of Medicine.
(2) Antonini, E.; Brunori, M. "Hemoglobin and Myoglobin in Their Reactions with Ligands", North-Holland Publishing Co.: Amsterdam, 1971. (a) p 224. (b) p 260. (c) p 310. (d) p 313.
(3) Sono, M.; Smith, P. D.; McKray, J. A.; Asakura, T. *J. Biol. Chem.* **1976**, *251*, 1418–1426.
(4) (a) Seybert, D. W.; Moffatt, K.; Gibson, Q. H. *J. Biol. Chem.* **1976**, *251*, 45–52. (b) Seybert, D. W.; Moffatt, K.; Gibson, Q. H.; Chang, C. K. *ibid.* **1977**, *252*, 4225–4231.
(5) Tamura, M.; Yonetani, T. *Biochim. Biophys. Acta* **1973**, *322*, 211–217.
(6) (a) Sharma, V. S.; Ranney, H. M.; Geibel, J. F.; Traylor, T. G. *Biochem. Biophys. Res. Commun.* **1975**, *66*, 1301–1306. (b) Sharma, V. S.; Schmidt, M. R.; Ranney, H. M. *J. Biol. Chem.* **1976**, *251*, 4267–4272.
(7) Debey, P.; Balny, C.; Douzou, P. *FEBS. Lett.* **1973**, *35*, 86–90.
(8) (a) McQuarrie, R.; Gibson, Q. H. *J. Biol. Chem.* **1971**, *246*, 5832–5835. (b) Gibson, Q. H. *Prog. React. Kinet.* **1964**, *2*, 328.
(9) (a) Chang, C. K.; Traylor, T. G. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 2647–2650. (b) Chang, C. K.; Traylor, T. G. *J. Am. Chem. Soc.* **1973**, *95*, 5810–5811. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 1166–1170. (d) Geibel, J.; Chang, C. K.; Traylor, T. G. *J. Am. Chem. Soc.* **1975**, *97*, 5924–5926. (e) Geibel, J.; Cannon, J.; Campbell, D.; Traylor, T. G. *ibid.* **1978**, *100*, 3575–3585. (f) Cannon, J.; Geibel, J.; Whipple, M.; Traylor, T. G. *ibid.* **1976**, *98*, 3395–3396. (g) Traylor, T. G.; Chang, C. K.; Geibel, J.; Berzinis, A.; Mincey, T.; Cannon, J., *J. Am. Chem. Soc.* In press. (h) White, D. K.; Cannon, J. B.; Traylor, T. G. *J. Am. Chem. Soc.*, **1979**, *101*, 2443–2454. (i) Berzinis, A.; White, D. K. Unpublished data. (j) Mincey, T.; Traylor, T. G. *J. Am. Chem. Soc.* **1979**, *101*, 765–766.
(10) Gibson, Q. H.; Roughton, F. J. W. *Hoppe-Seyler's Z. Physiol. Chem.*, **1957**, *136*, 507–526.
(11) Antonini, E.; Brunori, M.; Wyman, J. *Biochemistry* **1965**, *4*, 545–551.
(12) Berzinis, A. Unpublished work.
(13) (a) Collman, J. P.; Brauman, J. I.; Halbert, T. R.; Suslick, K. S. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 3333–3337. (b) Collman, J. P.; Brauman, J. I.; Doxsee, K. M.; Halbert, T. R.; Suslick, K. S. *ibid.* **1978**, *75*, 564–568.
(14) (a) Brault, D.; Rougee, M. *Biochemistry* **1974**, *13*, 4591–4597. *Biochem. Biophys. Res. Commun.* **1974**, *57*, 654–659.
(15) Heidner, E. J.; Ladner, R. C.; Perutz, M. F. *J. Mol. Biol.* **1976**, *104*, 707–722.
(16) (a) Caughey, W. S. *Ann. N.Y. Acad. Sci.* **1970**, *174*, 148–153. (b) Alben, J. O.; Caughey, W. S. *Biochemistry* **1968**, *7*, 175–183. (c) Caughey, W. S.; Alben, J. O.; McCoy S.; Boyer, S. H.; Charache, S.; Hathaway, P. *Biochemistry* **1969**, *8*, 59–62.
(17) Tidwell, T. T. *Tetrahedron* **1978**, *34*, 1855–1868.
(18) Alberding, N.; Austin, R. H.; Chan, S. S.; Eisenstein, L.; Frauenfelder, H.; Gunsalus, I. C.; Nordlund, T. M. *J. Chem. Phys.* **1976**, *65*, 4701–4711.
(19) We are grateful to Elizabeth Geibel for preparation of this compound.

Preparation and Characterization of Beaded Poly(*N*-acrylylpyrrolidine): Bidirectional Synthesis of Cys-, His-, Gln-, or Glu-Containing Polypeptides[1]

Glenn L. Stahl, Roderich Walter,* and Clark W. Smith

Contribution from the Department of Physiology and Biophysics, University of Illinois at the Medical Center, Chicago, Illinois 60612.
Received June 16, 1978

Abstract: The synthesis, characterization, and application of a poly(*N*-acrylylpyrrolidine) resin as an improved insoluble support for the preparation of peptides is described. The three-dimensional polymer was obtained by reverse-phase suspension copolymerization of the water-soluble monomers *N*-acrylylpyrrolidine, *N,N'*-bis(acrylyl)-1,2-diaminoethane, and *N*-acrylyl-1,6-diaminohexane hydrochloride to yield a resin in beads of predetermined size. This method of polymer preparation appears to be generally applicable for water-soluble monomers. The particular resin studied contained 4.4% cross-linking, 0.7 mmol of primary amine per g of polymer and was prepared with a monomer-to-solvent dilution ratio of 1:4. The resin has favorable swelling properties in solvents covering a broad range of polarities (CH_2Cl_2, alcohols, AcOH, DMF, and H_2O), which allow the adaptation of most of the techniques developed for the Merrifield method of solid-phase peptide synthesis as well as the use of procedures not previously compatible with polystyrene. The feasibility of amino acid and peptide attachment and detachment has been demonstrated with three types of polymer-to-peptide bridging groups: (1) a new *S*-carbamoyl group for the side-chain attachment of cysteine was used for a high yield bidirectional synthesis of deaminooxytocin, a potent biologically active analogue of oxytocin; (2) the dinitrophenylene group for the side-chain attachment of histidine was used for the bidirectional synthesis of thyrotropin-releasing hormone; (3) the benzyl ester group for COOH-terminal attachment and, moreover, for the side-chain attachment of glutamic acid. *N*-Benzyloxycarbonylprolylglutamyl-O^5-[4-(oxymethyl)phenylacetamidopoly(*N*-acrylylpyrrolidine)]glycinamide was assembled bidirectionally to demonstrate the feasibility of preparing both glutamic acid and glutamine-containing peptides from the same polymer-attached peptide intermediate; saponification and ammonolysis were the respective methods of choice for the liberation of the Z-Pro-Glu-Gly-NH_2 and Z-Pro-Gln-Gly-NH_2 model peptides. Reaction conditions used for detachment are nonacidic and different from those normally used for removal of N^{α}-amino group protection. Coupling reactions on the resin have been demonstrated in such diverse solvents as CH_2Cl_2, DMF, and aqueous media.

The introduction of the principle of peptide assembly on insoluble supports marked a milestone for the field of peptide synthesis.[2] When the term "solid-phase peptide synthesis" was originally introduced, it was generally felt that the polymer would act only as an inert, solid support. However, during the last decade extensive experience with polystyrene-based resin beads has shown that the insoluble support does actually have a dynamic influence on the synthesis of the peptide. Unfortunately, the influences on mass transport of reagents, solvation of polymers as well as peptide, and reaction rates of acylation as well as deprotection have been mostly negative because of physicochemical incompatibility of polystyrene with the attached peptide.[3,4] Emphasis to overcome these problems has focused on making the growing peptide more compatible with the polymer through the application and development of appropriate protecting groups.[5,6]

Instead of attempting to make the growing peptide chain conform to the requirements of a polystyrene-based resin, there appears to be a greater potential to improve the techniques of peptide synthesis by developing insoluble polymeric supports which are physicochemically compatible with the backbone structure of a peptide.[3,4] Ideally, a new polymer would influence the course of the synthetic reactions in a positive manner by providing an environment more favorable for the reactions.

Several proposals have been made for the development of insoluble polymeric supports with chemical structures resembling those of polypeptides. For example, Anfinsen[7] suggested the use of a polypeptide per se as a polymeric support. A cross-linked poly(L-lysine) has been reportedly used for oligonucleotide synthesis.[8] Inman and Dintzis proposed the use of cross-linked polyacrylamide for peptide synthesis,[9] but,

0002-7863/79/1501-5383$01.00/0

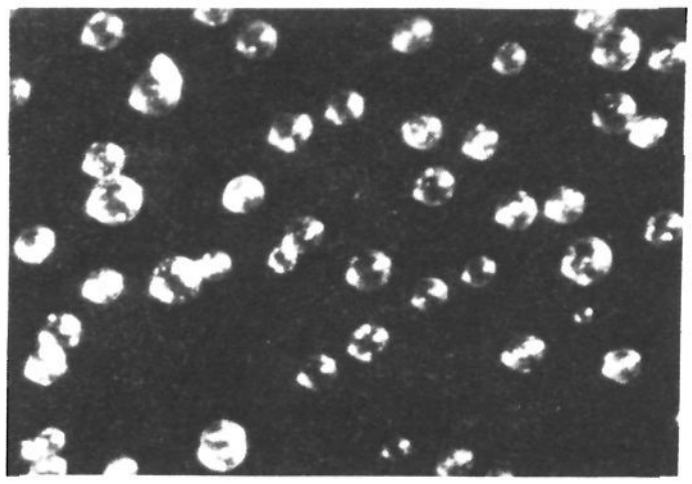


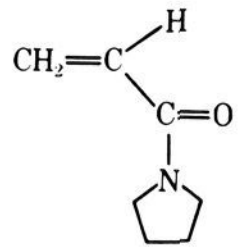
Figure 1. Poly(*N*-acrylylpyrrolidine)-*co*-4.4 mol % *N,N'*-bis(acrylyl)-1,2-diaminoethane-*co*-9.9 mol % *N*-acrylyl-1,6-diaminohexane hydrochloride (PAP) beads as obtained after drying to constant weight in vacuo at 40 °C over P_2O_5. Unsized beads photographed at 50× magnification.

since such a polymer only swells in water and highly polar media unsuited at the time of suggestion for peptide synthesis, the proposal was considered untenable. A poly(*N,N*-dimethylacrylamide) resin has been prepared and used for peptide synthesis.[10-12] However, this resin has been synthesized in only a partially beaded form[10] and attempts to scale up the procedure resulted in totally amorphous polymer.[11,†] While the physical form of the polymer does not change its chemical properties that make it useful for peptide synthesis, in our experience the handling of amorphous polymer poses problems and requires special techniques. Filtration of a polymer preparation containing even a small percentage of amorphous material was found to be impractically slow and alternate procedures such as centrifugation during the many steps of peptide synthesis are cumbersome at best.

Polymer Design

In our view two factors, chemical nature and topographical structure, determine the physicochemical properties that render a polymer matrix favorable for peptide synthesis. The present study deals primarily with the first factor; the evaluation of topographical contributions to peptide synthesis must be delayed and would become meaningful only after a suitable, beaded polymer has been obtained and studied in some detail. Three specific issues are the focal points of the current study: (1) search for a monomer which gives a polymer with a broad spectrum of solvent interactions. While polystyrene does not swell appreciably in polar solvents, acrylamide polymers have been reported to interact well only with polar solvents such as water, dimethylformamide (DMF), and certain alcohols but less well with CH_2Cl_2 and $CHCl_3$.[8,10] (2) Development of a reproducible procedure for obtaining beaded resin in large or small scale preparations. (3) Investigation of peptide-to-polymer bridging groups used previously and of new bridging groups. When the physicochemical properties of a new polymer differ dramatically from those of polystyrene, it is necessary to reexamine the behavior of well-established bridging groups as well as to develop new bridging groups which would take advantage of the ability of the new polymer to interact with both aqueous and nonaqueous media.

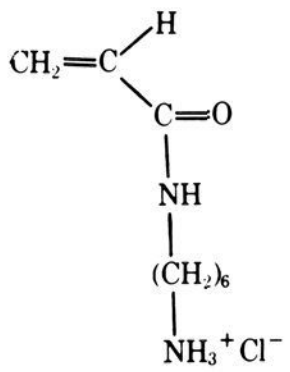
Addressing the first point of the above stated goals, the monomers desired should contain a carboxamide moiety, must be polymerizable by free radical initiation, and should be soluble both in organic and aqueous media so that the resulting polymer will have a wide range of solvent interactions. In addition, the solubility and reactivity of each monomer toward radical polymerization must be near those of the other monomers so that its uniform distribution in the polymer is likely.



1

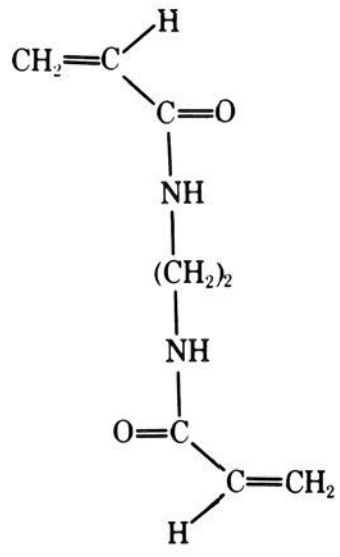
On this basis, *N*-acrylylpyrrolidine[13] (**1**) became the monomer of choice.

In order to introduce a chemical functional group into the polymer onto which the amino acid or peptide would eventually be attached, **1** was copolymerized with the functionalizing agent, *N*-acrylyl-1,6-diaminohexane hydrochloride (**2**), which is easily prepared in high purity and yield by a method recently developed in this laboratory.[14]



2

N,N'-Bis(acrylyl)-1,2-diaminoethane (**3**) was selected as the cross-linking agent because a conformationally flexible cross-link is required in order that all reaction sites in the



3

matrix are as sterically equivalent as possible and not subject to the constraints imposed by the cross-link.

The topography of the polymer matrix is determined by the chemical nature of the monomers, the molar percentage of cross-link and the monomer dilution ratio. In this study the molar percentage of cross-linking agent to base monomer was set at five. This degree of cross-linking (DC) provided the desired mechanical integrity for the resin. The monomer dilution ratio (MDR: ratio of the amount of inert solvent, i.e., water, to the amount of monomers by weight in the polymerization mixture) was set at four. When combined with a DC of five, a three-dimensional polymer resulted that posessed a high degree of flexibility as revealed by its good swelling properties.[15] The schematic structure of the polymer is de-

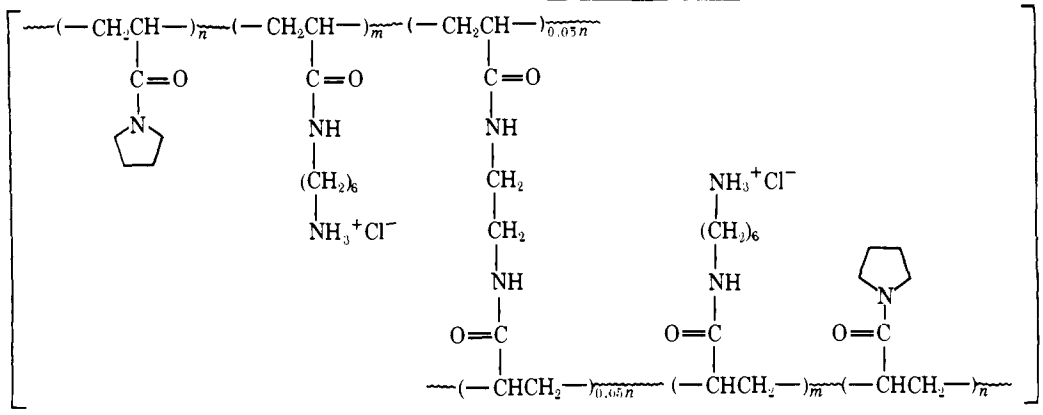
† Added in proof: A new procedure for the preparation of beaded polydimethylacrylamide resin has just been described (Arshady, R.; Atherton, E.; Gait, M. J.; Lee, K.; Sheppard, R. L. *J. Chem. Soc., Chem. Commun.* **1979**, 423.

Table I. Comparison of the Swelling Properties of Poly(*N*-acrylylpyrrolidine) (PAP) and Polystyrene Resin

solvent	spec vol (mL/g) PAP·HCl[a]	Boc-Gly-PAP[b]	Boc-Gly-polystyrene[c]
dry resin	1.5	1.5	1.6
acetone	1.5	2.4	3.8
methanol	9.5	6.6	1.6
ethanol	8.1	7.3	1.8
2-propanol	7.3	6.5	2.1
dimethylformamide	6.4	5.2	5.2
N-methylpyrrolidinone	5.4	6.2	6.5
CH_2Cl_2	6.4	7.5	7.6
$CHCl_3$	6.8	8.7	9.5
EtOAc	1.9	1.9	4.7
AcOH	7.9	8.5	2.5
H_2O	10.5	6.2	1.6
CF_3CH_2OH	14.1	11.2	1.6

[a] Poly(*N*-acrylylpyrrolidine) with DC 4.4, MDR 4, and 0.7 mmol of amine hydrochloride as determined by chloride titration.[18] [b] Same resin as in *a* fully acylated by Boc-Gly by using DCC/HBT coupling.[19] Amino acid analysis after 24, 48, and 72 h of hydrolysis at 110 °C in 6 M HCl gave 0.58 ± 0.01 mmol of Gly/g of resin. [c] Prepared from Lab-Systems polystyrene–1% divinylbenzene nominally chloromethylated to 0.75 mmol/g. Exhaustive acylation with Boc-Gly by using the cesium salt method of Gisin[20] gave 0.57 mmol of Gly/g of resin by amino acid analysis after hydrolysis of an aliquot in propionic acid/12 M HCl (1:1) for 24 h at 130 °C.

picted below and referred to hereafter as poly(*N*-acrylylpyrrolidine) (PAP), **4**.[17]



4

Polymer Synthesis

The synthesis of PAP was achieved by a system of reverse-phase suspension polymerization, which on the basis of our experience appears to be a general procedure for the preparation of polymers in beads of nearly any desired size starting with water-soluble monomers. An aqueous solution of monomer (**1**), functionalizing agent (**2**), and cross-linking agent (**3**) in appropriate ratios along with the first component of the redox initiator system, ammonium peroxydisulfate, was suspended in a mixture of hexane and carbon tetrachloride; importantly, this nonpolar solvent mixture prevents partitioning of the monomers into the organic phase. The density of the organic phase can be adjusted by addition of either the light or heavy solvent until the aqueous phase sinks only very slowly in the organic phase. Droplet size of the aqueous suspension is regulated by the addition of sorbitan sesquioleate and by the stirring rate of the mixture. Once the desired bead size has been obtained, the second half of the redox initiator system, *N,N,N′,N′*-tetramethyl-1,2-diaminoethane, was added. The beads thus secured in high yield are of such uniform size that they can be used directly for peptide synthesis without any sizing at all (Figure 1). Individual batches with average dry bead diameters of 200 μm down to 10 μm have been attained. Virtually quantitative conversions have been obtained with batch sizes varied from 2 to 30 g without deleterious effect on the bead quality of PAP.

Although the present composition and topography of PAP (DC of 4.4, MDR of four and having 0.7 mmol/g of amino group) may not yet be the optimal one for polymer-assisted peptide synthesis, the new resin shows remarkable promise. In either its protonated or acylated form, the resin exhibits favorable swelling characteristics with a variety of solvents ranging in polarity from water to CH_2Cl_2 (Table I). Boc-Gly-PAP is superior to Boc-Gly-polystyrene in its ability to interact with polar solvents such as CH_3OH, CH_3CH_2OH, CF_3CH_2OH, AcOH, and H_2O; all of these solvents are useful for peptide synthesis with PAP. In addition, Boc-Gly-PAP exhibits swelling properties that are nearly identical with those of Boc-Gly-polystyrene in DMF, *N*-methylpyrrolidinone, CH_2Cl_2, and $CHCl_3$ solvents commonly used in the Merrifield method. These especially favorable swelling properties of PAP should allow the use of most of the techniques developed for the solid-phase method as well as the development and employment of new techniques that rely on aqueous or other polar solvents (e.g., liquid NH_3), the use of which are precluded for polystyrene by its poor swelling properties in such solvents.

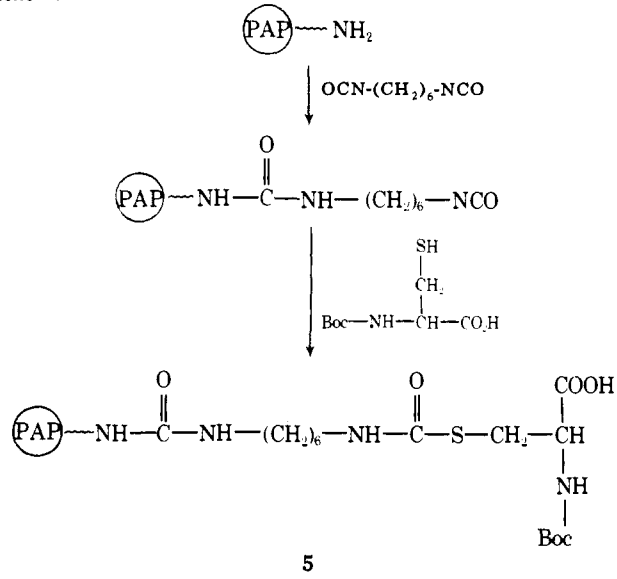
Peptide-to-Polymer Linking Groups for Amino Acid Attachment

Attachment of an amino acid to a resin via its side chain[21] was the first step in the development of the flexible bidirectional procedure of peptide synthesis on a polymer.[22] Side-chain functional groups offer an opportunity to use peptide-to-polymer linking groups that can be cleaved by methods distinct from those used during the elongation of the polypeptide.

S-Carbamoyl Group for Cysteine Side-Chain Attachment. A new polymer-to-peptide bridging group for the attachment of cysteine via its side-chain sulfhydryl moiety by using a *S*-carbamoyl (Scam) linkage to the hexamethylamino function of PAP was synthesized and its feasibility for bidirectional peptide synthesis was evaluated. The Scam group is a modification of the *S*-ethylcarbamoyl (Ec) side-chain protecting group introduced for cysteine by Guttmann.[23] On the basis of past experiences, the *S*-carbamoyl moiety has several features desirable for a polymer-to-peptide bridging group. It is acid-resistant, withstanding treatment with 2 M HBr/AcOH, does not undergo S → N migration or β-elimination when treated with tertiary amine.

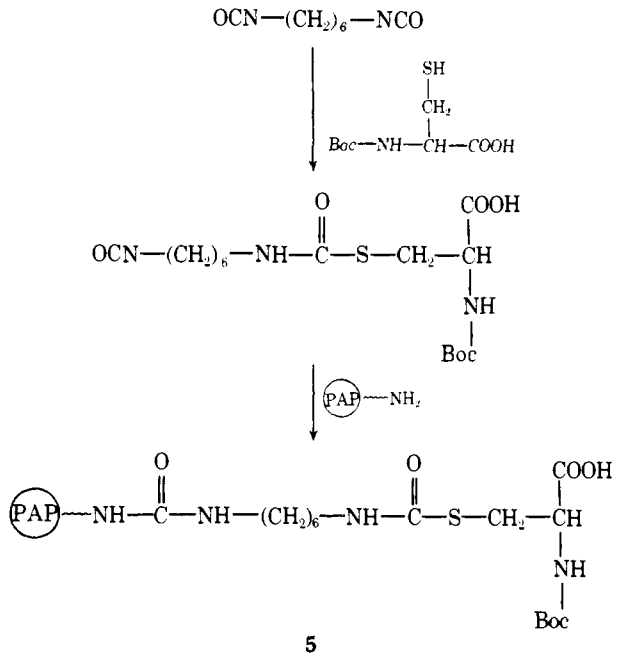
Scam was synthesized as depicted in Scheme I by treatment of the free base of PAP with a 10-fold excess of 1,6-diisocyanatohexane followed by the addition of a 3-fold excess of

Scheme I



5

Scheme II



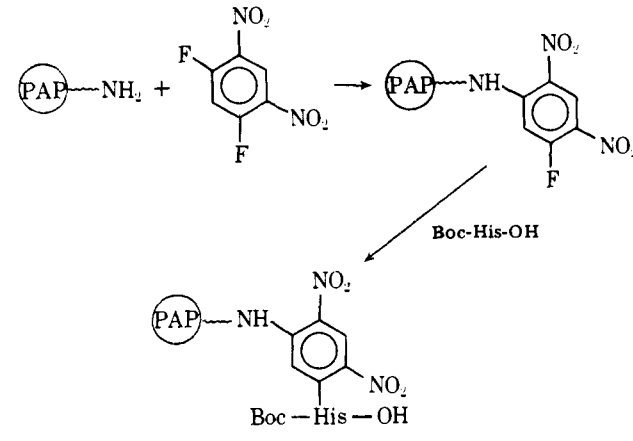
5

Boc-Cys. Boc-Cys(Scam-PAP) resin had a substitution of 0.23 mmol/g of Cys as determined by amino acid analysis after acid hydrolysis. In an attempt to eliminate intrachain cyclization,[24] Scam was also synthesized by an alternate procedure depicted in Scheme II. The fact that the resulting Boc-Cys(Scam-PAP) resin still had a substitution of only 0.25 mmol/g of Cys was most likely due to the fact that it proved impossible to completely remove all of the excess 1,6-diisocyanatohexane which competed for available amino groups.

Scam may be cleaved by a variety of base treatments such as 2 equiv of aqueous NaOH added to a methanol suspension of the resin or simply by refluxing the resin in liquid NH_3. It may also be cleaved by treatment with Na in liquid NH_3, if such treatment is desired to remove other protecting groups simultaneously.

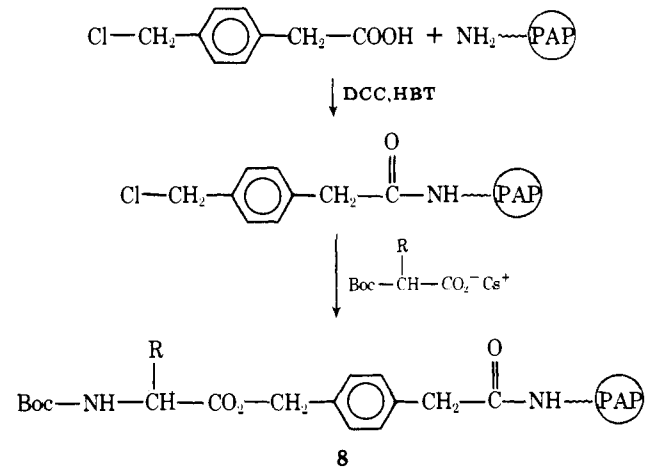
Dinitrophenylene Group for Histidine Side-Chain Attachment. The side chain of histidine has been protected and attached to resins for bidirectional peptide synthesis by the dinitrophenyl polymer-to-peptide linking group.[25] The versatility of this group[22,25] warranted its application to the new PAP resin as well. Scheme III illustrates the attachment of the group

Scheme III



6

Scheme IV



8

to the resin and the subsequent incorporation of Boc-His onto the resin. In brief, PAP·HCl was neutralized with 10% triethylamine and allowed to react with an excess of 1,5-difluoro-2,4-dinitrobenzene plus triethylamine. The resin was then reacted with Boc-His in the presence of diisopropylethylamine to yield Boc-His(DNP-PAP). The detachment of Boc-His from the resin by thiolysis[25] demonstrated that Boc-His(DNP-PAP) had a substitution of 0.47 mmol/g.

4-(Oxymethyl)phenylacetamido Bridging Group. In order to test the applicability of the more usual benzyl ester-type bridging group to PAP, the 4-(oxymethyl)phenylacetamido group[26-28] (OPA) was chosen. OPA was synthesized by two approaches. The pathway depicted in Scheme IV is similar to that used for the introduction of this group onto a polystyrene resin.[26] 4-(Chloromethyl)phenylacetic acid[29] (**7**) was coupled to the PAP resin by using dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole[19] (HBT). The resulting 4-(chloromethyl)phenylacetamido resin was reacted with the cesium salt of a *tert*-butyloxycarbonyl (Boc) amino acid in DMF[20] to yield Boc-aminoacyl-4-(oxymethyl)phenylacetamido-PAP resin (Table II). As discussed by Mitchell et al.,[28] this route either may lead to N-benzylation of some hexamethylamino sites or residual chloromethyl groups, which did not react with the first Boc-amino acid, or may participate in undesirable benzylation reactions at a later stage of a synthesis. Therefore, this route may not be desirable for an extensive synthesis, but it is most general and convenient for the attachment of a number of different amino acids for feasibility studies.

In a second approach Boc-aminoacyl-OPA-PAP was prepared via Boc-aminoacyl-4-(oxymethyl)phenylacetic acid

Table II. Percent of Substitution of *tert*-Butyloxycarbonyl Amino Acids on 4-(Oxymethyl)phenylacetamido-PAP

amino acid	substitution[a] (mmol of amino acid/g of resin)
Boc-Ala-	0.30
Boc-Glu(−)-O$^{\alpha}$Bu^{t}	0.35 (0.42)[b]
Boc-Gly-	0.26 (0.60)[b]
Boc-Ile-	0.35
Boc-Phe-	0.30
Boc-Val-	0.29

[a] Values obtained by treatment of 4-(chloromethyl)phenylacetamido-PAP with Boc-amino acid cesium salt. [b] Values obtained by coupling Boc-aminoacyl-4-(oxymethyl)phenylacetic acids onto PAP.

phenacyl ester intermediates.[28] It was found that coupling Boc-Gly-4-(oxymethyl)phenylacetic acid or Boc-Glu-O^5-(4-(oxymethyl)phenylacetic acid) *O-tert*-butyl ester to PAP gave higher substituted resins than by Scheme IV (Table IV). An inherent disadvantage of the second route is that it requires a number of synthetic steps for each individual amino acid with intermediates often difficult to purify.

The OPA bridge has been shown to be more stable to acid hydrolysis than the standard benzyl ester.[27,28] This was found also to be the case for this bridge attached to the PAP resin as well, since a 1:1 mixture of trifluoroacetic acid and methanesulfonic acid, which cleaved Gly-Ala from the standard Merrifield resin,[30] failed to cleave any amount of amino acid that could be detected by TLC from either Boc-Gly or Boc-Val-OPA-PAP resin. However, as the design of PAP was directed toward the removal of the product under mild reaction conditions, other methods were tried for the cleavage of the amino acid from the OPA-PAP resin.

Saponification. The inadequacy of OH^- to detach peptides from polystyrene polymer has been attributed to exclusion of these ions from this kind of polymer matrix.[31] Such is not the case for PAP, since its hydrochloride can easily be converted to the free base by treatment with an equivalent amount of aqueous NaOH. While cleavage of a Boc-amino acid from a Boc-aminoacyl-OPA-PAP by 2 equiv of NaOH in water was generally found to proceed too slowly to be of practical value, addition of methanol or ethanol to the reaction medium greatly enhanced the rate of cleavage. In the case of sterically hindered COOH-terminal amino acids, the cleaved product was contaminated with the corresponding methyl or ethyl ester. Use of branched chain alcohols eliminated this difficulty; *tert*-butyl alcohol or isopropyl alcohol–H_2O (7:3, v/v) was found to be the solvent of choice. Rates of saponification for Boc-Glu-(OPA-PAP)-O-Bu^t in these two solvents are shown in Figure 2A. Cleavage was quantitative in 4 h in either solvent by addition of 2 equiv of 1 M NaOH (0.07 M maximum initial concentration) and no detectable ester formation was seen when 50-μg samples of cleaved products were applied for TLC. The Boc-amino acids—Gly, Ala, and Phe—were all cleaved quantitatively by using the above conditions. Even the hydrophobic, sterically hindered dipeptide Boc-Leu-Ile-OPA-PAP was cleaved 72% and Boc-Leu-Val-OPA-PAP was cleaved 95% when 1 equiv more of 1 M NaOH was added after 4 h and the reaction allowed to proceed an additional 2 h. In all examples, no contamination by ester was detected. The extent of racemization of the Boc-Leu-Val thus obtained was determined by analyzing for L-Leu-D-Val[32] after removing the protecting groups with CF_3CO_2H. The dipeptide contained 0.7% of the L,D diasteroisomer. When in a control experiment crystalline Boc-L-Leu-L-Val-OMe[33] was subjected to identical saponification conditions, 0.2% L-Leu-D-Val was found after removal of the Boc protecting group. The additional 0.5% L-Leu-D-Val may be partially the result of the presence of the resin during saponification or may be formed during the attachment of Boc-Val to the resin or during subsequent synthetic procedures.

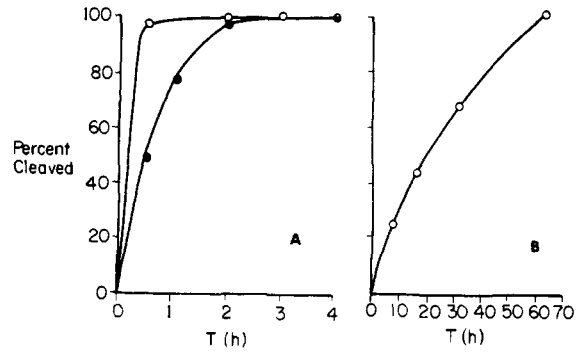


Figure 2. Rates of saponification and ammonolysis of Boc-Glu(OPA-PAP)OBu^t. (Panel A) Saponification in 70% 2-propanol in water by 2 equiv of 1 M NaOH (○—○). Saponification in *tert*-butyl alcohol by 2 equiv of 1 M aqueous NaOH (●—●). (Panel B) Ammonolysis in CF_3CH_2OH at 25 °C; solution was saturated with ammonia at 0 °C.

Reducing Techniques. The well-known cleavage reaction of benzyl ester with sodium in liquid ammonia[34] and the successful cleavage of Scam with this reagent prompted us to try the technique with the OPA linkage. Surprisingly it was found that neither Boc-Val-OPA-PAP nor Val-OPA-PAP was cleaved to any detectable degree by such treatment, despite the observation that PAP swells in ammonia and the characteristic blue color of dissolved sodium metal fades. A competitive reductive cleavage of the polylacrylylpyrrolidine amide bond, similar to that observed for X-Pro bonds,[35] is one possible explanation for this finding.

It has been demonstrated that Pd(II) acetate dissolved in DMF can penetrate polystyrene resin and be converted into an active catalyst for hydrogenolysis.[36] The favorable solvation properties of PAP in solvents that would be potentially useful for hydrogenolysis (e.g., alcohols, acetic acid, water, and secondary amides) lead us to test the feasibility of the technique for the detachment of protected amino acids from various Boc-aminoacyl-OPA-PAP resins. Table III shows the results of some of our experiments with various solvents and differing concentrations of Pd(II) salts. Pd(II) acetate was soluble in DMF and CF_3CH_2OH, but hydrogenolysis proceeded only in DMF. By using dimethylamine-free DMF with two additions of catalyst, Boc-Ala, Boc-Phe, and Boc-Glu-Oα-Bu^t have been successfully detached. The desirability of such a mild procedure as catalytic hydrogenolysis for peptide detachment and the positive results of these initial experiments warrant further investigation.

Ammonolysis. Boc-amino acids, in all but the most sterically hindered examples, were readily cleaved as their amides from Boc-aminoacyl-OPA-PAP resins quantitatively by ammonolysis in methanol[37] (Table IV). It was found that with some sterically hindered amino acids a significant contamination occurred due to concurrent formation of the methyl ester. This difficulty was circumvented through the use of CF_3CH_2OH as the solvent. This solvent swells Boc-amino-acyl-OPA-PAP resins almost 10-fold (Table I) and absorbs ammonia at 0 °C to cause a 50% increase in total solution volume. In addition, reaction temperatures may be increased to as high as 40 °C without dangerously increasing the pressure within the sealed reaction vessel. The rate of ammonolysis for Boc-Glu(OPA-PAP)-OBu^t in CF_3CH_2OH is shown in Figure 2B. Thus, even the sterically hindered hydrophobic protected dipeptide Boc-Leu-Val- was detached smoothly and free from ester contamination.

Table III. Percent Cleavage of *tert*-Butyloxycarbonyl Amino Acid from *tert*-Butyloxycarbonylaminoacyl-4-(oxymethyl)-phenylacetamido-PAP by Hydrogenolysis[a]

	conditions[b]					
amino acid	*N*-methylpyrrolidinone Pd(II)OAc[c]	DMF Pd(II)OAc[c]	DMF Pd(II)OAc[d]	DMF Pd(II)OAc[e]	10% HOAc-DMF Pd(II)OAc[c]	H_2O $PdCl_2$[f]
Boc-Gly	47	57	85		74	
Boc-Ile	46	58	78	70	24	48

[a] Amino acid analysis cannot be performed on the catalyst impregnated resins. Yields are of amino acids actually isolated. [b] Reactions were conducted at 1 atm of H_2 pressure at 45–50 °C for 24 h. [c] Catalyst concentration 20 mg/mL. [d] Initial catalyst concentration 20 mg/mL; second addition of 20 mg/mL catalyst after 8 h. [e] Initial catalyst concentration 20 mg/mL; doubled after 4 h and tripled after 6 h. [f] Resin initially wetted with EtOH, catalyst concentration 20 mg/mL, made acid with 1 drop of concentrated HCl.

Table IV. Percent Cleavage of *tert*-Butyloxycarbonyl Amino Acid or Peptide from *tert*-Butyloxycarbonylaminoacyl-4-(oxymethyl)-phenylacetamedo-PAP by Ammonolysis

amino acid or peptide	% cleaved (MeOH)	% cleaved (CF_3CH_2OH)	time (h) (at 25 °C)
Boc-Ala-	100	100	72
Boc-Glu(−)-OBu^t	100	100	48
Boc-Gly-	100	99	72
Boc-Leu-Ala	98	99	72
Boc-Leu-Val-	56[a]	74[b]	72
Boc-Phe-	100	100	72
Boc-Val	85	72[b]	72

[a] Reaction period was 24 h. Product contaminated with approximately 50% methyl ester. [b] Reaction temperature was 40 °C.

Table V. Sequence of Operations Used for Each Deprotection and Coupling Cycle

step no.	reagent	repetitions	time (min)
1	CH_2Cl_2	4	2
2	CF_3CO_2H/CH_2Cl_2/anisole (49:49:2, v/v/v)	1	30
3	CH_2Cl_2	5	2
4	DMF	3	2
5	preactivated Boc-amino acid plus diisopropylethylamine[a]	1	120
6	DMF	4	2
7	EtOH	3	2

[a] See Experimental Section.

Coupling Reactions in Aqueous and Nonaqueous Solvents

Coupling reactions to Ile-OPA-PAP in solvents with different polarities were investigated. First, the symmetrical anhydride[38] of Boc-Leu (3 equiv) was prepared and added to Ile-OPA-PAP suspended in CH_2Cl_2. The reaction was complete in less than 1 min as monitored by the ninhydrin[39] and picrylsulfonic acid[40] tests. Second, Boc-Leu·H_2O (3 equiv) in DMF was preactivated[41] by DCC, mediated by HBT,[19] and added to Ile-OPA-PAP suspended in DMF. The reaction was completed within 5 min. Third and most importantly, a suspension of Ile-OPA-PAP in 5% aqueous $NaHCO_3$, when treated with an equal volume of ethanolic Boc-Leu-OSu[42] (4 equiv), was also coupled in less than 5 min. The Ile-Leu ratio determined by amino acid analysis for all three Boc-Leu-Ile-OPA-PAP resins was 1:1. Thus, efficient coupling reactions even to sterically hindered Ile have been demonstrated on PAP in solvents of widely different polarities including aqueous media.

Bidirectional Synthesis of Polypeptides

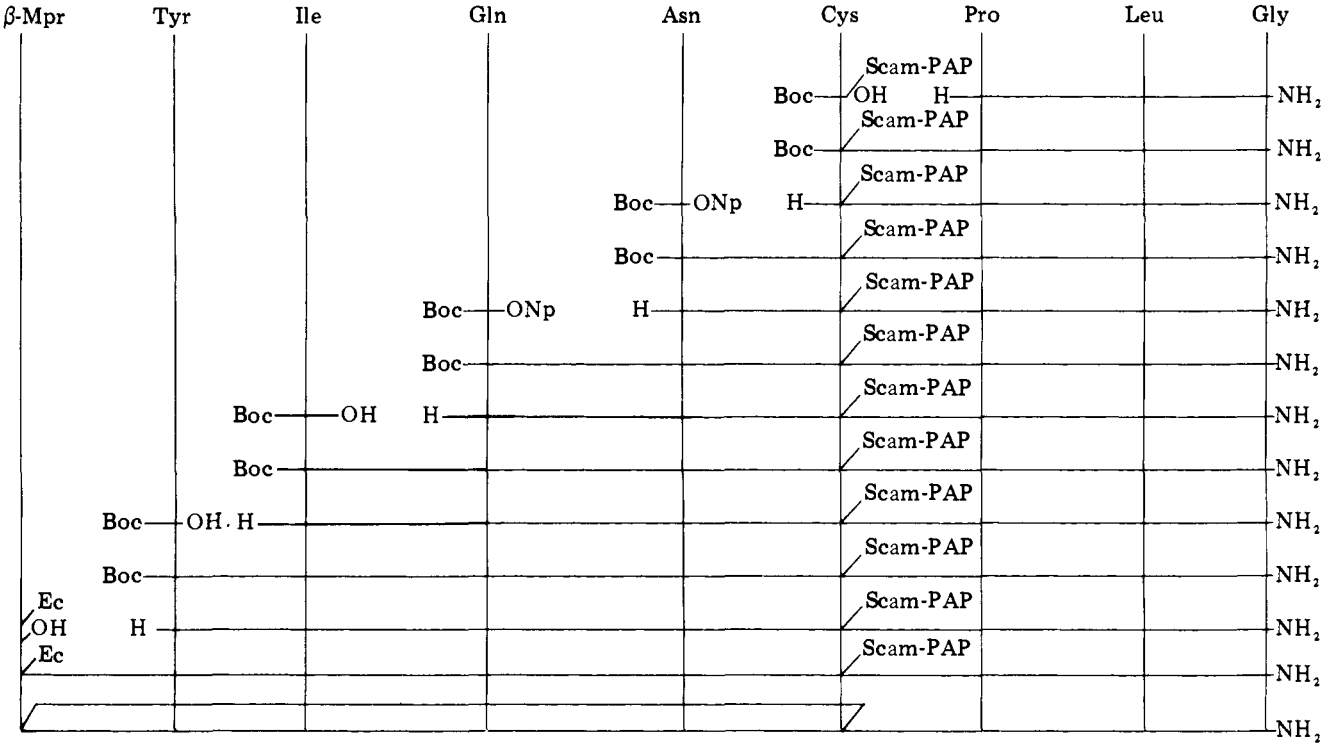
Some years ago the method of bidirectional solid-phase peptide synthesis[22] was introduced with the preparation of the tripeptide, thyrotropin-releasing hormone (TRH).[25] It was concluded that the attachment of an amino acid residue to an insoluble polymer by its side chain allows flexible bidirectional schemes of synthesis, usually restricted to classical solution techniques, to be combined with advantages of polymer-supported peptide synthesis. It is also recognized that the chemically different functionalities of amino acid side chains provide an opportunity to explore peptide-to-polymer linking groups that can be cleaved by methods specific and distinct from conditions used for protecting-group removal during peptide chain elongation.

Now we report on an extension of the bidirectional method of peptide synthesis to include the use of the newly developed poly(*N*-acrylylpyrrolidine) (PAP) type resin[17] in combination with the use of the Cys, His, and Glu amino acid side chains as anchoring moieties to the polymeric support, and the *S*-carbamoyl (Scam), dinitrophenyl (DNP),[25] and 4-(oxymethyl)phenylacetamido (OPA)[26,28] groups as peptide-to-polymer bridging groups.

Bidirectional Synthesis of Deaminooxytocin Using the Cysteine Side Chain as Attachment Point. By using the Scam moiety as peptide-to-polymer linking group, Boc-Cys was attached to PAP to yield Boc-Cys(Scam-PAP) (**5**). This intermediate served in this study as starting material for the synthesis of deaminooxytocin,[43] a potent analogue of the posterior pituitary hormone oxytocin.[44] The approach used in the bidirectional synthesis of the analogue is summarized in Scheme V.

Intermediate **5** was extended in the COOH-terminal direction by the coupling of Pro-Leu-Gly-NH_2·0.5H_2O[45] by using DCC in the presence of HBT.[19] Boc-Cys-(Scam-PAP)-Pro-Leu-Gly-NH_2, after removal of the Boc-protecting group, was elongated in the NH_2-terminal direction in a stepwise manner according to the schedule of procedures given in Table V. The usual step of washing with tertiary amine was omitted to ensure that no cleavage of the Scam group occurred by the hydroxide produced from trace amounts of water in the solvents. Instead, diisopropylethylamine was added during the coupling reaction. Boc-protected amino acids were coupled either as their *p*-nitrophenyl esters mediated by HBT[46] or as free acids preactivated by DCC/HBT.[41] The sulfhydryl group of β-mercaptopropionic acid (β-Mpr) was protected by the *S*-ethylcarbamoyl (Ec) group[23] and the tyrosine hydroxyl group was left unprotected. Coupling occurred rapidly; the nitrophenyl ester couplings were completed in 60 min, and most reactions were finished before 5 min, when the first test sample was withdrawn from the reaction mixture. Completeness of coupling was evaluated most reliably by a modified version of the procedure of Hancock and Battersby[40] rather than the ninhydrin test.[39,47] The amino acid composition of the peptide intermediate attached to the resin is given in Table VI (step 7). Note that the substitution of cysteine after the final coupling step corresponds to the degree of substitution expected for complete incorporation of all residues in the absence of any

Scheme V



cleavage of the Scam peptide-to-polymer linkage. The peptide was removed from the resin by treatment of a slurry of the polymer in methanol under a nitrogen atmosphere with 3 equiv of aqueous NaOH. The liberation of sulfhydryl groups was measured by the Ellman method[48] and it was found that cleavage was complete and quantitative after 15 min. The solution was then diluted with MeOH/H_2O (1:1) and the disulfide bond formed by oxidation with ICH_2CH_2I.[49] The product was purified by gel filtration on Sephadex G-15, first in 50% AcOH, then in 0.2 M AcOH, and was finally isolated by lyophilization. The overall yield of deaminooxytocin based on the initial substitution of cysteine from step 1 on the resin was 59%. The product possessed the full biological potency of deaminooxytocin lyophilizate of 690 ± 40 units/mg (lit.[43] 684 ± 32) uterotonic activity determined on the isolated rat uterus. The peptide produced a single spot on thin-layer chromatography in two solvent systems and had the expected amino acid analysis (Table VI). The yield of deaminooxytocin obtained with this PAP resin is higher than the overall yield obtained from the best solid-phase syntheses of neurohypophyseal

Table VI. Amino Acid Analysis Data for Bidirectional Deaminooxytocin Synthesis[a]

coupling cycle no.:		1	2		7			
residue no. in the peptide:		6	6–9		1–9		theory[b]	final product
Cys	substitution[c]	0.23[d,e]	0.19[e]		0.16[e]		0.16	
	ratio			0.82		0.89	1.00	1.03[e]
Pro	substitution		0.23		0.18		0.16	
	ratio			1.00		1.00	1.10	0.97
Leu	substitution		0.23		0.18		0.16	
	ratio			1.00		1.00	1.00	1.02
Gly	substitution		0.23		0.18		0.16	
	ratio			1.00		1.00	1.00	1.03
NH_3	substitution		0.22		0.58		0.48	
	ratio			0.94		3.22	3.00	2.83
Asp	substitution				0.17		0.16	
	ratio					0.94	1.00	1.00
Glu	substitution				0.18		0.16	
	ratio					1.00	1.00	1.05
Ile	substitution				0.16		0.16	
	ratio					0.89	1.00	0.95
Tyr	substitution				0.15		0.16	
	ratio					0.83	1.00	0.96[f]
β-Mpr | Cys	ratio							0.46
½ cystine	ratio							0.41

[a] Standard hydrolysis conditions (see Experimental Section) were used unless otherwise indicated. [b] Based on an initial substitution of 0.23 mmol of Cys/g of resin corrected for theoretical weight gain. [c] Substitution in mmol/g. [d] Determined as cysteine. [e] Determined as cysteic acid. [f] Phenol, 0.2%, added to hydrolysis mixture.

hormones.[50,51] Solution-phase synthesis of these hormones generally give poorer yields than those achieved by the solid-phase method.[51]

Synthesis of Thyrotropin-Releasing Hormone (TRH) with His Side-Chain Attachment. In this study the use of the dinitrophenylene polymer-to-peptide bridging group (**6**) was applied for bidirectional synthesis on PAP. N^{α}-Boc-N^{im}-(2,4-dinitro-5-PAP-phenyl)histidine [Boc-His(DNP-PAP)] was first extended in the COOH-terminal direction by the addition of proline amide. After removal of the Boc group with 50% CF_3CO_2H in CH_2Cl_2, the resino dipeptide was acylated at the NH_2 terminus by pyroglutamylpentachlorophenyl ester[52] (<Glu-OPhCl$_5$). The product was liberated from the resin by thiolysis,[25] precipitated, and purified by a single high performance liquid chromatography step on silica gel by using aqueous 70% ethanol. The purified TRH (48% yield based on the histidine content of the resin) released 14 873 ± 774 Δng/mL of medium of TSH from female rat pituitaries in vitro as compared with 15 167 ± 695 Δng/mL for the standard preparation of Bowers et al.[53]

Synthesis of a Model Tripeptide with Glutamyl Side-Chain Attachment. N^{α}-Boc-glutamyl-O^5-(4-(oxymethyl)phenylacetamido-PAP) *O-tert*-butyl ester (Boc-Glu-(OPA-PAP)-OBu^t) (**8**) was converted by treatment with CF_3CO_2H to Glu(OPA-PAP). Next the liberated NH_2 terminus of this intermediate was acylated by Boc-Pro-OSu. The resulting resino dipeptide was activated with DCC, mediated by HBT, and extended with glycinamide at the COOH terminus. This tripeptide resin served as a convenient intermediate for the synthesis of both Z-Pro-Gln-Gly-NH_2 and Z-Pro-Glu-Gly-NH_2. Z-Pro-Gln-Gly-NH_2 was obtained by ammonolysis of the substituted resin in CF_3CH_2OH in 74% yield after purification. The product was identical with a sample prepared by solution techniques. Purified Z-Pro-Glu-Gly-NH_2 was obtained in 53% yield after saponification of the resin with 0.14 M NaOH in *t*-BuOH-H_2O (6:1, v/v) for 4 h.

In summary, a general method is reported by which three-dimensional polymers may be prepared from water-soluble monomers (which may also possess an appreciable solubility in organic solvents) in a beaded form of predetermined size. The synthesis of poly(*N*-acrylylpyrrolidine) (PAP) allows precise control of the extent of substitution by regulation of the quantity of functionalizing agent, *N*-acrylyl-1,6-diaminohexane hydrochloride, which is copolymerized. The new resin possesses a broad range of solvent interactions enabling use of most of the techniques developed for the Merrifield method of solid-phase peptide synthesis as well as allowing the application of procedures not possible on polystyrene based supports. The feasibility of amino acid and peptide attachment and detachment has been demonstrated by using *S*-carbamoyl, dinitrophenyl, and benzyl ester polymer-to-peptide bridging groups. By using these bridging groups the bidirectional synthesis of deaminooxytocin, TRH, Glu- and Gln-containing model peptides has been achieved. Moreover, the conditions used for detachment take advantage of the physicochemical properties of the new polymer and are not only mild, but are different from the acidic conditions employed for the removal of N^{α}-protecting groups during the course of peptide chain elongation.

Experimental Section

General Procedures. All melting points were determined in open capillary tubes and are reported uncorrected. Thin-layer chromatography was performed on precoated plates of silica gel G-60 F-254 (E. Merck). Compounds were applied in loads of 20, 40, and 60 μg and chromatograms were developed for 10–15 cm in the following solvent systems (all by volume): (A) acetone-AcOH-H_2O (18:1:1); (B) acetone-AcOH (9:1); (C) $CHCl_3$-MeOH-AcOH (18:1:1); (D) $CHCl_3$-MeOH (18:1); (E) $CHCl_3$-MeOH-38% NH_4OH (10:4:0.4); (F) 1-BuOH-AcOH-H_2O (4:1:1); (G) EtOAc-pyridine-AcOH-H_2O (5:5:1:3); (H) EtOH-H_2O (7:3); (I) $CHCl_3$-MeOH-38% NH_4OH (5:5:1); (J) $CHCl_3$-MeOH-AcOH-H_2O (7:3:0.5:0.1); (K) $CHCl_3$-MeOH-AcOH (9:1:1). Visualization was performed by treatment with Cl_2 followed by starch-KI spray. Amino acid analyses[54] were performed on a Durrum Model 500 amino acid analyzer following hydrolysis in degassed 6 M HCl at 110 °C for 22 h. Most of the reactions on PAP resin were performed with a Schwarz/Mann automated peptide synthesizer. Optical rotations were taken on a Zeiss 0.01° circle polarimeter.

Completeness of coupling reactions was measured by a modified version of the procedure of Hancock and Battersby.[40] A 1% solution of picrylsulfonic acid (PSA) (Chemical Dynamics) was prepared in distilled *N*-methylpyrrolidinone or DMF. A 1–10-mg portion of peptide resin was placed in a small test tube and 2 drops (Pasteur pipet) each of PSA solution and 10% diisopropylethylamine in $CHCl_3$ were added. After 10 min at room temperature, 1 mL of EtOH was added and the resin beads were viewed through a 10× magnifier. All the color was on the beads and ranged from bright red for a very positive test (0.5 mmol of free amine/g) to faint yellow for approximately 0.001 mmol of free amine/g.

***N,N'*-Bis(acrylyl)-1,2-diaminoethane (3).** A solution of acrylyl chloride (16.6 mL, 0.20 mol) in $CHCl_3$ (150 mL) was cooled to −30 °C. A solution of 1,2-diaminoethane (6.1 mL, 0.091 mol) and triethylamine (26 mL, 0.19 mol) in 150 mL of $CHCl_3$ was added dropwise while keeping the reaction temperature at −30 to −40 °C. After 2 h at 23 °C, the mixture was filtered, the filtrate evaporated to 10 mL, and the product precipitated with hexane. The precipitate was dissolved in MeOH (200 mL) and passed through a 3 × 60 cm column of Rexyn 201 (OH^- cycle). The eluate was treated with decolorizing charcoal, the solvent evaporated, and the product crystallized from EtOAc–hexane: 10.5 g (62%); mp 142–143 °C. Recrystallization with 88% recovery; mp 144–145 °C; (lit. mp[55] 144–145 °C). Single spot on TLC R_f (A) 0.76. Anal. Calcd for $C_8H_{12}N_2O_2$: C, 57.1; H, 7.19; N, 16.6. Found: C, 56.9; H, 7.10; N, 16.6.

Poly(*N*-acrylylpyrrolidine) (PAP) (4). In a typical experiment, a solution of *N*-acrylylpyrrolidine[13] (22.3 g, 180 mmol), **3** (1.5 g, 9 mmol), *N*-acrylyl-1,6-diaminohexane hydrochloride[14] (4.3 g, 20.7 mmol), and $(NH_4)_2S_2O_8$ (0.48 g) in H_2O (110 mL) was added to an organic phase of hexane (575 mL) and CCl_4 (265 mL) in a 1-L wide-mouth reaction vessel fitted with a mechanical stirrer. The density of the organic phase was adjusted by addition of 20 mL more CCl_4 so that the aqueous phase sank slowly when stirring was stopped. With the stirrer at 600 rpm, 0.3 mL of sorbitan sesquioleate was added and the mixture flushed for 20 min with N_2-saturated with vapor of the same composition as the suspension. Finally, *N,N,N'N'*-tetramethyl-1,2-diaminoethane (0.96 mL) was added and, after an induction period of 3 min, the reaction temperature rose from 23 to 30 °C in a span of 5 min. Stirring was continued for 30 min; the beads were filtered and washed with 2-propanol, MeOH, H_2O, EtOH, $CHCl_3$, EtOAc, and dried in vacuo at 80 °C to constant weight: 28.0 g (99.6% conversion). The dry bead diameter of the batch was 150 ± 25 μm measured by a vernier equipped microscope. Titration for chloride[18] gave a substitution of 0.69 mmol/g. A 1.0-g portion of the resin was neutralized with 10% diisopropylethylamine in CH_2Cl_2. A solution of Boc-Gly (0.37 g, 2.1 mmol) and HBT·H_2O (0.64 g, 4.2 mmol) in DMF was preactivated with DCC (0.43 g, 2.1 mmol) for 50 min, filtered and added to a DMF suspension of the resin. After 15 min, diisopropylethylamine (0.1 mL, 0.6 mmol) was added and the reaction continued for 4 h. This procedure was repeated for an additional 4 h after which the resin was negative to the ninhydrin test and to PSA. Amino acid analysis after 24-, 48-, and 72-h hydrolyses of aliquots revealed a substitution of 0.58 ± 0.01 mmol of glycine per g of resin (94% of theoretical). The hydrochloride and Boc-glycine-PAP resin were used to determine the specific volume in various solvents shown in Table I. Dry resin (70 mg) was packed in a 3 × 170 mm column having 0.01-mL graduations. The volume of the packed resin was determined while dry, and after thorough equilibration with each solvent.

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-*S*-[carbamoyl-poly(*N*-acrylylpyrrolidine)]cysteine, Boc-Cys(Scam-PAP)-OH (5).** A 7.1-g portion of **4** (4.2 mmol of amino group) was washed thrice with 100-mL portions of the following: $CHCl_3$, $CHCl_3$–diisopropylethylamine (9:1), $CHCl_3$, and DMF. To an agitated suspension of the resin in DMF was added 1,6-diisocyanatohexane (Polysciences) (7.06 g, 42 mmol) and diisopropylethylamine (1.4 mL, 8.4 mmol). After 2 h, the reaction mixture

was drained and the resin washed with DMF (3 times, 100 mL). The resin was then treated for 18 h with a DMF solution (100 mL) of Boc-Cys[45] (13 mmol), after which the resin was washed with DMF, EtOH, and DMF again (thrice each, 100 mL). Finally the resin was suspended in DMF and acetic anhydride (1.0 mL) and diisopropylethylamine (0.7 mL, 4.2 mmol) were added. After 60 min, the reaction vessel was drained and the resin washed with DMF (100 mL), 10% aqueous AcOH (3 times, 100 mL), EtOH (three times, 100 mL), and $CHCl_3$ (three times, 100 mL). Amino acid analysis gave a substitution of 0.23 mmol of cysteine per g of resin.

Alternate Preparation for (5). A solution of Boc-Cys (10 mmol) in *N*-methylpyrrolidinone was added dropwise with stirring to a solution of 1,6-diisocyanatohexane (5.0 g, 30 mmol) in *N*-methylpyrrolidinone. After 30 min, the solvent was removed by evaporation (0.04 mmHg, 52 °C), and the resulting oil triturated with hexane (20 mL, four times). When the last hexane wash was evaporated, only a few drops of 1,6-diisocyanatohexane were present, but TLC in system B still showed significant contamination by the diisocyanate. When repeated attempts to crystallize *N*-Boc-*S*-(1-carbamoyl-6-isocyanatohexane)cysteine failed, the crude product was dissolved in DMF (450 mL) and added to 3.8 g of PAP which had been previously neutralized as for **5**. Diisopropylethylamine (0.43 mL) was added and the reaction allowed to proceed overnight. The resin was washed and acetylated as above; amino acid analysis gave 0.25 mmol of cysteine per g of resin.

Cleavage of Boc-Cys(Scam-PAP). A. Reaction with Sodium Hydroxide. Boc-Cys(Scam-PAP) (0.50 g, 125 μmol) was suspended in N_2-flushed MeOH (9 mL) and 0.25 mL of 1 M aqueous NaOH solution added. Aliquots of 10 μL were withdrawn at 3-min intervals and the amount of sulfhydryl was determined by the method of Ellman.[48] Cleavage was complete and quantitative in 15 min. TLC of the acidified solution in system C showed only Boc-Cys. Amino acid analysis of the resin showed <0.001 mmol Cys/g.

B. Reactions with Sodium in Liquid NH_3. Boc-Cys(Scam-PAP) (0.3 g, 69 μmol) was suspended in refluxing anhydrous liquid NH_3 (70 mL of freshly distilled from Na) and a glass-encased stick of sodium metal (freshly cut surface) was introduced until the blue color of dissolved sodium persisted for 30 s without additional sodium. The excess sodium was discharged with a drop of AcOH and the NH_3 was removed by evaporation and lyophilization. The residue was dissolved in 50 mL of MeOH–H_2O (1:1, v/v) and a 200-μL aliquot withdrawn for quantitative sulfhydryl group determination showed 60% of the theoretical amount. Amino acid analysis of the resin revealed 0.07 mmol of Cys/g (70% cleaved). Longer treatments with sodium (up to 30 min) did not substantially improve the yield. As above, TLC showed only Boc-Cys and a trace of $(Boc\text{-}Cys)_2$.

C. Reaction with Liquid NH_3. Boc-Cys(Scam-Pap) was suspended in anhydrous liquid NH_3 and refluxed for 3 h. The NH_3 was removed and, when the residue was dissolved in MeOH–H_2O (50 mL, 1:1, v/v), quantitative determination of sulfhydryl groups showed 74% of the theoretical amount.

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-*N*^{im}-(2,4-dinitro-5-PAP-phenyl)histidine, Boc-His(DNP-PAP) (6).** Pap·HCl (1.0 g, 0.7 mmol) was treated with 10-mL portions as follows: $CHCl_3$ (five times), 10% triethylamine in $CHCl_3$ (four times), and $CHCl_3$ (five times). A solution of 1,5-difluoro-2,4-dinitrobenzene (2.5 g, 12.2 mmol) in $CHCl_3$ (15 mL) was added to the resin and shaken. At 45-min intervals, 50 μL of triethylamine was added until 250 μL total. After shaking 18 h, the resin was ninhydrin negative. The reaction mixture was drained and the resin washed five times which 10-mL portions of $CHCl_3$ and DMF. A solution of Boc-His (0.9 g, 3.5 mmol) and diisopropylethylamine (0.6 mL, 3.5 mmol) was prepared in warm (50 °C) DMF (15 mL) and added to the resin. After shaking for 2 days, the reaction mixture was drained and the resin washed five times with 10-mL portions of DMF, $CHCl_3$, and EtOAc. Thiolysis with mercaptoethanol[25] showed the resin to contain 0.47 mmol of His per g of resin.

4-(Chloromethyl)phenylacetic Acid (7). The procedure of Bogdanov[29] was followed. A suspension of phenylacetic acid (32 g, 0.28 mol), $ZnCl_2$ (12.5 g, 0.08 mol), and aqueous 37% formaldehyde (110 mL, 1.5 mol) was warmed to 75 °C and HCl was slowly bubbled through the stirred solution for 12 h while maintaining a 75–80 °C temperature. The reaction mixture was cooled to room temperature and the insoluble material removed by filtration and washed with $CHCl_3$. The washed product (6.5 g) was crystallized from $CHCl_3$ (100 mL) to yield 4.7 g, mp 155–156 °C. From the mother liquor and previous washings, another 5.84 g of crystalline product was obtained: total yield, 9.84 g (23%); mp 155–156 °C (lit.[27,29] 152–153 °C, 153–154 °C).

***N*-*tert*-Butyloxycarbonylaminoacyl-4-(oxymethyl)phenylacetamido-poly(*N*-acrylylpyrrolidine) Resin (8).** The glycine resin is typical. A sample of **4** (2.0 g, 1.2 mmol of amine) was washed with $CHCl_3$ (20 mL, twice), 7% diisopropylethylamine in $CHCl_3$ (20 mL, thrice), and $CHCl_3$ (20 mL, four times). A solution of **7** (660 mg, 3.6 mmol), DCC (741 mg, 3.6 mmol), and HBT (550 mg, 3.6 mmol) in DMF (20 mL) was added and after shaking for 10 min diisopropylethylamine (0.21 mL, 1.2 mmol) was added. After 15 h, the resin was checked by the ninhydrin test and estimated to be 90% acylated.[39] The resin was washed with DMF (20 mL, twice), EtOH (20 mL, thrice), and DMF (20 mL, four times). The remaining amino groups were acetylated with acetic anhydride (2 mL) in DMF (20 mL) and diisopropylethylamine (0.2 mL) for 15 h. The ninhydrin negative resin was then washed with DMF and $CHCl_3$ and dried in vacuo at 35 °C. A sample (1.00 g) was suspended in a DMF (10 mL) solution of Boc-Gly Cs^+ [20] (307 mg, 3.0 mmol) and shaken for 24 h. The resin was filtered, washed with DMF, water, and MeOH, and dried in vacuo at 50 °C. Amino acid analysis gave a Gly value of 0.26 mmol/g of resin. Results for resins substituted by Boc-Ala, Boc-Glu(−)-O$^{\alpha}$-Bu^t, Boc-Ile, Boc-Phe, and Boc-Val, all prepared in the same manner, are given in Table II.

***N*-*tert*-Butyloxycarbonylglycyl-4-(oxymethyl)phenylacetic Acid, Boc-Gly-OPA (9).** This compound was prepared by the general method of Mitchell et al.[28] from Boc-Gly·DCHA (14.3 mmol) and 4-(bromomethyl)phenylacetic acid phenacyl ester (7.3 mmol). Boc-Gly-OPA phenacyl ester crystallized from EtOAc–hexane: 1.42 g (44% yield); mp 64–69 °C; TLC (D) single spot R_f 0.65 (R_f 0.70 for 4-(bromomethyl)phenylacetic acid phenacyl ester and R_f 0.09 for Boc-Gly). The phenacyl ester (1.10 g, 2.5 mmol) was reduced with zinc in aqueous 85% AcOH and isolated as its DCHA salt:[28] 1.06 g (84%, 37% overall); mp 149–151 °C; TLC (E) two spots R_f 0.76 for DCHA and R_f 0.34 for Boc-Gly-OPA. Anal. Calcd for $C_{28}H_{44}N_2O_6$: C, 66.6, H, 8.79; N, 5.55. Found: C, 66.5; H, 8.85; N, 5.17.

***N*-*tert*-Butyloxycarbonylglutamyl-*O*^{5}-(4-(oxymethyl)phenylacetic Acid) *O*-*tert*-Butyl Ester (10).** Boc-Glu(O^-Cs^+)-O-Bu^t (10 mmol) in DMF (50 mL) was treated with 4-(bromomethyl)phenylacetic acid phenacyl ester (7 mmol) at 50 °C for 5 h and overnight at room temperature. Precipitated CsBr was filtered off and the filtrate evaporated. The residue was dissolved in EtOAc and washed with H_2O, 5% $NaHCO_3$, saturated NaCl (thrice each). After drying the EtOAc solution with Na_2SO_4, the solvent was evaporated leaving the crude product. The phenacyl group was cleaved by reduction in aqueous 85% AcOH (90 mL) with Zn (147 mmol) and Boc-Glu(OPA·DCHA)-O-Bu^t isolated as in the general procedure described by Mitchell et al.[28]: 2.8 g (63%); mp 136 °C softening from 100 °C. TLC (C,E) showed contamination by two impurities. Recrystallization from isopropyl ether gave no improvement. The crude product was partitioned between EtOAc and 10% aqueous citric acid. After drying the EtOAc phase and evaporating the solvent, the residual oil was dissolved in 3 mL of $CHCl_3$ and applied to a Merck Lobar size C column which had been previously equilibrated with $CHCl_3$ for purification by high performance liquid chromatography.[57] The column was eluted at 5 mL/min first with $CHCl_3$ then with $CHCl_3$–MeOH–AcOH (20:1:1) and the eluate collected in 15-mL fractions after 335 mL had eluted. Two peaks were detected by monitoring at 254 nm, a minor peak in fractions 6–16 and a major one in fractions 19–26. Fractions 19–26 were pooled and evaporated; the residue was dissolved in benzene and evaporated thrice more. The straw-colored oil was dried in vacuo overnight over NaOH. The oil was dissolved in Et_2O (40 mL) and DCHA (4.1 mmol) added. The resulting crystals were filtered, washed with Et_2O, and dried: 1.72 g (39%); mp 138.5–141 °C; $[\alpha]^{26}_D$ −21° (*c* 1, DMF); TLC (C) R_f 0.58, (E) R_f 0.70. Anal. Calcd for $C_{35}H_{56}N_2O_8 \cdot 0.25H_2O$: C, 65.9; H, 8.94; N, 4.40. Found: C, 65.9; H, 8.67; N, 4.26.

***N*-*tert*-Butyloxycarbonylglutamyl-*O*^{5}-(4-(oxymethyl)phenylacetamido-PAP) *O*-*tert*-Butyl Ester: Alternate Preparation of 8.** Boc-Glu(OPA-PAP)-O-Bu^t is typical. PAP·HCl (1.0 g, 0.7 mmol amino group) was washed with 20-mL portions of solvent as follows: $CHCl_3$ (thrice); 10% triethylamine in $CHCl_3$ (four times), $CHCl_3$ (thrice), EtOH (thrice), CH_2Cl_2 (four times). Boc-Glu(OPA)-O-Bu^t (1.1 mmol freed of DCHA by aqueous citric acid) in DMF (10 mL) with HBT (185 mg, 1.21 mmol) was added to the resin followed by DCC (250 mg, 1.21 mmol) in DMF (8 mL). After 1 h, triethylamine (0.3 mmol) and DCC (0.7 mmol) were added, and after 18 h the reaction was complete as monitored by the ninhydrin test. The reaction mixture

was drained and the resin washed with CH_2Cl_2 (thrice), EtOH (thrice), EtOAc (thrice) and dried in vacuo. Amino acid analysis showed 0.42 mmol of Glu per g of resin. The results of Boc-Gly-OPA-PAP prepared this way are shown in Table II.

Saponification of Boc-Aminoacyl-OPA-PAP Resins. A portion of Boc-aminoacyl-OPA-PAP resin was suspended in *tert*-butyl alcohol (20 mL/g) or 70% aqueous isopropyl alcohol (10 mL/g) and 2 equiv of 1 M NaOH solution added. For a substitution of 0.4 mmol of amino acid per g of resin, a maximum concentration of 0.07 M is achieved. After the desired reaction times (most cleavages were 90% complete in 4 h), the reaction solution acidified with AcOH; samples were withdrawn for TLC and compared in system F with the respective authentic Boc-amino acids or peptides. The resin was then filtered and washed with H_2O, EtOH, EtOAc (thrice each). After drying in vacuo the resin was subjected to hydrolysis for amino acid analysis.

Test for Racemization. The filtrate obtained from the saponification of Boc-Leu-Val-OPA-PAP was evaporated and the residue redissolved in neat CF_3CO_2H for 30 min. The CF_3CO_2H was evaporated and the residue dissolved in 50% aqueous MeOH and neutralized with 1 M NaOH. Aliquots of the solution were applied to the amino acid analyzer and eluted with pH 4.25 buffer (0.2 M Na^+) at 65 °C and 8.3 mL/min. Under these conditions, L-Leu-D-Val and L-Leu-L-Val emerged at 35 min 39 s and 39 min 58 s, respectively, with a ratio of 0.007:1. The limit of detection for the L,D diasterioisomers was 0.1%. When Boc-L-Leu-L-Val-OMe was subjected to saponification as above, 0.2% of L,D diasterioisomer was detected.

Treatment of Boc-Val-OPA-PAP and Val-OPA-PAP with Sodium in Liquid Ammonia. A 100-mg portion of Boc-Val-OPA-PAP or Val-OPA-PAP was suspended in 25–50 mL of anhydrous refluxing liquid ammonia and treated with sodium metal as described for Boc-Cys(Scam-PAP). After removal of ammonia, the resin was resuspended in MeOH, acidified with AcOH, and an aliquot of the solution spotted for TLC (system F). No trace of Boc-Val or Val was detected. The resin was filtered and washed as described for the saponification procedure. After hydrolysis the substitution of sodium-treated resin was identical with that of untreated resin (0.29 mmol/g).

Hydrogenolysis. An aliquot of Boc-aminoacyl-OPA-PAP (50–150 mg) was suspended in 2 mL of solvent (for H_2O the resin was wetted with EtOH) and flushed with N_2 for 30 min at 45–50 °C. At that time, Pd(II) acetate (40 mg), or $PdCl_2$ for aqueous solvent, was added and the mixture stirred for another 30 min under N_2 before H_2 was started. In experiments where subsequent additions of catalyst were made, the vessel was flushed with N_2 for 15 min both before and after addition of another 40 mg of Pd(II) acetate. Additions of catalyst were performed after 8 h if only once and after 5 and 10 h if two additions were made. Total exposure of the reaction to H_2 was 24 h in all cases. The reaction was then flushed with N_2, the black resin filtered, washed with MeOH (four times, 5-mL portions each) and the filtrates were pooled. The solvents were removed under reduced pressure and the residue was treated with CF_3CO_2H (0.5 mL, 30 min). The solution was evaporated to dryness and the residue dissolved and diluted to 5.0 mL. The concentration of amino acid in solution was determined by quantitative amino acid analysis of an aliquot of the solution (Table III). In experiments where two additions of catalyst were made and the reaction solvent was DMF, the yields of other Boc-amino acids cleaved were: Boc-Ala, 72%; Boc-Glu-O-Bu^t, 54%; Boc-Phe, 54%.

Ammonolysis of Boc-Aminoacyl-OPA-PAP Resins. A portion of Boc-aminoacyl-OPA-PAP resin was suspended in MeOH or preferably CF_3CH_2OH (1 g of resin to 20–50 mL of solvent) and the suspension was saturated with anhydrous NH_3 at 0 °C. The reaction vessel was sealed and the suspension stirred or shaken for the desired reaction period (usually 72 h). The vessel was cooled before opening, the solvents were evaporated thoroughly, and the resin was resuspended in EtOH, filtered washed with EtOH, EtOAc, and dried in vacuo for hydrolysis and amino acid analysis (Table IV). The filtrate was evaporated, and, after several additional evaporations from EtOH to eliminate excess NH_3, the crude product was isolated by lyophilization from AcOH or dioxane. In system F, TLC showed the major portion of the crude product to correspond to the Boc-amino acid amide. After hydrolysis, amino acid analysis of crude amides all had 1:1 ratios of amino acid to ammonia.

Model Coupling Reactions. Boc-Ile-OPA-PAP (1.0 g, 0.35 mmol of Ile/g) was treated with 20-mL portions as follows: CH_2Cl_2 (thrice), CF_3CO_2H–CH_2Cl_2–anisole (49:49:2), CH_2Cl_2 (five times), 10% diisopropylethylamine in CH_2Cl_2 (twice), CH_2Cl_2 (four times). The resin was divided into three equal portions.

A. Coupling in CH_2Cl_2. To one portion of Ile-OPA-PAP was added 0.35 mmol of symmetrical anhydride of Boc-Leu[38] in 4 mL of CH_2Cl_2. Small portions of resin were withdrawn and washed immediately with EtOH at intervals of 1, 3, 5, 10, 20 min after addition of the anhydride. All of the test aliquots were negative to ninhydrin and PSA. The reaction mixture was drained and the resin washed thrice with 5-mL portions of CH_2Cl_2, EtOH, EtOAc and dried in vacuo. After hydrolysis, amino acid analysis of the resin showed a Leu to Ile ration of 0.96:1.00.

B. Coupling in DMF. To a second portion of Ile-OPA-PAP which had been washed thrice with DMF was added a filtered solution, which had been preactivated[41] for 45 min, of Boc-Leu·H_2O (87 mg, 0.35 mmol), HBT·H_2O (53 mg, 0.35 mmol) and DCC (74 mg, 0.36 mmol) in DMF (4 mL). Small portions were withdrawn as in A above with the 5-min sample being the first one to give negative ninhydrin and PSA tests. The reaction mixture was drained and the resin washed thrice with 5-mL portions of DMF, EtOH, and EtOAc. After drying and hydrolysis, the resin showed a Leu to Ile ratio of 0.96:1.00.

C. Coupling in Aqueous Solution. The third portion of Ile-OPA-PAP was washed thrice with 4-mL portions of EtOH and 5% aqueous $NaHCO_3$ (1:1). To the resin suspended in 2 mL of 5% aqueous $NaHCO_3$ was added Boc-Leu-OSu[42] (162 mg, 0.5 mmol) in EtOH (2 mL). Samples were withdrawn as in A above with the 5-min sample the first to give a negative PSA test. The reaction mixture was drained and the resin washed thrice with 4-mL portions of 50% aqueous EtOH, EtOH, EtOAc, and dried in vacuo. After hydrolysis, amino acid analysis gave a ratio of Leu to Ile of 0.96:1.00.

β-Mpr(Ec)-Tyr-Ile-Gln-Asn-Cys(Scam-PAP)-Pro-Leu-Gly-NH_2 (11). Compound **5** (2.03 g) was washed with DMF (three times, 40 mL). A solution of Pro-Leu-Gly-NH_2·0.5H_2O[10] (0.43 g, 1.5 mmol) and HBT·H_2O (0.37 g, 2.4 mmol) in DMF (30 mL) was dried over activated 4-Å molecular sieves and added to the resin. DCC (0.14 g, 0.67 mmol) in DMF (5 mL) was added and the mixture shaken 12 h. An additional 25 mg (0.12 mmol) of DCC was added and the reaction was allowed to continue 2 h longer. The mixture was drained and the resin washed with DMF, EtOH, and $CHCl_3$ (three times, 40 mL each). The amino acid analysis value at this step is shown in Table VI.

The synthesis was continued in a stepwise manner by using the procedures illustrated in Table V. Asparagine and glutamine were introduced as their *p*-nitrophenyl esters (2.4 mmol, each) with the reactions mediated by HBT·H_2O (2.4 mmol).[46] Boc-Ile·0.5H_2O, Boc-Tyr, and β-Mpr(Ec)[58] were coupled by preactivating[41] the protected acid (1.5 mmol) and HBT·H_2O (2.25 mmol) with DCC (1.5 mmol) for 60 min and adding the filtered solution to the resin. As soon as the activated amino acid solution was added to the resin, diisopropylethylamine (0.87 mmol) was added. Reactions were allowed to proceed for 2 h and in all cases were complete as measured by the modified picrylsulfonic acid test.[40] The amino acid analysis for the completed resino peptide is shown in Table VI.

Deaminooxytocin (12). Compound **11** (522 mg, corresponding to 94.0 μmol of peptide) was suspended in 9.0 mL of MeOH, flushed with MeOH-saturated N_2 and 1.0 mL of 1 M NaOH added. Aliquots of 10 μL were withdrawn at intervals and developed by the Ellman method.[48] Sulfhydryl group concentration was quantitative after 15 min. The solution was diluted with MeOH/H_2O (240 mL, 1:1, v/v) and ICH_2CH_2I (34 mg, 120 μmol) was added. After 5 min, the Ellman test demonstrated the absence of sulfhydryl groups; 5 mL of AcOH was added and the volume reduced to ~4 mL by rotary evaporation. AcOH (4 mL) was added and the solution applied to a 2.15 × 112 cm column of Sephadex G-15 (fine) equilibrated in 50% AcOH. The column was eluted with 50% AcOH and collected in 2.4-mL fractions. Peptide material was detected by monitoring the UV absorbance at 280 nm, fractions comprising the main peak were pooled, and the material was isolated by evaporation and lyophilization: wt 64 mg. The entire batch was subjected to a second gel filtration on the same column in 0.2 M AcOH. The product emerged as a single peak. Fractions (4.0 mL) comprising the peak area (76–96) were pooled and the product was isolated by lyophilization: wt 53 mg (59%); TLC R_f G 0.82, R_f F 0.43. Results of amino acid analyses are shown in Table VI.

Rat uterine assays were performed on isolated horns from virgin rats in natural estrus according to the method of Holton[59] as modified by Munsick[60] with the use of Mg^{2+}-free van Dyke–Hastings solutions as bathing fluid. The four-point design of Schild[61] was used to obtain

specific activities as compared with USP posterior pituitary reference standard. A value of 690 ± 40 units/mg was obtained.

<Glu-His(DNP-PAP)-Pro-NH_2 (13). A solution of HBT (92 mg, 0.6 mmol) in DMF (10 mL) was added to **6**, Boc-His(DNP-PAP) (1 g, 0.47 mmol of His per g), and after shaking 2 min DCC (97 mg, 0.47 mmol) was added. After 1 h HCl·Pro-NH_2 (219 mg, 1.4 mmol) in DMF (5 mL) was added followed by diisopropylethylamine (0.25 mL, 1.4 mmol). After shaking 1 h another equiv (0.4 mmol) of diisopropylethylamine was added and the mixture shaken overnight. The reaction mixture was drained, the resin was washed with DMF, and the coupling procedure was repeated. After hydrolysis in 6 M HCl, amino acid analysis showed the substitution of Pro and NH_3 to be 0.41 and 0.45 mmol per g resin, respectively.

The dipeptide resin was treated as shown in Table V, steps 1–4. A solution of *N*-methylmorpholine (0.1 mL, 0.89 mmol) in DMF (15 mL) was added followed by <Glu-$OPhCl_5$ (530 mg, 1.4 mmol). After 18 h the resin was ninhydrin negative.[39] The reaction mixture was drained and the resin was washed with DMF and EtOAc five times each and dried in vacuo: 1.17 g (uncorrected for samples withdrawn for analysis). After hydrolysis in 6 M HCl amino acid analysis showed the substitution of Glu, Pro, and NH_3 to be 0.40, 0.37, and 0.44 mmol per g of resin, respectively, for a ratio of 1.0:0.92:1.1.

<Glu-His-Pro-NH_2 (TRH, 14). To a suspension of **13** (1 g) in DMF (8 mL) was added triethylamine (0.05 ml) and 2-mercaptoethanol (0.8 mL). After stirring 12 h, the suspension was filtered and the resin washed with DMF, MeOH, and EtOAC. After drying and hydrolysis in 6 M HCl, amino acid analysis of the polymer showed a residual substitution of 0.07 mmol of peptide/g of resin indicating that 82% of TRH was liberated. The combined filtrates were evaporated under reduced pressure to ca. 1 mL and added to Et_2O (50 mL). The precipitate was collected by centrifugation, washed with Et_2O, and dried in vacuo: yield 139 mg. After hydrolysis amino acid analysis gave the following molar ratios: Glu, 1.00; Pro, 1.05; His, 0.98; NH_3, 1.03. The peptide (137 mg) was dissolved in MeOH (0.5 mL) and 0.04 mL of AcOH added to form the acetate salt for purification by high performance liquid chromatography.[57] This solution was applied to a Merck Lobar size B column which had been equilibrated with 95% EtOH–H_2O (7:3, v/v). The column was eluted with the same solvent at a rate of 2 mL/min. The product eluted witha peak maximum at 195 mL (R_f 0.46). Fractions containing TRH were pooled, the solvents were evaporated, and the residue was lyophilized from 0.2 M AcOH: 82 mg (48% yield based on His content of resin); TLC, R_f H 0.37, R_f I 0.48. Following hydrolysis amino acid analysis gave the following molar ratios: Glu, 1.00; Pro, 1.00; His, 1.00; NH_3, 0.97.

Z-Pro-Glu(OPA-PAP)-Gly-NH_2 (15). Boc-Glu(OPA-PAP)-OBu^t (1.0 g, 0.42 mmol of Glu per g of resin) was treated with 20-mL portions of the following: CH_2Cl_2 thrice, 2 min each; CF_3CO_2H–CH_2Cl_2–anisole (49:49:2) twice, 30 min each; CH_2Cl_2 five times, 2 min each; diisopropylethylamine–CH_2Cl_2 (1:9) twice 2 and 5 min; CH_2Cl_2 four times, 2 min each; and DMF thrice, 2 min each. A solution of Z-Pro-OSu (0.57 g, 1.65 mmol, 4 equiv) and HBT·H_2O (0.25 g, 1.65 mmol, 4 equiv) in DMF (20 mL) was added. After shaking 4 h, diisopropylethylamine (0.4 mL) was added and the pH (Fisher Indicator Solution) was found to be 5–6. After 12 h, the reaction mixture was drained and resin (ninhydrin negative) was washed with 20-mL portions of the following: DMF thrice, 2 min each; EtOH thrice, 2 min each; CH_2Cl_2 thrice, 2 min each; AcOH–CH_2Cl_2 (1:9) once, 10 min; CH_2Cl_2 thrice, 2 min each. A portion of the resin was withdrawn, dried, and after hydrolysis showed a ratio of Pro:Glu of 1.01:1.00 by amino acid analysis. The substitution of the resin was 0.41 mmol of Glu per g of resin.

After washing the resin with DMF (thrice, 20 mL, 2 min), a solution of Gly-NH_2 (90 mg, 1.2 mmol, 3 equiv), HBT·H_2O (275 mg, 1.8 mmol), and DCC (125 mg, 0.6 mmol, 1.5 equiv) in DMF (20 ml) was added. After 24 h, the reaction mixture was drained and the resin washed with DMF, EtOH, and CH_2Cl_2 thrice each. After drying, hydrolysis of a portion of the resin gave amino acid ratios as follows: Glu, 0.95; Pro, 1.02; Gly, 1.00; NH_3, 1.08. Substitution of Glu had remained at 0.41 mmol per g of resin.

Z-Pro-Gln-Gly-NH_2 (16). One portion of **15** (515 mg, 0.216 mmol of peptide) was suspended in CF_3CH_2OH (20 mL) and saturated with NH_3 at 0 °C. After 48 h at 23 °C the sealed flask was opened and the solvents evaporated. The resin was extracted with CF_3CH_2OH and filtered. Amino acid analysis of the resin showed that 95.6% of the tripeptide had been removed (residual substitution was 0.02 mmol Glu per g of resin). The filtrate was evaporated and the residue dissolved in 0.25 mL of $CHCl_3$ and applied to a Merck Lobar size A column that had been previously equilibrated with $CHCl_3$. The column was eluted at a rate of 2 ml/min with $CHCl_3$ for 12 min, then with $CHCl_3$–MeOH–AcOH–H_2O (8:2:0.5:0.1, by volume) at the same rate. The eluate was collected in 4-mL fractions and peptide material was detected by monitoring the absorbancy at 254 nm. Fractions comprising the major peak (14–16) were pooled, evaporated, and the residue isolated by lyophilization from AcOH: 69.5 mg (74%); $[\alpha]^{25}_D$ −37° (*c* 0.9, DMF); TLC R_f F 0.37, R_f J 0.43. Anal. Calcd for $C_{20}H_{27}N_5O_6$·0.5AcOH: C, 54.4; H, 6.31; N, 15.1. Found: C, 54.0; H, 6.15; N, 15.1. Amino acid analysis: Glu, 1.05; Pro, 0.98; Gly, 1.00; NH_3 2.10. A small portion crystallized from MeOH had mp 218–220 °C.

As a control, compound **16** was also prepared by solution techniques. To a cold (0 °C) solution of Z-Pro-Gln[62] (1.13 g, 3 mmol), HCl·Gly-OEt (0.56 g, 4 mmol), and HBT (6 mmol) in DMF (10 mL) was added diisopropylethylamine (0.68 mL, 4 mmol) and DCC (0.68 g, 3.3 mmol). After 22 h, the solution was filtered and the filtrate evaporated to dryness. The residue was slurried in Et_2O, filtered, washed with 5% $NaHCO_3$, H_2O, 1 M HCl, H_2O, and dried in vacuo. The precipitate was crystallized from MeOH to yield 0.75 g (54%) of Z-Pro-Gln-Gly-OEt: mp 201–202.5 °C; $[\alpha]^{25}_D$ −46° (*c* 0.5, DMF); single spot by TLC R_f K 0.5. Anal. Calcd for $C_{22}H_{30}N_4O_7$: C, 57.1; H, 6.54; N, 12.1. Found: C, 56.9; H, 6.47; N, 11.8. The tripeptide ester (0.73 g, 1.6 mmol) was suspended in MeOH saturated with NH_3 for 18 h. The NH_3 was evaporated and the solid amide isolated by filtration. Crystallization from MeOH gave 0.50 g (74%) of Z-Pro-Gln-Gly-NH_2: mp 220–222 °C; $[\alpha]^{25}_D$ −38° (*c* 1, DMF): TLC R_f F 0.37, R_f J 0.43. Amino acid analysis: Glu, 0.98; Pro, 1.02; Gly, 1.00; NH_3, 1.93. Note: products of both syntheses were also identical by TLC.

Z-Pro-Glu-Gly-NH_2 (17). A second portion of **15** (500 mg, 0.21 mmol of peptide) was suspended in *t*-BuOH (2.5 mL) and 1 M NaOH (0.42 mL, 2 equiv) was added. After 4 h, 70 μL of 6 M HCl was added and the suspension diluted with AcOH (2 mL). The resin was filtered and washed thrice with HOAc and the combined filtrates evaporated. The resin was washed further with EtOAc, dried in vacuo, and after hydrolysis amino acid analysis revealed only 0.036 mmol of residual Glu per g of resin corresponding to a liberation of 91.4% of **17.** The product was dissolved in AcOH (3 mL) and filtered to remove insoluble NaCl. After evaporation of the AcOH, the desalting process was repeated and finally the product was dissolved in 0.5 mL of $CHCl_3$–MeOH (1:1, v/v) and applied to the column described for the purification of **16.** The column was eluted with $CHCl_3$ at a rate of 2 mL/min for 18 min and then with $CHCl_3$–MeOH–AcOH–H_2O (8:2:0.5:0.1, by volume). The eluate was monitored at 254 nm and collected in 4-mL fractions. The fractions comprising the main peak (18 and 19) were pooled and evaporated. TLC (J) revealed a small inpurity. The product was rechromatographed as described above, but the column was preequilibrated and eluted with $CHCl_3$–MeOH–AcOH–H_2O (9:1:0.5:0.1, by volume). Fractions 14–18 were pooled and evaporated, and the product was isolated by lyophilization: 48.7 mg (53%); $[\alpha]^{25}_D$ −26° (*c* 1, DMF); TLC R_f J 0.31. Anal. Calcd for $C_{20}H_{26}N_4O_7$·AcOH: C, 53.4; H, 6.12; N, 11.3. Found: C, 53.7; H, 6.16; N, 11.7.

Acknowledgments. The authors thank Dr. C. Y. Bowers and Ms. G. Reynolds for kindly performing bioassays of TRH; Dr. R. Matsueda, Visiting Professor from Sankyo Co., Ltd., Tokyo, Japan, for helpful discussions and for suggesting an improved synthesis of *N,N'*-bis(acrylyl)-1,2-diaminoethane. We also thank Ms. Yuan Fen Ting, Mr. G. Skala, Mrs. E. Skala, and Mr. C. R. Botos of this laboratory for their skillful technical assistance. This work was supported by Grant AM-20314 from the U.S. Public Health Service and by the donors of the Petroleum Research Fund, administered by the American Chemical Society, and by the American Philosophical Society.

References and Notes

(1) Abbreviations follow the IUPAC-IUB Tentative Rules on Biochemical Nomenclature, *J. Biol. Chem.* **1972**, *247*, 977. Optically active amino acids are of the L configuration. In addition, the following abbreviations are used: PAP, poly(*N*-acrylylpyrrolidine); OPA, 4-(oxymethyl)phenylacetamido; DC,

degree of cross-linking in mole percent; MDR, monomer dilution ratio expressed as the ratio of solvent to monomer by weight; PSA, picrylsulfonic acid; Scam, *S*-carbamoyl; DCC, dicyclohexylcarbodiimide; HBT, 1-hydroxybenzotriazole; DNP, 2,4-dinitrophenyl; DCHA, dicyclohexylamine; Ec, *S*-ethylcarbamoyl; β-Mpr, β-mercaptopropionic acid; TRH, thyrotropin-releasing hormone.

(2) Merrifield, R. B. *Fed. Proc., Fed Am. Soc. Exp. Biol.* **1962,** *21,* 412. *J. Am. Chem. Soc.* **1963,** *85,* 2149.
(3) Fankhauser, P.; Brenner, M. "The Chemistry of Polypeptides"; Katsoyannis, P. G., Ed.; Plenum Press: New York, 1973; pp 389–411.
(4) Sheppard, R. C. "Peptides 1971"; Nesvadba, H., Ed., North-Holland Publishing Co.: Amsterdam, 1973; p 111.
(5) Merrifield, R. B. *Adv. Enzymol.* **1969,** *32,* 221.
(6) Meienhofer, J. "Hormonal Proteins and Peptides", Vol. 2; Li, C. H., Ed.; Academic Press: New York, 1973; pp 46–267.
(7) Anfinsen, C. B. "Chemistry of Natural Products"; IUPAC; Butterworths: London, 1968; p 461.
(8) Chapman, T. M.; Kleich, D. G. *J. Chem. Soc., Chem. Commun.* **1973,** 193.
(9) Inman, J. K.; Dintzis, H. M. *Biochemistry* **1969,** *8,* 4074.
(10) Atherton, E.; Clive, D. L. J.; Sheppard, R. C. *J. Am. Chem. Soc.* **1975,** *97,* 6584.
(11) Atherton, E.; Clive, D. L. J.; East, D. A.; Sheppard, R. C. "Peptides 1976"; Loffet, A., Ed.; University of Brussels: Brussels, 1976; pp 291–297.
(12) Atherton, E.; Sheppard, R. C. "Peptides: Proceedings of the Fifth American Peptide Symposium"; Goodman, M., Meienhofer, J., Eds.; Wiley: New York, 1977; pp 503–505; Atherton, E.; Caviezel, M.; Over, H.; Sheppard, R. C. *J. Chem. Soc., Chem. Commun.* **1977,** 819; Atherton, E.; Fox, H.; Harkiss, D.; Sheppard, R. C. *J. Chem. Soc., Chem. Commun.* **1978,** 539.
(13) Parrod, J.; Elles, J. *J. Polym. Sci.* **1958,** *29,* 411; Elles, *J. Chim. Mod.* **1959,** *4,* 53.
(14) Stahl, G. L.; Walter, R.; Smith, C. W. *J. Org. Chem.* **1978,** *43,* 2285.
(15) The importance of the MDR on polymer topography is often overlooked, but it plays as important a role as the DC in determining polymer–solvent interactions.[16]
(16) Morawetz, H.; "Peptides: Chemistry, Structure and Biology", Walter, R., Meienhofer, J., Eds.; Ann Arbor Science Publisher: Ann Arbor, Mich., 1975; pp 385–394; Morawetz, H. "Macromolecules in Solution", 2nd ed.; Interscience: New York, 1975; p 457.
(17) Smith, C. W.; Stahl, G. L.; Walter, R. *Int. J. Pept. Protein Res.* **1979,** *13,* 109.
(18) Stewart, J. M.; Young, J. D. "Solid Phase Peptide Synthesis", W. H. Freeman Co.: San Francisco, 1969; p 55.
(19) König, W.; Geiger, R. *Chem. Ber.* **1970,** *103,* 788.
(20) Gisin, B. F. *Helv. Chim. Acta,* **1973,** *56,* 1476.
(21) Sklyarov, L. Y.; Shaskova, I. V. *Zh. Obshch. Khim.* **1969,** *39,* 2779; Meienhofer, J.; Trzeciak, A. *Proc. Natl. Acad. Sci. U.S.A.* **1971,** *68,* 1006.
(22) Glass, J. D.; Walter, R.; Schwartz, I. L. "Peptides 1972"; Hanson, H., Jakubke, H. D., Eds.; North-Holland Publishing Co.: Amsterdam, 1973; pp 135–140; Glass, J. D.; Talansky, A.; Grzonka, Z.; Schwartz, I. L.; Walter, R. *J. Am. Chem. Soc.* **1974,** *96,* 6476.
(23) Guttmann, S. *Helv. Chim. Acta,* **1966,** *49,* 83.
(24) Three reactions are possible when the free base of PAP is treated with diisocyanate: (1) the desired reaction of one isocyanato group with the amino group giving a urea bond and a free isocyanato function; (2) intrachain cyclization, where two amino groups on the same polymer chain react with both ends of the 1,6-diisocyanatohexane; (3) cross-linking, where two amino groups from different polymer chains react with the 1,6-diisocyanatohexane. While reaction 2 uses up amino groups unproductively and results in lower substitutions, it should not adversely affect the polymer characteristics. A 400-fold excess of difunctional reagent is necessary to make reaction 2 insignificant. It is essential, however, that reaction 3 be suppressed. Kopecek, J. (*Makromol. Chem.,* **1977,** *178,* 2169) has shown that, during reaction of difunctional agents with reactive copolymers, reaction 3 is insignificant when a 10-fold excess of difunctional reagent is used.
(25) Glass, J. D.; Schwartz, I. L.; Walter, R. *J. Am. Chem. Soc.* **1972,** *94,* 6209.
(26) Sparrow, J. T. "Peptides, Chemistry, Structure and Biology"; Walter, R., Meienhofer, J., Eds.; Ann Arbor Science Publisher: Ann Arbor, Mich., 1975; pp 419–424.
(27) Mitchell, A. R.; Erickson, B. W.; Ryabtsev, M. N.; Hodges, R. S.; Merrifield, R. B. *J. Am. Chem. Soc.* **1976,** *98,* 7357.
(28) Mitchell, A. R.; Kent, S. B. H.; Engelhard, M.; Merrifield, R. B. *J. Org. Chem.* **1978,** *43,* 2845.
(29) Bogdanov, M. N. *J. Gen. Chem. USSR* (*Engl. Transl.*) **1958,** *28,* 1670.
(30) Yajima, H.; Kiso, Y.; Ogawa, H.; Fujii, N.; Irie, H. *Chem. Pharm. Bull.* (*Tokyo*) **1975,** *23,* 1164.
(31) Tesser, G. I.; Buis, J. T.; Wolters, E. T. M.; Bothe-Helmes, E. G. *Tetrahedron,* **1976,** *32,* 1069.
(32) Manning, J. M.; Moore, S. *J. Biol. Chem.* **1968,** *243,* 5591.
(33) Laufer, D. A.; Blout, E. R. *J. Am. Chem. Soc.* **1967,** *89,* 1246.
(34) Sifferd, R. H.; du Vigneaud, V. *J. Biol. Chem.* **1935,** *108,* 753; Photaki, I.; du Vigneaud, V. *J. Am. Chem. Soc.* **1965,** *87,* 908.
(35) Jošt, K.; Rudinger, J. *Collect. Czech. Chem. Commun.* **1967,** *32,* 1229; Katsoyannis, P. G.; Zalut, C.; Tometsko, A.; Tilak, M.; Johnson, S.; Trakatellis, A. C. *J. Am. Chem. Soc.* **1971,** *93,* 5871, and references therein; Bossert, H. W.; Jaquenoud, P.-A. "Peptides 1974", Wolman, Y., Ed.; Wiley: New York, 1975; pp 233–238.
(36) Schlatter, J. M.; Mazur, R. H.; Goodmanson, O. *Tetrahedron Lett.* **1977,** 2851; Jones, Jr., D. A. *Tetrahedron Lett.* **1977,** 2853; Goodmanson, O. J.; Scaros, M. G.; Schlatter, J. M.; Mazur, R. H. Presented at 7th Conference on Catalysis in Organic Synthesis, Chicago, Ill., 1978.
(37) Bodanszky, M.; Sheehan, J. T. *Chem. Ind.* (*London*) **1966,** 1597; Manning, M. *J. Am. Chem. Soc.* **1968,** *90,* 1348.
(38) Hagenmaier, H.; Frank, H. *Hoppe-Seyler's Z. Physiol. Chem.* **1972,** *353,* 1976; Lemaire, S.; Yamashiro, D.; Behrens, C.; Li, C. H. *J. Am. Chem. Soc.* **1977,** *99,* 1577.
(39) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970,** *34,* 595.
(40) Hancock, W. S.; Battersby, J. E. *Anal. Biochem.* **1976,** *71,* 260.
(41) Jäger, G.; König, W.; Wissmann, H.; Geiger, R. *Chem. Ber.* **1974,** *107,* 215; Nestor, J. J., Jr.; Ferger, M. F.; Chan, W. Y. *J. Med. Chem.* **1975,** *18,* 1022.
(42) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1964,** *86,* 1839.
(43) Hope, D. B.; Murti, V. V. S.; du Vigneaud, V. *J. Biol. Chem.* **1962,** *237,* 1563.
(44) du Vigneaud, V.; Ressler, C.; Swan, J. M.; Roberts, C. W.; Katsoyannis, P. G. *J. Am. Chem. Soc.,* **1954,** *76,* 3115.
(45) Zaorál, M.; Rudinger, J. *Collect. Czech. Chem. Commun.* **1955,** *20,* 1183.
(46) König, W.; Geiger, R. *Chem. Ber.* **1973,** *106,* 3626.
(47) Heating the Scam protected intermediate in the presence of reagents of the ninhydrin test[16] gave a faint green color, which made judging the degree of coupling reactions too subjective.
(48) Ellman, G. L. *Arch. Biochem. Biophys.* **1959,** *82,* 70.
(49) Weygand, F.; Zumach, G. *Z. Naturforsch. B.* **1962,** *17,* 807.
(50) Live, D. H.; Agosta, W. C.; Cowburn, D. *J. Org. Chem.* **1977,** *42,* 3556.
(51) Hruby, V. J.; Upson, D. A.; Agarwal, N. S. *J. Org. Chem.* **1977,** *42,* 3552. Spatola, A. F.; Cornelius, D. A.; Hruby, V. J.; Blomquist, A. T. *J. Org. Chem.* **1974,** *39,* 2207.
(52) Flouret, G. *J. Med. Chem.* **1970,** *13,* 843.
(53) Bowers, C. Y.; Sievertsson, H.; Chang, J.; Stewart, J.; Castensson, S.; Bjorkman, S.; Chang, D.; Folkers, K. "Thyroid Research", Robbins, J., Braverman, L. E., Eds.; Excerpta Medica: Amsterdam, 1975; pp 1–4.
(54) Moore, S. "Chemistry and Biology of Peptides"; Meienhofer, J., Ed., Ann Arbor Science Publisher: Ann Arbor, Mich., 1972; pp 629–653.
(55) Specht, E. H.; Newman, A.; Neher, H. T. U.S. Patent 2 773 063.
(56) Smith, C. W.; Ferger, M. F.; Chan, W. Y. *J. Med. Chem.* **1975,** *18,* 822.
(57) Gabriel, T. F.; Michalewsky, J.; Meienhofer, J. *J. Chromatogr.* **1976,** *129,* 287; Gabriel, T. F.; Jiminez, M. H.; Felix, A. M.; Michalewsky, J.; Meienhofer, J. *Int. J. Pept. Protein Res.* **1977,** *9,* 129.
(58) Lundell, E. O.; Ferger, M. F. *J. Med. Chem.* **1975,** *18,* 1045.
(59) Holton, P. *Br. J. Pharmacol. Chemother.* **1948,** *3,* 328.
(60) Munsick, R. A. *Endocrinology* **1960,** *66,* 541.
(61) Schild, H. O. *J. Physiol.* (*London*) **1942,** *101,* 115.
(62) Naithani, V. K. *Hoppe-Seyler's Z. Physiol. Chem.* **1972,** *353,* 1806.