Bioassays were carried out by the methods described in ref 1–4.

Enzymatic Degradations. For comparison of the synthetic and natural materials, VIII (4.6 mg, trifluoroacetate) was dissolved in 0.2 *M* acetic acid (1 ml) and passed through a 0.6 × 10 cm column of DEAE-Sephadex A-25, in acetate form. The peptide-containing fraction of the effluent was lyophilized. The lyophilized material weighed 2.4 mg. The natural material was prepared as described previously.[2]

Cleavage with Kallikrein. Two milligrams each of the synthetic and natural VIP were dissolved in 0.5 ml of water, and 0.5 ml of 2% NH_4HCO_3 was added, immediately followed by 20 μl of an 0.2% solution of kallicrein (sialidase-treated pig pancreatic kallikrein B, batch H376, was a gift from Professor E. Werle). On addition of ammonium bicarbonate, both solutions turned cloudy, that of the synthetic material rather more so than that of the natural. After 30 min at 21°, the solutions, both of which had clarified, were frozen and lyophilized. The residues were taken up in 0.25 ml of water and kept on the boiling water bath for 6 min. After cooling, the solutions were acidified (to about 0.2 *M*) with acetic acid and lyophilized. Aliquots of the lyophilized products were subjected to high voltage paper electrophoresis in parallel with the undegraded materials. It is seen from Figure 2 that the electrophoretic patterns given by the natural and synthetic products are essentially similar.

Separation of the Kallikreinic Fragments. The lyophilized kallikreinic degradation mixtures were dissolved in 0.25 ml of 0.02 *M* NH_4HCO_3, preadjusted to pH 6.4 with CO_2, and passed through a 0.6 × 12 cm column of carboxymethyl-cellulose (Whatman CM-22) equilibrated with this buffer. Elution with the same buffer was carried out until a 4-ml fraction had been collected. The elution was then continued with 4 ml each of 0.04, 0.08, 0.16, and 0.32 *M* NH_4HCO_3. The fractions were lyophilized and relyophilized from 0.2 *M* acetic acid. By analyzing aliquots of the fractions by means of high-voltage paper electrophoresis and by the Pauly reaction, it was found that fraction 1 contained the N-terminal kallikreinic fragment, V-K-N, and fraction 4 the C-terminal V-K-C. The yields were 0.64 mg of natural and 0.35 mg of synthetic V-K-N, and 0.52 mg of natural and 0.25 mg of synthetic V-K-C.

Comparison of Natural and Synthetic V-K-N. Synthetic and natural V-K-N (0.25 mg each) were degraded with trypsin (TRTPCK from Worthington) under the conditions given above for the degradation of VIP with kallikrein, for 2 hr. Figure 3 shows the electrophoretic patterns obtained on high-voltage paper electrophoresis.

The two tryptic fragments V-K-N-Tr-N and V-K-N-Tr-C, the latter identical with leucylarginine, were separated as follows. The degradation mixture was dissolved in 0.1 ml of 0.02 *M* NH_4HCO_3, preadjusted to pH 6.4 with CO_2, and followed by the same buffer, passed through a 0.3 × 8 cm column of carboxymethyl-cellulose. A fraction of 1.5 ml was collected, whereupon the buffer was changed to 0.2 *M* NH_4HCO_3, and a second 1.5-ml fraction was collected. Both fractions were lyophilized and relyophilized from 0.2 *M* acetic acid. Fraction 1 contained V-K-N-Tr-N and fraction 2, V-K-N-Tr-C.

Degradation of V-K-N-Tr-N with Chymotrypsin. The samples of natural and synthetic V-K-N-Tr-N were degraded with chymotrypsin (TLCK-treated chymotrypsin from Merck) under the conditions given above for the degradation of V-K-N with trypsin. Figure 5 shows the electrophoretic pattern at pH 6.4 of the degradation products.

Comparison of V-K-C from Natural and Synthetic VIP. Natural and synthetic C-terminal kallikreinic fragments, V-K-C, of VIP (150 μg each) were dissolved in 150 μl of 1% CNBr in 0.1 *M* HCl.[23] The solutions were kept for 16 hr at 21°, whereupon they were passed through 0.3 × 5 cm columns of DEAE-Sephadex equilibrated with 0.2 *M* acetic acid; 0.5-ml volumes of each effluent were lyophilized, and aliquots of the lyophilized material were subjected to paper electrophoresis and paper chromatography in the Waley–Watson system.[19] In neither case was any difference observed in the split products derived from the natural and synthetic materials. A paper chromatogram is shown in Figure 5. It may also be seen from this chromatogram that the fastest migrating product is indistinguishable in mobility from the natural and synthetic C-terminal hendecapeptides of VIP described previously.[7]

In another experiment, synthetic and natural preparations of V-K-C were degraded with chymotrypsin under conditions identical with those described above for the degradation of V-K-N-Tr-N with this enzyme, and with prolonged exposure to larger amounts of it. An electropherogram of aliquots of the lyophilized degradation products is shown in Figure 6.

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Removal of the N^{α}-Benzyloxycarbonyl Group from Cysteine-Containing Peptides by Catalytic Hydrogenolysis in Liquid Ammonia, Exemplified by a Synthesis of Oxytocin[1]

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Abstract: Cleavage of the benzyloxycarbonylamine protecting group from *S*-benzylcysteine-containing peptides can be attained by palladium-catalyzed hydrogenation when liquid ammonia is used as a solvent. The efficacy of the procedure is demonstrated by a synthesis of oxytocin *via* incremental chain elongation.

Development of adequate combinations of selectively removable protecting groups is still a major concern in peptide synthesis. For conventional synthesis in solution, combination of the *tert*-butyloxycarbonyl (Boc)[3] group[4] for α-amino and the benzyloxy-

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(3) Abbreviations for amino acids (all of L configuration) follow the rules of the IUPAC-IUB Commission on Biochemical Nomenclature in *Biochemistry*, **5**, 1445, 2885 (1966); **6**, 362 (1967); *J. Biol. Chem.*, **247**, 977 (1972). Boc, *tert*-butyloxycarbonyl; Bpoc, 2-(*p*-biphenylyl)isopropyloxycarbonyl; Bzl, benzyl; Fmoc, 9-fluorenylmethyloxycarbonyl; O-*t*-Bu, *tert*-butyl ester; ONph, *p*-nitrophenyl ester; Z, benzyloxycarbonyl.

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carbonyl (Z) group[5] for ω-amino group protection has been very effective.[6] This tactic proved to be inadequate[7,8] for solid-phase peptide synthesis[9] because acidolysis of N^{α}-Boc groups by trifluoroacetic acid[4,10] or 1–2 *N* hydrogen chloride in organic solvents[11] causes undesired fission of small but significant amounts of N^{ω}-Z groups.[12] Efforts to improve the approximate 3000:1 ratio of acid stability of Z over Boc led to the development of either more acid-labile (N^{α}) protecting groups, *e.g.*, the 2-(*p*-biphenylyl)isopropyloxycarbonyl (Bpoc) group,[13] or more acid-stable (N^{ω}) protecting groups, *e.g.*, the 2,4-dichlorobenzyloxycarbonyl[14] or the *p*-bromobenzyloxycarbonyl[15] groups. At present, selective deblocking of most protecting groups commonly used in peptide synthesis is essentially based on graded acidolysis which suffers from the inherent weakness that its selectivity is not total but more or less relative. A most effective alternative procedure has been the hydrogenolytic cleavage of N^{α}-benzyloxycarbonyl groups, developed by Bergmann and Zervas,[5] which offers absolute selectivity by quantitative liberation of α-amino groups and complete stability of all *tert*-butyl-derived protecting groups. This otherwise ideal combination has not been applicable to cysteine- or methionine-containing peptides which resist catalytic hydrogenolysis. Attempts have been reported to overcome this obstacle by addition of tertiary base[16] or of boron trifluoride etherate[17] to hydrogenolysis mixtures, or by development of amino protecting groups which may be hydrogenolyzed with partially poisoned catalysts, such as the 1,1-dimethyl-2-propynyloxycarbonyl group,[18] but general applicability of these procedures has, as yet, not been attained.

We have observed[19] that palladium-catalyzed hydrogenation in liquid ammonia effected quantitative cleavage of N^{α}-benzyloxycarbonyl groups from *S*-benzylcysteine and a few cysteine-containing model peptides. Under these reaction conditions *N*-*tert*-butyloxycarbonyl, *tert*-butyl ester, and *tert*-butyl ether protecting groups remained completely stable.[19] Experience has accumulated that liquid ammonia, which has been introduced to peptide chemistry by du Vigneaud,[20,21] is a powerful solvent for many protected peptides up to considerable chain length[22] which may possess very low solubility in most commonly used organic solvents. These observations suggested that application of the proven tactics of hydrogenolytic removal of N^{α}-Z blocking in the presence of Boc, *tert*-butyl ester, and *tert*-butyl ether protecting groups in side chains[23] might be extended to synthesis of cysteine- (or methionine-) containing peptides. As an example for probing the scope and limitations of catalytic hydrogenation in liquid ammonia for the cleavage of N^{α}-Z groups from *S*-benzylcysteine-containing peptides, a synthesis of oxytocin is reported herein.

The known protected tetrapeptide intermediate, Z-Cys(Bzl)-Pro-Leu-Gly-NH_2 (I), was prepared by standard procedures.[24,25] Catalytic hydrogenation of I in liquid ammonia in the presence of palladium black[26] was slow and produced only 10% Z-group scission after prolonged periods of time, as observed by thin-layer chromatography (solvent B). Addition of triethylamine (4 molar excess) to the liquid ammonia solution improved rate and extent of cleavage (*ca.* 50%). Addition of ammonium chloride (1.2 molar excess) afforded approximately 40–50% cleavage. Best results (up to 60% cleavage) were obtained in the presence of a 1.2 molar excess of ammonium acetate. We are at present unable to explain the observed resistance of the benzyloxycarbonyl group in I to hydrogenolysis in liquid ammonia. In the case of two other peptides possessing NH_2-terminal *N*-benzyloxycarbonyl-*S*-benzylcysteine residues, *i.e.*, Z-Cys(Bzl)-Gly-NH_2[27] and Z-Cys(Bzl)Tyr-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 (XIII),[24,29,30] and the amino acid [Z-Cys(Bzl)-OH][31] itself, Z-group removal was quantitative without additions of tertiary base or ammonium acetate. Perhaps an unfavorable folding of protected tetrapeptide I in liquid ammonia might impede hydrogenolysis of the Z group.

The incomplete cleavage observed with the Z group of I must be considered unsatisfactory and the use of an alternative amino protecting group, not requiring

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acidolytic cleavage, was studied for this step of the oxytocin synthesis. The 9-fluorenylmethyloxycarbonyl (Fmoc) group[32] has been reported to be readily cleaved by mild base treatment. The intermediate tetrapeptide, Fmoc-Cys(Bzl)-Pro-Leu-Gly-NH_2 (III), was prepared by dicyclohexylcarbodiimide–1-hydroxybenzotriazole mediated[33] condensation of Fmoc-Cys(Bzl)-OH (II) and H-Pro-Leu-Gly-NH_2.[29] The $N\alpha$-Fmoc group was quantitatively removed from III after standing in refluxing liquid ammonia for 8 hr; and subsequent condensation with Z-Asn-ONph[30,34] afforded the protected pentapeptide Z-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 (V)[30,35] in high yield.

Catalytic hydrogenolysis of the Z group in liquid ammonia was quantitative in each subsequent step, *i.e.*, for V and the ensuing hexa-, hepta-, and octapeptide intermediates, as well as for the final nonapeptide derivative, as mentioned above. Incorporation of all Z-amino acids was attained by the *p*-nitrophenyl ester method.[30,36] The protected nonapeptide, Z-Cys(Bzl)-Tyr-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 (XIII), was obtained in crystalline form. Removal of all protecting groups by treatment with Na in liquid ammonia,[21] oxidative disulfide bond formation,[37] and purification of the hormone by countercurrent distribution[38] and gel filtration[39] was carried out according to known procedures. Highly active oxytocin was obtained (48% yield, *ca.* 470 units/mg of oxytocic potency).

This successful synthesis suggests that catalytic hydrogenolysis might be generally applicable to the cleavage of *N*-benzyloxycarbonyl groups from *S*-benzylcysteine- and perhaps other sulfur-containing peptides when liquid ammonia is used as a solvent. The procedure might thus overcome a main obstacle to a more general use in peptide synthesis of the ideal combination of N^{α}-benzyloxycarbonyl protecting groups and side-chain protection by *tert*-butyl derived groups,[23] which are completely stable in liquid ammonia.[19]

Experimental Section

Details on materials and methods have been described previously.[40] Solvent systems for thin-layer chromatography (silica gel G) were (A) chloroform–methanol (5:1), (B) chloroform–methanol–acetic acid (8:1:1), and (C) 1-butanol–acetic acid–water (3:1:1).

General Procedure for Catalytic Hydrogenation in Liquid Ammonia. All glassware was dried prior to use. Anhydrous ammonia (Matheson Gas Products) was passed through a drying tube filled with KOH pellets and condensed in a three-necked round-bottomed flask immersed in Dry Ice–acetone. The cold bath was removed and the flask fitted with a magnetic stirrer. A Dry Ice reflux condenser was placed on the center neck. N^{α}-Benzyloxycarbonyl peptide derivative (1 mmol in approximately 150 ml) was dissolved with stirring. Freshly prepared palladium black[26] (0.2–0.5 g), freed from water by thorough washing with anhydrous methanol, was added in methanol-wet form under a nitrogen barrier.[41] This catalyst was more effective than palladium on charcoal.[19] A stream of dried (concentrated H_2SO_4) hydrogen was continuously passed through the magnetically stirred solution at the boiling point of ammonia (*ca.* −33°). Reaction progress was followed by thin-layer chromatography. After 6–8 hr, the ammonia was evaporated to dryness under nitrogen. The residue was immediately dissolved in distilled dimethylformamide or methanol and the solution filtered from the catalyst. Evaporation afforded products, which were in most cases homogeneous.

***S*-Benzyl-*N*-benzyloxycarbonyl-L-cysteinyl-L-prolyl-L-leucylglycinamide** (I) was prepared in 63% yield by the mixed anhydride method, essentially as described by Zaoral and Rudinger:[25] mp 169–170°; R_f 0.56 (A), 0.86 (B); $[\alpha]^{20}D$ −63.0° (*c* 2, dimethylformamide) (lit.[24] mp 170–171.5°; $[\alpha]^{21}D$ −60.0° (*c* 2, dimethylformamide); lit.[25] mp 170–171°).

***S*-Benzyl-*N*-9-fluorenylmethyloxycarbonyl-L-cysteine** (II). To a chilled solution of *S*-benzyl-L-cysteine (2.11 g, 10 mmol) in a mixture of 26.5 ml of 10% Na_2CO_3 and 10 ml of dioxane, Fmoc–Cl[32] (2.85 g, 11 mmol) in dioxane was added dropwise with vigorous stirring and ice-bath cooling. The mixture was stirred for 4 hr in an ice bath and for 8 hr at room temperature, and then poured into 500 ml of water. Excess reagent was extracted with ether and the aqueous layer was acidified with concentrated HCl to pH 2–3 at 0°. A white precipitate was extracted with ethyl acetate (150 ml × 3) and the organic layer was washed with water (three times), dried ($MgSO_4$), and evaporated *in vacuo* to give a white powder: yield, 4.07 g (94%); mp 118–120°; R_f 0.55 (A); $[\alpha]^{21}D$ −45.6° (*c* 1, dimethylformamide).

Anal. Calcd for $C_{25}H_{23}NO_4S$ (433.5): C, 69.3; H, 5.34; N, 3.23; S, 7.17. Found: C, 69.8; H, 5.35; N, 3.30; S, 7.41.

***S*-Benzyl-*N*-9-fluorenylmethyloxycarbonyl-L-cysteinyl-L-prolyl-L-leucylglycinamide** (III). Boc-Pro-Leu-Gly-NH_2[42] (1.85 g, 4.8 mmol) was treated with 1 *N* HCl in glacial acetic acid for 30 min at room temperature. The ensuing hydrochloride was dissolved in 20 ml of ethyl acetate. To the stirred solution, *N*-methylmorpholine (0.61 ml), II (2.0 g, 4.61 mmol) in dimethylformamide (15 ml), 1-hydroxybenzotriazole[33] (0.53 g, 5.53 mmol), and *N,N'*-dicyclohexylcarbodiimide[43] (1.045 g, 5.07 mmol) were successively added at 0°. Work-up was as usual by washing with 1 *N* HCl (three times) and 1 *M* $NaHCO_3$ (two times). The material was crystallized from ethyl acetate–hexane: yield, 2.12 g (66.3%); mp 164–165°; R_f 0.73 (A), 0.63 (B); $[\alpha]^{21}D$ −49.8° (*c* 1, dimethylformamide).

Anal. Calcd for $C_{38}H_{45}N_5O_6S$ (699.9): C, 65.2; H, 6.48; N, 10.0; S, 4.54. Found: C, 65.0; H, 6.39; N, 9.94; S, 4.23.

***S*-Benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide** (IV). **A. By Hydrogenolysis of I.** I (300 mg) was dissolved in 35 ml of anhydrous liquid ammonia and hydrogenated in the presence of freshly prepared palladium (*ca.* 0.1 g) and triethylamine (0.28 ml) at the boiling point of the solvent. The reaction was followed by thin-layer chromatography (solvent system B) which showed incomplete reaction (50–60%) after 6 hr. Evaporation of ammonia under nitrogen was followed by addition of methanol (10 ml) and stirring for 10 min. The catalyst was then removed by filtration and washed with methanol (10 ml). The combined filtrate and washings were passed through a column (1.2 × 10 cm) of Dowex 50-X8 (H^+ form) which was washed with methanol. The desired material was then eluted with 2 *N* ammonium hydroxide–methanol (1:1) and the eluate evaporated *in vacuo*. Crystallization from water gave 95.2 mg (42.3%); mp 130–134°; R_f 0.02 (B), 0.46 (C); $[\alpha]^{21}D$ −47.5° (*c* 1, ethanol) (lit.[25] mp 136–137°; $[\alpha]^{20}D$ −50.8° (*c* 3.3, ethanol); lit.[44] mp 69.5–71.5°; $[\alpha]^{22.6}D$ −47.7° (*c* 1, ethanol)).

B. By Hydrogen Bromide Treatment of I. I (6.01 g, 9.83 mmol) was treated with 4 *N* hydrogen bromide in acetic acid followed by Dowex 2-X8 (OH^- form).[30] The product (5.36 g, >100%) was chromatographically homogeneous, R_f 0.05 (B), 0.45 (C) and identical with IVA and was directly converted to the known protected pentapeptide V.

C. By Liquid Ammonia Treatment of III. A suspension of III (0.70 g, 1.0 mmol) in liquid ammonia (300 ml) was stirred at refluxing point of the solvent for 8 hr.[32] No clear solution was ob-

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tained, but the reaction was complete after 6 hr. The solution was concentrated to about 20 ml. The remaining ammonia was frozen and removed by lyophilization. Homogeneity of the crude material (0.502 g, 100%) was confirmed by thin-layer chromatography, R_f 0.05 (B), 0.46 (C). This material was identical with IVA. Treatment with Z-Asn-ONph afforded the known protected pentapeptide V in almost quantitative yield.

Benzyloxycarbonyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (V). To a solution of IV (0.502 g) in ethyl acetate (3 ml) Z-Asn-ONph[30,34] (0.387 g, 1.0 mmol) was added and the suspension stirred at room temperature for 48 hr. The precipitate which formed was collected by filtration and washed with ethyl acetate (20 ml) and ethanol (5 ml), and then dried. The crude material was recrystallized from 40% methanol: yield, 0.718 g (99%); mp 212–213.5°; R_f 0.46 (B), 0.84 (C); $[\alpha]^{20}D$ −60.5° (*c* 1, dimethylformamide) (lit.[30] mp 212–213°; $[\alpha]^{22}D$ −59.4° (*c* 1, dimethylformamide); lit.[35] mp 210.5–211.5°; $[\alpha]^{20.5}D$ −59.5° (*c* 2, dimethylformamide)).

In the following, the peptide amide derivatives with a free α-amino group, obtained by catalytic hydrogenolysis in liquid ammonia, are described. These were subsequently converted into the next higher known protected peptides as described for V.

L-Asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (VI). V (5.37 g, 7.40 mmol) was hydrogenated for 8 hr in liquid ammonia (800 ml) using methanol–wet palladium black as described above to afford colorless crystals from methanol–ether; 4.36 g (99%); mp 102–104° with softening at 86°; R_f 0.02 (B), 0.55 (C); $[\alpha]^{21}D$ −59.1° (*c* 1, dimethylformamide).

Anal. Calcd for $C_{27}H_{41}N_7O_6S$ (591.7): C, 54.8; H, 6.98; N, 16.6; S, 5.42. Found: C, 54.6; H, 7.25; N, 16.4; S, 4.99.

Condensation with Z-Gln-ONph[30,45] afforded the protected hexapeptide (VII) in 89% yield: mp 209.5–210°; R_f 0.02 (B), 0.76 (C); $[\alpha]^{21}D$ −57.0° (*c* 1, dimethylformamide); lit.[30] mp 233–234° dec; $[\alpha]^{21}D$ −54° (*c* 1, dimethylformamide); lit.[46] mp 209°; lit.[45] mp 209–210°).

L-Glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (VIII). VII (3.82 g, 4.47 mmol) was hydrogenated in liquid ammonia (600 ml) for 6.5 hr to give a colorless powder from methanol–ether: 3.25 g (100%); mp 136–138°; R_f 0.55 (C); $[\alpha]^{21}D$ −62.6° (*c* 1, acetic acid); lit.[46] mp 145° dec; $[\alpha]D$ −67.3° (*c* 2.3, acetic acid); lit.[47] mp 135°).

The protected heptapeptide (IX) was prepared by the coupling of VIII with Z-Ile-ONph[30] in 100% yield: mp 232–233°; R_f 0.85 (C); $[\alpha]^{21}D$ −50.2° (*c* 1, dimethylformamide) (lit.[30] mp 233–235°; $[\alpha]^{20}D$ −50.0° (*c* 1, dimethylformamide); lit.[35] mp 230–231.5°; $[\alpha]^{21}D$ −48.8° (*c* 1, dimethylformamide)).

L-Isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (X). IX (4.12 g, 4.26 mmol) was hydrogenated in liquid ammonia (600 ml) for 8.5 hr to produce colorless crystals from methanol–ether; 3.51 g (99%); mp 218–220° with softening at 168°; R_f 0.48 (C); $[\alpha]^{21}D$ −59.1° (*c* 1, dimethylformamide) (lit.[48] mp 211–213°; $[\alpha]^{20}D$ −57.1° (*c* 1, dimethylformamide).

This was coupled with Z-Tyr-ONph[49] to afford the protected octapeptide, Z-Tyr-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 (XI), in 82.4% yield: mp 237.5–238.5°; R_f 0.02 (B), 0.66 (C): $[\alpha]^{21}D$ −43.0° (*c* 1, dimethylformamide).

L-Tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (XII). XI (3.39 g, 3.0 mmol) was hydrogenated in liquid ammonia (500 ml) for 8 hr. Colorless crystals were obtained from methanol–ether: 2.98 g (100%); mp 179–180°; R_f 0.55 (C); $[\alpha]^{21}D$ −41.3° (*c* 1, dimethylformamide).

Anal. Calcd for $C_{47}H_{69}N_{11}O_{11}S$ (996.2): C, 56.7; H, 6.98; N, 15.5; S, 3.22. Found: C, 57.0; H, 6.62; N, 15.2; S, 2.88.

Condensation with Z-Cys(Bzl)-ONph[36,50] afforded the protected nonapeptide (XIII), Z-Cys(Bzl)-Tyr-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2, in 98% yield as colorless microscopic needles from dimethylformamide–formic acid (99:1): mp 235–236.5°; R_f 0.82 (C); $[\alpha]^{21}D$ −56.1° (*c* 1, dimethylformamide); $[\alpha]^{20}D$ −58.2° (*c* 2.5 acetic acid) (lit.[24] mp 243–245°; $[\alpha]^{22}D$ −43° (*c* 2, dimethylformamide); lit.[29] mp 224–225°; lit.[30] mp 245–248°; $[\alpha]^{20}D$ −50.5° (*c* 1, dimethylformamide); $[\alpha]^{20}D$ −64.5° (*c* 2.5, acetic acid); lit.[46] mp 241°; $[\alpha]^{20}D$ −51.5° (*c* 2.5, acetic acid)).

***S*-Benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (XIV).** Hydrogenation of XIII (52 mg) in liquid ammonia (20 ml) was carried out for 8 hr in the presence of palladium catalyst. Colorless crystals were obtained from ethanol: 44.1 mg (94.5%); mp 244–246° with softening at around 175°; R_f 0.28 (C); $[\alpha]^{21}D$ −48.8° (*c* 0.5, dimethylformamide).

Anal. Calcd for $C_{37}H_{80}N_{12}O_{12}S_2$ (1189.5): C, 57.6; H, 6.78; N, 14.1; S, 5.39. Found: C, 57.8; H, 6.62; N, 14.1; S, 5.83.

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Communications to the Editor

Facile Intramolecular Hydrolysis of Dipeptides and Glycinamide

Sir:

The enzymic hydrolysis of glycinamides and peptide derivatives continues to be of considerable interest, and some debate on the source of activation remains.[1] Previously, we have demonstrated activations of 10^4–10^6 when such substrates are directly coordinated *via* the carbonyl oxygen to octahedral metal ions such as Co(III)[2] and where solvolytic hydrolysis is involved. We now report the metal ion promoted *intramolecular* hydrolysis of a simple peptide which can give rise to accelerations comparable with those observed enzymically.

Treatment of *cis*-$[Co(en)_2Br(glyglyOC_3H_7)](NO_3)_2$ with *ca.* 1 *M* HOCl at 0° for 10 min, followed by chromatography on Sephadex (SP C-25, 0.5 *M* $NaClO_4$, pH 8, 2°) resulted in the separation of a violet-red 2+ band

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