1.76-1.84 (m, 1 H), 1.40-1.56 (m, 1 H), 1.01-1.18 (m, 1 H); ^{31}P NMR (121.4 MHz, $CDCl_3$) δ 63.5; ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.3 (J_{PC} = 13.2), 139.6 (J_{PC} = 12.9), 131.4 (J_{PC} = 8.4), 130.0, 129.9, 128.87, 128.64, 128.5, 128.3, 127.1, 126.5, 126.1, 44.5 (J_{PC} = 59.6), 39.3 (J_{PC} = 65), 35.5 (J_{PC} = 52.1), 34.8, 29.4 (J_{PC} = 7.5), 28.8 (J_{PC} = 10.5); MS (CI, CH_4) m/e 375 (MH^+, base peak). Anal. Calcd for $C_{25}H_{27}OP$: C, 80.19; H, 7.27. Found: C, 79.73; H, 7.07.

Acknowledgment. We gratefully acknowledge financial support for this project provided by the donors of the Petroleum Research Fund, administered by the American Chemical Society. We thank Mr. Stephen Belmont for carrying out the APT-HETCOR spectral experiments and Professor L. D. Quin of the University of Massachusetts for helpful discussions on preparation of the manuscript.

Supplementary Material Available: ^1H NMR spectra of compounds **2a**, **2b**, **2c**, **2d**, **2e**, **11a**, **11b**, and **11c** and ^{13}C spectra of compounds **2d** and **2e** (10 pages). Ordering information is given on any current masthead page.

Cysteine Alkylation in Unprotected Peptides: Synthesis of a Carbavasopressin Analogue by Intramolecular Cysteine Alkylation

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Received August 17, 1990

An expedient method that allows modification of a cysteine residue side chain through alkylation of unprotected peptides is described. In order to test the generality of the method, an unprotected cysteine-containing tetrapeptide possessing several potentially competing nucleophilic functionalities such as amino, hydroxy, and carboxyl groups was chosen as a model peptide. The model tetrapeptide, H-Ser-Lys-Cys-Phe-OH was synthesized by standard solid-phase methods, cleaved with HF, and purified by reverse-phase HPLC. Reaction of the unprotected peptide with 1.3 equiv of various alkylating agents in saturated ammonia in methanol at 0 °C proceeded cleanly to yield single alkylation products within one hour as shown by HPLC analysis. In most cases acidic, basic, and neutral side chains were introduced by this method in over 80% yield. The methodology is also applicable to the synthesis of cyclic peptides by intramolecular cysteine alkylation and provides a useful alternative to cyclizations that occur through disulfide or amine bond-forming reactions as illustrated by the synthesis of a carbavasopressin.

Introduction

The derivatization of natural products such as steroids and alkaloids is a well-developed and widely used approach in medicinal chemistry. However, selective modification of amino acid side chain functional groups in protected or unprotected peptides has been much less frequently reported. Instead, the unnatural amino acids or side chain modified amino acids are frequently prepared by separate syntheses and assembled into peptides. The synthesis of many unnatural amino acids can often be rather difficult and time consuming. We became interested in selective side chain derivatization of unprotected peptides because it allows the expedient preparation of many peptide analogues for structure–activity relationship studies. Selective modification of cysteine in unprotected peptides is most compelling since a considerable amount of thiol chemistry has been developed and extensively used in protein chemistry and affinity labeling.[1,2] Selective alkylation of the thiol functionality is possible due to its high nucleophilic reactivity relative to other amino acid side chains. Although much of the thiol chemistry applied to protein derivatization should also be applicable to cysteine in small peptides, derivatization of cysteine in unprotected peptides has not found widespread use. In this paper we report the selective thiol alkylation of an unprotected model tetrapeptide with various alkylation agents in good yields. The methodology is also applicable to the synthesis of cyclic peptides through intramolecular cysteine alkylation and provides a useful alternative to cyclizations that occur through disulfide or amide bond-forming reactions.[3] As an illustration of the cyclization methodology, we report the synthesis of a carbavasopressin.

Results and Discussion

In order to test the general applicability of the cysteine alkylation methodology, an unprotected cysteine-containing peptide possessing several potentially competing nucleophilic functionalities such as amino, hydroxy, and carboxyl groups was chosen as a model peptide. The model tetrapeptide, H-Ser-Lys-Cys-Phe-OH–2 trifluoroacetic acid (TFA), **1**, was synthesized by standard solid-phase methods using Merrifield resin, cleaved with anhydrous hydrogen fluoride (HF), and purified by reverse-phase high-performance liquid chromatography (RPHPLC). After lyophilization, the purified peptide was obtained as a white amorphous powder and could be stored under nitrogen in the freezer (−20 °C) for more than 6 months without any sign of disulfide formation by RPHPLC analysis.

For a typical alkylation, reaction of unprotected peptide **1** with 1.3 equiv of various alkylating agents in methanol saturated with ammonia at 0 °C proceeded cleanly to yield single monoalkylation products within 1 h as shown by RPHPLC analysis. For the less reactive alkylating agent ethyleneimine, a large excess of reagent was used in dimethylformamide (DMF) saturated with ammonia at room temperature. Basic, acidic, and neutral side chains were introduced by this method in over 80% isolated yield. All the alkylation products were characterized by NMR, FABMS, elemental, and amino acid analyses. The indication of selective thiol alkylation was provided by exam-

(1) Means, G. E.; Feeney, R. E. *Chemical Modifications of Proteins*; Holden-Day: San Fransico, 1971.
(2) Jacoby, W. B., Wilchek, M., Eds. *Methods in Enzymology, 46*; Academic Press: New York, 1977.
(3) Buku, A.; Schwartz, I. L. *J. Protein Chem.* **1985**, *4*(3), 163.

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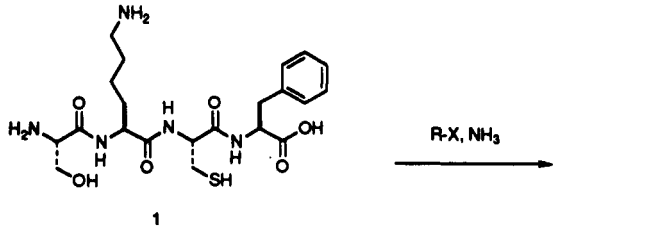
ining the NMR of the alkylated peptides and further verified by amino acid analysis.



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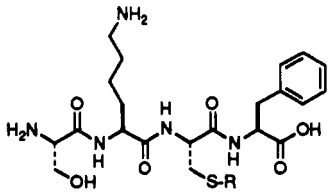

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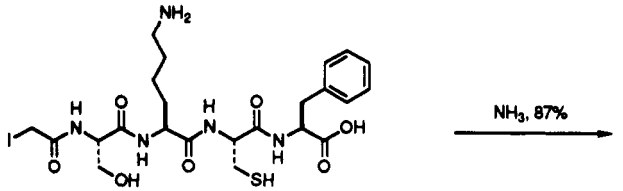
	R-X	R	Yield, %
a.	$ClCH_2CO_2H$	CH_2CO_2H	84
b.	$CH_2{=}CHCO_2Me$	$CH_2CH_2CO_2Me$	80
c.	$BrCH_2Ph$	CH_2Ph	84
d.		$CH_2CH_2NH_2$	81

The methodology is also applicable to the synthesis of cyclic peptides by intramolecular cysteine alkylation. Iodoacetyl-Ser-Lys-Cys-Phe-OH·TFA, **3**, prepared and purified as described before, was cyclized cleanly to **4** in refluxing liquid ammonia in 87% isolated yield. Confir-



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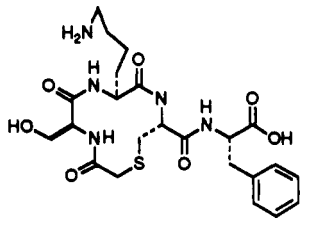

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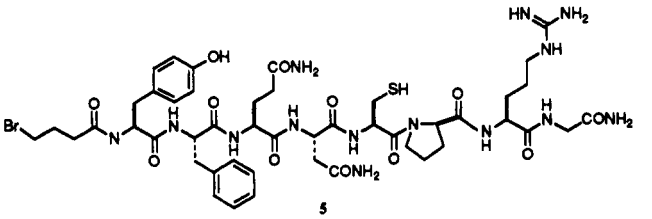
mation of selective intramolecular cysteine alkylation was provided by FABMS, NMR, and amino acid analyses. It is interesting to note that the intramolecular cyclization through cysteine alkylation to form a 12-membered ring is preferred over the formation of a 6-membered ring through serine alkylation. In order to test the applicability and limitations of the intramolecular cyclization methodology, a carbavasopressin analogue was synthesized. Carbavasopressin is an analogue of arginine-vasopressin (AVP) in which the disulfide bond of a 20-membered ring is replaced by the corresponding CH_2–S linkage. Carbavasopressin analogues have been prepared through solution methods[4] and more recently through cyclization on the peptide resin.[5] Both reported methods involved the cyclization of the linear peptide by intramolecular amide bond formation. The current synthesis of cyclic peptides using intramolecular cysteine alkylation provides a useful alternative. The linear peptide precursor, 4-bromobutyryl-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2·TFA **5** was prepared, purified, and cyclized as previously described. Carbavasopressin was prepared in 65% isolated yield by cyclization of **5** in refluxing liquid ammonia for 6 h. The product was characterized by high-resolution FABMS and amino acid analysis.



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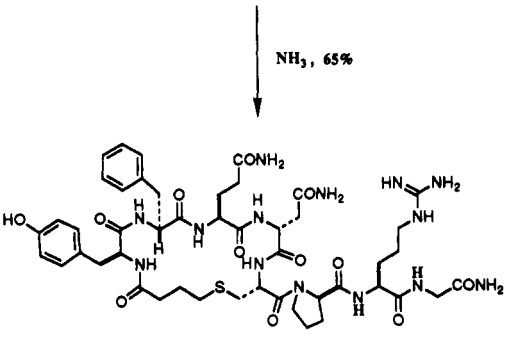

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Experimental Section

General Methods. Protected amino acids were purchased from Bachem (Torrance, CA). *N*-α-t-Boc-L-phenylalanine Merrifield resin (0.6 mequiv/g) was purchased from Bachem and *N*-α-t-Boc-glycine PAM resin (0.68 mequiv/g) was purchased from Peninsula Laboratories, Inc. Anisole, diisopropylethylamine, diisopropylcarbodiimide, trifluoroacetic acid, benzyl bromide, chloroacetic acid, methyl acrylate, and 4-bromobutyryl chloride were purchased from Aldrich Chemical Co. and used without further purification. *N,N*-Dimethylformide, methylene chloride, and acetonitrile were purchased from Fisher Scientific (Fairlawn, NJ). Ethyleneimine was purchased from Eastman Kodak and used without further purification. Solid-phase peptide synthesis was performed with an automated Biosearch 9500 Peptide Synthesizer.[5] Reverse-phase high-pressure chromatography was performed with a Waters Delta Prep 3000 System [column: 21.4 mm i.d. × 25cm, Dynamax (Rainin), 8μ silica, C-18 reverse-phase column]. The sample was purified by gradient elution (from 10% to 60% acetonitrile in water with 0.2% TFA (v/v) saturated with Helium, in 35 min) at a flow rate of 18 mL per minute. UV detection was run at 220 nm. Peptides **1** and **3** were purified by RPHPLC with Dynamax Preparative Column (i.d.: 41.1 mm, 8 m silica, C-18 reverse-phase column). The sample was purified by gradient elution (from 10% to 50% acetonitrile with 0.1% TFA (v/v) saturated with Helium, in 50 min) at a flow rate of 45 mL per minute. Care should be taken to exclude oxygen during the purification process in order to prevent the oxidation of sulfur in peptides. After purification, samples were frozen using a dry ice–2-propanol bath and lyophilized to give white powders. Proton magnetic resonance spectra were run at 300 MHz. Elemental analyses, mass spectra, and the above determinations were performed by the Analytical Research Department, Abbott Laboratories. Amino acid analyses were performed on a Beckman Model 6300 amino acid analyzer. The peptides were hydrolyzed with 6 N HCl containing 0.3% phenol at 150 °C for 2 h. The

(4) (a) Jost, K.; Prochazka, Z.; Cort, J. H.; Barth, T.; Skopkova, J.; Prusik, Z.; Sorm, F. *Collect. Czech. Chem. Commun.* **1974**, *39*, 2835. (b) Prochazka, Z.; Barth, T.; Cort, J. H.; Jost, K.; Sorm, F. *Ibid.* **1977**, *43*, 655.

(5) (a) Buku, A.; Gaqzis, D.; Schwartz, I. L. *Abstracts of Papers*, the Eighth American Peptide Symposium, Tuscon, AZ, 1983, Abstract 10-7, p 249. (b) Lebl, M.; Hruby, V. J. *Tetrahedron Lett.* **1984**, *104*, 2607.

content of serine was generally low. Abbreviations: *N*-α-t-Boc-L-*O*-benzyl-serine, Boc-Ser(Bzl); *N*-α-t-Boc-*N*-ε-(2-chloro-Cbz)-L-lysine, Boc-Lys(Cl-Z); *N*-α-t-Boc-*S*-*p*-methoxybenzyl-L-cysteine, Boc-Cys(MeOBzl); *N*-α-t-Boc-L-phenylalanine, Boc-Phe; *N*-α-t-Boc-L-glutamine, Boc-Gln; *N*-α-t-Boc-L-asparagine, Boc-Asn; *N*-α-t-Boc-L-proline, Boc-Pro; *N*-α-t-Boc-N^g-*p*-tosyl-L-arginine, Boc-Arg(Tos); *N*-α-t-Boc-*O*-benzyl-L-tyrosine, Boc-Tyr(Bzl); *S*-carboxymethylcysteine; Cys(Cm); *S*-($CH_2CH_2NH_2$)cysteine, thioLys; iodo acetyl, IAc. The number of TFA and H_2O in the formula of products were determined by CHN analysis.

H-Ser-Lys-Cys-Phe-OH·2TFA (1). The protected peptide resin intermediate H-Ser(Bzl)-Lys(Cl-Z)-Cys(MeOBzl)-Phe-Merrifield resin was prepared in four cycles of solid-phase synthesis with an automated Biosearch 9500 Peptide Synthesizer using 2 g of Boc-Phe-Merrifield resin (0.6 mequiv/g). The first cycle was the deprotection with 45% TFA in methylene chloride containing 2.5% anisole (v/v/v), neutralization with 10% diisopropylethylamine in methylene chloride (v/v), and coupling with the next amino acid. Coupling was accomplished using 0.2–0.4 M Boc-amino acid derivatives in DMF, with 0.2–0.4 M diisopropylcarbodiimide in methylene chloride over a reaction time of 1–2 h. Following the synthesis, the protected peptide resin was removed from the reaction vessel by washing with DMF (3×) into a fritted-glass funnel, followed by washing the resin with methanol (3×) and methylene chloride (3×). The resin was dried under vacuum for 2 h before cleavage. The protected peptide-resin intermediate in anisole (4 mL) was cleaved and deprotected using anhydrous HF (40 mL) at 0 °C for 1 h using a Teflon Kel-F vacuum line in a well-ventilated hood. The HF was evaporated, and the residue was extracted from the resin with 20% degassed aqueous acetic acid (4×30 mL), lyophilized to a dry amorphous powder, and purified by RPHPLC. After removal of solvent by lyophilization, 430 mg of the purified peptide was obtained as a white powder, 50.3% yield. FABMS: $(M + H)^+$ calcd for $C_{21}H_{34}N_5O_6S$ *m/e* 484.2230, measured 484.2225. Anal. Calcd for $C_{21}H_{33}N_5O_6S$·2TFA: C, 42.19; H, 4.92; N, 9.85. Found: C, 42.04; H, 4.91; N, 9.82. Amino acid Anal.: Ser, 0.51; Cys, 0.51; Phe, 1.01; Lys, 0.99. NMR (D_2O): δ 1.25–1.45 (m, 2 H), 1.60–1.85 (m, 4 H), 2.77–2.98 (m, 4 H), 3.04 (dd, *J* = 9, 13.8 Hz, 1 H), 3.24 (dd, *J* = 6, 13.8 Hz), 3.90–4.05 (m, 2 H), 4.16 (dd, *J* = 4.5, 6 Hz, 1 H), 4.37 (t, *J* = 7.5 Hz, 1 H), 4.47 (dd, *J* = 6, 7.5 Hz, 1 H), 4.61 (dd, *J* = 5, 9 Hz, 1 H), 7.23–7.43 (m, 5 H).

H-Ser-Lys-Cys(CM)-Phe-OH·2TFA·1.5H_2O (2a). Tetrapeptide 1 (22 mg, 0.031 mmol) was dissolved in methanol (1 mL) saturated with ammonia under nitrogen at room temperature and then cooled to 0 °C. The solution turned into a gellike suspension upon cooling. Chloroacetic acid (4 mg, 0.042 mmol) was added with stirring. After 1 h at 0 °C, the reaction mixture was allowed to warm to room temperature at which time the reaction was complete by RPHPLC analysis of a worked up aliquot. The reaction mixture was dissolved in 6 mL of water, and the pH was adjusted to 2 with TFA. Purification by RPHPLC and lyophilization provided **2a** as a white powder, 20 mg, 84% yield. FABMS: $(M + H)^+$ calcd for $C_{23}H_{36}N_5O_8S$ *m/e* 542.2284, measured 542.2227. Anal. Calcd for $C_{23}H_{35}N_5O_8S$·2TFA·1.5H_2O: C, 40.25; H, 5.06; N, 8.79. Found: C, 40.36; H, 4.66; N, 8.79. Amino acid Anal.: Ser, 0.50; Cys(Cm), 0.94; Phe, 1.00; Lys, 1.05. NMR (D_2O): δ 1.25–1.45 (m, 2 H), 1.60–1.90 (m, 4 H), 2.80–3.05 (m, 4 H), 3.05 (dd, *J* = 9, 15 Hz, 1 H), 3.25 (dd, *J* = 6, 15 Hz, 1 H), 3.40 (s, 2 H), 3.98 (ddd, *J* = 4.5, 6, 12 Hz, 2 H), 4.15 (dd, *J* = 4.5, 6 Hz, 1 H), 4.38 (t, *J* = 7.5 Hz, 1 H), 4.51 (dd, *J* = 6, 9 Hz, 1 H), 4.62 (dd, *J* = 5.5, 9 Hz, 1 H), 7.20–7.45 (m, 5 H).

H-Ser-Lys-Cys($CH_2CH_2CO_2CH_3$)Phe-OH·2TFA (2b). Tetrapeptide 1 (22 mg, 0.031 mmol) was dissolved in methanol saturated with ammonia (1 mL) at room temperature and then cooled to 0 °C. Methyl acrylate (4 μL, 3.8 mg, 0.044 mmol, neat) was added, and the reactioon mixture was stirred with cooling under nitrogen. The reaction was complete within 1 h at 0 °C as shown by RPHPLC analysis. Compound **2b** was purified and characterized as described before. Yield: 19.8 mg, 80.2%. FABMS: $(M + H)^+$ calcd for $C_{25}H_{40}N_5O_8S$ *m/e* 570.2597, measured 570.2601. Anal. Calcd for $C_{25}H_{39}N_5O_8S$·2TFA: C, 42.70; H, 5.31; N, 8.59. Found: C, 42.74; H, 5.03; N, 8.58. Amino acid Anal.: Ser, 0.54; Cys($CH_2CH_2CO_2H$), 0.95; Phe, 1.05; Lys, 1.00. NMR (D_2O): δ 1.30–1.40 (m, 2 H), 1.60–1.80 (m, 4 H), 2.63–2.73 (m, 2 H), 2.73–2.85 (m, 3 H), 2.90–2.98 (m, 3 H), 3.02 (dd, *J* = 7.5, 15 Hz, 1 H), 3.20 (dd, *J* = 6, 15 Hz, 1 H), 3.70 (s, 3 H), 3.90–4.05 (m, 2 H), 4.46 (t, *J* = 7.5 Hz, 1 H), 4.48 (dd, *J* = 6, 9 Hz, 1 H), 4.56 (dd, *J* = 6, 9 Hz, 1 H), 7.22–7.41 (m, 5 H).

H-Ser-Lys-Cys(Bzl)-Phe-OH·2TFA (2c). Tetrapeptide 1 (22 mg, 0.031 mmol) was dissolved in methanol (1 mL) saturated with ammonia under nitrogen at room temperature and cooled to 0 °C with an ice–water bath. Benzyl bromide (5 μL, 7.15 mg, 0.041 mmol, neat) was added and the reaction mixture stirred at 0 °C for 1 h. The reaction was monitored and purified as described previously. Yield: 21 mg, 84%. FABMS: $(M + H)^+$ calcd for $C_{28}H_{40}N_5O_6S$ *m/e* 574.2699, measured 574.2704. Anal. Calcd for $C_{28}H_{39}N_5O_6S$·2TFA: C, 47.91; H, 5.12; N, 8.74. Found: C, 47.71; H, 5.11; N, 8.76. Amino acid Anal.: Ser, 0.60; Phe, 1.01; Cys(Bzl), 0.90; Lys, 1.04. NMR (D_2O): δ 1.25–1.45 (m, 2 H), 1.58–1.85 (m, 4 H), 2.68 (dd, *J* = 9, 15 Hz, 1 H), 2.81 (dd, *J* = 6, 15 Hz, 1 H), 2.92 (t, *J* = 7.5 Hz, 2 H), 3.02 (dd, *J* = 8.5, 15 Hz, 1 H), 3.20 (dd, *J* = 6, 15 Hz, 1 H), 3.77 (s, 2 H), 3.95 (ddd, *J* = 4.5, 6, 12 Hz, 1 H), 4.30–4.40 (m, 2 H), 4.55 (dd, *J* = 5, 9 Hz, 1 H), 7.20–7.40 (m, 10 H).

H-Ser-Lys-Cys($CH_2CH_2NH_2$)-Phe-OH·3TFA·0.5H_2O (2d). Tetrapeptide 1 (22 mg, 0.031 mmol) was dissolved in saturated ammonia in DMF (0.2 mL) at room temperature under nitrogen and cooled to 0 °C with an ice–water bath. Ethyleneimine (100 μL) was added and stirred for 5 min at 0 °C and 2 h at room temperature. RPHPLC analysis of aliquot workup showed the presence of a major new peak and a minor peak which was identified as the disulfide dimer of 1 by FABMS. The reaction mixture was poured into H_2O (18 mL) and the pH adjusted to 2 with TFA and purified as described before. Yield: 22 mg, 81%. FABMS: $(M + H)^+$ calcd for $C_{23}H_{39}N_6O_6S$ *m/e* 527.2652, measured 527.2653. Anal. Calcd for $C_{23}H_{38}N_6O_6S$·3TFA·0.5H_2O: C, 39.68; H, 4.82; N, 9.57. Found: C, 39.57; H, 4.72; N, 9.69. Amino acid Anal.: Ser, 0.51; Phe, 1.00; Lys and thioLys, 1.91. NMR (D_2O): δ 1.21–1.40 (m, 2 H), 1.57–1.80 (m, 4 H), 2.73–3.06 (m, 7 H), 3.06–3.26 (m, 3 H), 3.98 (ddd, *J* = 4.5, 6, 9 Hz, 2 H), 4.15 (t, *J* = 4.5 Hz, 1 H), 4.35 (t, *J* = 7.5 Hz, 1 H), 4.45 (dd, *J* = 4.5, 7.5 Hz, 1 H), 4.51 (dd, *J* = 6, 7.5 Hz, 1 H), 7.20–7.40 (m, 5 H).

IAc-Ser-Lys-Cys-Phe-OH·TFA·0.5H_2O (3). The protected peptide resin, iodoacetyl-Ser(Bzl)-Lys(Cl-Z)-Cys(MeOBzl)-Phe-Merrifield resin was prepared by coupling iodoacetic acid to the resin H-Ser(Bzl)-Lys(Cl-Z)-Cys(MeOBzl)-Phe-Merrifield resin for 1 h until it gave a negative Kaiser test. Tetrapeptide **3** was deprotected, cleaved from the resin, and purified by RPHPLC as described before. Yield, 515 mg, 56% from Boc-Phe-Merrifield resin (2 g, 0.6 mequiv/g). FABMS: $(M + H)^+$ calcd for $C_{23}H_{35}IN_5O_7S$ *m/e* 652, measured 652. Anal. Calcd for $C_{23}H_{34}IN_5O_7S$·TFA·0.5H_2O: C, 38.77; H, 4.66; N, 9.04. Found: C, 38.80; H, 4.49; N, 8.91. NMR (D_2O): δ 1.28–1.50 (m, 2 H), 1.60–1.90 (m, 4 H), 2.83 (ddd, *J* = 6, 7.5, 15 Hz, 2 H), 2.96 (t, *J* = 7.5 Hz, 2 H), 3.05 (dd, *J* = 9, 13.5 Hz, 1 H), 3.25 (dd, *J* = 5, 13.5 Hz, 1 H), 3.70–3.95 (m, with masked singlet at 3.82, 5 H), 4.30–4.40 (m, 2 H), 4.45 (dd, *J* = 6, 7.5 Hz, 1 H), 4.67 (dd, *J* = 6, 9 Hz, 1 H), 7.30–7.40 (m, 5 H).

Cyclo-Ser-Lys-Cys(CH_2CO-)-Phe-OH·1.5TFA (4). Liquid ammonia (30 mL) was condensed into a 250-mL 3-neck round-bottom flask equipped with a dry ice condenser and cooled with a dry ice–2-propanol bath. A solution of tetrapeptide **3** (30 mg, 0.039 mmol) in degassed methanol (5 mL) was added dropwise into the stirred liquid ammonia at −78 °C under nitrogen. The cooling bath was removed after the completion of addition, and the reaction mixture was allowed to reflux for 2 h. RPHPLC analysis of a worked up aliquot showed the total disappearance of starting material. The ammonia was allowed to evaporate under a slow stream of nitrogen overnight. Methanol and residual ammonia were removed in vacuo; the solid residue was dissolved in 0.2% TFA in water and purified with RPHPLC. Removal of solvents by lyophilization gave cyclic peptide **4** as a white powder, 24 mg, in 87% yield. FABMS: $(M + H)^+$ calcd for $C_{23}H_{34}N_5OS$ *m/e* 524.2179, measured 524.2180. Anal. Calcd for $C_{23}H_{34}N_5OS$·1.5TFA: C, 44.96; H, 5.01; N, 10.08. Found: C, 45.08; H, 5.12; N, 10.37. Amino acid Anal.: Ser, 0.52; Lys, 1.00; Cyc(Cm), 1.04; Phe, 0.96. NMR (D_2O): δ 1.30–1.49 (m, 2 H), 1.52–1.83 (m, 3 H), 1.90–2.10 (m, 1 H), 2.86–3.10 (m, 4 H), 3.15–3.30 (m, 4 H), 3.90 (ddd, *J* = 6, 6, 15 Hz, 2 H), 4.18–4.26 (m, 1 H), 4.42 (dd, *J* = 4.5, 9 Hz, 1 H), 4.64 (dd, *J* = 4.5, 7.5 Hz, 1 H), 7.20–7.40 (m, 5 H).

4-Bromobutyryl-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2·TFA (5). The protected peptide resin, bromobutyryl-Tyr(Bzl)-Phe-Gln-Asn-Cys-Pro-Arg(Tos)-Gly-PAM resin was prepared by coupling 4-bromobutyryl chloride to the resin, H-Tyr(Bzl)-Phe-Gln-Asn-Cys-Pro-Arg(Tos)-Gly-PAM resin, until it gave a negative Kaiser test. Peptide 5 was deprotected, cleaved from the resin, and purified by RPHPLC as described before. Yield: 75 mg, 8% from Boc-Gly-PAM resin (1.2 g, 0.68 mequiv/g). FABMS: $(M + H)^+$ calcd for $C_{47}H_{67}BrN_{14}O_{12}S$ m/e 1133, measured 1133. The peptide was used in the next step without further characterization.

δ[1-Carba,Gly^9]vasopressin (6). Liquid ammonia (50 mL) was condensed into a 250-mL 3-neck round bottom flask equipped with a dry ice condenser and cooled with a dry ice–2-propanol bath under an atmosphere of argon. A small piece of sodium was added, and the solution turned blue. A crystal of ammonium acetate hydrate was then added to decolorize the solution. A suspension of peptide 5 (10 mg) in degassed absolute ethanol was added into the stirred liquid ammonia at −78 °C under argon. The cooling bath was removed after the completion of addition, and the reaction mixture was allowed to reflux for 6 h. The ammonia was evaporated under a slow stream of argon overnight. Ethanol and residual ammonia were removed in vacuo. The solid residue was dissolved in degassed 0.2% TFA in water (7 mL), the pH was adjusted to 2 with TFA, and the residue was purified with RPHPLC. Removal of solvents by lyophilization gave peptide 6 as a white powder, 6.5 mg, in 65% yield. High-resolution FABMS (using deoxygenated ethanol): $(M + H)^+$ calcd for $C_{47}H_{67}N_{14}O_{12}S$ m/e 1051.4783, measured 1051.4780. Amino acid Anal.: Tyr, 0.98; Phe, 1.05; Glx, 1.08; Asx, 1.10; Cys-X, 0.90; Pro, 1.05; Arg, 1.05; Gly, 0.94.

Registry No. 1, 132126-26-2; **2a**, 132126-28-4; **2b**, 132126-30-8; **2c**, 132126-32-0; 3, 132155-35-2; 4, 132126-34-2; 5, 132126-36-4; 6, 64156-79-2; BOC-Ser(Bzl)-OH, 23680-31-1; BOC-Lys(Cl-Z)-OH, 54613-99-9; BOC-Cys(MeOBzl)-OH, 18942-46-6; $ClCH_2COOH$, 79-11-8; CH_2=CHCOOMe, 96-33-3; $PhCH_2Br$, 100-39-0; $Br(CH_2)_3COCl$, 927-58-2.

Peptide Synthesis Catalyzed by Modified α-Chymotrypsin in Low-Water Organic Media

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Received May 21, 1990

Enzyme-catalyzed synthesis of peptide bonds in organic solvents has been investigated by using α-chymotrypsin either modified with poly(ethylene glycol) or immobilized on different supports, in order to find out the importance of water content in the reaction. High yields of peptide synthesis were obtained whatever the type of enzyme derivative used. By varying the type of support, a modification in the enzyme environment was observed and resulted in a significant increase in the reaction yield when nucleophiles with poor affinity for the enzyme were used. Since organic solvents also affected substrate specificity with respect to the donor ester, a general methodology was proposed for the enzymatic synthesis of peptides in low-water organic media.

Introduction

The enzymatic synthesis of active peptides such as Leu- and Met-enkephalins (H_2N-Tyr-Gly-Gly-Phe-Leu(Met)-COOH) may be considered as an interesting example of the synthetic applicability of proteases to the preparation of naturally occurring peptides.[1-3] Other reactions such as esterification,[4] one-pot tripeptide synthesis,[5] and synthesis of isopeptides[6] or peptides containing D-amino acids[7] have been described. Two strategies are generally used in protease-catalyzed peptide formation: kinetics and equilibrium-controlled synthesis.[8] The enzymatic synthesis of peptides has the advantage of taking place under mild experimental conditions, and epimerization of chiral carbons is not observed. However, in many cases, the addition of water-soluble organic solvents to increase substrate solubility is accompanied by dramatic changes in the enzyme catalytic properties. A high percentage of organic solvent would be advantageous in suppressing competitive hydrolysis of the acyl-enzyme intermediate as well as secondary hydrolysis of the newly synthesized peptides. Such processes have been investigated in biphasic systems,[9] reversed micelles,[10] but in recent years catalysis in low-water organic media has been given increasing attention.[11] The enzyme can be directly suspended in the organic media, although it is preferable to stabilize enzymes through their immobilization on inert supports when water-miscible solvents are used. The enzyme can also be derivatized with poly(ethylene glycol) (PEG), an amphiphilic polymer, in order to make it soluble in organic solvents with most if not all of its original activity.[12] Modified enzymes have been used in the synthesis of peptides in benzene or 1,1,1-trichloroethane.[13,14] In the

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0022-3263/91/1956-3149$02.50/0