(20 mm); *m/e* 156; ir 1720 (C=O), 1650 (C=C), and 1110 cm^{-1} (ether); nmr (CCl_4) δ 1.22 (3 H, d, J = 7.0 Hz), 1.28 (3 H, d, J = 7.0 Hz), 1.72 (3 H, m), 2.11 (3 H, s), 3.70 (1 H, q, J = 7.0 Hz), 3.93 (1 H, q, J = 7.0 Hz), and 4.87 (2 H, m).

Anal. Calcd for $C_9H_{16}O_2$: C, 69.19; H, 10.32. Found: C, 69.29; H, 10.58.

The second component was identified as 2,3,3,4-tetramethyl-4-acetyloxetane: bp 70° (20 mm); *m/e* 156; ir 1720 (C=O) and 990^{-1} (oxetane ring); nmr (CCl_4) δ 0.95 (3 H, s), 1.10 (3 H, s), 1.18 (3 H, d, J = 7.0 Hz), 1.33 (3 H, s), 2.15 (3 H, s), and 4.40 (1 H, q, J = 7.0 Hz).

Anal. Calcd for $C_9H_{16}O_2$: C, 69.19; H, 10.32. Found: C, 69.47; H, 10.13.

Biacetyl–2,3-Dimethyl-2-butene (5e) Photoadducts.—A mixture of biacetyl and **5e** was irradiated for 48 hr. After the removal of unreacted materials, the fraction boiling at 73–84° (13 mm) (8.1 g) was collected; residues, 0.6 g. Vpc analysis (3-m PEG 6000 or UCON LB 550 X, 140°) indicated that the photoproduct mixture contained one major component **6e** and several minor components (**6e**:others = 5.0:1.0). The ir spectrum of the fraction mixture indicated that the minor components mainly consisted of alcohol compounds. Separation by preparative vpc (3-m PEG 6000, 140°) gave pure **6e**: bp 74° (13 mm); *m/e* 170; ir 1728 (C=O), 1650 (C=C), and 1110 cm^{-1} (ether); nmr (CCl_4) δ 1.16 (3 H, d, J = 7.0 Hz), 1.23 (3 H, s), 1.28 (3 H, s), 1.73 (3 H, m), 2.08 (3 H, s), 3.61 (1 H, q, J = 7.0 Hz), and 4.86 (2 H, m).

Anal. Calcd for $C_{10}H_{18}O_2$: C, 70.54; H, 10.66. Found: C, 70.39; H, 10.57.

Irradiation of Biacetyl to α-*methyl*-d_3-Styrene (5b').—**5b'** (D = 87%) was prepared by the Wittig reaction of methyltriphenylphosphonium bromide and trideuteriomethyl phenyl ketone.[26] A mixture of 2.6 g (0.03 M) of biacetyl and 3.6 g (0.03 M) of **5b'** in 180 ml of benzene was irradiated for 60 hr. After the recovery of **5b'** (1.6 g), a boiling fraction at 110–120° (6 mm) (0.7 g) was collected; residues, 0.4 g. Vpc analysis and isolation of the products done by the same conditions as for **5b**. The ratio of **6b**:**7b**:**8b** = 2.0:5.8:1.0. The % D of starting and recovered **5b'** and the photoproducts were determined by nmr. No deuterium was introduced into position C_α of the allyl moiety of **6b'** (% D of C_γ and methine protons, 87%).

Biacetyl–Isobutene Photoaddition at Room Temperature.—A mixture of biacetyl (0.01 M) and isobutene (0.01 M) in benzene was irradiated at room temperature in a Pyrex test tube for 6 hr. The reaction mixture was analyzed as described above. It was shown that the ratio of **6c**:oxetane was 1.62:1.0.

Registry No.—**1**, 26995-37-9; **2**, 26995-38-0; **3**, 26959-33-1; **4**, 26959-34-2; **5a**, 926-66-9; **5b**, 98-83-9; **5b'**, 16914-16-2; **5c**, 115-11-7; **5d**, 513-35-9; **5e**, 563-79-1; **6a**, 40519-21-9; **6b**, 40519-22-0; **6c**, 40519-23-1; **6d**, 26959-35-3; **6e**, 40519-25-3; **7a**, 40519-26-4; **7b**, 40519-27-5; **8a**, 40580-22-1; **8b**, 40519-28-6; **10**, 40519-29-7; biacetyl, 431-03-8; indene, 95-13-6; furan, 110-00-9; ethyl vinyl ether, 109-92-2; 3,3,4-trimethyl-4-acetyloxetane, 40519-30-0; 2,3,3,4-tetramethyl-4-acetyloxetane, 26959-36-4; methylphenylphosphonium bromide, 1779-49-3; trideuteriomethyl phenyl ketone, 17537-31-4.

A Simple, High Yield Synthesis of Arginine Vasopressin

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Biologically fully active arginine vasopressin has been synthesized *via* the stepwise active ester and fragment condensation methods. The synthesis was begun with proline at the carboxyl terminus utilizing the trityl group for sulfhydryl protection of cysteine and the Boc group for amino nitrogen protection. Synthesis of Boc-Cys(Trt)-Tyr-Phe-Gln-Asn-Cys(Trt)-Pro was followed by cyclization of the cysteine moiety in 70% yield with I_2 in 80% acetic acid. The remaining dipeptide unit, Arg-Gly-NH_2, was attached to proline by means of the hydroxysuccinimide ester of the cyclized heptapeptide. The guanidyl group was protected as a picrate. The Boc group was then removed from protected vasopressin with 90% TFA to give, after final purification, vasopressin in an overall yield of 11%.

Many syntheses of the antidiuretic hormone arginine vasopressin, (**18**) have been published over the past 18 years. Most of these syntheses[1-5] have involved the fragment condensation method, the exception being the guanylation of a protected ornithine nonapeptide[6] and a solid phase synthesis.[7]

In all of these methods, however, the benzyl group has been utilized for the protection of the sulfhydryl group of cysteine. Treatment of a fully protected nonpeptide with sodium in liquid ammonia and subsequent oxidation to form the disulfide bridge has afforded the desired hormone in varying degrees of yield and purity. The main disadvantage of such an approach has been the rather low yield of pure material obtained in the final cyclization step.

In an effort to minimize side reactions during the cyclization to form the disulfide bridge and in order to obtain intermediates for biological testing, we have used a different approach in synthesizing this hormone. We achieved sulfhydryl protection by means of the easily removed trityl group, masking of the α-amino nitrogen with the *tert*-butoxycarbonyl (Boc)[8] group and blocking of the guanidyl group of arginine by protonation. The complete synthesis is outlined in Chart I and was achieved with an overall yield of 11% [based on Boc-Cys(Trt)-OCP] of biologically fully active hormone.

All coupling reactions were performed in DMF *via* active esters and all intermediates, other than **15**, have been characterized. Initially, 90% trifluoroacetic acid (TFA) was used to remove the Boc group. Although trityl groups apparently were removed from sulfur atoms to some extent during the procedure,[9] subsequent removal of the solvent *in vacuo* at 40° reversed the equilibrium and retritylated the peptide. Addition of ether to the resulting oily residue afforded a solid TFA

(1) V. du Vigneaud, D. T. Gish, and P. G. Katsoyannis, *J. Amer. Chem. Soc.*, **76**, 4751 (1954).

(2) P. G. Katsoyannis, D. T. Gish, and V. du Vigneaud, *ibid.*, **79**, 4516 (1957).

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(4) R. O. Studer and V. du Vigneaud, *ibid.*, **82**, 1499 (1960).

(5) R. O. Studer, *Helv. Chim. Acta*, **46**, 421 (1963).

(6) M. Bodanszky, M. A. Ondetti, C. A. Birkhimer, and P. L. Thomas, *J. Amer. Chem. Soc.*, **86**, 4452 (1964).

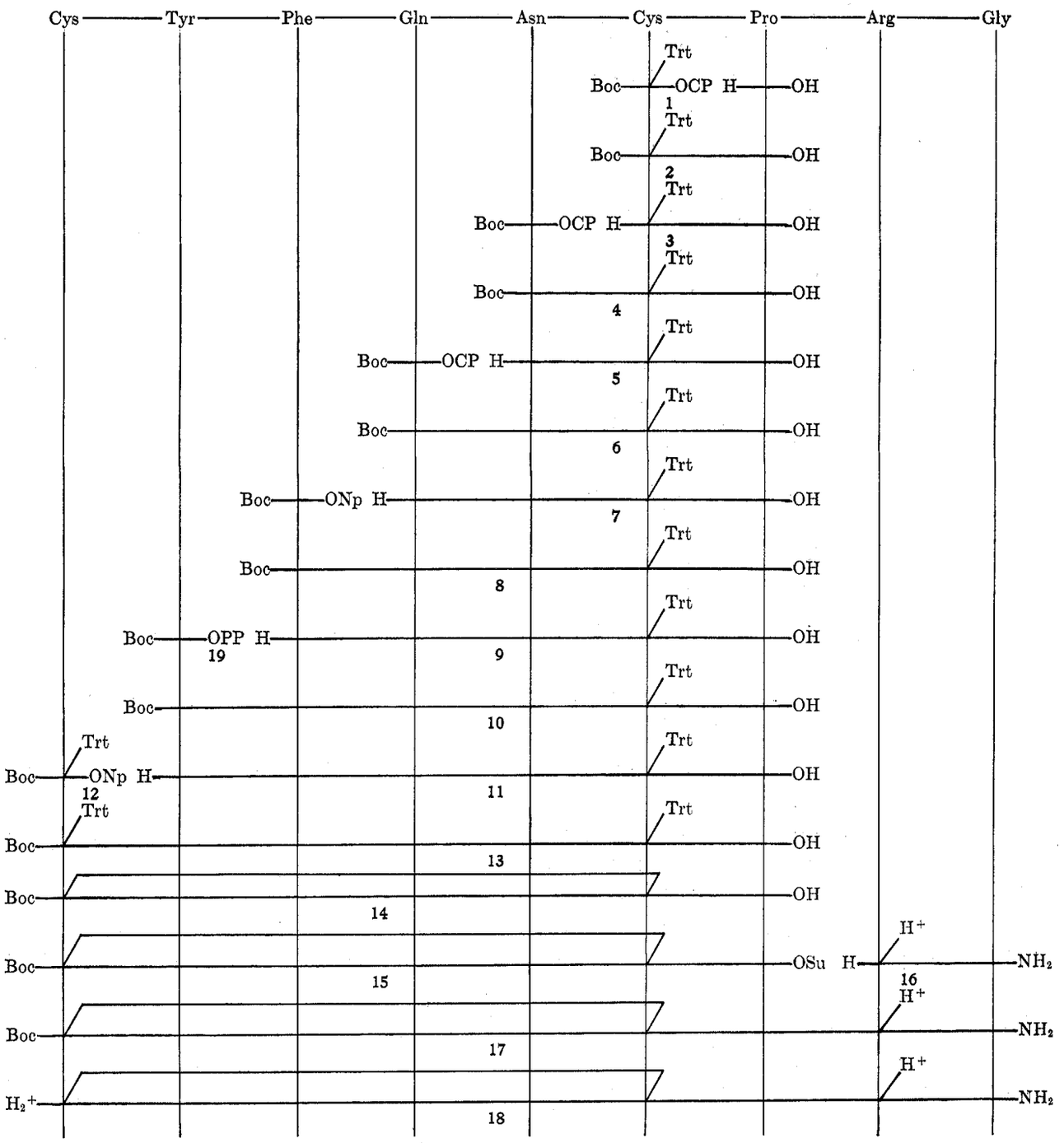
(7) J. Meienhofer, A. Trzeciak, R. T. Havran, and R. Walter, *ibid.*, **92**, 7199 (1970).

(8) Abbreviations according to IUPAC-IUB recommendations: *Biochem. J.*, **126**, 773 (1972). In addition, OCP = 2,4,5-trichlorophenoxy and OPP = pentachlorophenoxy. All amino acids have the L configuration.

(9) I. Photaki, J. Taylor-Papadimitriou, C. Sakarellos, P. Mazarakis, and L. Zervas, *J. Chem. Soc. C*, 2683 (1970).

CHART I

SYNTHESIS OF ARGININE VASOPRESSIN



salt which was <2% detritylated (based on recovered triphenylcarbinol). This method of deblocking was successfully used up to the preparation of **7**, at which step an approximately 1:1 mixture of **7** and <Glu-Asn-Cys(Trt)-Pro was obtained. We then turned to a tenfold excess of 2 *M* HCl in a 1:2 dioxane–acetic acid system as a means of deprotection and this afforded **7** in 90% yield with almost no pyroglutaminyl tetrapeptide being formed. The method of work-up was exactly as described for the Boc removal with TFA. Except for the final step in preparing **18** from **17**, the HCl method was used throughout the synthesis with excellent results.

One interesting observation should be briefly mentioned at this point. During the coupling of Boc-Asn-OCP with **3**, the usual excess of active ester (10–15%) was found to be insufficient to react completely with the dipeptide. It was apparent from thin layer chromatography data that the active ester was itself decomposing during the coupling reaction. This decomposition is currently being studied by us in order to determine the products formed by Boc-Asn-OCP under normal coupling conditions. Preliminary results have indicated that there is more than one decomposition product and the amounts of these products are de-

pendent on whether *N*-methylmorpholine is present in a DMF solution of the active ester.

Cyclization of protected heptapeptide **13** was accomplished by the addition of a 5×10^{-3} *M* solution of **13** in 80% acetic acid to an excess of I_2 (5×10^{-3} *M* in 80% acetic acid) in a manner similar to those previously described.[10,11]

To avoid removal of the Boc group during work-up, 1 *N* NaOH (equivalent to the HI formed) was added. The solvent was removed *in vacuo* and ether was added to give a solid which, after countercurrent distribution, gave a 70% yield of pure **14**.

Formation of the protected vasopressin **17** was accomplished by the *in situ* preparation of the *N*-hydroxysuccinimide active ester and coupling to H-Arg-Gly-NH_2·picrate (prepared *in situ* from the dipicrate[12]). Although crude **17** can be used satisfactorily in the final step, a small amount was purified by countercurrent distribution and characterized as a monopicrate. It was most interesting that the picrate salt remained intact during countercurrent distribution in the system *n*-BuOH–HOAc–H_2O (4:1:5) without formation of the expected acetate.

Crude **17** was deprotected in 90% TFA and then precipitated with ether. Purification of the vasopressin **18** and removal of picric acid were accomplished by means of gradient elution with 0.1 *N* to glacial acetic acid from an IRC-50 ion-exchange column. Fractions containing vasopressin were then collected; the solvent was removed *in vacuo* at 45° and the product lyophilized. Drying *in vacuo* at about 105° for 16 hr over magnesium perchlorate afforded the pure hormone as a diacetate·$^1/_2$ hydrate, homogeneous by thin layer chromatography. After standing in air for several days, subsequent elemental analysis showed the compound to be a diacetate·$3^1/_2$ hydrate. Bioassay[13] on the ethanol–saline loaded rat indicated an activity of 454 IU/mg.

Several advantages of this synthetic method are evident: (1) oxidation of a protected intermediate gives a high yield of easily purified disulfide; (2) the overall yield obtained is excellent; (3) it should be easy to scale-up this synthesis to allow preparation of gram quantities of the hormone or analogs; (4) the use of a cyclic disulfide as an intermediate does not pose any particular problems as **14** is stable to TFA, coupling conditions, countercurrent distribution, and ion-exchange chromatography.

We are currently optimizing yields at all stages of the reaction sequence, investigating the use of more stable sulfhydryl-protecting groups, and continuing to study the cyclization reaction under various conditions.

Experimental Section

Thin layer chromatograms (tlc) were run on silica gel G with 1-butanol–HOAc–H_2O (7:1:2) (R_{fA}), $CHCl_3$–MeOH–HOAc–H_2O (64:30:2:4) (R_{fB}), $CHCl_3$–MeOH (95:5) (R_{fC}), $CHCl_3$–MeOH (98:2) (R_{fD}), and $CHCl_3$–MeOH (90:10) (R_{fE}). Spots were revealed with *tert*-butyl hypochlorite followed by KI (1%)–starch (1%).[14] Purifications by means of countercurrent distribution (CCD) were performed in $CHCl_3$–CCl_4–MeOH–H_2O (26:27:37:10) (system 1) or 1-butanol–HOAc–H_2O (4:1:5) (system 2). Melting points[15] were obtained on a Mel-Temp capillary melting point apparatus and were uncorrected, and optical rotations were obtained with a Perkin-Elmer Model 141 polarimeter.

Boc-Cys(Trt)-OCP (**1**).—A solution of 28.1 g (60.6 mmol) of Boc-Cys(Trt)-OH[16] and 13.7 g (69.7 mmol) of 2,4,5-trichlorophenol in 110 ml of EtOAc was cooled to 4° in an ice bath. To the stirred solution was added 13.2 g (66.7 mmol) of dicyclohexylcarbodiimide (DCCD) in 30 ml of EtOAc; the reaction mixture was stirred in ice for 1.5 hr and then allowed to warm to room temperature over a 2.5-hr period. The precipitate of dicyclohexylurea (DCU) was removed by filtration, treated with boiling acetone, and filtered again. The combined filtrates were stripped *in vacuo* to leave a solid which was purified by crystallization from *i*-PrOH–EtOAc: yield 29.2 g (75%) of white compound; mp 166–167°; $[\alpha]^{24}D$ +24° (*c* 1, DMF); R_{fC} 0.78.

Anal. Calcd for $C_{33}H_{30}Cl_3NO_4S$: C, 61.64; H, 4.70; S, 4.99; Cl, 16.54. Found: C, 61.84; H, 4.64; S, 5.17; Cl, 16.24.

Boc-Cys(Trt)-ONp[17] (**12**).—A solution of 28.5 g (61.5 mmol) of Boc-Cys(Trt)-OH and 11.1 g (80.0 mmol) of *p*-nitrophenol in 225 ml of EtOAc was cooled to 20° and treated dropwise, with stirring, over a 15-min period with a solution of 14.0 g (68.0 mmol) of DCCD in EtOAc. The reaction mixture was stirred 1 hr at 20° and then at room temperature for 2 hr. The DCU was filtered and the filtrate was washed successively with 3 × 100 ml of 1 *M* K_2CO_3 and 3 × 100 ml of water and then dried (Na_2SO_4). Evaporation of the dried solvent *in vacuo* gave a solid which was dissolved in a minimum quantity of warm benzene and filtered into 1 l. of stirred Skellysolve B, yield 25.9 g (72.0%) of tan crystals, mp 160.5–164°. An analytical sample, recrystallized from xylene, had mp 164–167°, R_{fD} 0.64, $[\alpha]^{28}D$ +37° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{33}H_{32}N_2O_6S$: C, 67.79; H, 5.52; N, 4.79; S, 5.48. Found: C, 67.91; H, 5.40; N, 4.66; S, 5.27.

Boc-Cys(Trt)-Pro (**2**).—A 2.53-g (22.0 mmol) sample of (L)-Pro was dissolved in 75 ml of DMF with 3.64 ml of 6.04 *N* HCl in dioxane, and then 12.9 g of **1** was added. The reaction was initiated by the addition of 5.0 ml (45 mmol) of *N*-methylmorpholine and the reaction mixture stirred for 24 hr at room temperature, after which it was cooled. Unreacted proline was removed by filtration and the filtrate added to 800 ml of rapidly stirred, cold 1 *N* HCl. The resulting white precipitate was filtered, washed with cold water, and air-dried. Purification was effected by dissolving the crude protected dipeptide in 50 ml of EtOAc and adding the solution to 600 ml of cold, rapidly stirred Skellysolve B. After drying *in vacuo* at 40° for 1.5 hr, 10.8 g (91.4%) of **2** was obtained as a $1^1/_2$ hydrate: R_{fB} 0.89, R_{fC} 0.18; $[\alpha]^{29}D$ +31° (*c* 1, DMF), +26° (*c* 1, MeOH).

Anal. Calcd for $C_{32}H_{36}N_2O_5S \cdot 1^1/_2H_2O$: C, 65.39; H, 6.69; N, 4.77; S, 5.46. Found: C, 65.45; H, 6.23; N, 4.74; S, 5.20.

H-Cys(Trt)-Pro·HCl (**3**).—A 2.80-g (5.00 mmol) sample of **2** was dissolved in 17 ml of HOAc and then treated at room temperature for 5 min with 8.2 ml of 6.2 *N* HCl in dioxane. The solvents were removed *in vacuo* at 45°, and anhydrous ether was added to the residual oil to give a white precipitate. After filtering, washing several times with ether, and drying *in vacuo* at 75° for 1.5 hr, 2.21 g (89.0%) of **3** was obtained, R_{fA} 0.31, $[\alpha]^{27}D$ +46° (*c* 1, MeOH).

Anal. Calcd for $C_{27}H_{25}N_2O_3S \cdot HCl \cdot H_2O$: C, 62.96; H, 6.07; N, 5.44; S, 6.22; Cl, 6.88. Found: C. 62.98; H, 5.81; N, 5.66; S, 6.41; Cl, 6.48.

Boc-Asn-Cys(Trt)-Pro (**4**).—A solution of 19.8 mmol of **3**, 23.8 mmol (20% excess) of Boc-Asn-OCP,[18] and 4.5 ml (40 mmol) of *N*-methylmorpholine in 90 ml of DMF was stirred overnight at room temperature. A routine tlc of the reaction mixture showed some **3** still remaining and no active ester, which has R_{fE} 0.58. An additional 0.82 g (2.0 mmol) of Boc-Asn-

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(11) R. Geiger and W. König, German Patent 1917939 (1970).

(12) D. T. Gish and V. du Vigneaud, *J. Amer. Chem. Soc.*, **79**, 3579 (1957). Other salts of this dipeptide are extremely hygroscopic and difficult to store and manipulate.

(13) L. M. Hofmann, *Arch. Int. Pharmacodyn. Ther.*, **169**, 189 (1967).

(14) R. H. Mazur, B. W. Ellis, and P. S. Cammarata, *J. Biol. Chem.*, **237**, 1619 (1962).

(15) In many instances, the melting points of various peptide intermediates were undefined and therefore are not reported.

(16) H. Zahn and K. Hammerström, *Chem. Ber.*, **102**, 1048 (1969).

(17) E. Schnabel, H. Klostermeyer, and H. Berndt, *Justus Liebigs Ann. Chem.*, **749**, 90 (1971).

(18) W. Broadbent, J. S. Morley, and B. E. Stone, *J. Chem. Soc. C*, 2632 (1967).

OCP was added and the reaction mixture again stirred at room temperature overnight. At the end of this time, tlc showed no **3** present and some Boc-Asn-OCP, which was decomposed with 1 ml (9 mmol) of $(CH_3)_2NCH_2CH_2NH_2$ at room temperature for 1 hr. After cooling to 4°, the reaction mixture was added to 1250 ml of cold 1 *N* HCl with rapid stirring. The resulting white precipitate was filtered, washed with water, and air-dried. Dissolving the crude protected tripeptide in 90 ml of EtOAc, boiling, and cooling afforded a 78% yield of pure **4**: R_{fA} 0.78, R_{fE} 0.19; $[\alpha]^{27}D$ −4.2° (*c* 1, DMF); mp 199–200° dec.

Anal. Calcd for $C_{36}H_{42}N_4O_7S \cdot {}^1/_2H_2O$: C, 63.23; H, 6.48; N, 8.19; S, 4.69. Found: C, 63.49; H, 6.42; N, 8.48; S, 4.74.

H-Asn-Cys(Trt)-Pro·HCl (5).—It was prepared in 98% yield in the same manner as previously described for **3**, R_{fA} 0.45, $[\alpha]^{27}D$ +32° (*c* 1, MeOH).

Anal. Calcd for $C_{31}H_{34}N_4O_5S \cdot HCl \cdot H_2O$: C, 59.18; H, 5.93; N, 8.90; S, 5.10; Cl, 5.64. Found: C, 59.12; H, 5.89; N, 8.64; S, 5.34; Cl. 5.44.

Boc-Gln-Asn-Cys(Trt)-Pro (6).—It was prepared as described previously for **4** using a 10% excess of Boc-Gln-OCP.[18] Crude product was triturated with Skellysolve B and dried *in vacuo* to a glassy foam, R_{fA} 0.77, with faint impurities at R_{fA} 0.27 and R_{fA} 0.83. An analytically pure sample was obtained by stirring the impure product for 2 hr at room temperature in 1% HCl, filtering, drying, again triturating and washing with Skellysolve B and drying *in vacuo*, $[\alpha]^{28}D$ −13° (*c* 1, HOAc).

Anal. Calcd for $C_{41}H_{50}N_6O_9S$: C, 61.33; H, 6.28; N, 10.45; S, 3.99. Found: C, 61.21; H, 6.35; N, 10.26; S, 4.24.

H-Gln-Asn-Cys(Trt)-Pro·HCl (7).—Crude **6** was deprotected as previously described for **3** to give a 92% yield of tetrapeptide salt (based on two steps from **5**): R_{fA} 0.35; $[\alpha]^{28}D$ +9° (*c* 1, DMF), +7° (*c* 1, MeOH).

Anal. Calcd for $C_{36}H_{42}N_6O_7S \cdot HCl \cdot 3H_2O$: C, 54.50; H, 6.22; N, 10.59; S, 4.04; Cl, 4.47. Found: C, 54.40; H, 5.81; N, 10.31; S, 3.98; Cl, 4.55.

Attempted Preparation of H-Gln-Asn-Cys(Trt)-Pro·CF_3CO_2H.—A 402-mg (0.500 mmol) sample of **6** was stirred at room temperature for 5.5 hr with 1.5 ml of a 1:1 TFA–HOAc solution and then added to cold ether. The white precipitate was filtered and dried to give 358 mg of a compound which showed two spots at R_{fA} 0.25 and R_{fA} 0.42. The reaction was repeated on a larger scale using anhydrous TFA at room temperature for 5 min with the same results. Since it was suspected that one of the two products might be <Glu-Asn-Cys(Trt)-Pro resulting from the cyclization of glutamine, the following procedure was adopted.

A 1.51-g sample of the mixed products was dissolved with 0.91 g of Boc-Phe-ONp and 0.42 ml of *N*-methylmorpholine in 10 ml of DMF and the reaction followed by tlc. Over a period of 6 hr, the material at R_{fA} 0.25 gradually disappeared while the material having an R_{fA} of 0.42 remained unchanged. After 24 hr at room temperature, there was no change in the tlc other than what had taken place during the first 6 hr of reaction time. After work-up in the usual manner, the crude material (1.95 g) was purified *via* CCD (system 1). After 400 transfers, two products were obtained: 360 mg of a compound having K = 3.9 and R_{fA} 0.42, and the second (500 mg) having K = 0.9, R_{fA} 0.78. The compound having the higher R_f value and lower partition coefficient was identified by nmr and analysis as Boc-Phe-Gln-Asn-Cys(Trt)-Pro (**8**), $[\alpha]^{26}D$ +0.5° (*c* 1, DMF).

Anal. Calcd for $C_{50}H_{59}N_7O_{10}S \cdot 2H_2O$: C, 60.90; H, 6.44; N, 9.94; S, 3.25. Found: C, 60.56; H, 6.14; N, 9.78; S, 3.41.

The compound with the R_f value of 0.42 and K = 3.9 was tentatively identified by nmr and elemental analysis as <Glu-Asn-Cys(Trt)-Pro.

Anal. Calcd for $C_{36}H_{39}N_5O_7S \cdot H_2O$: C, 61.43; H, 5.87; N, 9.95; S, 4.56. Found: C, 61.62; H, 6.31; N, 9.97; S, 4.42.

Comparison of the ir and nmr spectra and tlc's of this material with those of authentic <Glu-Asn-Cys(Trt)-Pro (see following experiment) satisfactorily proved its structure.

<Glu-Asn-Cys(Trt)-Pro.—It was prepared in 75% yield in the usual manner from <Glu-OPP,[19] **5**, and *N*-methylmorpholine in DMF. The crude product was treated with Darco G-60 in a MeOH–EtOAc solution and added to a tenfold excess of ether. The resulting white powder was filtered, washed with ether, and dried *in vacuo* at 65°. Tlc showed one spot, R_{fA} 0.45 and R_{fB} 0.64; mp decomposes gradually from 160–200°, $[\alpha]^{28}D$ −5.2° (*c* 1, DMF).

Anal. Calcd for $C_{36}H_{39}N_5O_7S \cdot H_2O$: C, 61.43; H, 5.87; N, 9.95; S, 4.56. Found: C, 61.17; H, 5.92; N, 9.49; S, 4.59.

The compound had identical ir and nmr with those of the by-product isolated by CCD after the coupling of impure H-Gln-Asn-Cys(Trt)-Pro·CF_3CO_2H with Boc-Phe-ONp.

Boc-Phe-Gln-Asn-Cys(Trt)-Pro (8).—It was synthesized from 9.45 g (11.9 mmol) of **7**, 5.06 g (13.1 mmol) of Boc-Phe-ONp,[20] and 2.8 ml (25.2 mmol) of *N*-methylmorpholine in 50 ml of DMF as described for **2**. To remove <Glu-Asn-Cys(Trt)-Pro as a by-product, the crude material was purified *via* CCD (system 1). After 400 transfers, 9.30 g (80% yield) of pure pentapeptide (as a $1^1/_2$ hydrate) was collected from tubes 135–200 (K = 0.7). Tlc showed one spot, R_{fA} 0.78, $[\alpha]^{28}D$ −2.5° (*c* 1, DMF).

Anal. Calcd for $C_{50}H_{59}N_7O_{10}S \cdot 1^1/_2H_2O$: C, 61.46; H, 6.40; N, 10.04; S, 3.28. Found: C, 61.22; H, 6.13; N, 9.91; S, 3.20.

H-Phe-Gln-Asn-Cys(Trt)-Pro·HCl (9).—The protected pentapeptide **8** (1.65 g) was dissolved in 6 ml of HOAc and stirred with 3 ml of 6 *N* HCl in dioxane solution for 4 min. After removing the solvents *in vacuo* at 40°, adding ether to solidify the product, washing, and drying, a quantitative yield of the hydrochloride salt was obtained. Tlc showed one spot, R_{fA} 0.33, $[\alpha]^{28}D$ +3.0° (*c* 1, DMF).

Anal. Calcd for $C_{45}H_{51}N_7O_8S \cdot HCl \cdot 2H_2O$: C, 58.59; H, 6.12; N, 10.63; S, 3.48; Cl, 3.84. Found: C, 58.55; H, 6.17; N, 10.49; S, 3.72; Cl, 3.74.

Boc-Tyr-OPP (19).—To 10.1 g (35.8 mmol) of Boc-Tyr-OH[21] and 14.3 g (53.7 mmol) of pentachlorophenol in 50 ml of cold EtOAc was added a cooled solution of 8.12 g (39.4 mmol) of DCCD in 15 ml of EtOAc, and the reaction mixture was stirred in an ice bath for 1.5 hr. At that time an additional 50 ml of EtOAc was added to enhance stirring of the thick reaction mixture. After stirring overnight at room temperature, the precipitated DCU was removed by filtration and washed with acetone; the filtrate was dried over anhydrous $MgSO_4$ and stripped to a tan solid. Purification by crystallization from EtOAc–Skellysolve B afforded 10.4 g (54.7%), mp 167.5–168.5°, R_{fC} 0.53, $[\alpha]^{28}D$ −42° (*c* 1, MeOH).

Anal. Calcd for $C_{20}H_{18}Cl_5NO_5$: C, 45.35; H, 3.43; N, 2.64; Cl, 33.47. Found: C, 45.53; H, 3.42; N, 2.60; Cl, 33.70.

Boc-Tyr-Phe-Gln-Asn-Cys(Trt)-Pro (10).—It was synthesized as previously described for **2** from 8.67 g (9.22 mmol) of **9**, 5.36 g (10.1 mmol) of **19**, and 2.2 ml (19.8 mmol) of *N*-methylmorpholine in 60 ml of DMF. The crude product was boiled in 15 parts of EtOAc, the solution cooled, and ether added. After filtering, washing with ether, and drying, 9.45 g (89%) of pure hexapeptide was obtained. Tlc showed one spot, R_{fA} 0.72; $[\alpha]^{28}D$ −9.0° (*c* 1, DMF), −26.5° (*c* 1, MeOH).

Anal. Calcd for $C_{59}H_{68}N_8O_{12}S \cdot 2.5H_2O$: C, 61.17; H, 6.35; N, 9.67; S, 2.77. Found: C, 61.08; H, 6.07; N, 9.60; S, 3.08.

H-Tyr-Phe-Gln-Asn-Cys(Trt)-Pro·HCl (11).—Deprotection of 9.33 g (8.06 mmol) of **10** in 27 ml HOAc and 13.5 ml of 6.04 *M* HCl in dioxane as described for **3**, afforded a quantitative yield of the desired hydrochloride salt. Tlc showed one spot, R_{fA} 0.45, $[\alpha]^{28}D$ −9.0° (*c* 1, DMF).

Anal. Calcd for $C_{54}H_{60}N_8O_{10}S \cdot HCl \cdot 3H_2O$: C, 58.76; H, 6.12; N, 10.15; S, 2.90; Cl, 3.21. Found: C, 58.87; H, 5.91; N, 10.10; S, 2.82; Cl, 3.53.

Boc-Cys(Trt)-Tyr-Phe-Gln-Asn-Cys(Trt)-Pro (13).—It was synthesized in the usual manner from 3.40 g (3.16 mmol) of **11**, 2.07 g (3.54 mmol) of **12**, and 0.70 ml (6.30 mmol) of *N*-methylmorpholine in 25 ml of DMF. The crude product was dissolved in five parts of boiling EtOAc which, upon cooling, gave an oil. The oil solidified after trituration with cold EtOAc, and the product was filtered and dried, wt 3.76 g. An additional 0.63 g was obtained upon dilution of the filtrate with Skellysolve B. Tlc data on both crops showed identical results, R_{fA} 0.90, with a faint impurity at R_{fA} 0.40; both crops showed one spot at R_{fB} 0.87. Overall yield was 93% (as a dihydrate), $[\alpha]^{26}D$ −15° (*c* 1, MeOH).

Anal. Calcd for $C_{81}H_{87}N_9O_{13}S_2 \cdot 2H_2O$: C, 65.08; H, 6.14; N, 8.43; S, 4.29. Found: C, 65.35; H, 6.45; N, 8.35; S, 4.40.

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Boc-Cys-Tyr-Phe-Gln-Asn-Cys-Pro (14).—To 475 ml of 4.8 $\times 10^{-3}$ *M* I_2 (2.28 mmol) in 80% aqueous HOAc was added at room temperature over a 75-min period a solution of 3.15 g (2.13 mmol) of **13** in 425 ml of 80% aqueous HOAc. After stirring at room temperature for an additional 30 min, the excess I_2 was reduced with 1 *N* Na_2SO_3 and 5.1 ml of 1 *N* NaOH was added to neutralize HI. The reaction mixture was stripped to an oil which solidified upon trituration with cold ether. The solid was washed several times with ether and purified *via* CCD (system 2). After 480 transfers, the contents of tubes 170–206 (K = 7.9) were collected, the solvents were evaporated, and the resulting solid was dried *in vacuo* at 75° for 3 hr, wt 1.81 g. Tlc indicated some starting material still present, R_{fA} 0.76 with the desired product R_{fA} 0.47. Therefore, the compound was treated again with 120 ml of I_2 solution and worked up as previously described after stirring for 45 min at room temperature after the addition was completed. Another CCD purification (240 transfers) afforded 1.44 g (69.5%) of **14**, R_{fA} 0.47, $[\alpha]^{26}D$ −74° (*c* 1, DMF). Recovered triphenylcarbinol from the ether washings of both cyclization reactions amounted to 985 mg or 88.7% of theory after recrystallization from MeOH.

Anal. Calcd for $C_{48}H_{57}N_9O_{13}S_2 \cdot 3H_2O$: C, 50.32; H, 6.19; N, 12.28; S, 6.25. Found: C, 50.09; H, 5.68; N, 12.64; S, 6.24.

Subsequently, it has been found in the synthesis of analogs that using a 15–20% excess of I_2 will give complete cyclization the first time.

H-Arg-Gly-NH_2·dipicrate (16).—A solution of 24.5 g (60.0 mmol) of Z-Arg(NO_2)-Gly-NH_2[22] in 250 ml of 90% acetic acid containing 2.5 g of Pd (black) was hydrogenated at 25 psi and room temperature for 6.5 hr. Removal of the catalyst and evaporation of the filtrate *in vacuo* left 36.1 g of a viscous oil containing the crude dipeptide and ammonium acetate. The residue was taken up in 125 ml of water and treated with 39.8 g of picric acid in 250 ml of warm ethanol. The resulting precipitate was recrystallized from 1 l. of 8:1 water–ethanol to give 17.9 g (44% yield) of the dipicrate, mp 210.0–210.5° dec, $[\alpha]^{27}D$ +15.3° (*c* 1, 50% aqueous acetone) [lit.[12] mp 209–210°, $[\alpha]^{24}D$ +15.9° (*c* 1, 50% aqueous acetone)].

Boc-Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2·picrate (17).—A 1.54-g (1.53 mmol) sample of **14** and 196 mg (1.70 mmol) of *N*-hydroxysuccinimide were dissolved in 6 ml of DMF and treated for 2.5 hr at room temperature with 350 mg (1.70 mmol) of DCCD, after which was added 1.17 g (1.70 mmol) of **16** and 0.20 ml (1.8 mmol) of *N*-methylmorpholine. After stirring overnight at room temperature, the reaction mixture was cooled to 0° and the DCU removed by filtration (recovered 327 mg or 85.7% of theory). The filtrate was concentrated at 40° (oil pump) and the gummy solid triturated at −70° with ether. The resulting yellow solid was washed with ether several times and dried *in vacuo* (70°, 4 hr) to give 3.18 g of crude **17**. The sample was divided into two equal portions and 1.59 g purified *via* CCD (system 2). After 240 transfers, the contents of tubes 176–190 (K = 3.4) were collected and solvents evaporated. The product was precipitated with ether in the cold. The filtered nonapeptide was dried *in vacuo* at 55°, yield 576 mg. Tlc showed product, R_{fA} 0.29, picric acid, R_{fA} 0.62, and some impurity still remaining at the origin. A repeat purification on 410 mg for 240 transfers afforded 229 mg of pure **17**, $[\alpha]^{27}D$ −79° (*c* 0.5, MeOH), after isolation and drying *in vacuo* (3 hr, 75°) as described above, R_{fA} 0.29, homogeneous.

Anal. Calcd for $C_{51}H_{73}N_{15}O_{14}S_2 \cdot C_6H_3N_3O_7$: C, 48.43; H, 5.42; N, 17.84; S, 4.54. Found: C, 48.53; H, 5.65; N, 17.62; S, 4.54.

Arg^8-vasopressin (18).—To the remaining 1.59 g of crude **17** was added 25 ml of 90% aqueous TFA and the resulting solution stirred at room temperature for 2 hr. Cooling to 0° and the addition of 125 ml of cold ether gave a yellow solid, which weighed 1.17 g after drying *in vacuo* at room temperature. Purification of the crude vasopressin was performed by ion exchange on IRC-50 (100:1 weight ratio) using a gradient elution of 0.1 *N* to glacial acetic acid. Flow rate was 2 ml/min and 15-ml fractions were collected. The purified product was found in tubes 95–160, which were collected and the solvents evaporated. The resulting oily residue was lyophilized and then dried *in vacuo* over magnesium perchlorate for 16 hr at 105°: wt 414 mg or 44.5% yield (as diacetate hemihydrate) in two steps from **14**: R_{fA} 0.1; $[\alpha]^{25}D$ −26° (*c* 0.5, 1 *N* HOAc).

Anal. Calcd for $C_{46}H_{65}N_{15}O_{12}S_2 \cdot 2CH_3CO_2H \cdot {}^1/_2H_2O$: C, 49.47; H, 6.15; N, 17.32; S, 5.28. Found: C, 49.25; H, 5.97; N, 17.17; S, 5.63.

Amino acid analysis[23] showed $^1/_2$ Cys 1.65, Phe 1.01, Tyr 0.89, Glu 1.02, Asp 1.07, Pro 0.98, Arg 0.99, Gly 1.05, NH_3 2.95.

An eight-point bioassay was performed in the saline–alcohol loaded rat.[13] Results showed our arginine vasopressin to have an activity of 454 IU/mg as a free base (95% confidence limits being 340–534 IU/mg). Natural arginine vasopressin has a potency of *ca.* 450 IU/mg as free base.

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