This difference in behavior has been accounted for by the higher affinity of the polarizable nucleophilic reagent CH_3S^- for the reaction center bound to the more polarizable iodine atom.[11] The less reactive iodo derivative shows in both reactions a higher activation enthalpy than the fluoro derivative; however, the difference $\Delta\Delta H^{\ddagger} = (\Delta H^{\ddagger}_I - \Delta H^{\ddagger}_F)$ becomes smaller[10] in going from the reaction with CH_3O^- ($\Delta\Delta H^{\ddagger} = 3.9$ kcal/mol) to the reaction with the more polarizable CH_3S^- ($\Delta\Delta H^{\ddagger} = 1.8$ kcal/mol).

Another striking example of the role of the polarizability of the ring-leaving group bond is given by the comparison of reactivity of methoxide and benzenethiolate ions with 2-halobenzothiazoles.[12] In the reaction of otherwise unsubstituted 2-halobenzothiazoles, where the polarizability factor is relatively unimportant, methoxide ion is more reactive than PhS^-; the reverse is true in the reaction of 2-halo-6-nitrobenzothiazoles, where the polarizability of the Hal–C bond becomes stronger, owing to the conjugation with the nitro group.

Therefore, we suggest that the observed inversion of reactivity between the pyrrole and the benzene derivatives may be associated with the polarizability of the leaving groups on the different substrates. In the pyrrole derivative, as well as in the other five-membered heteroaromatic substrates, the bond between the reaction center and the nitro groups should be more polarizable than in 1,4-dinitrobenzene because of the possibility of an extended conjugation between the heteroatom and both nitro groups. The reaction of the benzene compound with the polarizable thiolate ion could not benefit from this possibility, thus becoming slower than the reaction of pyrrole compound 1. However, at the moment, a detailed evaluation among the heteroaromatic substrates of the role of this factor, as compared to other important factors[1] (electronegativity of the heteroatom, aromaticity of the ring), is not yet feasible.

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Registry No.—Methanol, 67-56-1; *p*-toluenethiol, 106-45-6; 1-methyl-2-nitro-5-(*p*-tolylthio)pyrrole, 63059-30-3; 2-nitro-5-(*p*-tolylthio)furan, 63059-31-4; 2-nitro-5-(*p*-tolylthio)thiophene, 19991-81-2; 1-nitro-4-(*p*-tolylthio)benzene, 22865-48-1.

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Comparative Use of Benzhydrylamine and Chloromethylated Resins in Solid-Phase Synthesis of Carboxamide Terminal Peptides. Synthesis of Oxytocin Derivatives[1,2]

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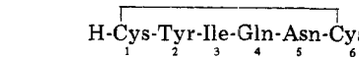
Specifically deuterated derivatives of the peptide hormone oxytocin were synthesized by the solid-phase method of peptide synthesis using either the standard chloromethylated resin or the benzhydrylamine resin as the support for the syntheses, and a comparison of the overall efficiency of the syntheses on the two resins was made. [1-Hemi-DL-[β,β-$^2H_2$]cystine]oxytocin was synthesized using the standard chloromethylated resin, and the two diastereomers were separated and purified by partition chromatography and gel filtration in an overall yield of about 30%. [1-Hemi-DL-[α-$^2H_1$]cystine]oxytocin was prepared using the benzhydrylamine resin to prepare the nonapeptide resin precursor, but otherwise using essentially identical conditions as used for the synthesis on the chloromethylated resin. Again the two diastereomers were separated and purified by partition chromatography and gel filtration. The overall yield of purified diastereomers under the best conditions was about 49%. For the synthesis of the latter compounds, *S*-3,4-dimethylbenzyl protecting groups were used to introduce the cysteine residues. The overall yields of the peptide hormone derivatives prepared on the benzhydrylamine resin were substantially improved if HF reactions were run at lower temperatures (0 °C rather than 25 °C), and if the *S*-3,4-dimethylbenzyl rather than the *S*-benzyl group was used for cysteine protection. Reproducible procedures for preparing benzhydrylamine resins with amino substitution levels of 0.15–0.45 mmol of amino group/g of resin were developed.

Since the introduction of the solid-phase synthesis of peptides by Merrifield,[3] the primary resin support has been chloromethylated polystyrene cross-linked with 1–2% divinylbenzene.[4,5] With this resin, the C-terminal amino acid is attached to the resin to afford a C-terminal resin benzyl ester. Subsequent synthesis of the remaining peptide chain is then accomplished with the resin benzyl ester serving as the C-terminal protecting group. This group is reasonably stable to the usual conditions of solid-phase peptide synthesis, but losses of 1–2% have been observed during each coupling procedure.[6,7] If a carboxamide C-terminal residue is desired, as is the case for many small biologically active peptides, it is generally necessary to first cleave the peptide from the resin as the protected carboxamide terminal derivative and then remove the other protecting groups. The former is usually done by treatment of the peptide resin with ammonia in an-

hydrous methanol[8] using the above resin or the corresponding nitrated resin,[9] or by transesterification followed by ammonolysis.[10] Displacement of the peptide from the resin is not always achieved[11] and even in favorable cases, the peptide is not quantitatively cleaved from the resin by ammonolysis. In addition, the methodology is generally not compatible with aspartic acid and glutamic acid containing peptides. To help circumvent some of these problems, Pietta and Marshall[12] and others[13,14] have used the benzhydrylamine resin for the preparation of carboxamide terminal peptides, and it has found considerable use in peptide synthesis.[4b] Several alternative resins for solid-phase synthesis of carboxamide terminal peptides have appeared.[15-18] However, little has been done to evaluate the comparative merits of the benzhydrylamine resin or its optimum use in solid-phase peptide synthesis except for the work of Orlowski et al.,[15] using a *p*-methoxybenzhydrylamine resin to prepare di- and tripeptides. We report here the synthesis of derivatives of the nonapeptide hormone oxytocin,



which posseses a C-terminal glycinamide residue, using the benhydrylamine resin and the conventional chloromethylated resin, and compare the syntheses under a variety of conditions. We have found the benzhydrylamine resin more advantageous. In the course of these studies we have also prepared benzhydrylamine resins with reproducible levels of amino substitution, and synthesized a derivative of *S*-3,4-dimethylbenzyl-DL-[α-$^2H_1$]cysteine for use in the synthesis of the oxytocin derivatives on the benzhydrylamine resin.

The solid-phase synthesis on the chloromethylated resin was accomplished by standard procedures used in our laboratory to prepare oxytocin derivatives.[19,20] [1-Hemi-DL-[β,β-$^2H_2$]cystine]oxytocin was prepared, and the diastereomeric hormone derivatives were separated and purified[19,20] by partition chromatography,[21,22] followed by gel filtration chromatography on Sephadex G-25. The overall yield of purified oxytocin derivatives is 30% (see Experimental Section),[23] which is a typical yield obtained by this procedure in the synthesis of oxytocin and derivatives.[8,19,20]

For the solid-phase synthesis on the benzhydrylamine resin, polystyrene resin, 1% cross-linked with divinylbenzene, was converted to the benzhydrylamine resin by a slight modification of procedures previously reported.[14a] The polystyrene cross-linked resin was converted to a phenyl ketone resin, followed by reductive amination using ammonium formate at 150–160 °C for various lengths of time. A highly reproducible level of amino substitution on the resin could be obtained, with the substitution being 0.15 ± 0.02, 0.35 ± 0.04, and 0.45 ± 0.05 mmol of amine/g of resin after 20, 36, and 48 h, respectively. All of these resins retained excellent mechanical and swelling properties for peptide synthesis. In the syntheses of oxytocin derivatives reported here we have used a resin substituted at a level of 0.37 mmol of glycinamide/g of resin. In this way a direct comparison with the chloromethylated resin (in which the substitution level was 0.36 mmol of glycinate/g of resin—see Experimental Section) was made. Amino and peptide resins from the chloromethylated and benzhydrylamine resins showed similar structural and mechanical behavior throughout the syntheses.

For the synthesis on the benzhydrylamine resin, we chose the synthesis of [1-hemi-DL-[α-$^2H_1$]cystine]oxytocin, in which the diastereomeric derivatives were separated from one another and purified by partition chromatography on Sephadex G-25. This permitted a direct comparison of the use of the two different starting resins under essentially identical conditions of purification as well as peptide synthesis. The rationale for preparing partially deuterated peptide hormone derivatives and their uses in biochemical and biophysical studies have been discussed elsewhere.[19,24,25]

The first synthesis on the benzhydrylamine resin followed the same procedures as the synthesis on the chloromethylated resin, except that no benzyl protecting group was used on the hydroxyl group of tyrosine. Previous studies have shown that when peptides with *O*-benzyl protected tyrosine residues are treated with HF, an undesirable side reaction involving alkylation of the tyrosine aromatic nucleus obtains,[26] and we wished to avoid this in our syntheses. The protected specifically deuterated amino acid Boc-*S*-benzyl-DL-[α-$^2H_1$]cysteine[19] was used to introduce the *N*-terminal amino acid residue (see Experimental Section). The peptide resin Boc-DL-[α-$^2H_1$]Cys(Bzl)-Tyr-Ile-Gln-Asn-Cys(DMB)-Pro-Leu-Gly-NH-resin was treated with anhydrous HF containing 10% anisole at 20 °C for 1 h, and the residual *S*-benzyl protecting groups[27] were removed by treatment of the peptide material obtained from the HF treatment with sodium in anhydrous liquid ammonia.[28] The peptide was oxidized with 0.01 N $K_3Fe(CN)_6$[29] under nitrogen.[30] The isomers were separated from each other and from by-products by partition chromatography on Sephadex G-25, followed by gel filtration of the separated diastereomers on Sephadex G-25.[31] The overall yield of purified peptides was 29%, which was essentially the same as was obtained using the chloromethylated resin.

The need to utilize two separate reaction steps to completely remove the *S*-benzyl protecting group led us to investigate the use of the 3,4-dimethylbenzyl group for sulfhydryl protection, since previous studies had shown that the group was completely removed by treatment with HF.[32,33] Again we synthesized [1-hemi-DL-[α-$^2H_1$]cystine]oxytocin and separated and purified the diastereomers. The synthesis of the desired peptide precursor on the benzhydrylamine resin followed the same procedures as before, except that a *S*-3,4-dimethylbenzyl derivative of the specifically deuterated amino acid DL-[α-$^2H_1$]cysteine was prepared and used to incorporate the cysteine residue at position 1 (see Experimental Section).

The protected peptide resin Boc-DL-[α-$^2H_1$]Cys(DMB)-Tyr-Ile-Gln-Asn-Cys(DMB)-Pro-Leu-Gly-NH-resin was treated with HF containing 10% anisole at 0 °C for 60 min. After the usual workup, oxidation, and purification the purified diastereomeric hormone derivatives [1-hemi-L-[α-$^2H_1$]cystine]oxytocin and [1-hemi-D-[α-$^2H_1$]cystine]oxytocin were obtained in an overall yield of 49%. On the other hand, a similar cleavage run at 25 °C in HF for 1 h gave only a 26% overall yield of the two purified diastereomeric peptides and large amounts of by-products.

The hormone derivatives from the various syntheses were assessed for purity and found to be pure by several criteria, including single spots and identical behavior with authentic oxytocin or [1-hemi-D-cystine]oxytocin on TLC using at least three different solvent systems, amino acid analysis, optical rotation, carbon-13 and proton NMR, and by their milk-ejecting activities.[34]

These comparative studies of solid-phase synthesis of oxytocin derivatives using chloromethylated and benzhydrylamine resin suggest that somewhat greater overall yields of oxytocin derivatives (from starting Gly resins to purified peptide hormone derivatives) can be obtained on benzhydrylamine resins than on chloromethylated resins. Under the best synthetic and cleavage conditions for using the benzhydrylamine resin (HF at 0 °C for 60 min, *S*-3,4-dimethylbenzyl protecting groups) our overall yield of purified hormone derivatives was 49%. Very similar yields in the synthesis of oxytocin (55%) have also been obtained on benzhydrylamine resins by Live, Agosta, and Cowburn[35] using similar procedures to those reported here. The overall yields (glycine-

substituted resin to final purified oxytocin derivatives) on chloromethylated resins as reported here (see Experimental Section) and elsewhere[8,19,20] are generally about 30%.

However, it must be pointed out that if the HF cleavage of the protected peptide–benzhydrylamine resin is run at 25 °C rather than 0 °C, or *S*-benzyl groups are used rather than *S*-3,4-dimethylbenzyl groups, the overall yields of pure oxytocin derivatives (26 and 29%, respectively) are about the same as obtained on the chloromethylated resin. Hence, proper choice of HF cleavage conditions and protecting groups are crucial to obtaining any advantages for the benzhydrylamine resin. The exact nature of the side reactions responsible for the decrease in yields in running the HF reaction at 25 °C rather than 0 °C (both reactions were run with the same precursor peptide resin) was not determined, though it undoubtedly involved reactions at the sulfur atom(s) of the cysteine residue(s). This was indicated by the large increase in dimeric and other high molecular weight products observed under the latter condition, and the large decrease in half-cystine content in these side products on amino acid analysis (see Experimental Section).

We have used the Leuckart reductive amination procedure in the preparation of the benzhydrylamine resin, which Orlowski et al.[15] have shown to be the preferred method of synthesis, since it leads to little or no undesirable secondary amine formation. We obtained an overall yield of about 49% of the purified oxytocin derivates (an octa- or nonapeptide), which compares well with the overall yields of 65 and 75% for two purified tripeptides which Orlowski et al.[15] obtained using the *p*-methoxybenzhydrylamine resin. Since the length and the properties of the peptides are quite different, it is difficult to compare the relative merits of the two resins. However, the results obtained do suggest that the benzhydrylamine resin (and/or its substituted derivatives) is particularly attractive for the syntheses of carboxamide terminal peptides containing specifically labeled derivatives, such as the ^{2}H compounds reported here, and those containing ^{14}C, ^{3}H, ^{13}C, ^{15}N, ^{125}I, and other labels which are of increasing importance in studies of peptide hormone structure and function, and which often must be incorporated using very precious and expensive amino acid derivatives.

Experimental Section

Thin layer chromatography (TLC) was done on silica gel G glass plates using the following solvent systems: (A) 1-butanol–acetic acid–water (4:1:5, upper phase only); (B) 1-butanol–acetic acid–pyridine–water (15:3:10:12); (C) 1-pentanol–pyridine–water (7:7:6). The peptides were detected on the TLC plates using ultraviolet light, iodine vapors, ninhydrin, and fluorescamine. Capillary melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were obtained using a Varian T-60 spectrometer or a Bruker WH-90 FT spectrometer. Optical rotation values were measured at the mercury green line (547 nm) using a Zeiss Old 4 polarimeter. Elemental analyses were performed by Spang Microanalytical Laboratory. Amino acid analyses were obtained by the method of Spackman, Stein, and Moore[36] on a Beckman 120C amino acid analyzer after hydrolysis in 6 N HCl for 22–24 h at 110 °C. Partition chromatography purification and separation of oxytocin diastereomers was accomplished on Sephadex G-25 (block polymerizate).[20–22] Following partition chromatography, detection of peptides in eluents was made using UV spectroscopy (280 or 260 nm) or by the Folin–Lowry method.[37] The desired peptides were isolated by addition of deionized water to the organic solvents, followed by rotary evaporation in vacuo at 25–30 °C and lyophilization of the aqueous solution.

Solid-Phase Peptide Synthesis Procedures. Solid-phase peptide syntheses were done using either the Merrifield resin of chloromethylated polystyrene beads cross-linked with 1% divinylbenzene substituted with Boc-glycine[4] or using the benzhydrylamine resin substituted with Boc-glycine (vide infra). The *tert*-butyloxycarbonyl (Boc) group was used for protection of a α-amino groups of amino acids. Protection of side-chain functional groups was *O*-benzyl or no hydroxyl protection for Tyr, and *S*-benzyl (Bzl) or *S*-3,4-dimethylbenzyl (DMB) for cysteine. All protected amino acids were monitored for purity by TLC in at least three solvent systems and by mixed melting points. Asparagine and glutamine were coupled with a four-fold excess of the protected amino acid *p*-nitrophenyl ester and 1-hydroxybenzotriazole as catalyst.[19] In general, *two* coupling steps were used for dicyclohexylcarbodiimide (DCC) mediated coupling of the protected amino acid in methylene chloride (CH_2Cl_2) using a 1.5–3 molar excess of the protected amino acid and a 1.2–2.4 molar excess of DCC in each coupling step. Coupling times generally were for 30 min. A typical synthesis program for coupling an amino acid residue to the growing peptide chain was as previously detailed.[19] All coupling steps were monitored by the ninhydrin method[38] to ensure complete coupling, and coupling reactions were repeated if a positive test (<99% coupling) was indicated.

3,4-Dimethylbenzyl Mercaptan (2). The general procedure of Urquhart et al.[39] for preparing alkyl mercaptans was used. From 30.93 g (0.2 mol) of 3,4-dimethylbenzyl chloride (1) and 15.2 g (0.2 mol) of thiourea, 24.9 g (82%) of the title compound was obtained: bp 94 °C (7 mm) [lit.[40] bp 112 °C (14 mm)]; NMR (neat) δ 1.35 (t, 1 H), 1.80 (s, 6 H), 3.20 and 3.22 (d of d, 2 H), 6.62 (s, 3 H).

***S*-3,4-Dimethylbenzyl-DL-[α-$^{2}H_1$]cysteine (5).** The title compound was prepared by a slight modification of published methods.[19,41,42] From 3.04 g (20 mmol) of 3,4-dimethylbenzyl mercaptan (2) and 8.37 g (21 mmol) of diethyl α-acetamido-α-dimethylaminomethylmalonate methiodide in 75 mL of ethanol-*d*, 2.64 g (73%) of the title compound was obtained: mp 202–203 °C (lit. mp for protio compound, 184–186 °C;[33] 195–197 °C[32]); TLC in solvent systems A and B gave single spots identical to authentic *S*-3,4-dimethylbenzyl-L-cysteine; NMR (CD_3CO_2D) δ 2.25 (s, 6 H), 3.10 (s, br, 2 H), 3.75 (s, br, 2 H), 4.3–4.7 (α-CH, undetectable), 7.05 (s, 1.2 H—an exchange of about two deuterium atoms into aromatic ring has occurred).

Anal. Calcd for $C_{12}H_{14}D_3NO_2S$: C, 59.48; H, 8.17; N, 5.78. Found: C, 59.15; H, 7.89; N, 6.16.

***N*-Boc-*S*-3,4-Dimethylbenzyl-DL-[α-$^{2}H_1$]cysteine.** The title compound was prepared by the procedure of Schnabel.[43] Treatment of 2.0 g of **5** gave 2.02 g (72%) of the title compound, mp 120.5–121.5 °C. Single uniform spots were obtained on TLC using systems A and B with R_f values identical with those of the L-protio analogue: NMR ($CDCl_3$) δ 1.45 (s, 9 H), 2.20 (s, 6 H), 2.8–2.9 (br s, 2 H), 3.65–3.75 (br s, 2 H), 4.40–4.60 (α-CH undetectable), 7.0 (s, 1.2 H), 11.1 (s, 1 H).

Synthesis of Benzhydrylamine Resin. A highly reproducible synthesis of benzhydrylamine resin[12–14] has been obtained as outlined below. The copolystyrene–1% divinylbenzene phenyl ketone resin was prepared as previously outlined,[14a] except that more extensive washings were performed using EtOH and 50% aqueous EtOH. From 30 g of polystyrene resin cross-linked with 1% divinylbenzene (Biobeads S-X1, 200–400 mesh) there was obtained 33.7 g of pale cream colored phenyl ketone resin, IR (KBr pellet) 1660 cm^{-1}.

The Leuckart reaction was run under similar conditions to those previously reported.[14a] The reactions were run with 5-g portions of the ketone resin for 20, 36, or 48 h at 150–160 °C (oil bath temperature, 170–180 °C). The mixture was cooled and the resin filtered off and washed with four 50-mL portions of H_2O, CH_3OH, and CH_2Cl_2. The resin was dried in vacuo and then hydrolyzed with 80 mL of 12 N HCl in propanoic acid (1:1) at reflux for 5 h. The resin salt was filtered off, washed with four 50-mL portions of H_2O, 50% aqueous EtOH, EtOH, and CH_2Cl_2, and then neutralized with two 50-mL portions of 10% diisopropylethylamine in CH_2Cl_2. After washing thoroughly with CH_2Cl_2 the resin was dried in vacuo, yield ~4.7 g.

In several separate preparations, the degree of amino substitution was found to be reasonably constant by the preceding procedures, being 0.15 ± 0.02 mmol/g of resin after 20 h of reductive amination, 0.35 ± 0.04 mmol/g of resin after 36 h, and 0.45 ± 0.05 mmol/g of resin after 48 h. The degree of substitution was determined by a direct aldimine test[39,40] and by substitution with a Boc amino acid (Gly or Val) to completion (negative ninhydrin test[37]), and then removing the Boc protecting group and measuring the amino acid substitution by the modified[44] aldimine test,[45] or by amino acid analysis.

Solid-Phase Synthesis of [1-Hemi-DL-[β,β-$^{2}H_2$]cystine]oxytocin Using Chloromethylated Resin and Separation of the Diastereomers. The synthesis of the protected nonapeptide precursor to the title compound was accomplished as previously reported.[19] Starting with 3.4 g of Boc-glycine-*O*-resin with a substitution of 0.36 mmol/g of resin (1.23-mmol scale) there was obtained 1.4 g (89%) of crude H-DL-[β,β-$^{2}H_2$]Cys(Bzl)-Tyr(Bzl)-Ile-Gln-Asn-Cys(DMB)-Pro-Leu-Gly-NH_2, mp 220–225 °C. A 325-mg portion of the nonapeptide (0.25 mmol) was deprotected and purified as described previously.[19] The all-L diastereomer (R_f 0.23) [1-hemi-[β,β-$^{2}H_2$]cystine]oxytocin was obtained as a white powder (40 mg, 32%) after gel filtration on Sephadex G-25, $[\alpha]^{22}_{547}$ −22° (*c* 0.5, 1 N HOAc). Amino

acid analysis: Asp, 1.0; Gly, 1.0; Pro, 0.92; Gly, 1.0; Half-Cys, 2.0; Ile, 1.0; Leu, 1.0; Tyr, 1.0. On TLC a single uniform spot identical with authentic oxytocin was seen in solvent systems A, B, and C. The compound had identical carbon-13 and proton NMR spectral and milk-ejecting[34] activities as previously reported. The diastereomer [1-hemi-D-[β,β-$^2H_2$]cystine]oxytocin (R_f 0.32) was obtained as a white powder (36 mg, 29%) after gel filtration on Sephadex G-25, [α^{22}_{547} −69° (c 0.5, 1 N HOAc). Amino acid analysis: Asp, 1.0; Glu, 1.0; Pro, 1.0; Gly, 1.0; Half-Cys, 2.0; Ile, 1.0; Leu, 1.0; Tyr, 0.90. On TLC a single uniform spot identical with authentic [1-hemi-D-cystine]oxytocin was seen using solvent systems A, B, and C. The compound had identical milk-ejecting activity[34] and ^{13}C NMR spectra as previously reported. The combined yield of the diastereomers based on starting glycine substituted resin is 29%.

Boc-Glycine–Benzhydrylamine Resin. Benzhydrylamine resin (36 h, 0.38 mmol of amino group/g of resin), prepared as discussed above (8.0 g), was treated with CH_2Cl_2 to swell the resin and filtered. The resin was stirred with 1.39 g (7.9 mmol) of Boc-glycine and 1.63 g (7.9 mmol) of DCC in 70 mL of methylene chloride for 30 min, and then filtered. The resin was washed with three 30-mL portions of CH_2Cl_2, EtOH, and CH_2Cl_2 and gave a negative ninhydrin test.[38] After removal of the Boc protecting group and neutralization, the modified aldimine test established the glycine substitution level to be 0.37 mmol/g of resin.

Synthesis of Boc-DL-[α-$^2H_1$]Cys(DMB)-Tyr-Ile-Gln-Asn-Cys(DMB)-Pro-Leu-Gly-NH-resin. The title compound was synthesized using 2.7 g (1.0 mmol) of the above benzhydrylamine resin and the standard solid-phase methodology. The title compound was obtained as 3.9 g of a pale cream resin.

Synthesis of [1-Hemi-DL-[α-$^2H_1$]cystine]oxytocin Using the *S*-3,4-Dimethylbenzyl Protecting Group and Separation of the Diastereomers. HF Treatment at 0 °C. A 0.98-g portion (0.25 mmol) of the protected peptide resin from above was treated with 20 mL of anhydrous HF (freshly distilled from CoF_3) and 2 mL of anisole at 0 °C for 1 h. The solvents were removed in vacuo at 0 °C. Under nitrogen, the residue was washed with four 30-mL portions of ethyl acetate, and the peptide was extracted from the resin with 10 mL of HOAc, two 20-mL portions of 30% HOAc, and three 30-mL portions of 0.2 N HOAc. The combined extracts were concentrated to about 80 mL in vacuo by rotary evaporation and lyophilized. The white powder (280 mg) was dissolved in 600 mL of 0.1% aqueous acetic acid under nitrogen,[30] oxidized in the usual manner,[29] and the products were separated and purified by partition chromatography on Sephadex G-25 using the solvent system 1-butanol–3.5% aqueous HOAc in 1.5% pyridine (1:1). Analysis of the fractions at 280 nm on a Gilford spectrophotometer showed a small by-product peak at R_f 0.6 (yield 25 mg) and well-resolved peaks for [1-hemi-D-[α-$^2H_1$]cystine]oxytocin (R_f 0.33) and [1-hemi-L-[α-$^2H_1$]cystine]oxytocin (R_f 0.23). The fractions corresponding to each diastereomer were separately pooled and lyophilized, and then each was separately purified by gel filtration chromatography on Sephadex G-25. There was obtained 68 mg (54%) of [1-hemi-[α-$^2H_1$]cystine]oxytocin, [$\alpha]^{24}_{547}$ −21° (c 0.498, 1 N HOAc). TLC in solvent systems A, B, and C gave single uniform spots identical with authentic oxytocin. Amino acid analysis: Asp, 1.0; Glu, 1.0; Pro, 1.0; Gly, 1.0; Half-Cys, 2.0; Ile, 1.0; Leu, 1.0; Tyr, 0.90. The carbon-13 NMR spectrum was identical with that of authentic oxytocin except for the peak corresponding to the C-2 carbon of the half-cystine-1 residue, which was greatly reduced in intensity. The milk-ejecting activity was determined and found to be 480 ± 55 units/mg, identical with authentic oxytocin. Also obtained was 54 mg (43%; the overall combined yield of purified oxytocin derivatives was 49%) of [1-hemi-D-[α-$^2H_1$]cystine]oxytocin, [$\alpha]^{24}_{547}$ −62° (c 0.504, 1 N HOAc). TLC in solvent systems A, B, and C gave single uniform spots, identical with authentic [1-hemi-D-cystine]oxytocin. Amino acid analysis: Asp, 1.0; Glu, 1.0; Pro, 1.0; Gly, 1.0; Half-Cys, 2.0; Ile, 0.93; Leu, 1.0; Tyr, 0.90. The milk-ejecting activity[34] was 35 ± 10 units/mg, identical with other 1-hemi-D-cystine derivatives.[19] Anal. Calcd for $C_{43}H_{65}DN_{12}O_{12}S_2 \cdot CH_3CO_2H$: C, 50.7; H, 6.39; N, 15.8 Found: C, 50.8; H, 6.37; N, 16.2. The entire procedure was repeated with essentially identical results (48% overall yield).

HF Treatment at 25 °C. A 0.98-g portion (0.25 mmol) of the protected peptide resin 1 was treated with 20 mL of anhydrous HF (freshly distilled from CoF_3) and 2 mL of anisole at room temperature (25 °C) for 1 h. The peptide material was extracted, oxidized, and purified by partition chromatography as before. Folin–Lowry analysis of the fractions indicated that a very large component was the by-product peak at R_f 0.60 (yield 72.3 mg) and the poorly resolved diastereomers [1-hemi-D-[α-$^2H_1$]cystine]oxytocin and [1-hemi-[α-$^2H_1$]cystine]oxytocin. The polymer peak gave the following amino acid analysis: Asp, 1.0; Glu, 0.9; Pro, 1.1; Gly, 1.0; Half-Cys, 1.0; Ile, 0.9; Leu, 1.1; Tyr, 0.8; NH_3, 3.1. TLC analysis indicated several components were present. The combined diastereomer peak (145 mg) was resubjected to partition chromatography, and an excellent separation of the diastereomers was obtained with [1-hemi-D-[α-$^2H_1$]cystine]-oxytocin (44 mg) at R_f 0.33 and [1-hemi-L-[α-$^2H_1$]cystine]oxytocin (61 mg) at R_f 0.22. Gel filtration of the 1-hemi-D-cystine diastereomer on Sephadex G-25 gave 26.1 mg (21%) of the pure derivative with a small broad peak of higher molecular weight material preceding it. Gel filtration of the all-L diastereomer on Sephadex G-25 gave 37.6 mg (30%; the overall yield of the two diastereomers was 25.5%) of the pure diastereomer and a significant amount of a broad peak of higher molecular weight material (yield 15.3 mg). Amino acid analysis of the latter peak gave: Asp, 1.0; Glu, 1.0; Pro, 1.1; Gly, 1.0; Half-Cys, 1.6; Ile, 1.0; Leu, 1.2; Tyr, 0.9; NH_3, 3.2. Both of the purified oxytocin diastereomers had identical properties with those prepared above as determined by TLC in solvent systems A, B, and C, amino acid analysis, optical rotation, carbon-13 NMR spectroscopy, and milk-ejecting activity. The procedure was repeated with about the same results (overall yield of 24%).

Synthesis of [1-Hemi-DL-[α-$^2H_1$]cystine]oxytocin on Benzhydrylamine Resin Using the *S*-Benzyl Protecting Group. The synthesis of the protected nonapeptide resin to the title compound was as before using 4.03 g (1.49 mmol) of Boc-Gly-NH resin, except that Boc-*S*-3,4-dimethylbenzylcysteine[19,32,33] was used to introduce the cysteine-6 residue and Boc-*S*-benzyl-DL-[α-$^2H_1$]cysteine[19] was used to introduce the cysteine-1 residue (1.1 and 0.5 equiv at the two coupling steps) to give 6.07 g of Boc-DL-[α-$^2H_1$]Cys(Bzl)-Tyr-Ile-Gln-Asn-Cys(DMB)-Pro-Leu-Gly-NH-resin. A 1.06-g (0.25 mmol) portion of the resin was treated with 20 mL of anhydrous HF and 2 mL of anisole at 20 °C for 1 h, and the crude lyophilized peptide product was obtained as before (334 mg). To remove the remaining *S*-benzyl protecting groups, the crude product was dissolved in 150 mL of anhydrous ammonia (freshly distilled from sodium) and treated with sodium until a blue color persisted for 45 s. The solvents were removed by evaporating under nitrogen and lyophilization. The residue was oxidized and the diastereomers were separated and purified as before to give 34 mg (27%) of [1-hemi-[α-$^2H_1$]cystine]oxytocin, [$\alpha]^{22}_{547}$ −22° (c 0.5, 1 N HOAc). TLC on silica gel plates gave single uniform spots in solvent systems A, B, and C, identical with the compounds as previously prepared. Amino acid analysis: Asp, 1.0; Glu, 1.1; Pro, 0.94; Gly, 1.0; Half-Cys, 1.9; Ile, 1.0; Leu, 1.0; Tyr, 1.0. Also obtained was 39 mg (31%; overall yield of both purified oxytocin derivatives 29%) of [1-hemi-D-[α-$^2H_1$]cystine]oxytocin, [$\alpha]^{22}_{547}$ −63° (c 0.5, 1 N HOAc). TLC on silica gel plates gave single uniform spots in solvent systems A, B, and C identical with the compound as previously prepared. Amino acid analysis: Asp, 1.0; Glu, 1.0; Pro, 1.0; Gly, 1.0; Half-Cys, 2.0; Ile, 0.93; Leu, 1.0; and Tyr, 0.90.

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Registry No.—**1**, 102-46-5; **2**, 4496-95-1; **5**, 63527-92-4; thiourea, 62-56-6; diethyl 2-acetamido-α-dimethylaminomethylmalonate methiodide, 7689-61-4; *N*-Boc-*S*-3,4-dimethylbenzyl-DL-[α-$^2H_1$]-cysteine, 63527-93-5; [1-hemi-DL-[β,β-$^2H_2$]cystine]oxytocin, 57866-62-3; H-DL-[β,β-$^2H_2$]Cys(Bzl)-Tyr(Bzl)-Ile-Gln-Asn-Cys-(DMB)-Pro-Leu-Gly-NH_2, 63527-94-6; 1-hemi[β,β-$^2H_2$]cystine]-oxytocin, 57866-63-4; [1-hemi-D-[β,β-$^2H_2$]cystine]oxytocin, 57866-64-5; [1-hemi-DL-[α-$^2H_1$]cystine]oxytocin, 63527-95-7; [1-hemi-L-[α-$^2H_1$]cystine]oxytocin, 63527-96-8; [1-hemi-D-[α-$^2H_1$]cystine]-oxytocin, 63527-97-9; Boc-*S*-3,4-dimethylbenzylcysteine, 41117-66-2; Boc-*S*-benzyl-DL-[α-$^2H_1$]cysteine, 57866-75-8.

References and Notes

(1) Financial support from the U.S. Public Health Service and the National Science Foundation is gratefully ackonwledged. Taken in part from the Ph.D. Thesis of Donald A. Upson, University of Arizona, 1975.
(2) All amino acids except glycine are of the L configuration unless otherwise noted. Standard abbreviations for amino acids, protecting groups, and peptides as recommended by the IUPAC–IUB Commission on Biochemical Nomenclature [*J. Biol. Chem.*, **247**, 977 (1972)] are used. Other abbreviations include DCC, dicyclohexylcarbodiimide; DIEA, diisopropylethylamine; TFA, trifluoroacetic acid; DMB, 3,4-dimethylbenzyl.
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A Rapid, Efficient Synthesis of Oxytocin and 8-Arginine-vasopressin. Comparison of Benzyl, *p*-Methoxybenzyl, and *p*-Methylbenzyl as Protecting Groups for Cysteine

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Oxytocin and 8-arginine-vasopressin have been synthesized in high yields in a short time using 1.5-fold molar excesses of protected amino acids by means of solid-phase synthesis on a benzhydrylamine resin. Coupling of each residue to the peptide resin was measured by automated picrate monitoring. High-pressure liquid partition chromatography was found to be extremely useful in characterizing products and by-products. Benzyl, *p*-methoxybenzyl, and *p*-methylbenzyl were compared as cysteine protecting groups in the syntheses, with the last two being preferred.

Introduction and Strategy

The neurohypophyseal hormones and their synthetic analogues have been the subject of many studies aimed at elucidating their physiological properties and the correlation between their structures and functions,[1] with oxytocin (I, Figure 1) receiving particular attention in these investigations. We are presently concerned with developing an approach to the unequivocal determination of the conformations and dynamic properties of peptides in solution, principally concentrating our investigations on oxytocin. The technique which we are employing in these investigations is nuclear magnetic resonance (NMR), measuring three-bond homo- and heteronuclear coupling constants from which dihedral angles and their rotational isomerism can be deduced. To extract such data from the spectra, a variety of isotopic isomers is needed. Selective deuteration is required to simplify overlap and coupling in the proton NMR spectra, and selective enrichments in ^{13}C and ^{15}N facilitate the observation of couplings to these less abundant nuclei.[2] Because of the number of isomers needed for a complete study and the expense of enriched precursors, we have undertaken development of methods for the rapid and efficient synthesis of oxytocin. Our strategy for these studies is to synthesize a number of isomers of oxytocin with simultaneous isotopic enrichments in several of the residues. Our synthetic goal is a generally optimized procedure using all the amino acid reagents with maximum