Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron
Tetrahedron 60 (2004) 1875–1886

Solid-phase peptide synthesis in water. Part 3: A water-soluble N-protecting group, 2-[phenyl(methyl)sulfonio]ethoxycarbonyl tetrafluoroborate, and its application to solid phase peptide synthesis in water☆

Keiko Hojo, Mitsuko Maeda and Koichi Kawasaki*

Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Ikawadani-cho, Nishi-ku, Kobe 651-2180, Japan

Received 26 September 2003; accepted 11 December 2003

Abstract—Chemical synthesis of peptides has been performed in various organic solvents, but the safe disposal of organic solvents is now an important environmental issue. Our aim is to be able to perform solid-phase peptide synthesis in water. For this, we have designed a new water-soluble N-protecting group, 2-[phenyl(methyl)sulfonio]ethoxycarbonyl (Pms), and have studied its introduction onto amino acids. Pms-amino acids were prepared by treating 2-(phenylthio)ethoxycarbonyl amino acids with methyl iodide in the presence of silver tetrafluoroborate. Because sulfur-containing amino acids, such as Met and Cys, were modified by the reaction, we designed a new reagent, 2-[phenyl(methyl)sulfonio]ethyl-4-nitrophenyl carbonate, to introduce the Pms group on amino acids. This reagent is a stable crystalline material and its introduction onto amino acids (including sulfur-containing amino acids) was successful. The solid-phase synthesis of Leu- and Met-enkephalin amides using Pms-protected amino acids was successfully achieved in water.

1. Introduction

Chemical synthesis of peptides has been performed in various organic solvents by both solid-phase and solution methods. Advances in HPLC accelerated the development of the solid-phase method, which in turn accelerated the development of combinatorial chemistry. The solid-phase method is now the principal method for peptide synthesis, but it requires a large amount of organic solvent. Because the safe disposal of organic solvent waste is an important environmental issue, a method for peptide synthesis in water using low toxic reagents would be desirable.

To perform the coupling reaction in water, protected amino acids that are soluble in water are needed. Various polar N-protecting groups that enhance solubility in polar solvents including water have been reported, including a methylsulfonylethoxycarbonyl group by Tesser and Balvert-Geers,[2] a 2-(triphenylphosphonio)ethoxycarbonyl group by Kunz,[3] and a 9-(2-sulfo)fluorenylmethoxycarbonyl group by Merrifield and Bach.[4] These protecting groups are removable under basic conditions by a β-elimination mechanism. Kunz also reported the use of 2-(methylthio)ethoxycarbonyl[5] and 2-(4-pyridyl)ethoxycarbonyl[6] as a two-step-protecting group (Zweistufen–Schutzgruppe), which is removable under mild basic conditions after its methylation or oxidation. In 1978, Kunz[7] reported preparation of a tripeptide, 2-[diphenyl(methyl)phosphonio]ethoxycarbonyl-Leu-Phe-Phe-O*t*Bu, by the solution method in water.

In previous papers, we reported the preparation of water-soluble active esters (4-trimethylammoniophenyl ester and sulfophenyl ester) and their application to peptide synthesis by the solution method.[8] Here, with the aim of achieving solid phase peptide synthesis in water, we have designed a new water-soluble N-protecting group. We designed the 2-[phenyl(methyl)sulfonio]ethoxycarbonyl tetrafluoroborate (Pms) group (Fig. 1) as a water-soluble and easily removable N-protecting group. Introduction of the Pms group onto amino acids with various reagents was studied, and Leu- and Met-enkephalin amides were synthesized by the solid-phase method in water to evaluate the utility of Pms-amino acids.

☆ See Ref. 1.

Keywords: 2-[Phenyl(methyl)sulfonio]ethoxycarbonyl group; Synthesis in water; Water-soluble protecting group; Peptide synthesis; Solid phase synthesis.

* Corresponding author. Tel.: +81-78-974-4794; fax: +81-78-974-5689; e-mail address: kawasaki@pharm.kobegakuin.ac.jp

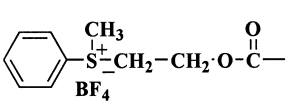


Figure 1. Structure of Pms group.

0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2003.12.036

2. Results and discussion

Pms-amino acids were prepared according to Route 1, as shown in Scheme 1. 2-(Phenylthio)ethyl chloroformate **2** was prepared from 2-(phenylthio)ethanol **1** and phosgene (prepared from triphosgen[9]) in dichloromethane. Compound **2** was reacted with an amino acid to give 2-(phenylthio)ethoxycarbonyl (Pte) amino acid **3**, which was extracted by ethyl acetate from the reaction mixture and showed a single spot on TLC. Compound **3** was used without further purification and was treated with methyl iodide and silver tetrafluoroborate to give Pms-amino acid **4**. Pms-amino acids **4a–j** (Table 1) were synthesized by Route 1. These Pms-amino acids were obtained as amorphous materials and identified by time-of-flight mass spectrometry (Tof-MS) and NMR spectra (see Section 4). In the ^{1}H NMR spectra of Pms-amino acid **4**, the methyl proton adjacent to the sulfonium moiety showed a clear singlet at δ 3.30. The signals due to the methylene region of the Pms group appeared as highly complex multiplets at δ 4.00–4.40, which is much lower than this region in **3**.

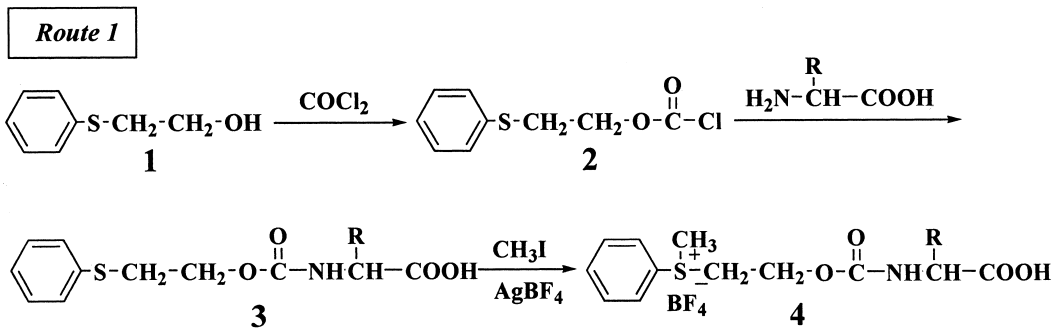
As expected, elimination of the Pms group from Pms-amino acids proceeded according to a base-promoted β-elimination mechanism. The electron-withdrawing sulfonium region of the Pms group renders hydrogens on the ethylene portion labile, and thus the hydrogens are susceptible to removal with weak bases to form the 2-[phenyl(methyl)-sulfonio]ethylene **5**. The mechanism of Pms elimination from a Pms-amino acid was studied by treating Pms-Phe-OH **4a** with a 5% aqueous solution of $NaHCO_3$ for 30 min. The reaction mixture was checked by HPLC and Tof-MS (Fig. 2). New peaks corresponding to 2-[phenyl(methyl)-sulfonio]ethylene **5** and its water adduct, 2-[phenyl (methyl)sulfonio]ethanol **6**, were detected by Tof-MS and HPLC, which verified that the Pms group was removed through a β-elimination mechanism.

Route 1 is an easy and efficient method by which to prepare Pms-amino acids; however, Pms sulfur-containing amino acids (Met and Cys) cannot be prepared, because the sulfurs of Met and Cys are converted to the onium salt by treatment with methyl iodide. In addition, it is not possible to prepare Pms-amino acids with an acid-labile group [such as *t*-butyl (*t*Bu) group, trityl (Trt) group and 2,2,5,7,8-pentamethyl-chroman-6-sulfonyl (Pmc) group] at their side chain could not be prepared by Route 1, because the acid labile group was cleaved by methylation procedure with methyl iodide and silver tetrafluoroborate. To overcome these limitations, we designed preparation Routes 2–5 using various *N*-Pms acylating agents, 2-[phenyl(methyl)sulfonio]ethyl chloroformate (Pms-Cl) **7**, 2-[phenyl(methyl)sulfonio]-ethoxycarbonylsuccinimide (Pms-OSu) **9**, 2-[phenyl(methyl) sulfonio]ethoxycarbonyl-5-norbornene-*endo*-2,3-dicarboximide (Pms-ONB) **11**, and 2-[phenyl(methyl)sulfonio]ethyl-4-nitrophenyl carbonate (Pms-ONp) **13**.[1b]

Pms-Cl **7** was prepared from phosgene[9] and the onium salt alcohol 2-[phenyl(methyl)sulfonio]ethanol **6**, which was converted from **1** by treatment with methyl iodide and silver tetrafluoroborate. The resulting Pms-Cl **7** was unstable even at low temperature, so it was used without purification to prepare Pms-amino acid (Route 2). Introduction of the Pms group onto Phe by using **7** at −10 °C in aqueous acetonitrile resulted in Pms-Phe-OH **4a** in low yield (27%) (Table 2). HPLC analysis of the reaction mixture showed major contaminant peaks of 2-[phenyl(methyl)sulfonio]ethanol **6** and its dehydrated product **5** (both might be derived from decomposition of **7**). These results indicate that **7** is so unstable that it decomposes readily before reaction in aqueous media such as aqueous acetonitrile. Thus, efficient Pms acylating agents with a more stable leaving group than chloride were required.

Because *N*-hydroxysuccinimide (HOSu) ester[10] is hydrophilic, has good reactivity and might be more stable than chloride, the preparation of Pms-OSu **9** was designed in two different ways, as shown in Scheme 2. In method A, the preparation of **9** from **7** and HOSu was attempted, but **9** could not be obtained because of the instability of **7**. In method B, **9** was prepared via Pte-OSu **8**, which was easily purified by column chromatography. Next, **8** was treated with methyl iodide and silver tetrafluoroborate to give **9**. Although crude **9** was obtained in over 90% purity, further purification was difficult because of its instability. The **9** was used without purification to react with Phe in aqueous acetonitrile to give Pms-Phe-OH **4a** in 68? yield. (Route 3).

Next, the *N*-hydroxy-5-norbornene-endo-2,3-dicarboximide (HONB)[11] carbonate (Pms-ONB) **11** (Fig. 3) was examined as an acylating agent (Route 4 in Scheme 3). Compound **11** was prepared via Pte-ONB **10** in the same way as the preparation of **9**. Compound **11** was obtained in over 90% purity but, like **9**, it could not be purified further without its decomposition. The crude **11** was reacted with Phe (Route 4) in aqueous acetonitrile to give Pms-Phe-OH **4a** in 66%



Scheme 1. Preparation of Pms-amino acid (Route 1).

Table 1. Analytical data for Pms amino acids

	Amino acid	MW.	Tof-Ms (*m/z*)	$[\alpha]_D^{24a}$	HPLC[b] room temperature (min)	Yield (%)
4a	Phe	360.45	360.4	−9.8	23.4	81[c]
4b	Gly	270.33	270.2	—	14.3[d]	73[c]
4c	Ala	284.35	284.4	−17.5	17.8[d]	63[c]
4d	Val	312.41	312.4	+5.7	16.7	73[c]
4e	Ile	326.43	326.5	+2.1	19.2	68[c]
4f	Leu	326.43	326.5	−9.8	20.4	72[c]
4g	Ser	300.35	300.3	+3.0	18.9[d]	56[c]
4h	Asp	328.36	328.6	+11.4	17.6	46[c]
4i	Pro	310.39	310.1	−19.2	20.1	69[c]
4j	Tyr	376.45	376.4	−2.3	12.2[d]	86[c]
14a	Met	344.47	344.3	−33.5	21.2	65[e]
14b	Cys(Acm)	387.50	387.6	−20.1	18.7	47[e]
14c	Cys(Trt)	558.73	558.9	+7.3	32.7[f]	68[e]
15a	Tyr(*t*Bu)	432.55	432.9	−3.8	24.7[f]	69,[e] 65[g]
15b	Ser(*t*Bu)	356.46	356.9	+2.1	22.8	61,[c] 86[g]
15c	Asp(O*t*Bu)	384.47	384.4	+12	24.6	49,[e] 50[g]
15d	Glu(O*t*Bu)	398.49	398.16	−5.8	28.1	62[e]
15e	Thr(*t*Bu)	370.48	370.3	+4.9	25.9	74[e]
15f	Asn(Trt)	569.69	570.0	−13.2	30.1[f]	69,[e] 61[g]
15g	Gln(Trt)	583.72	583.5	−9.2	30.7[f]	55,[e] 59[g]
15h	His(Trt)	592.23	592.3	−3.6	25.5[f]	72,[e] 42[g]
15i	Trp(Boc)	499.60	499.7	−4.3	36.5[f]	59,[e] 62[g]
15j	Arg(Pmc)	635.82	635.8	−7.3	40.9[f]	63,[e] 41[g]
15k	Lys(Boc)	441.53	442.3	−3.8	28.3	57,[e] 43[g]

[a] c=1.0, CH_3CN.
[b] Column, DAISOPAK SP-120-5-ODS-B (2.5×250 mm). Flow rate, 1 ml/min. Eluent, CH_3CN/H_2O containing 0.05% TFA. Gradient: 10/90–50/50 (40 min).
[c] Route 1.
[d] Gradient: 1/99–50/50 (49 min).
[e] Route 5.
[f] Gradient: 10/90–70/30 (30 min).
[g] Route 4.

Table 2. Yields and reaction times of Routes 1–5 for preparation of Pms-Phe-OH

	Route 1	Route 2, Pms-Cl	Route 3, Pms-Osu	Route 4, Pms-ONB	Route 5, Pms-Onp
Yield (%)	81	27	68	66	88
Reaction time	—	<5 min	4 h	4 h	>1 day

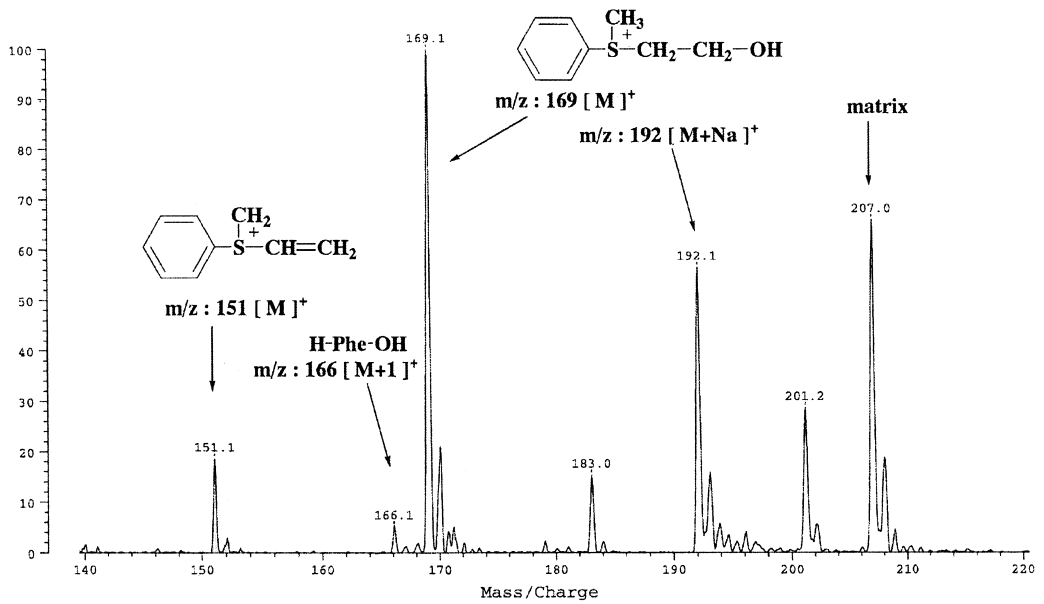
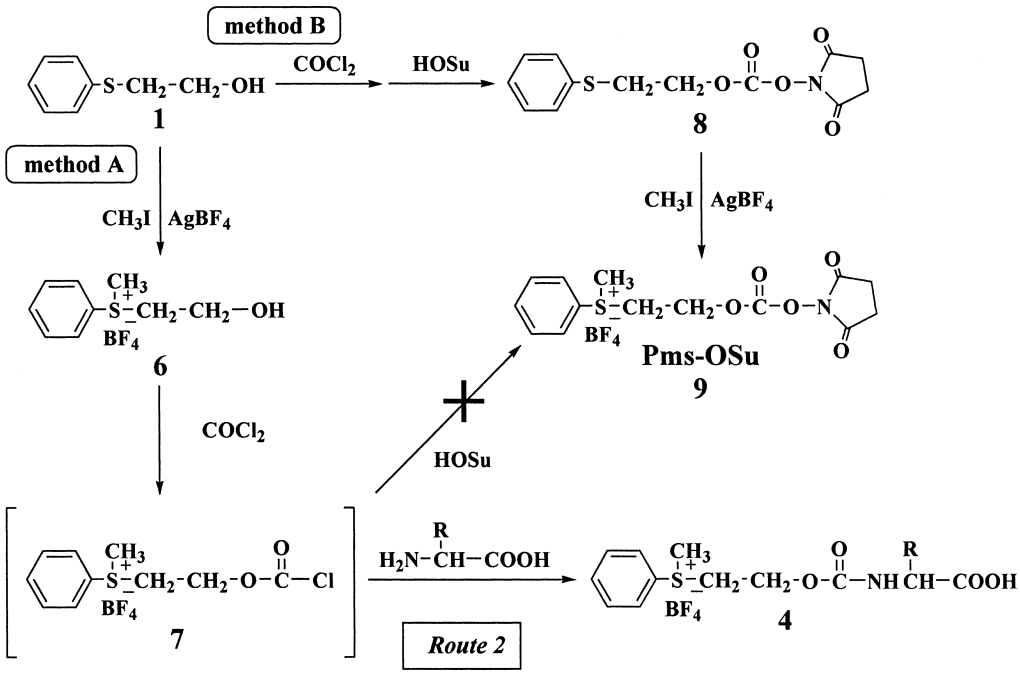


Figure 2. Tof-MS spectrum of the deprotection mixture of Pms-Phe-OH.



Scheme 2. Synthesis of Pms-OSu.

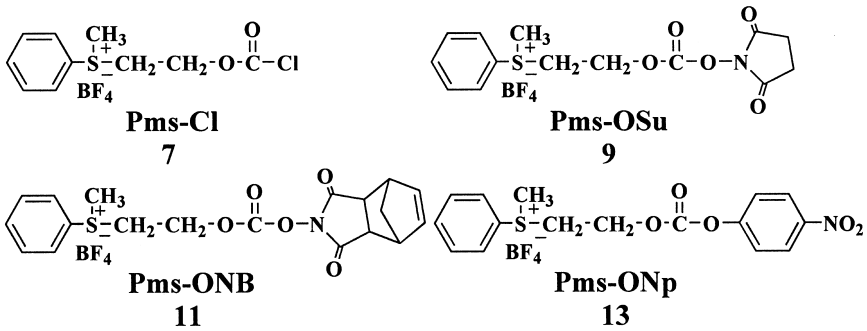
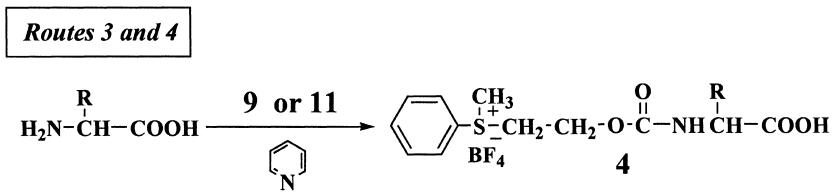


Figure 3. Reagents for preparing Pms-amino acids.

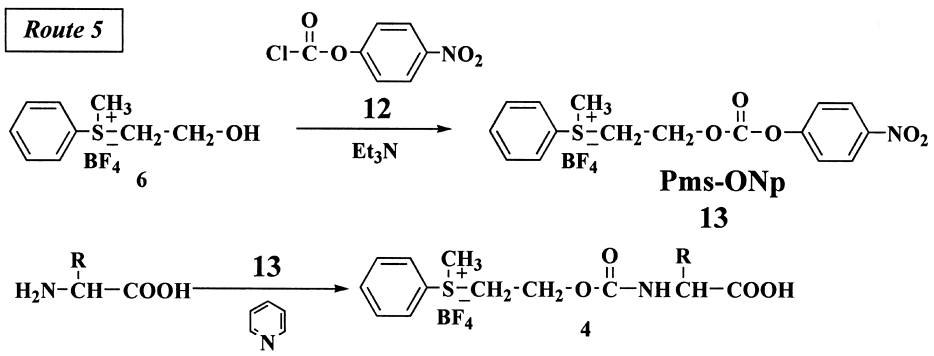


Scheme 3. Preparation of Pms-amino acid (Routes 3 and 4). Route 3: **9**, Pms-OSu. Route 4: **11**, Pms-ONB.

yield. Compound **11** has a high reactivity, similar to that of **9**.

Looking for a more stable acylating agent, we examined the Pms-4-nitrophenyl carbonate (Pms-ONp) **13** (Route 5 in Scheme 4). Compound **6** was reacted with 4-nitrophenyl chloroformate **12** at room temperature in acetonitrile to give **13** in 71% yield. Compound **13** was obtained as colorless crystal and could be stored for a few months in a refrigerator. Acylation of Phe with **13** in the presence of pyridine in aqueous acetonitrile (Route 5) was slow as compared to that with **9** and **11**. The acylation with **13** took more than 1 day at room temperature, while the acylation with **9** and **11** took 4 h at room temperature. However, the yield of **4a** was 88% by acylation with **13**, which was better than that obtained with either **9** (68%) or **11** (66%). Acylation of Phe with **7**, **9**, **11** and **13** (Routes 1–5) is summarized in Table 2. The three acylating agents **9**, **11** and **13**, but not **7**, gave a satisfactory coupling yield.

The procedure for introducing the Pms group onto sulfur-containing amino acids, such as Met, Cys(Trt) and



Scheme 4. Preparation of Pms-amino acid (Route 5).

Cys(Acm), was examined through Route 5. Pms-Met-OH **14a** was prepared using **13** in a mixture of aqueous 0.1% Triton X-100 solution and acetonitrile (1/1) in the presence of pyridine with a yield of 65% (Table 1). Pms-Cys(Acm)-OH **14b** and Pms-Cys(Trt)-OH **14c** were also prepared by the same procedure. The yields of **14b** and **14c** were 47 and 68%, respectively (Table 1). Whereas **14a** and **14b** were readily soluble, **14c** was sparingly soluble in water. Compound **14c** was soluble in aqueous organic solvents, such as 50% acetonitrile and 50% dimethylformamide (DMF), and also soluble in aqueous 5% Triton X-100 solution. Thus the hydrophobicity of the trityl group is greater than the hydrophilicity of the Pms group in **14c**.

Pms-amino acids with an acid-labile group [such as *t*-butyl (*t*Bu) group, trityl (Trt) group and 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Pmc) group] at their side chain could not be prepared by Route 1, **11** and **13** were used to prepare these Pms-amino acids (Schemes 3 and 4) and results were passable as shown in Table 1.

To evaluate Pms-amino acids, Leu-enkephalin amide (H-Tyr-Gly-Gly-Phe-Leu-NH_2) was synthesized by the solid-phase method in water. Before the synthesis, removal of the Pms group by various base treatments was examined using Pms-Phe-Leu-TentaGel resin. Because removal of the Pms group on Phe-Leu-TentaGel resin with base was slower than that on Phe itself, the Pms-Phe-Leu-TentaGel resin was preferentially used as the test compound. Hydrophobic or bulky sequence like Phe-Leu may have influence on removal of the Pms group in water. The resin was treated with various base solutions to remove the Pms group in water and then reacted with 9-fluorenylmethoxycarbonyl glycine (Fmoc-Gly-OH) with diisopropylcarbodiimide/1-hydroxybenzotriazole[12] in DMF until the resin gave a negative result in the Kaiser test[13] (ninhydrin test). The resulting Fmoc-Gly-Phe-Leu-TentaGel resin was treated with 20% piperidine/DMF to remove the Fmoc group. The resin was hydrolyzed, and the amino acids in the acid hydrolysate were analyzed. Removal of the Pms group from the Pms-Phe-Leu-TentaGel resin was calculated from the amino acid ratio of Gly and Phe in the acid hydrolysate. The Pms group was removable with mild bases, such as an aqueous solution of either 5% $NaHCO_3$ or 5% Na_2CO_3, as shown in Table 3. Treatment with an aqueous solution of 5% $NaHCO_3$ for 30 min was not strong enough to remove the Pms group completely, but a double treatment (30 min×2) gave a satisfactory result.

An important factor to consider in solid-phase synthesis is the swelling ability of the resin. To perform solid-phase synthesis in water, a resin is required to swell in water. However, the most common core resin, polystyrene resin, does not swell in polar solvents such as methanol and water. A poly(ethylene glycol)-grafted polystyrene resin, TentaGel resin,[14] has been reported as a resin designed to have increased swelling ability in both polar and non-polar solvents. This TentaGel resin was used for synthesis of Leu-enkephalin amide using a water-soluble carbodiimide [WSCD, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride[15]] as a coupling reagent in water. Because the introduction of Phe onto the resin and the subsequent coupling reaction for peptide synthesis was slow under the above conditions, an aqueous solution of 0.2% Triton X-100 was used as a solvent to increase the swelling ability of the resin and to increase the solubility of reactants. Furthermore, HOSu, which is soluble in water, was used as an additive to accelerate the coupling reactions.[16] Although the coupling reactions were accelerated under this condition, β-Ala was found in the acid hydrolysate of the synthetic Leu-enkephalin amide. Pure synthetic Leu-enkephalin amide could not be separated from by-products which contained β-Ala by HPLC. The amino acid ratios in the acid hydrolysate

Table 3. Deprotection studies on the Pms-Phe-Leu-TentaGel resin

Entry	Reagents	Time (min)	Yield (%)
1	5% $NaHCO_3/H_2O$	30	80
2	5% $NaHCO_3/H_2O$	30	96
3	0.01 mol/l $NaOH/H_2O$	1, 1, 1	100
4	0.01 mol/l $NaOH/H_2O$	3, 3, 3	100
5	0.005 mol/l $NaOH/EtOH-H_2O$ (1/1)	3, 3, 3	86
6	0.001 mol/l $NaOH/EtOH-H_2O$ (1/1)	3, 3, 3	77
7	2.5% $NaHCO_3/EtOH-H_2O$ (1/1)	3, 3, 3	81
8	2.5% $NaHCO_3/EtOH-H_2O$ (1/1)	5, 5, 5	100
9	2.5% $Na_2CO_3/EtOH-H_2O$ (1/1)	3, 3, 3	75
10	2.5% $Na_2CO_3/EtOH-H_2O$ (1/1)	5, 5, 5	100

Table 4. Synthetic protocol for the solid phase peptide synthesis in water

Step	Reagents	Time
1	H_2O	3 min×2
2	Aq. 5.0% $NaHCO_3$ or aq. 0.01 mol/l NaOH	30 min×2 or 3 min×2
3	H_2O	3 min×2
4	Aq. 0.2% Triton X	3 min×3
5	Pms-amino acid, WSCD, HONB, in aq. 0.2% Triton X	3 h
6	Aq. 0.2% Triton X	3 min×3

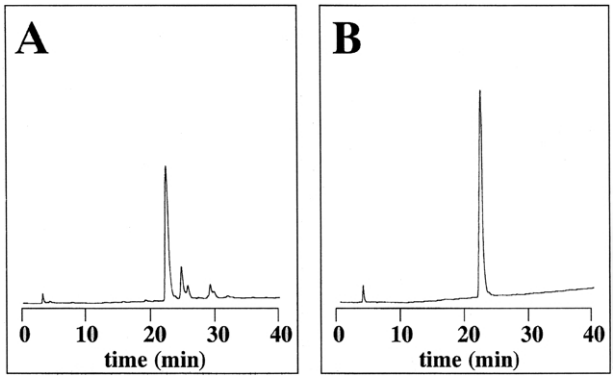


Figure 4. HPLC profiles of synthetic Leu-enkephalin amide. (A) Preparative HPLC Column, DAISOPAK SP-120-5-ODS-B (20×250 mm). Flow rate, 10 ml/min. Eluent, CH_3CN/H_2O containing 0.05% TFA. Gradient: 10/90–50/50 (40 min). (B) Analytical HPLC of the purified sample. Column, DAISOPAK SP-120-5-ODS-B (2.5×250 mm). Flow rate, 1 ml/min. Eluent, CH_3CN/H_2O containing 0.05% TFA. Gradient: 10/90–50/50 (40 min).

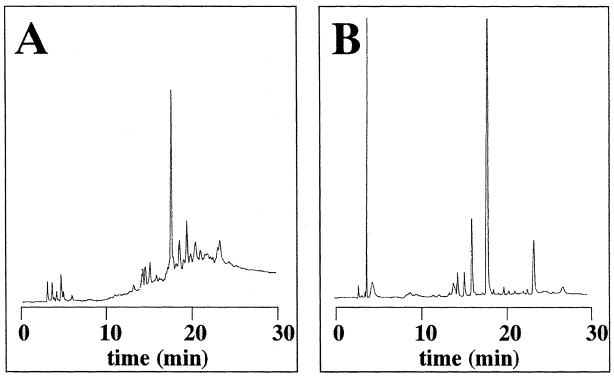


Figure 5. HPLC profile of synthetic crude Met-enkephalin amide. (A) Crude Met-enkephalin amide prepared on CLEAR resin. (B) Crude Met-enkephalin amide prepared on TentaGel resin. Column, DAISOPAK SP-120-5-ODS-B (2.5×250 mm). Flow rate, 1 ml/min. Eluent, CH_3CN/H_2O containing 0.05% TFA. Gradient: 10/90–50/50 (20 min).

of synthetic crude Leu-enkephalin separated by HPLC was; Tyr 0.82, Gly 1.92, Phe 1.06, β-Ala 4.69. Formation of β-Ala has been reported as a product of a side reaction (Lossen rearrangement reaction[17]) when HOSu is used as an additive. We did not examine this side reaction (Lossen rearrangement reaction) further, but it might occur more easily in aqueous media than in non-polar solvents.

Next, HONB,[11] which is also water-soluble, was used as an additive instead of HOSu and Leu-enkephalin was synthesized according to the protocol shown in Table 4. The Pms group was removed by treatment with an aqueous solution of 5% $NaHCO_3$ for 30 min, or by two lots of this treatment for Phe, because removal of the Pms group on Phe was slower than that on other Pms-amino acids, as described above. The synthetic Pms-Tyr-Gly-Gly-Phe-Leu-TentaGel resin was treated with an aqueous solution of 5% $NaHCO_3$ and then treated with trifluoroacetic acid (TFA) to cleave the peptide from the resin. The product was purified by HPLC on an ODS column. The HPLC profile of the synthetic Leu-enkephalin amide is shown in Figure 4. The retention time of the synthetic Leu-enkephalin amide was identical to that of Leu-enkephalin amide prepared by the Fmoc-based solid-phase method using organic solvents. The total yield calculated from the amino group content of the starting TentaGel resin was 61%.

In addition to the TentaGel resin, a cross-linked ethoxylate acrylate resin (CLEAR resin which has been also reported[18] to swell not only with organic solvents but also with water) was examined as a solid support for synthesis in water. Met-enkephalin amide was synthesized using CLEAR resin in water according to the same procedure (shown in Table 4) except for the base treatment. The Pms group was removed by two 3-min treatments with an aqueous solution of 0.01 mol/l NaOH, which yielded a more rapid and complete removal of the Pms group. The synthetic H-Tyr-Gly-Gly-Phe-Met-CLEAR-resin was treated with TFA to cleave the peptide from the resin, and then purified by HPLC on an ODS column. The crude HPLC profile of the synthetic Met-enkephalin amide is shown in Figure 5A. The yield calculated from amino group content of the starting CLEAR resin was 29%. Minor peaks were observed before and after the main peak of Met-enkephalin amide, which corresponded to that of Met-enkephalin amide prepared by the Fmoc-based solid-phase method using organic solvents. The early peaks contained deletion peptides (such as H-Tyr-Gly-Phe-Met-NH_2 and H-Gly-Gly-Phe-Met-NH_2) and oxidized peptide (such as H-Tyr-Gly-Gly-Phe-Met(O)-NH_2 and H-Tyr-Gly-Phe-Met(O)-NH_2), whereas the later peaks contained non-peptide compounds that probably derived from the used resin.

For comparison, Met-enkephalin amide was also synthesized in water using TentaGel resin, and the HPLC profile of the crude product is shown in Figure 5B. Again, minor peaks of deletion peptides (such as H-Tyr-Gly-Phe-Met-NH_2 and H-Gly-Gly-Phe-Met-NH_2) and oxidized peptides (such as H-Tyr-Gly-Gly-Phe-Met(O)-NH_2 and H-Tyr-Gly-Phe-Met(O)-NH_2) were observed before and after the main peak of Met-enkephalin amide. The yield of Met-enkephalin amide prepared on TentaGel resin was 32% and was just a little better than that of Met-enkephalin amide prepared on CLEAR resin (29%).

The yield of Leu-enkephalin and Met-enkephalin using TentaGel resin was 61 and 32%, respectively. Synthetic protocol for preparation of these 2 enkephalins was different (deprotection with 5% $NaHCO_3$ and 0.01 mol/l NaOH), but this difference might be mainly derived from properties of Leu and Met. The synthesis of both Met and Leu-enkephalin amide was performed under atmosphere (i.e. not under inert gas). Oxidation of Met during synthesis was observed in the profile of HPLC and might cause the lower yield.

3. Conclusion

Pms, a new water-soluble N-protecting group with high base lability and high polarity, has been developed and its application to solid-phase peptide synthesis in water has been evaluated. The derivative Pms-ONp was designed to prepare all types of Pms-amino acids including sulfur-containing amino acids. This reagent is a crystalline compound and can be kept stable in a refrigerator for a few months. Leu- and Met-enkephalin amides were successfully synthesized by the solid-phase method in

water using Pms-amino acids and, as such, this study may be the first to report the successful synthesis of peptides by the solid-phase method in water. In addition, we evaluated the potential of CLEAR resin and TentaGel resin for solid-phase synthesis in water. Future work should aim to develop a new resin that swells more than these two resins.

4. Experimental

Optical rotations were determined with an automatic polarimeter, model DIP-360 (Japan Spectroscopic Co.) Tof-Mass spectra were measured with a KRATOS-MALDI mass spectrometer (SiMADZU Co.) and ESI-Mass spectra were measured with a Waters ZQ2000 mass spectrometer. Reversed phase HPLC was performed using a Waters model 600 equipment with a DISOPAK column and gradient system of acetonitrile/water containing 0.05% TFA. Open column chromatography was performed on Silica gel 60 (BW-127ZH, Fuji Silicia Chemical Co.). Solvent system for ascending thin-layer chromatography on Silica gel G (type 60, Merck) is indicated as follows: R_f^1=$CHCl_3$–MeOH–H_2O (8/3/1, lower phase). ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrometer. 2-(phenylthio)ethanol and 4-nitorphenyl chloroformate were purchased from Tokyo Kasei Co., Ltd, Japan. HOSu, HONB and amino acid derivatives were purchased from Watanabe Chemical Industries, Ltd, Japan.

4.1. Route 1. Preparation of Pte-amino acids

4.1.1. Pte-Phe-OH (3a). To a solution of 2-(phenylthio)ethanol (4.4 ml, 30 mmol) and triphosgen (2.96 g, 10 mmol) in tetrahydrofuran (100 ml), Et_3N (4.18 ml, 30 mmol) in tetrahydrofuran (40 ml) was added dropwise at room temperature and the reaction mixture was stirred for 1.5 h. The mixture was filtered and the solvent was evaporated. The residue was dissolved in acetonitrile (30 ml) and added to a solution of Phe (4.95 g, 30 mmol) and Et_3N (4.18 ml, 30 mmol) in a mixture of acetonitrile and H_2O (1/1, 100 ml) at 0 °C. The mixture was stirred for 2 h at room temperature keeping its pH at 8 by addition of Et_3N and the solvent was removed in vacuo. The residue was dissolved in AcOEt and extracted with aqueous 5% $NaHCO_3$. The aqueous layer was acidified with 1.0 mol/l HCl, and the resulting precipitate was extracted with AcOEt. The extract was washed with saturated NaCl solution and concentrated in vacuo to leave an oily material. Yield 890 mg, 86%. ^{1}H NMR (400 MHz, CD_3CN) δ 7.41 (d, J=7.4 Hz, 2H), 7.29 (t, J=7.4 Hz, 2H), 7.25 (m, 5H), 7.21 (t, J=7.4 Hz, 1H), 4.43 (dd, J=5, 14 Hz, 1H), 4.18 (t, J=6.9 Hz, 2H) 3.18 (t, J=6.9 Hz, 3H), 3.05 (m, 1H), 2.92 (m, 1H). R_f^1 0.60.

The following Pte-amino acids were prepared according to the procedure described above.

4.1.2. Pte-Gly-OH (3b). Colorless solid. Yield 78%. ^{1}H NMR (400 MHz, D_2O) δ 7.39 (d, J=7.4 Hz, 2H), 7.29 (t, J=7.4 Hz, 2H), 7.20 (t, J=7.4 Hz, 1H), 4.17 (t, J=6.9 Hz, 2H), 3.76 (s, 2H), 3.16 (t, J=6.9 Hz, 3H). R_f^1 0.20.

4.1.3. Pte-Ala-OH (3c). Oily material. Yield 70% ^{1}H NMR (400 MHz, CD_3CN) δ 7.40 (d, J=7.4 Hz, 2H), 7.29 (t, J=7.4 Hz, 2H), 7.20 (t, J=7.4 Hz, 1H), 4.30 (s, 1H), 4.17 (t, J=6.9 Hz, 2H), 3.16 (t, J=6.9 Hz, 2H), 1.37 (d, J=7.2 Hz, 3H) R_f^1 0.28.

4.1.4. Pte-Val-OH (3d). Oily material. Yield 82%. ^{1}H NMR (400 MHz, CD_3CN) δ 7.39 (d, J=7.4 Hz, 2H), 7.29 (t, J=7.4 Hz, 2H), 7.19 (t, J=7.4 Hz, 1H), 4.19 (t, J=6.9 Hz, 2H), 4.06 (s, 1H), 3.16 (t, J=6.9 Hz, 3H), 2.16 (m, 1H), 0.97 (d, J=6.8 Hz, 3H), 0.94 (d, J=6.8 Hz, 3H). R_f^1 0.51.

4.1.5. Pte-Ile-OH (3e). Oily material. Yield 76%. ^{1}H NMR (400 MHz, CD_3CN) δ 7.39 (d, J=7.4 Hz, 2H), 7.29 (t, J=7.4 Hz, 2H), 7.19 (t, J=7.4 Hz, 1H), 4.18 (t, J=6.9 Hz, 2H), 4.08 (s, 1H), 3.16 (t, J=6.9 Hz, 2H), 1.87 (m, 1H), 1.52 (m, 1H), 1.25 (m, 1H), 0.95 (d, J=7.0 Hz, 3H), 0.92 (t, J=7.0 Hz, 3H). R_f^1 0.57.

4.1.6. Pte-Leu-OH (3f). Oily material. Yield 80%. ^{1}H NMR (400 MHz, CD_3CN) δ 7.40 (d, J=7.4 Hz, 2H), 7.29 (t, J=7.4 Hz, 2H), 7.19 (t, J=7.4 Hz, 1H), 4.17 (t, J=6.9 Hz, 2H), 4.16 (s, 1H), 3.16 (t, J=6.9 Hz, 2H), 1.72 (m, 1H), 1.59 (m, 1H), 0.95 (d, J=5.3 Hz, 3H), 0.93 (t, J=5.3 Hz, 3H). R_f^1 0.56.

4.1.7. Pte-Ser-OH (3g). Oily material. Yield 65%. ^{1}H NMR (400 MHz, CD_3CN) δ 7.40 (d, J=7.3 Hz, 2H), 7.28 (t, J=7.3 Hz, 2H), 7.20 (t, J=7.3 Hz, 1H), 4.53 (br s, 1H), 4.20 (t, J=6.9 Hz, 2H), 3.89 (dd-like, 1H), 3.83 (dd-like, 1H), 3.16 (t, J=6.9 Hz, 2H). R_f^1 0.24.

4.1.8. Pte-Asp-OH (3h). Colorless solid. Yield 66%. ^{1}H NMR (400 MHz, CD_3CN) δ 7.41 (d, J=7.4 Hz, 2H), 7.29 (t, J=7.4 Hz, 2H), 7.21 (t, J=7.4 Hz, 1H), 4.50 (s, 1H), 4.19 (t, J=6.9 Hz, 2H), 3.16 (t, J=6.9 Hz, 2H), 2.75 (m, 1H). R_f^1 0.12.

4.1.9. Pte-Pro-OH (3i). Colorless solid. Yield 78%. ^{1}H NMR (400 MHz, CD_3CN) δ 7.39 (d, J=7.3 Hz, 2H), 7.30 (t, J=7.3 Hz, 2H), 7.20 (t, J=7.3 Hz, 1H), 4.24 (m, 1H), 4.20 (m, 2H), 3.47 (m, 1H), 3.20 (m, 1H), 3.18 (t, J=6.9 Hz, 2H) 2.43 (m, 1H), 2.00 (m, 1H), 1.89 (m, 2H). R_f^1 0.51.

4.1.10. Pte-Tyr-OH (3j). Oily material. Yield 68%. ^{1}H NMR (400 MHz, CD_3CN) δ 7.40 (d, J=7.4 Hz, 2H), 7.32 (t, J=7.4 Hz, 2H), 7.22 (t, J=7.4 Hz, 1H), 7.07 (d, J=8.6 Hz, 2H), 6.74 (d, J=8.6 Hz, 2H), 4.32 (m, 1H), 4.14 (t, J=6.9 Hz, 2H), 3.16 (t, J=6.9 Hz, 2H), 3.13 (m, 1H), 2.83 (m, 1H). R_f^1 0.34.

4.2. Preparation of Pms-amino acids

Yields, MS spectra data and rotations of synthetic Pms-amino acids are shown in Table 1.

4.2.1. Pms-Phe-OH (4a). To an acetonitrile (15 ml) solution of **3a** (400 mg, 1.15 mmol) and silver tetrafluoroborate (389 mg, 2.0 mmol), methyl iodide (0.8 ml, 2.0 mmol) was added and the mixture was refluxed overnight at 40 °C. After cooling, the yellow precipitates were filtered off and the solvent was removed in vacuo. The residue was purified by preparative HPLC. Amorphous material. ^{1}H NMR (400 MHz, D_2O) δ 7.95 (d, J=7.5 Hz, 2H), 7.81 (t, J=7.3 Hz, 1H), 7.71 (dd, J=7.5, 7.3 Hz, 2H), 7.25 (m, 5H), 4.45 (m, 1H), 4.39 (m, 1H), 4.20 (m, 1H), 3.99

(m, 2H), 3.34 (s, 3H), 3.23 (m, 1H), 2.91 (m, 1H). Anal. Calcd for $C_{19}H_{22}BF_4NO_4S\cdot 1/3TFA$: C, 48.68; H, 4.64; N, 2.89. Found: C, 48.62; H, 4.32; N, 2.89.

The following Pms-amino acids were prepared according to the procedure described above (Route 1).

4.2.2. Pms-Gly-OH (4b). Amorphous material. ^{1}H NMR (400 MHz, D_2O) δ 7.95 (d, J=7.8 Hz, 2H), 7.82 (t, J=7.2 Hz, 1H), 7.72 (dd, J=7.8, 7.2 Hz, 2H), 4.52 (dt, J=13, 5.1 Hz, 1H), 4.32 (dt, J=13, 5.1 Hz, 1H), 4.00 (t, J=5.1 Hz, 2H), 3.74 (s, 1H), 3.31 (s, 3H). Anal. Calcd for $C_{12}H_{16}BF_4NO_4S\cdot 1/2TFA$: C, 37.70; H, 4.02; N, 3.38. Found: C, 37.92; H, 4.23; N, 3.36.

4.2.3. Pms-Ala-OH (4c). Amorphous material. ^{1}H NMR (400 MHz, D_2O) δ 7.96 (d, J=7.8 Hz, 2H), 7.84 (t, J=7.2 Hz, 1H), 7.74 (dd, J=7.8, 7.2 Hz, 2H), 4.50 (m, 1H), 4.34 (m, 1H), 4.30 (m, 1H), 4.01 (m, 2H), 3.31 (s, 3H), 1.31 (m, 3H). Anal. Calcd for $C_{13}H_{18}BF_4NO_4S\cdot 1/2TFA\cdot CH_3CN$: C, 40.15; H, 4.49; N, 4.68. Found: C, 40.41; H, 4.22; N, 4.29.

4.2.4. Pms-Val-OH (4d). Amorphous material. ^{1}H NMR (400 MHz, D_2O) δ 7.97 (d, J=7.5 Hz, 2H), 7.84 (t, J=7.3 Hz, 1H), 7.75 (dd, J=7.5, 7.3 Hz, 2H), 4.51 (m, 1H), 4.34 (m, 1H), 4.01 (t, J=5.2 Hz, 2H), 3.92 (m, 1H), 3.33 (s, 3H), 2.11 (m, 1H), 0.93 (d-like, 3H), 0.92 (d-like, 3H). Anal. Calcd for $C_{15}H_{22}NBF_4O_4S\cdot 2/5TFA$: C, 40.59; H, 4.69; N, 3.68. Found: C, 40.97; H, 4.21; N, 3.56.

4.2.5. Pms-Ile-OH (4e). Amorphous material. ^{1}H NMR (400 MHz, D_2O) δ 7.96 (d, J=7.5 Hz, 2H), 7.83 (t, J=7.3 Hz, 1H), 7.73 (dd, J=7.5, 7.3 Hz, 2H), 4.54 (m, 1H), 4.34 (m, 1H), 4.01 (br s, 2H), 3.95 (m, 1H), 3.31 (s, 3H), 1.82 (m, 1H), 1.38 (dquint., J=14.0, 7.0 Hz, 1H), 1.17 (dquint., J=14.0, 7.0 Hz, 1H), 0.90 (3H, d, J=7.0 Hz, βC–CH_3), 0.89 (3H, t, J=7.0 Hz, γC–CH_3). Anal. Calcd for $C_{16}H_{24}BF_4NO_4S\cdot 2/3TFA$: C, 39.06; H, 4.40; N, 2.40. Found: C, 38.67; H, 4.20; N, 2.22.

4.2.6. Pms-Leu-OH (4f). Amorphous material. ^{1}H NMR (400 MHz, D_2O) δ 7.96 (d, J=7.5 Hz, 2H), 7.83 (t, J=7.3 Hz, 1H), 7.73 (dd, J=7.5, 7.3 Hz, 2H), 4.54 (m, 1H), 4.34 (m, 1H), 4.01 (br s, 2H), 3.98 (m, 1H), 3.31 (s, 3H), 1.62 (m, 1H), 1.48 (m, 2H), 0.93 (d, J=5.5 Hz, 3H), 0.90 (t, J=5.5 Hz, 3H). Anal. Calcd for $C_{16}H_{24}BF_4NO_4S\cdot TFA$: C, 41.51; H, 4.68; N, 2.59. Found: C, 41.00; H, 4.78; N, 2.66.

4.2.7. Pms-Ser-OH (4g). Amorphous material. ^{1}H NMR (400 MHz, D_2O) δ 7.96 (d, J=7.5 Hz, 2H), 7.83 (t, J=7.3 Hz, 1H), 7.73 (dd, J=7.5, 7.3 Hz, 2H), 4.54 (m, 1H), 4.34 (m, 1H), 4.22 (m, 1H), 4.01 (br s, 2H), 3.31 (s, 3H), 3.94 (m, 1H), 3.86 (m, 1H). Anal. Calcd for $C_{13}H_{18}BF_4NO_5S$: C, 40.33; H, 4.69; N, 3.62. Found: C41.01; H, 4.36; N, 3.59.

4.2.8. Pms-Asp-OH (4h). Amorphous material. ^{1}H NMR (400 MHz, D_2O) δ 7.96 (d, J=7.7 Hz, 2H), 7.83 (t, J=7.5 Hz, 1H), 7.73 (dd, J=7.7, 7.5 Hz, 2H), 4.50 (m, 1H), 4.47 (m, 1H), 4.34 (m, 1H), 4.01 (br s, 2H), 3.32 (s, 3H), 2.78 (m, 2H). Anal. Calcd for $C_{14}H_{18}BF_4NO_6S\cdot 2/3TFA\cdot 1/2CH_3CN$: C, 35.94; H, 3.42; N, 3.40. Found: C, 35.63; H, 3.49; N, 3.46.

4.2.9. Pms-Pro-OH (4i). Amorphous material. ^{1}H NMR (400 MHz, D_2O) δ 7.96 (d, J=7.5 Hz, 2H), 7.84 (t, J=7.3 Hz, 1H), 7.75 (dd, J=7.5, 7.3 Hz, 2H), 4.57 (m, 1H), 4.38 (m, 1H), 4.16 (m, 1H), 4.00 (m, 2H), 3.30 (s, 3H), 3.47–3.01 (m, 2H), 2.22 (m, 1H), 2.00 (m, 1H), 1.84 (m, 2H). Anal. Calcd for $C_{15}H_{20}BF_4NO_4S\cdot TFA$: C, 39.86; H, 4.33; N, 2.73. Found: C, 39.54; H, 4.10; N, 2.74.

4.2.10. Pms-Tyr-OH (4j). Amorphous material. ^{1}H NMR (400 MHz, D_2O) δ 7.96 (d, J=7.5 Hz, 2H), 7.80 (t, J=7.3 Hz, 1H), 7.71 (dd, J=7.5, 7.3 Hz, 2H), 7.07 (d, J=8.4 Hz, 2H), 6.73 (d, J=8.4 Hz, 2H), 4.41 (m, 1H), 4.34 (m, 1H), 4.19 (m, 1H), 3.97 (m, 1H), 3.88 (m, 1H), 3.31 (s, 3H), 3.13 (m, 1H), 2.81 (m, 1H). Anal. Calcd for $C_{19}H_{22}BF_4NO_5S\cdot 1/2TFA$: C, 46.17; H, 4.36; N, 2.69. Found: C, 46.43; H, 4.52; N, 2.60.

4.3. Route 2

4.3.1. 2-[Phenyl(methyl)sulfonio]ethanol (6). To a solution of 2-(phenylthio)ethanol (1.34 ml, 10 mmol) and silver tetrafluoroborate (2.32 g, 12 mmol) in acetonitrile (60 ml), methyl iodide (1.24 ml, 20 mmol) was added and the mixture was stirred overnight at 40 °C. After cooling, the yellow precipitate was filtered off and the solvent was evaporated in vacuo. The residue was flash chromatographed on silica gel, eluted with CH_3Cl–MeOH–H_2O (8/3/1, lower phase). The collected fractions were combined and concentrated in vacuo to leave a colorless oil. Yield 2.26 g, 88%. ^{1}H NMR (400 MHz, D_2O) δ 7.97 (dd-like, J=8.0, 1.7 Hz, 2H), 7.84 (tt, J=7.5, 1.7 Hz, 1H), 7.75 (dd-like, J=8.0, 7.5 Hz, 2H), 4.03 (m, 1H), 4.34 (m, 1H), 3.90 (m, 2H), 3.82 (s, 3H), 3.78 (m, 1H), 3.31 (s, 3H). Tof-MS m/z 169.14 (M^+, $C_9H_{13}OS$ requires 169.26).

4.3.2. Pms-Phe-OH (4a). To a suspension of **6** (513 mg, 2.0 mmol) and triphosgen (394 mg, 1.33 mmol) in dichloromethane (30 ml), Et_3N (0.56 ml, 4.0 mmol) in dichloromethane (10 ml) was added dropwise at −10 °C, and the mixture was stirred at −10 °C for 1.5 h. After evaporation of the solvent in vacuo at 10 °C, the residue was dissolved in acetonitrile (15 ml), and this solution was added to a solution of Phe (330 mg, 2.0 mmol) and Et_3N (0.35 ml, 2.5 mmol) in a mixture of acetonitrile and water (1/1, 50 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h and the solvent was removed in vacuo. The product was purified by preparative HPLC. Yield: 241 mg. The product was identified with Pms-Phe-OH prepared through Route 1 by NMR and Mass spectra.

4.4. Route 3

4.4.1. Pte-OSu (8). To a solution of 2-(phenylthio)ethanol (1.34 ml, 10 mmol) and triphosgen (1.96 g, 6.6 mmol) in tetrahydrofuran (40 ml), Et_3N (2.79 ml, 20 mmol) in tetrahydrofuran (15 ml) was added dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 1.5 h and the mixture was filtered. The solvent was evaporated off in vacuo and the residue was dissolved in acetonitrile (15 ml). The solution was added to a solution of HOSu (1.15 g, 10 mmol) and Et_3N (1.39 ml, 10 mmol) in a mixture of acetonitrile (30 ml) at 0 °C, and the mixture was stirred at room temperature for 3 h keeping its pH at 8. The solvent was removed in vacuo and the residue was purified by flash chromatography on silica gel, using hexane–ethyl acetate

(2/1) as an eluate. The collected fractions were combined and concentrated in vacuo to leave a colorless oil. Yield. 2.12 g, 72%. ^{1}H NMR (400 MHz) δ (CD_3CN): 7.42 (d, J=7.1 Hz, 2H), 7.34 (dd, J=7.3, 7.1 Hz, 2H), 7.25 (dd, J=7.3 Hz, 1H), 4.43 (t, J=6.5 Hz, 2H), 3.27 (t, J=6.5 Hz, 2H), 2.76 (s, 4H). ESI-MS m/z 313.2[$(M+NH_4)^+$, $C_{13}H_{13}NO_5{\cdot}NH_4$ requires 313.3]. R_f [(hexane–ethyl acetate (2/1)] 0.38.

4.4.2. Pms-OSu (9). To an acetonitrile solution (15 ml) of **8** (295 mg, 1.0 mmol) and silver tetrafluoroborate (232 mg, 1.2 mmol), methyl iodide (1.24 ml, 20 mmol) was added, and the mixture was stirred overnight at 40 °C. After cooling, the yellow precipitate was filtered off and the solvent was removed in vacuo to leave a colorless oil. Yield 384 mg, 97%. ^{1}H NMR (400 MHz, CD_3CN) δ 7.96 (d, J=7.5 Hz, 2H), 7.84 (t, J=7.4 Hz, 1H), 7.74 (dd, J=7.5, 7.4 Hz, 2H), 4.73 (m, 1H), 4.49 (m, 1H), 4.04 (m, 1H), 3.93 (m, 1H), 3.29 (s, 3H), 2.76 (s, 4H). Tof-MS m/z 310.41 (M^+, $C_{14}H_{16}NO_5S$ requires 310.35).

4.4.3. Pms-Phe-OH (4a). To a solution of Phe (83 mg, 0.5 mmol) and pyridine (40.43 ml, 0.5 mmol) in aqueous 0.1% Triton X-100 solution–acetonitrile (1/1, 20 ml), **9** [prepared from **8** (146 mg, 0.5 mmol)] in acetonitrile (10 ml) was added at 0 °C, and the mixture was stirred at room temperature for 4 h. The solvent was removed in vacuo, the product was purified by preparative HPLC to give a colorless oil. The product was identified with Pms-Phe-OH prepared through Route 1 by NMR and Mass spectra.

4.5. Route 4

4.5.1. Pte-ONB (10). To a solution of 2-(phenylthio)ethanol (1.34 ml, 10 mmol) and triphosgen (1.96 g, 6.6 mmol) in tetrahydrofuran (40 ml), Et_3N (2.79 ml, 20 mmol) in tetrahydrofuran (15 ml) was added dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 1.5 h and filtered. The solvent was removed filtrate in vacuo and the residue was dissolved in acetonitrile (15 ml) and added to a solution of HONB (1.79 g, 10 mmol) and Et_3N (1.39 ml, 10 mmol) in acetonitrile (30 ml) at 0 °C. The reaction mixture was stirred at room temperature for 3 h keeping it pH at 8. The solvent was removed in vacuo and the residue was purified by flash chromatography on silica gel using hexane–ethyl acetate (2/1) as an eluate. The collected fractions were combined and concentrated in vacuo to leave a colorless solid. Yield 2.72 g, 76%. ^{1}H NMR (400 MHz, CD_3CN) δ 7.42 (d, J=7.1 Hz, 2H), 7.34 (dd, J=7.3, 7.1 Hz, 2H), 7.25 (dd, J=7.3 Hz, 1H), 6.18 (s, 2H), 4.36 (t, J=6.5 Hz, 2H), 3.45 (d-like, 2H), 3.30 (d-like, 2H) 3.18 (t, J=6.5 Hz, 2H), 1.78 (dd, J=9.0, 1.6 Hz, 1H), 1.52 (dd, J=9.0, 1.6 Hz, 1H). ESI-MS m/z 377.3 [$(M+NH_4)^+$, $C_{18}H_{17}NO_5S{\cdot}NH_4$ requires 377.3]. R_f [hexane–AcOEt (2/1)] 0.45.

4.5.2. Pms-ONB (11). To a solution of **10** (359 mg, 1.0 mmol) and silver tetrafluoroborate (232 mg, 1.2 mmol) in acetonitrile (15 ml), methyl iodide (1.24 ml, 20 mmol) was added and the mixture was stirred overnight at 40 °C. The resulting yellow precipitate was removed by filtration and the solvent was evaporated in vacuo to leave a colorless oil. Yield 450 mg, 98%. ^{1}H NMR (400 MHz, CD_3CN) δ 7.96 (d, J=7.5 Hz, 2H), 7.84 (t, J=7.4 Hz, 1H), 7.74 (dd, J=7.5, 7.4 Hz, 2H), 6.18 (s, 2H), 4.73 (m, 1H), 4.49 (m, 1H), 4.04 (m, 1H), 3.93 (m, 1H), 3.45 (d-like, 2H), 3.30 (d-like, 2H), 3.29 (s, 3H), 1.78 (dd, J=9.0, 1.6 Hz, 1H), 1.52 (dd, J=9.0, 1.6 Hz, 1H). Tof-MS m/z 374.5 (M^+, $C_{19}H_{20}NO_5S$ requires 374.43).

4.5.3. Pms-Phe-OH (4a). To a solution of Phe (83 mg, 0.5 mmol) and pyridine (40.43 ml, 0.5 mmol) in aqueous 0.1% Triton X-100 solution–acetonitrile (1/1, 20 ml), **11** [prepared from **10** (179 mg, 0.5 mmol)] in acetonitrile (10 ml) was added at 0 °C and the mixture was stirred at room temperature for 4 h. The solvent was removed in vacuo and the residue was purified by preparative HPLC to give a colorless oil. The product was identified with Pms-Phe-OH prepared through Route 1 by NMR and Mass spectra.

The following Pms-amino acids were prepared from Pms-ONB and a corresponding amino acid according to the procedure described above (Route 4).

4.5.4. Pms-Tyr(*t*Bu)-OH (15a). Amorphous material. ^{1}H NMR (400 MHz, CD_3CN) δ 7.86 (d, J=7.6 Hz, 2H), 7.80 (t, J=7.6 Hz, 1H), 7.69 (dd, J=7.5, 7.3 Hz, 2H), 7.14 (d, J=8.4 Hz, 2H), 6.93 (d, J=8.4 Hz, 2H), 4.35 (m, 1H), 4.34 (m, 1H), 4.13 (m, 1H), 3.80 (m, 1H), 3.72 (m, 1H), 3.18 (s, 3H), 3.14 (m, 1H), 2.89 (m, 1H), 1.29 (s, 9H). Anal. Calcd for $C_{23}H_{30}BF_4NO_5S{\cdot}$TFA: C, 47.41; H, 4.93; N, 2.21. Found: C, 47.85; H, 4.54; N, 2.40.

4.5.5. Pms-Ser(*t*Bu)-OH (15b). Amorphous material. ^{1}H NMR (400 MHz, CD_3CN) δ 7.96 (d, J=7.7 Hz, 2H), 7.84 (t, J=7.4 Hz, 1H), 7.72 (dd, J=7.7, 7.4 Hz, 2H), 4.54 (m, 1H), 4.34 (m, 1H), 4.28 (br m, 1H), 4.02 (br s, 2H), 3.77 (m, 1H), 3.68 (m, 1H), 3.32 (s, 3H), 1.19 (s, 9H). Anal. Calcd for $C_{17}H_{26}BF_4NO_5S{\cdot}1/2$TFA: C, 43.21; H, 5.34; N, 2.80. Found: C, 43.35; H, 4.86; N, 2.66.

4.5.6. Pms-Asp(O*t*Bu)-OH (15c). Amorphous material. ^{1}H NMR (400 MHz, CD_3CN) δ 7.96 (d, J=7.7 Hz, 2H), 7.84 (t, J=7.5 Hz, 1H), 7.72 (dd, J=7.7, 7.5 Hz, 2H), 4.53 (m, 1H), 4.45 (br m, 1H), 4.34 (m, 1H), 4.01 (br s, 2H), 3.32 (s, 3H), 2.79 (d, J=5.5 Hz, 2H) 1.44 (s, 9H). Anal. Calcd for $C_{18}H_{26}BF_4NO_6S{\cdot}1/2$TFA: C, 43.20; H, 5.06; N, 2.65. Found: C, 43.89; H, 4.78; N, 2.65.

4.5.7. Pms-Glu(O*t*Bu)-OH (15d). Amorphous material. ^{1}H NMR (400 MHz, CD_3CN) δ 7.92 (d, J=7.7 Hz, 2H), 7.82 (t, J=7.5 Hz, 1H), 7.72 (dd, J=7.7, 7.5 Hz, 2H), 4.43 (m, 1H), 4.21 (m, 1H), 4.11 (m, 1H), 3.87 (m, 1H), 3.78 (m, 1H), 3.23 (s, 3H), 2.29 (m, 2H), 2.07 (m, 1H), 1.86 (m, 1H), 1.43 (s, 9H). Anal. Calcd for $C_{19}H_{28}BF_4NO_6S{\cdot}1/5$TFA: C, 45.86; H, 5.59; N, 2.76. Found: C, 46.15; H, 5.38; N, 2.63.

4.5.8. Pms-Thr(*t*Bu)-OH (15e). Amorphous material. ^{1}H NMR (400 MHz, CD_3CN) δ 7.96 (d, J=7.7 Hz, 2H), 7.84 (t, J=7.5 Hz, 1H), 7.72 (dd, J=7.7, 7.5 Hz, 2H), 4.46 (m, 1H), 4.23 (m, 1H), 4.22 (br m, 1H), 4.22 (m, 2H), 4.07 (m, 1H), 3.89 (m, 1H), 3.80 (m, 1H), 3.23 (s, 3H), 1.16 (s, 12H). Anal. Calcd for $C_{18}H_{28}BF_4NO_5S{\cdot}1/2$TFA: C, 44.37; H, 5.59; N, 2.72. Found: C, 44.35; H, 5.36; N, 2.51.

4.5.9. Pms-Asn(Trt)-OH (15f). Amorphous material. ^{1}H NMR (400 MHz, CD_3CN) δ 7.87 (d, J=7.7 Hz, 2H), 7.74 (t, J=7.5 Hz, 1H), 7.72 (br s, 1H), 7.63 (dd, J=7.7, 7.5 Hz, 2H), 7.24 (m, 15H), 4.37 (m, 1H), 4.18 (m, 1H), 4.05 (m, 1H), 3.82 (m, 1H), 3.74 (m, 1H), 3.20 (s, 3H), 2.88 (m, 1H), 2.77 (m, 1H). Anal. Calcd for $C_{34}H_{35}BF_4N_2O_5S$·1/3TFA·CH_3CN: C, 57.38; H, 4.95; N, 5.63. Found: C, 57.74; H, 4.91; N, 6.08.

4.5.10. Pms-Gln(Trt)-OH (15g). Amorphous material. ^{1}H NMR (400 MHz, CD_3CN) δ 7.87 (d, J=7.7 Hz, 2H), 7.77 (t, J=7.5 Hz, 1H), 7.66 (dd, J=7.7, 7.5 Hz, 2H), 7.54 (br s, 1H), 7.25 (m, 15H), 4.37 (m, 1H), 4.18 (m, 1H), 4.05 (m, 1H), 3.79 (m, 1H), 3.74 (m, 1H), 3.20 (s, 3H), 2.42 (m, 2H), 2.11 (m, 1H), 1.81 (m, 1H). Anal. Calcd for $C_{33}H_{33}BF_4N_2O_5S$·2/3TFA·1/3CH_3CN·1/3H_2O: C, 56.43; H, 4.91; N, 4.27. Found: C, 56.26; H, 4.67; N, 4.42.

4.5.11. Pms-His(Trt)-OH (15h). Amorphous material. ^{1}H NMR (400 MHz, CD_3CN) δ 8.34 (s, 1H), 7.87 (d, J=7.7 Hz, 2H), 7.79 (m, 1H), 7.70 (dd, J=7.7, 7.5 Hz, 2H), 7.43–7.15 (m, 15H), 7.08 (s, 1H), 4.44 (m, 1H), 4.34 (m, 1H), 4.18 (m, 1H), 3.87 (m, 1H), 3.75 (m, 1H), 3.29 (d, J=14.7, 4.3 Hz), 3.21 (s, 3H), 3.06 (dd, J=14.7, 9.0 Hz, 1H). Anal. Calcd for $C_{35}H_{34}BF_4N_3O_4S$·2TFA·H_2O: C, 50.61; H, 4.14; N, 4.54. Found: C, 50.47; H, 3.82; N, 4.41.

4.5.12. Pms-Trp(Boc)-OH (15i). Amorphous material. ^{1}H NMR (400 MHz, CD_3CN) δ 8.09 (d, J=8.3 Hz, 1H), 7.84–7.72 (m, 3H), 7.67–7.61 (m, 3H), 7.53 (d-like, 1H), 7.33 (t-like, 1H), 7.26 (t-like, 1H), 4.49 (m, 1H), 4.35 (m, 1H), 4.14 (m, 1H), 3.78 (m, 1H), 3.71 (m, 1H), 3.32 (m, 1H), 3.15 (m, 1H), 3.12 (m, 1H), 1.63 (s, 9H). Anal. Calcd for $C_{26}H_{31}BF_4N_2O_6S$·1/2TFA: C, 51.29; H, 5.06; N, 4.49. Found: C, 51.24; H, 5.04; N, 4.21.

4.5.13. Pms-Arg(Pmc)-OH (15j). Amorphous material. ^{1}H NMR (400 MHz, CD_3CN) δ 7.92 (d, J=7.7 Hz, 2H), 7.78 (t, J=7.1 Hz, 1H), 7.70 (dd, J=7.7, 7.1 Hz, 2H), 6.21 (br s, 1H), 4.43 (m, 1H), 4.21 (m, 1H), 4.04 (br m, 1H), 3.87 (m, 1H), 3.79 (m, 1H), 3.59 (m, 1H), 3.23 (s, 3H), 2.65 (t, J=6.8 Hz, 2H), 2.52 (s, 3H), 1.82 (t, J=6.8 Hz, 2H), 1.78 (br m, 1H), 1.62 (br m, 1H), 1.58 (br m, 2H), 1.30 (s, 6H). Anal. Calcd for $C_{30}H_{43}BF_4N_4O_7S_2$·TFA·1/2$CH_3CN$: C, 46.24; H, 5.35; N, 7.35. Found: C, 46.49; H, 5.17; N, 7.30.

4.5.14. Pms-Lys(Boc)-OH (15k). Amorphous material. ^{1}H NMR (400 MHz, CD_3CN) δ 7.93 (d, J=7.7 Hz, 2H), 7.82 (t, J=7.5 Hz, 1H), 7.72 (dd, J=7.7, 7.5 Hz, 2H), 4.43 (m, 1H), 4.21 (m, 1H), 4.06 (m, 1H), 3.81 (m, 1H), 3.24 (s, 3H), 3.02 (t-like, 2H), 1.79 (br m, 2H), 1.67 (br m, 2H), 1.39 (s, 11H). Anal. Calcd for $C_{21}H_{33}BF_4N_2O_6S$·4/3TFA: C, 42.11; H, 5.13; N, 4.71. Found: C, 42.11; H, 5.13; N, 4.75.

4.6. Route 5

4.6.1. Pms-ONp (13). To a solution of **6** (256 mg, 1.0 mmol) in acetonitrile (15 ml, a solution of 4-nitrophenyl chloroformate (402 mg, 2.0 mmol) in acetonitrile (10 ml) and a solution of Et_3N (279 μl, 2.0 mmol) in acetonitrile (10 ml) were added alternately at 0 °C, and the mixture was stirred at room temperature for 2 h. The reaction mixture was filtered and concentrated in vacuo, and the residue was washed with water and crystallized from ether-hexane to give colorless crystal. Yield 298 mg, 71%. Mp 140 °C (decomp.). ^{1}H NMR (400 MHz, D_2O) δ 8.31 (dd, J=9.4 Hz, 2H), 7.98 (d, J=7.5 Hz, 2H), 7.86 (t, J=7.3 Hz, 1H), 7.75 (dd, J=7.5, 7.3 Hz, 2H), 7.43 (dd, J=9.4 Hz, 2H), 4.67 (m, 1H), 4.45 (m, 1H), 4.04 (m, 1H), 3.95 (m, 1H), 3.29 (s, 3H). Tof-MS m/z 334.5 (M^+, $C_{16}H_{16}NO_5S$ requires 334.37). Anal. Calcd for $C_{16}H_{16}BF_4NO_5S$: C, 45.63; H, 3.83; N, 3.33. Found: C, 45.68; H, 3.91; N, 3.39.

4.6.2. Pms-Phe-OH (4a). To a solution of Phe (83 mg, 0.5 mmol) and pyridine (40.4 μl, 0.5 mmol) in aqueous 0.1% Triton X-100 solution–acetonitrile (1/1, 20 ml), **13** (211 mg, 0.5 mmol) in acetonitrile (10 ml) was added at 0 °C, and the mixture was stirred at room temperature for 4 h. After removal of the solvent, the residue was dissolved in water and washed with AcOEt. The aqueous layer was concentrated in vacuo and the residue was purified by preparative HPLC to give a colorless oil. The product was identified by NMR and Mass spectra with Pms-Phe-OH prepared through Route 1.

The following Pms-amino acids were prepared from Pms-ONp and a corresponding amino acid according to the procedure described above.

4.6.3. Pms-Met-OH (14a). Amorphous material. ^{1}H NMR (400 MHz, D_2O) δ 7.98 (d, J=7.7 Hz, 2H), 7.85 (t, J=7.5 Hz, 1H), 7.75 (dd, J=7.7, 7.5 Hz, 2H), 4.52 (m, 1H), 4.34 (m, 1H), 4.34 (m, 1H), 4.01 (m, 2H), 3.32 (s, 3H), 2.91 (m, 2H), 2.21 (s, 3H). Anal. Calcd $C_{14}H_{19}BF_4NO_4S_2$·TFA: C, 37.44; H, 4.25; N, 2.57. Found: C, 37.71; H, 4.43; N, 2.60.

4.6.4. Pms-Cys(Acm)-OH (14b). Amorphous powder. ^{1}H NMR (400 MHz, D_2O) δ 7.94 (d, J=7.5 Hz, 2H), 7.80 (t, J=7.5 Hz, 1H), 7.72 (t, J=7.5 Hz, 2H), 4.52 (m, 1H), 4.34 (m, 1H), 4.34, 4.27 (ABq, J=14 Hz, 1H), 4.31 (m, 1H), 4.00 (m, 2H), 3.31 (s, 3H), 2.92 (dd, J=14, 4.7 Hz, 1H), 2.88 (dd, J=14, 4.7 Hz, 1H), 1.99 (s, 3H). Anal. Calcd for $C_{16}H_{23}BF_4N_2O_5S_2$·1/2TFA: C, 38.43; H, 4.46; N, 5.27. Found: C, 38.39; H, 4.21; N, 4.98.

4.6.5. Pms-Cys(Trt)-OH (14c). Amorphous powder. ^{1}H NMR (400 MHz, CD_2CN) δ 7.87 (d, J=7.5 Hz, 2H), 7.75 (t, J=7.5 Hz, 1H), 7.65 (t, J=7.5 Hz, 2H), 7.26–7.39 (m, 15H), 4.40 (m, 1H), 20 (m, 1H), 3.94 (m, 1H), 3.85 (br d, J=14 Hz, 1H), 3.75 (br d, J=14 Hz, 1H), 3.19 (s, 3H), 2.64 (dd like, 1H), 2.54 (dd like, 1H) Anal. Calcd for $C_{32}H_{32}BF_4NO_4S_2$: C, 59.54; H, 5.00; N, 2.17. Found: C, 59.79; H, 4.77; N, 1.88.

4.7. Examination in deprotection rate of Pms group on Pms-Phe-Leu-TentaGel resin with various bases in water

Pms-Phe-OH (89.4 mg, 0.2 mmol) and the H-Leu-TentaGel resin (192 mg, 50 μmol) was reacted with WSCD (38.2 mg, 0.2 mmol) in a presence of HONB (35.8 mg, 0.2 mmol) in aqueous 0.2% Triton X solution. The resulting Pms-Phe-Leu-TentaGel resin was washed with DMF and dichloromethane and dried in vacuo. Yield 210 mg (98%). Amino acid ratio in an acid hydrolysate: Phe 1.00, Leu 0.96. The resin (10 mg each) was treated with various base solutions

(aqueous 5% $NaHCO_3$, aqueous 0.01 mol/l NaOH, 0.005 mol/l NaOH in 50% aqueous EtOH, 2.5% $NaHCO_3$ in aqueous 50% EtOH, 2.5% Na_2CO_3 in aqueous 50% EtOH). After base treatment, the resin was washed with H_2O and DMF. Then Fmoc-Gly-OH (3.0 mg, 10 μmol) was coupled on the resin with diisopropylcarbodiimide (1.6 μl, 10 μmol) and HOBt (1.4 mg, 10 μmol) in DMF until the resin gave negative Kaiser test. The resulting resin was washed with DMF and dichloromethane, and dried in vacuo. After removal of the Fmoc group with 20% piperidine/DMF, the resin was hydrolyzed and Gly, Phe and Leu contents in the hydrolysate were analyzed. Each deprotection yield was calculated from the amino acid ratio of Gly and Leu. Results are summarized in Table 3.

4.7.1. Synthesis of Leu-enkephalin amide on TentaGel resin in water. The Fmoc-Rink amide-TentaGel resin (100 mg, 25 μmol) was swelled with dichloromethane and DMF, and then Fmoc group on the resin was removed by treatment with 20% piperidine/DMF. After washed with DMF and H_2O, the resin was swelled with aqueous 0.2% Triton X-100 solution. The synthesis was carried out according to the protocol shown in Table 4. Pms-Leu-OH 41.3 mg (0.1 mmol), Pms-Phe-OH 44.7 mg (0.1 mmol), Pms-Gly-OH 35.7 mg (0.1 mmol), and Pms-Tyr-OH 46.3 mg (0.1 mmol) were coupled in turn with WSCD 19.1 mg (MW: 191.7, 0.1 mmol) and HONB 17.9 mg (0.1 mmol). Deprotection was carried out with an aqueous solution of 5.0% $NaHCO_3$. After completion of the synthetic reaction, the peptide resin (H-Tyr-Gly-Gly-Phe-Leu-TentaGel resin) was washed with H_2O, DMF and dichloromethane, and dried in vacuo. Yield: 107.5 mg, 97%. The whole resin was treated with TFA–thioanisole–ethanedithiol (94/3/3, 15 ml) for 2 h at room temperature. The resin was removed by filtration and the TFA was removed in vacuo to leave a yellowish oil. The residue was dissolved in water, washed with ether, and lyophilized. The crude product was purified by HPLC to give an amorphous powder. Yield (based on amino group content of the resin): Yield 10.3 mg, 61%. $[\alpha]_D^{24}=+8.2°$ (c=0.2, H_2O). Tof-MS m/z 555.0 [$(M+1)^+$, $C_{28}H_{39}N_6O_6$ requires 555.64]. Amino acid ratios in an acid hydrolysate: Tyr 0.99, Gly 2.00, Phe 0.96, Leu 0.98 (average recovery: 83%).

4.7.2. Synthesis of Leu-enkepharin amide by the Fmoc strategy. The Fmoc group on the Rinkamide resin (100 mg, 60 μmol) was removed by treatment with 20% piperidine/DMF. Synthesis was carried out according to a general Fmoc strategy[19] Fmoc-Leu-OH 63.6 mg (0.18 mmol), Fmoc-Phe-OH 69.7 mg (0.18 mmol), Fmoc-Gly-OH 53.5 mg (0.18 mmol), and Fmoc-Tyr(*t*Bu)-OH 82.7 mg (0.18 mmol) were coupled in turn by diisopropylcarbodiimide (0.18 mmol) and 1-hydroxybenzotriazole 24.3 mg (0.18 mmol). Deprotection of Fmoc group was carried out with 20% piperidine/DMF. After completion of the synthetic reaction, the peptide resin (H-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-Rinkamide resin was washed with DMF and dichloromethane, and dried in vacuo. Yield 123 mg, 99%. The whole resin was treated with TFA–thioanisole–ethanedithiol (94/3/3, 15 ml) for 2 h at room temperature. The resin was filtered off, and the TFA was removed in vacuo to leave a yellowish oil. The residue was dissolved in water, washed with ether, and lyophilized. The crude product was purified by HPLC to give a white amorphous powder. Yield 34 mg (based on amino group content of the resin), 86%. $[\alpha]_D^{24}=+9.0°$ (c=0.2, H_2O). Tof-MS m/z 555.3 [$(M+1)^+$, $C_{28}H_{39}N_6O_6$ requires 555.64]. Amino acid ratios in an acid hydrolysate: Tyr 0.97, Gly 2.00, Phe 0.98, Leu, 1.01 (average recovery 97%).

4.7.3. Synthesis of Met-enkephalin amide on CLEAR resin in water. CLEAR resin (86 mg, 25 μmol) was swelled with dichloromethane and DMF and Fmoc group on the resin was removed with 20% piperidine/DMF. After washing with DMF and H_2O, the resin was swelled with aqueous 0.2% Triton X-100 solution. Synthetic reaction was carried out according to the protocol shown in Table 4. Pms-Met-OH 41.3 mg (0.1 mmol), Pms-Phe-OH 44.7 mg (0.1 mmol), Pms-Gly-OH 35.7 mg (0.1 mmol), and Pms-Tyr-OH 46.3 mg (0.1 mmol) were coupled in turn by WSCD 19.1 mg (0.1 mmol) and HONB 17.9 mg (0.1 mmol). Deprotection was carried out by treatment (twice) with an aqueous solution of 0.01 mol/l NaOH for 3 min. After completion of the synthetic reaction, the peptide resin (H-Tyr-Gly-Gly-Phe-Met-CLEAR resin) was washed with H_2O, DMF and dichloromethane, and dried in vacuo. Yield 92 mg, 86%. The whole resin was treated with TFA–thioanisole–ethanedithiol (94/3/3, 15 ml) for 2 h at room temperature. The resin was filtered off and the TFA was removed in vacuo to leave a yellowish oil. The residue was dissolved in water, washed with ether, and lyophilized. The crude product was purified by HPLC to give an amorphous powder. Yield (based on amino group content of the resin): Yield 4.9 mg, 29%. $[\alpha]_D^{24}=+6.7°$ (c=0.8, 20% CH_3CN/H_2O). Tof-MS m/z: 573.7 [$(M+1)^+$, $C_{27}H_{36}N_6O_6S$ requires 573.68]. Amino acid ratios in an acid hydrolysate: Tyr 1.01, Gly 2.00, Phe 0.93; Met 0.95 (average recovery: 94%).

4.7.4. Synthesis of Met-enkephalin amide on TentaGel resin in water. Performed in the same manner as described in Section 4.7.1. Yield (based on amino group content of the resin): yield 5.8 mg, 32% (amorphous powder). $[\alpha]_D^{24}=+7.1°$ (c=0.8, 20% CH_3CN/H_2O). Tof-MS m/z 573.4 [$(M+1)^+$, $C_{27}H_{36}N_6O_6S$ requires 573.68]. Amino acid ratios in an acid hydrolysate: Tyr 0.99, Gly 2.00, Phe 0.96, Met 0.98 (average recovery 92%).

References and notes

1. Preliminary communications were reported on: (a) Hojo, K.; Maeda, M.; Kawasaki, K. *J. Pept. Sci.* **2001**, *7*, 615–618. (b) Hojo, K.; Maeda, M.; Takahara, Y.; Yamamoto, S.; Kawasaki, K. *Tetrahedron Lett.* **2003**, *44*, 2849–2851.
2. Tesser, G. I.; Balvert-Geers, I. C. *Int. J. Pept. Protein Res.* **1975**, *7*, 295–305.
3. Kunz, H. *Chem. Ber.* **1976**, *109*, 2670–2683.
4. Merrifield, R. B.; Bach, A. F. *J. Org. Chem.* **1978**, *43*, 4808–4816.
5. Kunz, H. *Chem. Ber.* **1976**, *109*, 3693–3706.
6. Kunz, H.; Birnbach, S. *Tetrahedron Lett.* **1984**, *25*, 3567–3570.
7. Kunz, H. *Angew. Chem. Int.* **1978**, *17*, 67–68.
8. (a) Tsuji, T.; Okusada, S.; Maeda, M.; Kawasaki, K. *Chem.*

Pharm. Bull. **1986**, *34*, 2214–2217. (b) Kawasaki, K.; Tsuji, T.; Maeda, M.; Matsumoto, T.; Hirase, K. *Chem. Pharm. Bull.* **1987**, *35*, 1044–1048.
9. (a) Eckert, H.; Forster, B. *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 894–895. (b) Livius, C.; Pietro, D.; Alfonso, N.; Vitomir, S. *Synthesis* **1996**, 553–576.
10. Raquet, A. *Can. J. Chem.* **1976**, *54*, 733–733. Raquet, A. *Can. J. Chem.* **1982**, *60*, 976–980.
11. (a) Fujino, M.; Kobayashi, S.; Obayashi, M.; Fukuda, T.; Shinagawa, S.; Nishimura, O. *Chem. Pharm. Bull.* **1974**, *22*, 1857–1863. (b) Kitada, C.; Fujino, M. *Chem. Pharm. Bull.* **1978**, *26*, 585–590.
12. Konig, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788–798.
13. Kaiser, E.; Colescitti, R. L.; Bossinger, C. D.; Cook, R. I. *Anal. Biochem.* **1970**, *34*, 595–598.
14. (a) Bayer, E.; Rapp, W. In *Chemistry of peptides and proteins*; Volter, E., Bayer, E., Ovchinikov, Y. A., Ivanov, V. T., Eds.; Walter de Gryyter: Berlin, 1986; pp 3–8. (b) Bayer, E. *Angew. Chem. Int, Ed. Engl.* **1991**, *30*, 113–129.
15. Sheehan, J. C.; Cruickshank, P. A.; Boshrt, G. L. *J. Org. Chem.* **1983**, *26*, 2525–2528.
16. (a) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1964**, *86*, 1839–1842. (b) Zimmerman, J. E.; Anderson, G. W. *J. Am. Chem. Soc.* **1967**, *89*, 7151–7152.
17. Gross, H.; Bilk, L. *Tetrahedron* **1968**, *24*, 6935–6939.
18. Kempe, M.; Barany, G. *J. Am. Chem. Soc.* **1996**, *118*, 7083–7093.
19. Fields, G.; Noble, R. L. *Int. J. Pept. Protein Res.* **1990**, *35*, 161–214.