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2-(4-Sulfophenylsulfonyl)ethoxycarbonyl group: a new water-soluble *N*-protecting group and its application to solid phase peptide synthesis in water

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Abstract—Solid phase peptide synthesis is carried out in organic solvents, creating environmental problems after disposal. To avoid this problem, we aimed to perform solid phase peptide synthesis in water. A new water-soluble *N*-protecting group, 2-(4-sulfophenylsulfonyl)ethoxycarbonyl (Sps) group, was designed and Sps-amino acids were prepared. To evaluate the utility of this technique, Leu-enkephalin amide was prepared by solid phase synthesis using Sps-amino acids in water.

Solid phase synthetic methods have made peptide synthesis simple, rapid, and easily subject to automation. However this procedure requires a large amount of organic solvents, raising environmental concerns over disposal. In this report we have investigated how to perform peptide synthesis in water. To carry out peptide synthesis in water, water-soluble protected amino acids are needed. Various water-soluble *N*-protecting groups have been reported, including the methylsulfonylethoxycarbonyl group described by Tesser and Balvert-Geers,[1] and the 2-(triphenylphosphonio)ethoxycarbonyl,[2] 2-(triphenylphosphonio)isopropyloxycarbonyl,[3] and 2-(4-pyridyl)ethoxycarbonyl[4] groups reported by Kunz et al. Kunz has prepared a tripeptide, 2-[diphenyl(methyl)phosphonio]ethoxycarbonyl-Leu-Phe-Phe-O-*t*-Bu by a solution method in water.[2] Kunz also reported that the 2-(methylthio)ethoxycarbonyl group could be removed under mild basic conditions after treatment with methyl iodide.[5] We previously reported the preparation of water-soluble *N*-protected amino acids, 2-[phenyl(methyl)sulfonio]ethoxycarbonylamino acids (Pms-amino acids)[6] and 2-ethanesulfonylethoxycarbonylamino acids (Esc-amino acids)[7] and their application to solid phase peptide synthesis in water. We also reported the preparation of water-soluble active esters, 4-sulfophenyl ester derivatives.[8] Here, we have designed a new water-soluble *N*-protecting group, 2-(4-sulfophenylsulfonyl)ethoxycarbonyl (Sps) group, based on this water-soluble active ester (Fig. 1).

Sps-amino acids were prepared as shown in Figure 2. 2-Phenylthioethanol (**1**) was reacted with 4-nitrophenyl chloroformate to form 2-phenylthioethyl 4-nitrophenyl carbonate (Pte-ONp, **2**),[9a] which was then subjected to sulfonation to give 2-(4-sulfophenylthio)ethyl 4-nitrophenyl carbonate (Spt-ONp, **3**).[9b] **3** was converted to its sodium salt and then reacted with an amino acid to form 2-(4-sulfophenylthio)ethoxycarbonylamino acid (Spt-amino acid), which was then subjected to oxidation with hydrogen peroxide/trifluoroacetic acid to form Sps-amino acid (Route 1). Since methionine and cysteine contain sulfur, Sps-derivative of these amino acids cannot be obtained via Route 1. Alternatively, **3** was oxidized to form 2-(4-sulfophenylsulfonyl)ethyloxycarbonyl 4-nitrophenyl carbonate (**6**)[9c] which was converted to its sodium salt, and then coupled with an amino acid to give the Sps-amino acid (Route 2).

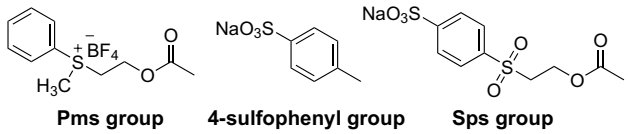

Figure 1. Pms-,[6] 4-sulfophenyl-[8] and Sps-groups.

Keywords: 2-(4-Sulfophenylsulfonyl)ethoxycarbonyl group; Water-soluble *N*-protecting group; Peptide synthesis in water.
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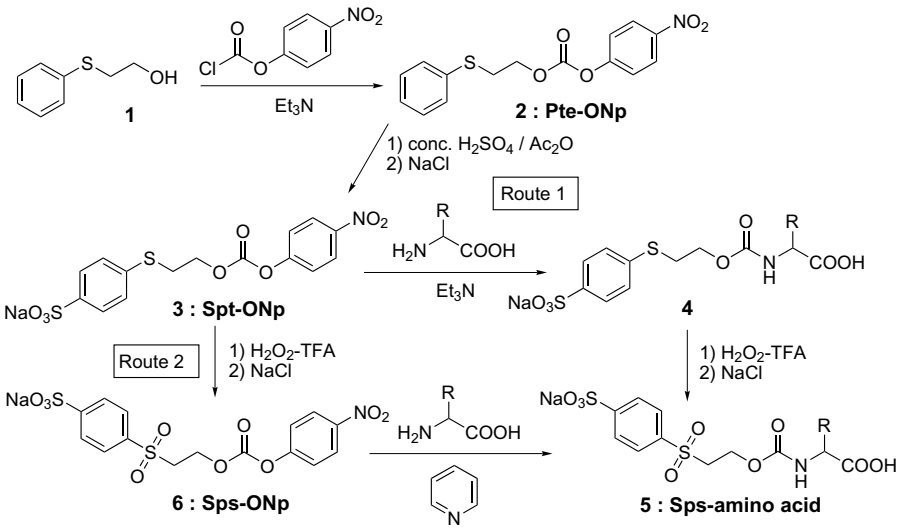

Figure 2. Synthetic scheme for Sps-amino acids.

Sps-Phe-OH,[10a] Sps-Gly-OH,[10b] and Sps-Leu-OH[10c] were prepared via Route 1, and Sps-Tyr(*t*-Bu)-OH[10d] was prepared via Route 2. All synthetic Sps-amino acids and their corresponding sodium salts are soluble in water. The sodium salts are stable crystalline solids. We previously reported the preparation of water-soluble *N*-protected amino acids, Pms-[6] and Esc-amino acids,[7] and their application to solid phase peptide synthesis in water. However, Pms-amino acids were rather unstable due to their onium form and Esc-amino acids (except Esc aromatic amino acids) were not detectable by measurement of optical absorption. In contrast, the Sps group is stable and can be detected by UV absorption. Therefore Sps-amino acids display characteristics suitable for automatic solid phase peptide synthesis in water.

The Sps group can be removed by treatment with a mild base, such as aqueous 5% Na_2CO_3, in water. Deprotection of Sps-amino acids was tested by treating Sps-Phe-OH either with aqueous 5% Na_2CO_3 or aqueous 5% $NaHCO_3$ or aqueous 0.025 M NaOH at room temperature, as shown in Figure 3. The Sps group on Phe was totally removed within 5 min by treatment with 5% Na_2CO_3 or 0.025 M NaOH, whereas removal was incomplete using 5% $NaHCO_3$ even after 40 min.

To evaluate the utility of Sps-amino acids, Leu-enkephalin amide (H-Tyr-Gly-Gly-Phe-Leu-NH_2) was prepared according to the protocol shown in Table 1 on a TentaGel resin [poly(ethylene glycol) grafted polystyrene resin][11] that swelled well in water. Sps-amino acids were used as their sodium salt and coupling reactions were performed with a water-soluble carbodiimide [WSCD, 1-ethyl-3(3-dimethylaminopropyl)carbodiimide hydrochloride][12] in the presence of *N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB)[13] to accelerate the reaction. Aqueous 0.2% Triton X solution was used as a solvent to increase swelling ability of the resin and solubility of Sps-amino acids. Removal of the Sps protection group was performed with 0.025 M NaOH in aqueous 50% ethanol for 3 min(× 2). The synthetic H-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-TentaGel resin was treated with trifluoroacetic acid to cleave the peptide from the resin. The liberated peptide was purified by HPLC using

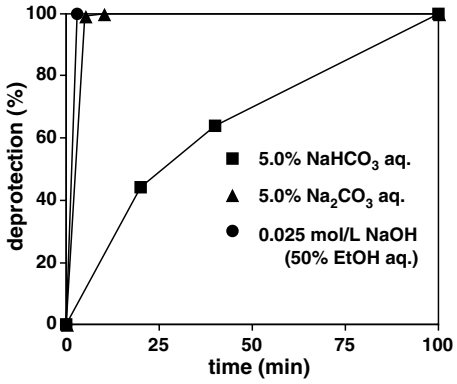

Figure 3. Deprotection of Sps-Phe-OH. Sps-Phe-OH (4.8 mg, 10 μmol) was dissolved in aqueous 5.0% $NaHCO_3$ (400 μL) or aqueous 5.0% Na_2CO_3 or aqueous 0.025 M NaOH and the solution was stirred at room temperature. An aliquot (10 μL) was taken periodically for analysis by HPLC. The rate of deprotection was calculated by measurement of the peak area of Sps-Phe-OH.

Table 1. Synthetic protocol for the solid phase synthesis of Leu-enkephalin amide in water

Step		Reagents	Time
1	Wash	H_2O	3 min × 2
2	Deprotection	0.025 M NaOH/aq 50% EtOH	3 min × 2
3	Wash	H_2O	3 min × 2
4	Wash	Aq 0.2% Triton X	3 min × 3
5	Coupling reaction	Sps-amino acid, WSCD, HONB, DIEA in aq 0.2% Triton X	2 h × 2
6	Wash	Aq 0.2% Triton X	3 min × 3

Four times molar quantity of each reagent (Sps-amino acid, WSC, DIEA and HONB) calculated from amino content of the used resin was used.

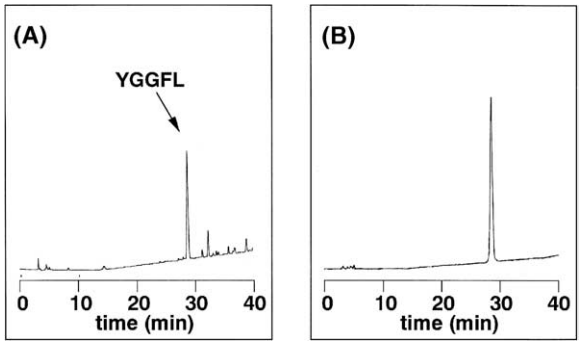

Figure 4. HPLC profiles of synthetic Leu-enkephalin amide. (A) Crude Leu-enkephalin amide. Column, DAISOPAK SP-120-5-ODS-B (20 × 250 mm). Flow rate, 10 mL/min. Eluent, CH_3CN/H_2O containing 0.05% TFA. Gradient: 10/90–50/50 (40 min). (B) Analytical HPLC of purified sample. Column, DAISOPAK SP-120-5-ODS-B (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). OD at 220 nm.

an ODS column. The yield of synthetic Leu-enkephalin amide[14] was 61% as calculated from the amino group content of the starting resin (Fig. 4).

In conclusion, we have designed a new water-soluble protecting group, the Sps group, and verified its utility by successful solid phase synthesis of Leu-enkephalin amide in water. We have compared the Sps group with our previously reported water-soluble *N*-protecting groups. The Sps group is more stable than the Pms group and the UV absorption properties are preferable to those of the Esc group for detection. Further work should aim to develop application of Sps-amino acids to preparation of large peptides.

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9. (a) Pte-ONp: Yield 85%. mp 43–45 °C. ^{1}H NMR δ (CD_3OD): 8.29 (2H, d-like, J = 9.3 Hz), 7.44 (2H, d-like, J = 7.1 Hz), 7.43 (2H, d-like, J = 9.3 Hz), 7.31 (2H, dd, J = 7.3, 7.1 Hz), 7.22 (1H, t, J = 7.3), 4.40 82H, t, J = 6.7 Hz), 3.28 (2H, t, J = 6.7 Hz). ESI-Mass *m/z*: Calcd for $C_{15}H_{17}N_2O_5S$ ($[M + NH_4]^+$) 337.09, Found: 337.1.
(b) Spt-ONp: Yield: 79.7%. mp 193–194 °C (dec.) amorphous material. ^{1}H NMR δ (CD_3OD): 8.30 (2H, d, J = 9.3 Hz), 7.77 (2H, d, J = 8.4 Hz), 7.47 (2H, d, J = 8.3 Hz), 7.43 (2H, d, J = 9.3 Hz), 4.45 (2H, t, J = 6.6 Hz), 3.70 (2H, t, J = 6.6 Hz), TOF-MS *m/z*: Calcd $C_{15}H_{12}NO_8S_2$ ($[M - H]^-$) 398.39. Found: 398.48.
(c) Sps-ONp: Yield: 80.3%. mp 171–174 °C (dec.). ^{1}H NMR δ (CD_3OD): 8.29 (2H, d, J = 8.2 Hz), 8.15 (2H, m), 8.03 (2H, m), 7.31 (d, J = 8.2 Hz), 4.70 (2H, t-like), 3.96 (2H, t-like). TOF-MS *m/z*: Calcd $C_{15}H_{12}NO_{10}S_2$ ($[M - H]^-$) 430.40. Found: 430.57.
10. (a) Sps-Phe-OH: Yield 77%. mp 242–245 °C (dec.). $[\alpha]_D^{26}$ −5.2 (c = 1.0, H_2O). ^{1}H NMR δ (CD_3OD): 8.04 (2H, dd, J = 8.5, 1.1 Hz), 7.95 (2H, dd, J = 8.5, 1.1 Hz), 7.23 (5H, m), 4.34 (1H, m), 4.28 (1H, m), 4.28 (1H, m), 3.25 (2H, t-like), 3.25 (1H, m), 2.90 (1H, m). TOF-MS *m/z*: Calcd for $C_{18}H_{18}NO_9S_2$ ($[M - H]^-$) 456.48. Found: 456.37.
(b) Sps-Gly-OH: Yield 68%. mp 219–222 °C (dec.). ^{1}H NMR δ (CD_3OD): 8.04 (2H, d, J = 8.6 Hz), 8.00 (2H, d, J = 8.6 Hz), 4.38 (2H, t, J = 5.7 Hz), 3.70 (2H, s), 3.64 (2H, t, J = 5.7 Hz). TOF-MS *m/z*: Calcd for $C_{11}H_{12}NO_9S_2$ ($[M - H]^-$) 366.35. Found: 366.24.
(c) Sps-Leu-OH: Yield 70%. mp 238–242 °C (dec.). $[\alpha]_D^{26}$ −22.7 (c = 1.0, H_2O). ^{1}H NMR δ (CD_3OD): 8.05 (2H, d, J = 8.2 Hz), 8.01 (2H, d, J = 8.2 Hz), 4.37 (2H, m), 4.10 (1H, m), 3.62 (2H, t, J = 5.8 Hz), 1.67 (1H, m), 1.55 (1H, m), 0.92 (6H, m). TOF-MS *m/z*: Calcd for $C_{15}H_{20}NO_9S_2$ ($[M - H]^-$) 422.46. Found: 422.29.
(d) Sps-Tyr(*t*-Bu)-OH: Yield 68%. mp 248–250 °C (dec.). $[\alpha]_D^{26}$ −5.6 (c = 1.0, H_2O). ^{1}H NMR δ : 8.04 (1H, dd, J = 8.4, 1.1 Hz), 7.95 (2H, d, J = 8.4 Hz), 7.03 (2H, J = 8.5, 1.1 Hz), 6.71 (2H, J = 8.5, 1.1 Hz), 4.26 (1H, m), 4.24 (2H, m), 3.55 (2H, m), 3.03 (1H, m), 2.80 (1H, m), 1.25 (9H, m). TOF-MS *m/z*: Calcd for $C_{22}H_{26}NO_{10}S_2$ ($[M - H]^-$) 528.59. Found: 528.56.
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14. $[\alpha]_D^{24}$ +8.2 (c = 0.2, H_2O). TOF-MS *m/z* 577.0 ($[M + Na]^+$, $C_{28}H_{39}N_6O_6Na$ requires 577.64). Amino acid ratios in an acid hydrolysate: Tyr, 0.98; Gly, 2.00, Phe, 0.96; Leu, 1.01. (average recovery: 88%).