Journal of Peptide Science
J. Peptide Sci. **7**: 615–618 (2001)
DOI: 10.1002/psc.361

Rapid Communication

A new water-soluble *N*-protecting group, 2-(phenyl(methyl)sulfonio)ethyloxycarbonyl tetrafluoroborate, and its application to solid phase peptide synthesis in water

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Received 16 August 2001
Accepted 7 September 2001

Abstract: A new water-soluble *N*-protecting group, 2-[phenyl(methyl)sulfonio]ethyloxycarbonyl tetrafluoroborate, has been prepared and its application to solid phase peptide synthesis in water has been studied. Leu-enkephalin amide was successfully synthesized in water by the solid phase method using this protecting group. Copyright © 2001 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: *N*-protecting group; water-soluble; SPPS in water; 2-[phenyl(methyl)sulfonio]ethyloxycarbonyl tetrafluoroborate protection

INTRODUCTION

Solid phase peptide synthesis [1] has many advantages compared with solution peptide synthesis (e.g. easy handling, rapid synthesis, and automation), but it requires a large amount of organic solvents. Since safe organic solvent waste disposal is an important environmental problem, we aimed to perform peptide synthesis in water. To perform the coupling reaction in water, protected amino acids must be soluble in water. In previous papers, we have reported the preparation of water-soluble active esters and their application to peptide synthesis by the solution method [2]. Here we report the preparation of water-soluble *N*-protected amino acids and their application to solid phase peptide synthesis in water. Various water-soluble *N*-protecting groups have been reported: methylsulfonylethyloxycarbonyl, reported by Tesser and Balvert-Geers [4]; the 2-phosphonioethyloxycarbonyl [5,6], 2-(triphenylphosphonio)isopropyloxycarbonyl [7], and 2-(4-pyridyl)ethyloxycarbonyl [8] groups of Kunz; the 9-(2-sulfo)fluorenyloxycarbonyl group of Merrifield and Bach [9]. Kunz described the preparation of a tripeptide in water by the solution method using a water soluble *N*-protecting group. These protecting groups were removable under mild basic conditions via a β-elimination mechanism. Kunz also reported that the 2-(methylthio)ethyloxycarbonyl group was removable under mild basic conditions after methylation or oxidation [10] and the 2-(4-pyridyl)ethyloxycarbonyl group similarly under mild basic conditions after methylation [8]. In 1978, Kunz successfully prepared tripeptides by the solution method using the 2-phosphonioethyloxycarbonyl group as an *N*-protecting group in water [11]. We designed the 2-[phenyl(methyl)sulfonio]ethyloxycarbonyl tetrafluoroborate (Pms) group as a water-soluble and easily removable *N*-protecting group. An amino acid was reacted with 2-(phenylthio)ethyl chloroformate to give a 2-(phenylthio)ethyloxycarbonyl (Pte) amino acid, followed by treatment with methyl iodide and silver tetrafluoroborate to give a Pms-amino acid.

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To evaluate the Pms-amino acids, Leu-enkephalin amide was prepared by the solid phase method in water.

MATERIALS AND METHODS

General

Amino acid compositions of acid hydrolysates were determined with a Waters Pico TAG amino acid analyser, and RP-HPLC was performed using Waters 600 equipment with a DAISOPAK column and gradient system of CH_3CN/water containing 0.05% trifluoroacetic acid. TOF-mass spectra were obtained from a Shimadzu/Kratos Kompact MALDI IV spectrometer. 2-(Phenylthio)ethanol was purchased from Tokyo Kasei Kogyo Co., Ltd, Japan. Poly(ethylene glycol) grafted Rink amide resin was purchased from Watanabe Chemical Industries, Ltd, Japan.

General Procedure for Preparation of Pms-amino Acids

2-(Phenylthio)ethyl chloroformate was prepared from 2-(phenylthio)ethanol and phosgene in dichloromethane. 2-(Phenylthio)ethyl chloroformate was reacted with an amino acid to give a Pte-amino acid. This was then converted to a Pms-amino acid by the reaction of methyl iodide in the presence of silver tetrafluoroborate in acetonitrile.

Pms-Tyr-OH: amorphous material. Yield 86%, $[\alpha]_D^{24}$–2.3($c = 1.0$ CH_3CN), TOF-MS m/z: 376.4(M^+). ^{1}H-NMR(D_2O): 3.24(3H, s, S^+–CH_3).

Pms-Phe-OH: amorphous material. Yield 81%, $[\alpha]_D^{24}$–9.8($c = 1.0$ CH_3CN), TOF-MS m/z: 360.3(M^+). ^{1}H–NMR(D_2O): 3.24(3H, s, S^+–CH_3).

Pms-Gly-OH: amorphous material. Yield 73%, TOF-MS m/z: 270.1(M^+).^{1}H-NMR(D_2O): 3.24(3H, s, S^+–CH_3).

Pms-Leu-OH: amorphous material. Yield 72%, $[\alpha]_D^{24}$–8.6($c = 1.0$ CH_3CN), TOF-MS m/z:326.5(M^+). ^{1}H-NMR(D_2O): 3.24(3H, s, S^+-CH_3).

General Protocol for Peptide Synthesis by Solid Phase Synthesis

The fluorenylmethyloxycarbonyl (Fmoc) group on the starting resin was removed by treatment with 20% piperidine/dimethylformamide. Solid phase synthesis was performed according to the protocol in Table 1. *N*-Hydroxy-5-norbornene-2,3-dicarboximide (HONB) [12] was added as an additive to stimulate coupling (Table 1).

Table 1 Synthetic Protocol for the Solid Phase Synthesis of Leu-enkephalin Amide in Water

Steps	Reagents	Reaction time
1	0.2% Triton X/H_2O	3 min × 6
2	Pms-amino acid (4 eq), WSCD (4 eq) and HONB (4 eq) in 0.2% Triton X/H_2O	3 h
3	0.2% Triton X/H_2O	3 min × 6
4	H_2O	3 min × 2
5	5% $NaHCO_3$	3 min × 2 30 min × 1
6	H_2O	3 min × 3

Purification of Synthetic Leu-enkephalin Amide

Synthetic H-Tyr-Gly-Gly-Phe-Leu-resin was treated with TFA containing 3% triisopropylsilane and 3% water for 2 h, and the TFA was removed *in vacuo*. The residue was dissolved in water and washed with ethyl acetate, followed by lyophilization. The crude product was purified by HPLC using a mixture of acetonitrile and water containing 0.05% TFA as an eluent. Yield 61%, $[\alpha]_D^{27}$+8.2($c = 0.2$, H_2O). Amino acid ratios in an acid hydrolysate: Tyr 0.99, Gly 2.00, Phe 0.96 Leu 0.98 (average recovery 83%). TOF-MS m/z: 555.0(M+1)$^+$, 577.0(M+Na)$^+$.

RESULTS AND DISCUSSION

2-(Phenylthio)ethyl chloroformate was prepared from 2-(phenylthio)ethanol by a reaction with phosgene and was reacted with an amino acid to give a Pte-amino acid. The resulting Pte-amino acid was extracted by ethyl acetate from the reaction mixture and showed a single spot on TLC. The Pte-amino acid was used without further purification and was treated with methyl iodide and silver tetrafluoroborate to give a Pms-amino acid. The protecting group, Pms, was removable by treatment with a mild base (such as 5% $NaHCO_3$ or 5% Na_2CO_3) for 30 min. Since [phenyl(methyl)sulfonio]ethylene and 2-[phenyl(methyl)sulfonio]ethanol were detected in a deprotection reaction mixture by mass spectroscopy, the mechanism of the deprotection reaction might be *via* β-elimination. The product of the β-elimination reaction, [phenyl(methyl)-sulfonio]ethylene tetrafluoroborate, could be converted to 2-[phenyl(methyl)sulfonio]ethanol tetrafluoroborate in water (Figure 1).

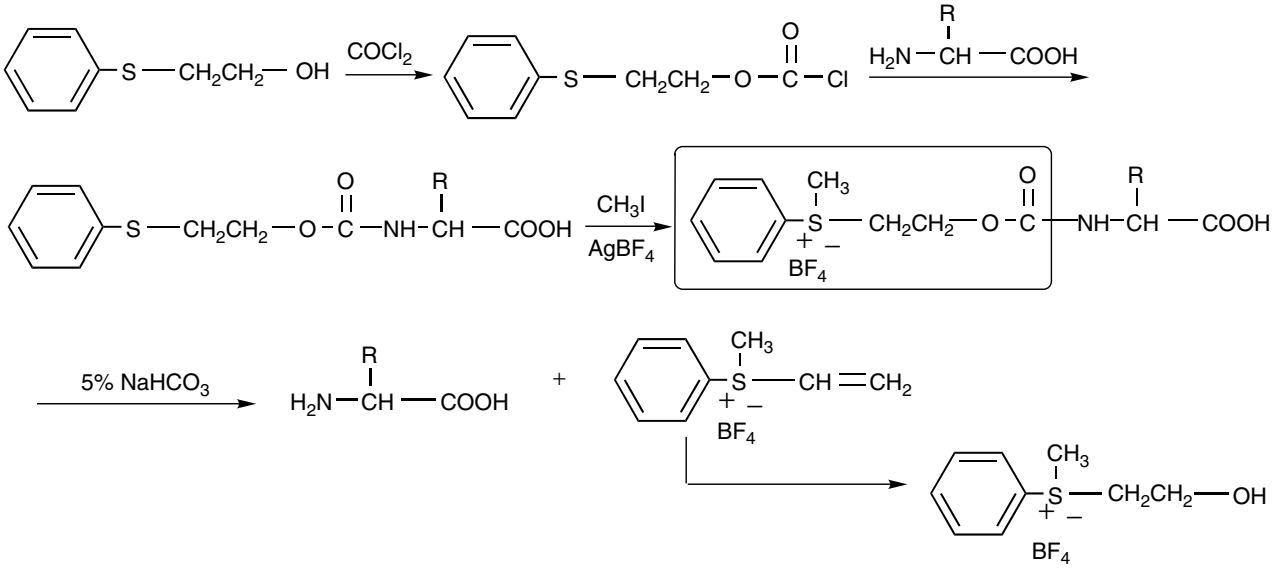

Figure 1 Synthetic scheme for the preparation and deprotection of Pms-amino acids.

To evaluate the protecting group, Leu-enkephalin amide was prepared by the solid phase method in water. Pms-Tyr-OH, Pms-Gly-OH, Pms-Phe-OH and Pms-Leu-OH were prepared. These Pms-amino acids were readily soluble in water. For solid phase synthesis in water, a resin which swells with water is necessary. Recently, poly(ethylene glycol) grafted polystyrene resins [13] were developed to increase the swelling of the resin in various solvents, including water. The poly(ethylene glycol) grafted Rink amide polystyrene resin was used for the present synthesis. Coupling reactions were performed with a water-soluble carbodiimide (WSCD, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride) [14] in the presence of HONB in water, containing 0.2% Triton X (Triton X was added to increase swelling of the resin). *N*-Hydroxysuccinimide (HOSu) was also used as an additive instead of HONB for coupling reactions, but the reaction was accompanied by a side reaction. Since β-Ala was always found in an acid hydrolysate of synthetic Leu-enkephalin amide when HOSu was used, a Lossen rearrangement reaction might be happening as the side reaction [15,16]. The Pms group was removed by treatment with aqueous 5% $NaHCO_3$ solution for 30 min. It was also removable by treatment with aqueous 5% Na_2CO_3 and aqueous 5% piperidine. The synthetic H-Tyr-Gly-Gly-Phe-Leu-resin was treated with TFA to cleave the peptide from the resin, followed by HPLC purification with an ODS column. The HPLC profile of the synthetic Leu-enkephalin amide is shown in Figure 2.

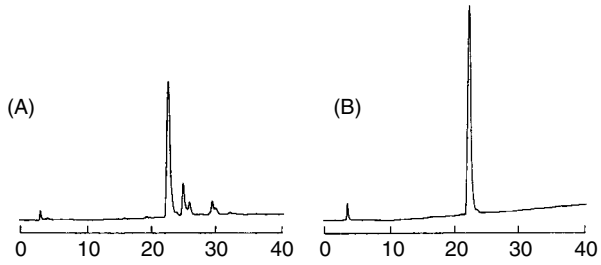

Figure 2 HPLC profile of the crude (A) and the purified (B) Leu-enkephalin amide. Column, DAISOPAK SP-120-5-ODS-B (4.6 × 250 mm). Flow rate, 1 mL/min. Eluent, CH_3CN/H_2O containing 0.05% TFA. Gradient: 10/90 → 50/50(40 min). OD at 220 nm.

The retention time of the synthetic Leu-enkephalin amide corresponded 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 resin was 61%. Leu-enkephalin amide was successfully prepared using Pms-amino acids by the solid phase method in water. However one problem remains. Using the synthesis method outlined here for Pms-amino acids, Pms-Met and Pms-Cys cannot be prepared. The preparation of Pms-amino acids including Met and Cys *via* a coupling of 2-[phenyl(methyl)sulfonio]ethyl chloroformate tetrafluoroborate and an amino acid is currently being investigated.

CONCLUSIONS

A new water soluble *N*-protecting group, Pms group, has been developed and its application to solid phase peptide synthesis has been evaluated. We were successful in preparing Leu-enkephalin amide by the solid phase method in water. This report may be the first report of successful solid phase peptide synthesis in water, and the Pms group is likely to be useful not only for peptide synthesis but also for general organic synthesis in aqueous media. Synthesis in water using reagents of low toxicity is likely to have future environmental advantages, in that it will cause a decrease in organic waste produced from organic synthesis techniques.

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