl (Mts)[3)] group has made possible the widespread use of methanesulfonic acid (MSA)[4)] as a deprotecting reagent in the final step of peptide synthesis. However, an unwanted side reaction, the formation of succinimide at Asp and Asn peptide residues, has been observed during the deblocking step with MSA.[5)] We recently reported the use of the 4-methoxy-2,6-dimethylbenzenesulfonyl (Mds)[6)] group as a new N^G-protecting group for Arg; it can be removed with trifluoroacetic acid (TFA)-thioanisole at 50°C in 1—2 hours.[7)] This paper deals with further studies on other multi-substituted benzenesulfonyl groups for the protection of the quanidino function of Arg.

First, we examined the formation of succinimide under various acidic conditions using MSA, hydrogen fluoride (HF),[8)] 1 M trifluoromethanesulfonic acid (TFMSA)–TFA,[9)] and TFA,

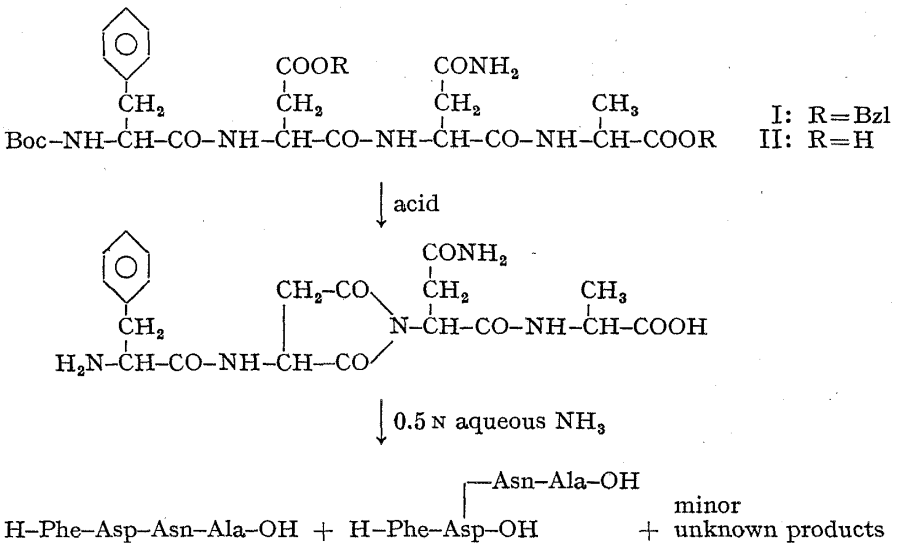

Chart 1

with two model peptides, Boc–Phe–Asp(OBzl)–Asn–Ala–OBzl [I], and Boc–Phe–Asp–Asn–Ala–OH [II] (shown in Chart 1). The products of acid treatment were examined by HPLC[10] and the results are listed in Table I. MSA treatment (20°C, 60 min) caused essentially complete transformation of both peptides into the aminosuccinyl derivatives, in spite of the free carboxyl group on the Asp residue in Peptide II. The structure of the imide was confirmed by infrared (IR) spectroscopy and paper electrophoresis. HF treatment (0°C, 60 min) gave 38.8% of the imide for peptide I and 18.8% for pepride II. Treatment with 1 M TFMSA–TFA (0°C, 90 min) gave 48.8% of the imide for peptide II. In order to obtain the α-peptide from the imide, the material obtained by treatment with MSA was then treated with 0.5 N aqueous ammonia at 0°C for 30 min.[11] The products, however, contained only 15.5% of the α-peptide and 75.4% of the β-peptide. This result clearly shows that succinimide formation is a serious side reaction and should be avoided. On the other hand, TFA treatment gave only a few percent of imide for peptide II, even at 50°C for 2 hours. From these results, TFA seems to be an excellent deblocking reagent compared to other acid reagents, at least for these model peptides.

Table I. Formation of Aminosuccinyl Peptides under Various Acidic Conditions

Materials and conditions			Intact peptide	Aminosuccinyl peptide
Peptide I	HF	0°C 60 min	56.7%	38.8%
	MSA[a]	20°C 60 min	2.1	87.3
Peptide II	HF	0°C 60 min	67.7	18.8
	MSA	20°C 60 min	3.0	92.6
	1 M TFMSA-TFA	0°C 90 min	50.1	48.8
	TFA	20°C 10 min	96.5	1.0
	TFA	26°C 72 h	91.9	6.6
	TFA	50°C 2 h	95.1	1.7

a) The reaction mixture obtained was treated with 0.5 N aqueous ammonia at 0°C for 30 min, but the products included 15.5% of the α-peptide and 75.4% of the β-peptide [checked by HPLC: column, Toyosoda LS-410; solvent, CH_3CN-0.1 M $AcONH_4$ (1:99); flow rate, 0.9 ml/min; elution times, α-peptide=11.6 min, β-peptide=9.2 min].

Recently, we introduced the 4-methoxy-2,6-dimethylbenzenesulfonyl (Mds)[6] group for the protection of arginine (this group is removable with TFA-thioanisole[12] at 50°C for 1—2 hours), and used it in syntheses of Substance P,[6] two LH–RH analogs,[6] and dynorphin [1—13].[13] During synthetic studies of dynorphin [1—13], we noticed that some Mds isomer, carbobenzoxy-N^G-2-methoxy-4,6-dimethylbenzenesulfonyl arginine [Z–Arg(iMds)-OH] was contained in crude Z–Arg(Mds)–OH. After isolation of this isomer as H–Arg(iMds)–OH,

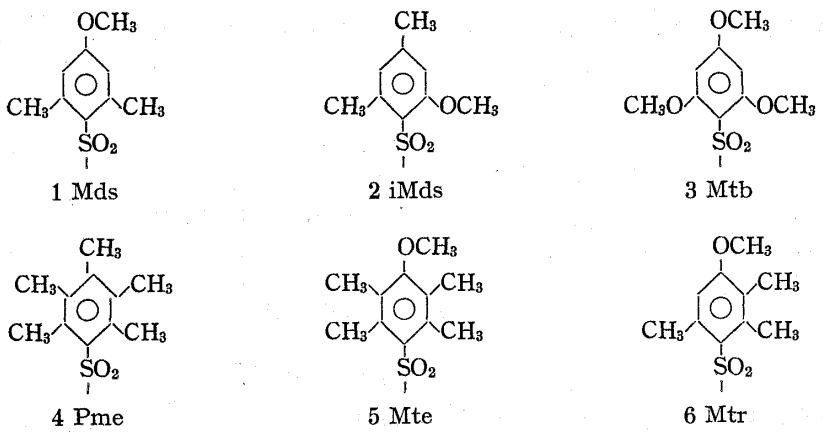

Fig. 1. Various N^G-Protecting Groups for Arginine

we examined its sensitivity to TFA-thioanisole at 50°C, and found that the iMds group is far more stable than the Mds group. This result prompted us to investigate the sensitivity of other substituted benzenesulfonyl groups to TFA-thioanisole. The various N^G-substituted benzenesulfonyl arginine derivatives that we synthesized are shown in Fig. 1.

1 is the Mds group and 2 is an isomer of it (iMds). Mds-Cl was prepared according to the method described in the previous paper[13] and obtained as crystals. 3 is a 2,4,6-trimethoxybenzenesulfonyl (Mtb) group, and 4 is a 2,3,4,5,6-pentamethylbenzenesulfonyl (Pme) group. Sulfonyl chlorides of 3 and 4 were synthesized according to the method of Pareos *et al.*[14] and obtained as crystals. Pme–Cl was obtained in 93.5% yield, but Mtb–Cl was obtained in only 6.3% yield from 1,3,5-trimethoxybenzene. 5 is a 4-methoxy-2,3,5,6-tetramethylbenzenesulfonyl (Mte) group, and 6 is a 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr) group. Sulfonyl chlorides of 5 and 6 were also prepared by a procedure similar to that of Pareos *et al.*, and could be obtained in good yields as crystals. In synthesizing Mtr–Cl, 2,3,5-trimethylanisole was treated with chlorosulfonic acid, and 4-methoxy-2,3,6-trimethylbenzenesulfonyl chloride alone was obtained without formation of an isomer.

The sulfonyl chlorides were introduced into Z–Arg–OH according to the procedure described for the preparation of Z–Arg(Mbs)–OH.[2] N^G-Protected arginine derivatives were obtained from the corresponding material by hydrogenation using Pd-black as a catalyst. These arginine derivatives were deprotected with TFA-thioanisole (9: 1) at 50°C, and the results are shown in Table II.

Table II. Deprotection of N^G-Protected Arginine Derivatives in TFA-thioanisole

Protecting group	0.5 h	1.0 h	4.0 h
Mtr	88.9%[a]	89.9%[a]	94.2%[a]
Mds	81.7	92.5[a]	94.4[a]
Mtb	84.2	89.9[a]	91.1[a]
Pme	40.1	63.7	89.3[a]
Mte	19.1	30.4	81.0
Mts	—	—	50.7
Mbs	—	—	43.7
iMds	7.0	11.1	28.4

N^G-Protected arginine derivatives were treated with TFA-thioanisole (9: 1) at 50°C for 0.5—4 h. The reaction products were subjected to quantitative amino acid analysis to estimate the content of regenerated arginine (%).
a) The starting material could not be observed on TLC.

The acid lability of these protecting groups to TFA-thioanisole was in the order 6(Mtr)>1(Mds)~3(Mtb)>4(Pme)>5(Mte)>Mts~Mbs>2(iMds). The Mtr group is the most labile protecting group and could be removed within 30 minutes (checked by TLC). Both the Mds and the Mtb groups could be removed in 1—2 hours. Succinimide formation was limited to a few percent under these conditions. The Pme group required 4 hours, but the Mte group could not be removed in 4 hours. The three acid-labile protecting groups, Mds, Mtb, and Mtr, were also examined in TFA-thioanisole (9: 1) at 25°C (checked by TLC). Both Mds and Mtb required 4—6 hours, while Mtr could be removed in about one hour. Interestingly, the Pme group, in which two methyl groups were introduced into the 3 and 5 positions of Mts, is far more labile than the Mts group, and the Mte group, in which two methyl groups were introduced into the 3 and 5 positions of Mds, is less labile than Mds. The reason why Mte is less labile than Mds may be the inhibition of resonance between the oxygen atom of the methoxy group and the phenyl ring. Therefore, we synthesized the Mtr group with only one methyl group introduced into the 3 position of Mds, and found that this group is the most acid labile protecting group of those presented here. These results clearly indicate that the

lability of the Mtr group is not only due to the absence of steric hindrance observed in the Mte group, but also to the methyl group effect observed in the Pme group.

From the standpoint of practical peptide synthesis, protecting groups that are resistant to both TFA treatment and catalytic hydrogenation and that can also be removed under mild conditions, such as with TFA-thioanisole, are desired. In synthesizing sulfur-containing peptides, TFA-resistant protecting groups are especially desirable. Therefore, we next examined the stability of these N^G-protected arginine derivatives to TFA at 25°C for 1 hour, and the results are shown in Table III. About 50% of the Mtr group could be removed and about 20% of the Mds and Mtb groups could be removed. However, the Pme group was rather stable to TFA treatment.

Table III. Cleavage of Various Protecting Groups in TFA

Mtr[a)]	Mds	Mtb	Pme	Mte
52.0%	22.3%	19.7%	2.0%	1.6%

N^G-Protected arginine derivatives were treated with TFA at 25°C for 60 min. The reaction products were subjected to quantitative amino acid analysis to estimate the content of regenerated arginine (%).
a) The Mtr group could be removed completely in 4—6 h.

These results suggest the use of the Pme group when TFA treatment is to be repeated frequently, such as in solid phase peptide synthesis, although the conditions of the deprotection are somewhat severe compared to those for the Mds group. When the TFA treatment is not required repeatedly, the Mds or Mtb group is suitable. The Mtr group cannot be used when TFA treatment is necessary, although in other cases, it seems to be a most useful protecting group. Practical peptide synthesis using the protecting groups presented here will be reported in the future.

Experimental

All melting points were taken by the capillary method and are uncorrected. Rotations were determined with a Perkin-Elmer model 141 polarimeter. Acid hydrolyses were carried out in 6 N HCl at 110°C for 24 h. Amino acid analyses were performed in a Hitachi 835 amino acid analyzer. Solutions were concentrated in a rotary evaporator under reduced pressure at a temperature of 20—40°C. Catalytic hydrogenations were performed at room temperature with palladium (Pd) black as a catalyst. The purity of the products was tested by thin-layer chromatography on silica gel (precoated silica gel plate $60F_{254}$, Merck) or cellulose (Avicel, Funakoshi Yakuhin Co. Ltd.) plates. Solvent systems used were *n*-hexane-$CHCl_3$ (1: 1, Rf^1), $CHCl_3$–MeOH–AcOH (9: 1: 0.5, Rf^2), AcOEt–pyridine–AcOH–H_2O (60: 20: 6: 10, Rf^3), *n*-BuOH–pyridine–AcOH–H_2O (30: 20: 6: 24, Rf^4), and AcOEt–*n*-BuOH–AcOH–H_2O (1: 1: 1: 1, Rf^5). *Rf* values are given for silica gel plates unless otherwise stated.

Boc-Asn-Ala-OBzl——H–Ala–OBzl·*p*-toluenesulfonic acid salt (40.6 g) was suspended in THF (300 ml) together with TEA (15.4 ml) under ice-cooling, and then Boc–Asn–OH (23.3 g), HONB (19.7 g) and DCC (22.7 g) were added. The mixture was stirred for 15 h, the DCU formed was filtered off, and the filtrate was concentrated. The residue was dissolved in AcOEt (500 ml) and the solution was washed with 4% $NaHCO_3$ solution, 10% citric acid, and water, then dried over anhydr. Na_2SO_4. After removal of the solvent by evaporation, the crystals formed were filtered off and recrystallized from AcOEt. Yield 29.1 g (74.0%) mp 145—146°C, $[\alpha]_D^{26}$ −32.8° (c=1.0 in MeOH), Rf^2 0.67. *Anal.* Calcd for $C_{19}H_{27}N_3O_6$: C, 58.00; H, 6.92; N, 10.68. Found: C, 58.55; H, 7.02; N, 10.64.

Boc-Asp(OBzl)-Asn-Ala-OBzl——Boc–Asn–Ala–OBzl (10.0 g) was treated with TFA (50 ml) at room temperature for 10 min, and the solvent was evaporated off. The residue was triturated with ether to give a precipitate, which was dissolved in THF (200 ml) together with TEA (5.0 ml) under ice-cooling. To this was added Boc–Asp(OBzl)–ONB prepared from Boc–Asp(OBzl)–OH (7.40 g), HONB (4.55 g), and DCC (5.24 g) in THF (100 ml), and the mixture was stirred for 15 h. After the usual work-up, the material was recrystallized from acetonitrile. Yield 12.8 g (90.4%) mp 102—104°C, $[\alpha]_D^{26}$ −25.1° (c=1.1 in MeOH), Rf^2 0.68. *Anal.* Calcd for $C_{30}H_{38}N_4O_9 \cdot 1/2CH_3CN$: C, 60.13; H, 6.43; N, 10.18. Found: C, 60.48; H, 6.53; N, 10.30.

Boc-Phe-Asp(OBzl)-Asn-Ala-OBzl [I]——Boc–Asp(OBzl)–Asn–Ala–OBzl (6.0 g) was treated with TFA (40 ml) in the same manner as described above, and the material was coupled with Boc–Phe–ONB prepared from Boc–Phe–OH (2.66 g), HONB (1.97 g), and DCC (2.27 g) in the presence of TEA (1.60 ml) in THF (20

ml). The solution was stirred for 15 hr then worked up as usual, and the product was crystallized from AcOEt. Yield 5.90 g (78.9%) mp 176—177°C, $[\alpha]_D^{26}$ −16.8° (c=1.0 in DMF), Rf^2 0.69. *Anal.* Calcd for $C_{39}H_{47}N_5O_{10}$: C, 62.80; H, 6.35; N, 9.39. Found: C, 63.25; H, 6.59; N, 9.46.

Boc-Phe-Asp-Asn-Ala-OH [II]——Boc–Phe–Asp(OBzl)–Asn–Ala–OBzl (1.0 g) was hydrogenated over Pd-black as a catalyst in AcOH (30 ml). After removal of the catalyst by filtration, the filtrate was concentrated, and the residue was triturated with ether to give a precipitate. Yield 0.75 g (85.7%) mp 189—190°C, $[\alpha]_D^{22}$ −19.9° (c=1.2 in DMF), Rf^3 0.16. *Anal.* Calcd for $C_{25}H_{35}N_5O_{10}\cdot H_2O$: C, 51.45; H, 6.39; N, 12.00. Found: C, 51.77; H, 6.11; N, 12.04.

H-Phe-Asp-Asn-Ala-OH——Boc–Phe–Asp–Asn–Ala–OH (100 mg) was treated with TFA (5 ml) at 20°C for 10 min. The TFA was evaporated off, and the residue was triturated with ether to give a precipitate. This powder was dissolved in 30% AcOH and passed through a column (1×10 cm) of Amberlite IRA-410 (acetate form), and the solution was lyophilized. To this was added 0.5 N AcOH (2 ml) and the crystals formed were collected by filtration. Yield 77 mg (96.7%) $[\alpha]_D^{21}$ −18.6° (c=0.5 in 50% AcOH), Rf^4 (cellulose) 0.39. Paper electrophoresis (pH 6.5 pyridine–acetate buffer, 500 volt, 2 h) 0.67×Asp. Amino acid ratios in acid hydrolysate: Asp 1.98(2); Ala 1.00(1); Phe 0.97(1) (average recovery 91%).

H-Phe-Asc-Asn-Ala-OH——Boc–Phe–Asp(OBzl)–Asn–Ala–OBzl (150 mg) was treated with MSA-anisole (1 ml—0.2 ml) at room temperature for 60 min and the reaction mixture was triturated with ether to give an oily precipitate, which was dissolved in water. The solution was passed through a column (1×10 cm) of Amberlite IRA-410 (acetate form), and lyophilized. The material was further applied to a column (2.2×115 cm) of Sephadex LH-20 (1 N AcOH). The desired fractions (235—265 ml) were pooled and lyophilized. Yield 83 mg (71.8%). $[\alpha]_D^{21}$ +2.6° (c=0.4 in 50% AcOH), Rf^4 (cellulose) 0.52. IR ν_{max}^{KBr} cm^{-1}: 1790 and 1710 (imide). Paper electrophoresis (pH 6.5 pyridine–acetone buffer, 500 v, 2 h) 0.46×Asp. Amino acid ratios in acid hydrolysate: Asp 2.00(2); Ala 1.00(1); Phe 0.94(1) (average recavery 86%).

H-Arg(Mds)-OH and H-Arg(iMds)-OH——A suspension of Z–Arg(Mds)–OH. CHA[13] (3.03 g) in AcOEt (40 ml) was washed with 0.2 N H_2SO_4 (12 ml), dried over anhydr. Na_2SO_4, and concentrated. The residue was hydrogenated over Pd black as a catalyst. The oily material obtained was crystallized from water. Yield 1.70 g (89.1%) mp 120—122°C (dec.), $[\alpha]_D^{23}$ −7.8° (c=0.7 in MeOH), Rf^3 0.12, Rf^5 0.49. *Anal.* Calcd for $C_{15}H_{24}N_4O_5S\cdot 1/2H_2O$: C, 47.23; H, 6.61; N, 14.69; S, 8.41. Found: C, 47.68; H, 6.58; N, 14.69; S, 8.47. NMR of the Mds group (DMSO-d_6) δ: 2.61 (6H, s), 3.95 (3H, s), 6.65 (2H, s). Crude Z–Arg(Mds)–OH·CHA (78.0 g) prepared using crude Mds–Cl was hydrogenated in the same manner as described above, and the oily material was crystallized from water. Yield 25.3 g (52.7%). The mother liquor was concentrated and the residue was applied to a column (7.5 cm×12 cm) of silica gel (AcOEt–pyridine–AcOH–H_2O=60: 20: 6: 10). The fractions containing the desired material (Rf^3 0.08) were pooled and concentrated. Yield 2.80 g (5.8%). The material (500 mg) thus obtained was further purified by column chromatography on a Sephadex LH-20 column (2.2×120 cm, 1 N AcOH). The fractions (345—445 ml) were pooled and lyophilized. Yield 380 mg $[\alpha]_D^{23}$ −6.7° (c=0.8 in MeOH), Rf^3 0.08, Rf^5 0.46. *Anal.* Calcd for $C_{15}H_{24}N_4O_5S\cdot AcOH$: C, 47.21; H, 6.53; N, 12.96; S, 7.41. Found: C, 47.49; H, 6.53; N, 13.09; S, 7.03. NMR of the iMds group (DMSO-d_6) δ: 2.26 (3H, s), 2.53 (3H, s), 3.76 (3H, s), 6.40 (1H, s), 6.76 (1H, s).

2,4,6-Trimethoxybenzenesulfonyl Chloride (Mtb-Cl)[14]——1,3,5-Trimethoxybenzene (5.05 g) was dissolved in CH_2Cl_2 (500 ml) and the solution was cooled to −5°——10°C. To this was added chlorosulfonic acid (6.0 ml) in CH_2Cl_2 (400 ml) and the solution was stirred for 3 h. The reaction mixture was then poored onto crushed ice containing 5% $NaHCO_3$ (300 ml). The organic layer was washed with water, dried over anhydr. $MgSO_4$, and concentrated. The residue was crystallized from CCl_4. Yield 0.61 g (6.3%). mp 130—133°C (Ref 13, mp 134—136°C), Rf^1 0.08. *Anal.* Calcd for $C_9H_{11}ClO_5S$: C, 40.53; H, 4.16; Cl, 13.30; S, 12.02. Found: C, 40.79; H, 4.16; Cl, 13.28; S, 11.84.

Z-Arg(Mtb)-OH——Z–Arg–OH (0.77 g) was dissolved in 4 N NaOH–acetone (2.5 ml—10 ml) and cooled with ice. To this was added Mtb-Cl (1.0 g) in acetone (4 ml) and the mixture was stirred for 2 h. After acidification with 10% citric acid, the solvent was evaporated off and the material was extracted with AcOEt. The AcOEt layer was washed with satd. NaCl solution, dried aver anhydr. Na_2SO_4, and concentrated. The residue was triturated with ether to give a precipitate, which was collected and further purified by column chromatography on silica gel (column: 4×10 cm, $CHCl_3$–MeOH–AcOH=9: 0.7 :0.35). The fractions (110—210 ml) were pooled and concentrated, and the residue was triturated with ether to give a precipitate. Yield 0.50 g (36.0%). mp 89—93°C, $[\alpha]_D^{23}$ +0.8° (c=0.5 in MeOH), Rf^2 0.27. *Anal.* Calcd for $C_{23}H_{30}N_4O_9S\cdot H_2O$: C, 49.63; H, 5.80; N, 10.17; S, 5.76. Found: C, 49.67; H, 5.57; N, 9.89; S, 5.81.

H-Arg(Mtb)-OH——Z–Arg(Mtb)–OH (0.15 g) was hydrogenated over Pb black in MeOH (30 ml). After the usual work-up, the material was triturated with ether to give a precipitate. Yield 0.10 g (84.9%). mp 115—120°C, $[\alpha]_D^{23}$ −8.9° (c=0.6 in MeOH), Rf^3 0.03, Rf^5 0.39. *Anal.* Calcd for $C_{15}H_{24}N_4O_7S\cdot CH_3OH$: C, 44.02; H, 6.47; N, 12.84; S, 7.35. Found: C, 43.55; H, 6.33; N, 12.84; S, 6.99.

2,3,4,5,6-Pentamethylbenzenesulfonyl Chloride (Pme-Cl)[14]——Pentamethylbenzene (17.8 g) was dissolved in CH_2Cl_2 (500 ml) and the solution was cooled to −5°——10°C. To this was added chlorosulfonic acid (24 ml) in CH_2Cl_2 (400 ml). After the usual work-up, the material was obtained as crystals from *n*-hexane. Yield 27.7 g (93.5%). mp 80—81°C (Ref 14, mp 80—81°C), Rf^1 0.61. *Anal.* Calcd for $C_{11}H_{15}$-

ClO_2S: C, 53.54; H, 6.13; Cl, 14.37; S, 13.00. Found: C, 53.78; H, 6.09; Cl, 14.39; S, 13.00.

Z-Arg(Pme)-OH·CHA——Z–Arg–OH (3.10 g) was dissolved in 4 N NaOH–acetone (10 ml—40 ml) and cooled with ice. To this was added Pme–Cl (4.32 g) in acetone (10 ml), and the whole was stirred for 3 h. After the usual work-up, the oily material obtained was dissolved in AcOEt (30 ml) together with cyclohexylamine (1.08 ml). The crystals that formed were collected by filtration and recrystallized from MeOH–AcOEt. Yield 4.20 g (67.5%). mp 173—175°C, $[\alpha]_D^{23}$ +5.8° (c=1.3 in MeOH), Rf^2 0.43. *Anal.* Calcd for $C_{31}H_{47}N_5O_6S$: C, 60.26; H, 7.67; N, 11.34; S, 5.19. Found: C, 60.15; H, 7.84; N, 11.25; S, 5.30.

H-Arg(Pme)-OH——A suspension of Z–Arg(Pme)–OH·CHA (1.24 g) in AcOEt (30 ml) was washed with 0.2 N H_2SO_4 (15 ml), dried over anhydr. Na_2SO_4, and concentrated. The residue was hydrogenated in MeOH in the usual manner, and the product was obtained as crystals from MeOH–ether. Yield 0.77 g (97.8%) mp 153—156°C, $[\alpha]_D^{23}$ −5.5° (c=0.9 in MeOH), Rf^3 0.14. *Anal.* Calcd for $C_{17}H_{28}N_4O_4S\cdot 1/2H_2O$: C, 51.89; H, 7.43; N, 14.28; S, 8.15. Found: C, 51.60; H, 7.74; N, 13.80; S, 8.12.

2,3,5,6-Tetramethylanisole——2,3,5,6-Tetramethylphenol (15.0 g) was dissolved in DMSO (150 ml) together with methyl iodide (28 ml), and the solution was cooled with ice. To this was added 60% NaH in oil (6.2 g), and the mixture was stirred for 10 h. After addition of water, the whole was extracted with ether, and the extract was dried over anhydr. Na_2SO_4. After concentration, the residue was crystallized from MeOH. A small amount of impurities was still present, but the material was used without further purification. Yield 10.2 g (62.1%) Rf^1 0.61.

4-Methoxy-2,3,5,6-tetramethylbenzenesulfonyl Chloride (Mte-Cl)——Chlorosulfonic acid (12 ml) in CH_2Cl_2 (400 ml) was added to a solution of 2,3,5,6-tetramethylanisole (10.0 g) in CH_2Cl_2 (500 ml) in the same manner as described for Pme-Cl. After the usual work-up, the product was obtained as crystals from *n*-hexane. Yield 10.0 g (62.5%). mp 58—59°C, Rf^1 0.48. *Anal.* Calcd for $C_{11}H_{15}ClO_3S$: C, 50.28; H, 5.75; Cl, 13.50; S, 12.21. Found: C, 50.52; H, 5.56; Cl, 13.42; S, 11.92.

Z-Arg(Mte)-OH-CHA——Z–Arg–OH (1.85 g) was dissolved in 4 N NaOH–acetone (6 ml—25 ml) and cooled with ice. To this was added Mte-Cl (2.50 g) in acetone (5 ml). The whole was stirred for 2 h and worked up as usual. The oily material obtained was dissolved in ether (20 ml) together with cyclohexylamine (0.58 ml). The crystals that formed were collected and washed with ether. Yield 2.40 g (63.1%). mp 127—129°C, $[\alpha]_D^{23}$ +5.9° (c=0.8 in MeOH), Rf^2 0.43. *Anal.* Calcd for $C_{31}H_{47}N_5O_7S$: C, 58.74; H, 7.48; N, 11.05; S, 5.06. Found: C, 58.84; H, 7.30; N, 11.25; S, 5.06.

H-Arg(Mte)-OH——Z–Arg(Mte)–OH·CHA (2.0 g) was suspended in AcOEt (40 ml). The suspension was washed with 0.2 N H_2SO_4 (20 ml) and concentrated. The residue was hydrogenated in MeOH in the usual manner, and the material obtained was triturated with ether to give a precipitate. Yield 1.20 g (92.7%) mp 150—152°C, $[\alpha]_D^{23}$ −4.5° (c=0.9 in MeOH), Rf^3 0.14. *Anal.* Calcd for $C_{17}H_{28}N_4O_5S\cdot 1/2H_2O$: C, 49.86; H, 7.14; N, 13.68; S, 7.83. Found: C, 50.38; H, 7.57; N, 13.48; S, 7.60.

2,3,5-Trimethylanisole——2,3,5-Trimethylphenol (10.0 g) was dissolved in DMSO (100 ml) together with methyl iodide (10.4 ml), and the solution was cooled with ice. To this was added 60% NaH in oil (5.6 g) and the mixture was stirred at room temperature for 10 h. After the usual work-up, the product was obtained as an oil. Yield 11.0 g (99.8%) Rf^1 0.77.

4-Methoxy-2,3,6-trimethylbenzenesulfonyl Chloride (Mtr-Cl)——Chlorosulfonic acid (24 ml) in CH_2Cl_2 (400 ml) was added to a solution of 2,3,5-trimethylanisole (18.0 g) in CH_2Cl_2 (600 ml) in the same manner as described for Pme-Cl. After the usual work-up, the material was obtained as crystals from *n*-hexane. Yield 21.6 g (72.5%) mp 56—58°C, Rf^1 0.50. *Anal.* Calcd for $C_{10}H_{13}ClO_3S$: C, 48.29; H, 5.27; Cl, 14.26; S, 12.89. Found: C, 48.42; H, 5.21; Cl, 14.25; S, 12.61.

Z-Arg(Mtr)-OH·CHA——Z–Arg–OH (2.83 g) was dissolved in 4 N NaOH–acetone (10 ml—40 ml) and the solution was cooled with ice. To this was added Mtr–Cl (4.0 g) in acetone (10 ml). The whole was stirred for 3 h and worked up as usual to afford an oily material. This product was dissolved in AcOEt (30 ml) together with cyclohexylamine (1.04 ml). The crystals that formed were collected and recrystallized from MeOH–AcOEt. Yield 4.10 g (72.1%). mp 195—197°C, $[\alpha]_D^{23}$ +6.5° (c=1.2 in MeOH), Rf^2 0.38. *Anal.* Calcd for $C_{30}H_{45}N_5O_7S$: C, 58.14; H, 7.32; N, 11.30; S, 5.17. Found: C, 58.08; H, 7.34; N, 11.58; S, 5.32.

H-Arg(Mtr)-OH——A suspension of Z–Arg(Mtr)–OH·CHA (1.50 g) in AcOEt (30 ml) was washed with 0.2 N H_2SO_4 (15 ml), and concentrated. The residue was hydrogenated in MeOH and the material obtained was crystallized from water. Yield 0.77 g (81.1%). mp 100—103°C, $[\alpha]_D^{23}$ −4.8° (c=1.3 in MeOH), Rf^3 0.14. *Anal.* Calcd for $C_{16}H_{26}N_4O_5S\cdot 1/2H_2O$: C, 48.59; H, 6.88; N, 14.18; S, 8.11. Found: C, 48.78; H, 7.16; N, 13.88; S, 8.29.

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References and Notes

1) Amino acids peptides and their derivatives in this paper are of the L-configuration. The following abbreviations are used: Z=benzyloxycarbonyl, Boc=*tert*-butoxycarbonyl, OBzl=benzyl ester, Mbs=*p*-methoxybenzenesulfonyl, Mts=mesitylene-2-sulfonyl, Mds=4-methoxy-2,6-dimethylbenzenesulfonyl,

iMds=2-methoxy-4,6-dimethylbenzenesulfonyl, Mtb=2,4,6-trimethoxybenzenesulfonyl, Pme=2,3,4,5,6-pentamethylbenzenesulfonyl, Mte=4-methoxy-2,3,5,6-tetramethylbenzenesulfonyl, Mtr=4-methoxy-2,3,-6-trimethylbenzenesulfonyl, TEA=triethylamine, CHA=cyclohexylamine, TFA=trifluoroacetic acid, HF=hydrogen fluoride, MSA=methanesulfonic acid, TFMSA=trifluoromethanesulfonic acid, DCC=N,N′-dicyclohexylcarbodiimide, DCU=N,N′-dicyclohexylurea, HONB=N-hydroxy-5-norbornene-2,3-dicarboximide, THF=tetrahydrofuran, DMF=dimethylformamide, DMSO=dimethylsulfoxide.

2) O. Nishimura and M. Fujino, *Chem. Pharm. Bull.*, **24**, 1568 (1976).
3) H. Yajima, M. Takeyama, J. Kanaki, and K. Mitani, *Chem. Commun.*, **1978**, 482.
4) H. Yajima, Y. Kiso, H. Ogawa, N. Fujii, and H. Irie, *Chem. Pharm. Bull.*, **23**, 1164 (1975).
5) M. Takeyama, K. Koyama, K. Inoue, T. Kawano, H. Adachi, T. Tobe, and H. Yajima, *Chem. Pharm. Bull.*, **28**, 1873 (1980).
6) M. Fujino, O. Nishimura, M. Wakimasu, and C. Kitada, *Chem. Commun.*, **1980**, 668.
7) Kiso *et al.* reported the deprotection of Arg(Mts) by treatment with TFA-thioanisole at 25°C for 3 days. [Y. Kiso, K. Ukawa, M. Satomi, S. Nakamura, K. Kitagawa, and T. Akita, "Peptide Chemistry 1979," ed. by H. Yonehara, Protein Research Foundation, Osaka, Japan, p. 193; Y. Kiso, M. Satomi, K. Ukawa, and T. Akita, *Chem. Commun.*, **1980**, 1063].
8) S. Sakakibara and Y. Shimonishi, *Bull. Chem. Soc. Jpn*, **38**, 1412 (1965).
9) M. Takeyama, K. Koyama, H. Yajima, M. Moriga, M. Aono, and M. Murakami, *Chem. Pharm. Bull.*, **28**, 2265 (1980).
10) Column: Nucleosil $5C_{18}$ (0.4×20 cm); solvent, CH_3CN–0.1 M $AcONH_4$ (1:9); flow rate, 0.5 ml/min; elution times, H–Phe–Asp–Asn–Ala–OH=5.2 min, H–Phe–Asc–Asn–Ala–OH=13.2 min.
11) M. Fujino, M. Wakimasu, S. Shinagawa, C. Kitada, and H. Yajima, *Chem. Pharm. Bull.*, **26**, 539 (1978).
12) Y. Kiso, K. Ukawa, and T. Akita, *Chem. Commun.*, **1980**, 101; Y. Kiso, K. Ukawa, S. Nakamura, K. Ito, and T. Akita, *Chem. Pharm. Bull.*, **26**, 673 (1980).
13) M. Wakimasu, C. Kitada, and M. Fujino, *Chem. Pharm. Bull.*, **29**, 2592 (1981).
14) C.M. Pareos, F.S. Varveri, and G.A. Gregoriou, *J. Org. Chem.*, **39**, 3594 (1974).