An Improved Polystyrene Support for Solid Phase Peptide Synthesis

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By placing a long spacer chain between the point of attachment of the first amino acid and the polystyrene support, an improved resin was obtained for use in solid phase peptide synthesis. A three-fold improvement in the overall yield of a 19-residue peptide from apolipoprotein C-III was realized.

Chloromethylpolystyrene cross-linked with 1% or 2% divinylbenzene has been widely used as the solid support for the synthesis of peptides by the Merrifield procedure.[1] Inherent problems in the use of this support have been encountered by many workers as well as the author. Hancock et al.[2] in a synthesis of acyl carrier protein from *E. coli* attempted to overcome some of these difficulties by repeated swelling, shrinking, and reswelling of the resin during the synthetic procedure. When this procedure was used during the synthesis[3] of peptide fragments of apolipoprotein C-III (apoC-III) from the human very low density lipoproteins,[4] a slight improvement was observed, but the yields were still relatively low, being in the range of 5–10% based on the initial loading of amino acid on the resin. Thus, studies were initiated to develop an improved resin support to overcome these difficulties.

Several approaches have been reported in the past[5,6] using shorter spacer molecules between the point of attachment of the first residue and the polymer matrix. Since these resins offered little advantage over the unmodified resin, we chose to incorporate a long aliphatic spacer molecule as has been done in affinity chromatography[7] to displace both small and large molecules from solid supports. This report describes the synthetic approach (Scheme I) used to obtain a support having a long spacer chain between the resin matrix and the point of attachment of the first residue. Its use in the synthesis of one 19-residue fragment (Figure 1) from apoC-III is presented and compared to the results obtained previously.

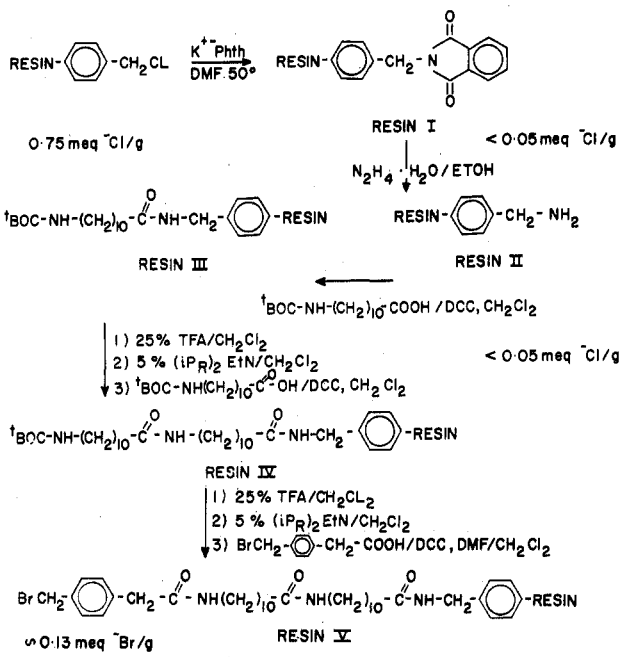
Phe-Ser-Glu-Phe-Trp-Asp-Leu-Asp-Pro-
Glu-Val-Arg-Pro-Thr-Ser-Ala-Val-Ala-Ala

Figure 1. Amino acid sequence of residues 61 to 79 of apolipoprotein C-III from the human serum very low density lipoproteins.

Results and Discussion

The displacement of chloride from commercial chloromethylated polystyrene (0.75 mequiv Cl^-/g) with potassium phthalimide in dimethylformamide at 50 °C proceeded to give resin I which contained <0.05 mequiv Cl^-/g of resin and whose ir spectrum displayed bands at 1710 and 1775 cm^{-1}, characteristic of the phthalimide ring. Treatment of resin I with hydrazine hydrate in refluxing ethanol overnight gave resin II whose ir spectrum after washing lacked the bands at 1710 and 1775 cm^{-1} and was highly fluorescent when treated with fluorescamine in methylene chloride according to the procedure of Felix and Jimenez.[8] The first residue of Boc-11-aminohendecanoic acid was coupled to resin II using dicyclohexylcarbodiimide (DCCI) in CH_2Cl_2. Although the resulting resin II was negative in the fluorescamine test after two couplings, it was treated with acetic anhydride in pyridine to acetylate any amino groups unavailable for coupling or to the fluorescamine reagent. Resin II was treated with 25% trifluoroacetic acid in CH_2Cl_2 and upon neutralization with 5% *i*-Pr_2EtN in CH_2Cl_2 displayed a very positive fluorescence after treatment with fluorescamine. A second residue of Boc-11-aminohendecanoic acid was introduced via DCCI coupling to give resin IV, which was also acetylated. Deprotection with 25% TFA–CH_2Cl_2 and neutralization with 5% *i*-Pr_2EtN–CH_2Cl_2 followed by DCCI coupling of (*p*-bromomethylphenyl)acetic acid in 50% DMF–CH_2Cl_2 gave resin V, which contained approximately 0.13 mequiv Br^-/g of resin. Resin V was acetylated after the introduction of the first Boc-amino acid to preclude formation of quaternary amine sites. Boc-amino acids were introduced quantitatively on resin V by the procedure of Gisin[9] using a two- to five-fold excess of their cesium salts. These amino acid resins contained <0.05 mequiv halide/g.

Scheme I



Using a Boc-alanine resin prepared by the above procedure, a 19-residue peptide (Figure 1) was synthesized using the same automatic synthesizer program as described for the earlier synthesis.[3] Throughout the synthesis increased incorporation of amino acid residues was observed as determined by amino acid analysis of the hydrolyzed peptide resins and peptide losses from the resin had diminished. A spin label attached to the NH_2 terminus of a peptide on this improved resin (Figure 2B) exhibited a narrower line EPR spectrum[10] than was observed with the unmodified commercial resin (Figure 2A). This result strongly suggested that at least the NH_2-terminal residue of the growing peptide (and perhaps even the complete peptide) on the modified resin was significantly more mobile and by inference provided greater steric accessibility of the reactive amino group for the incoming Boc-amino acid anhydride.

Cleavage of the peptide from the resin at 0 °C with anhydrous hydrogen fluoride and gel filtration on Bio-Gel P-10

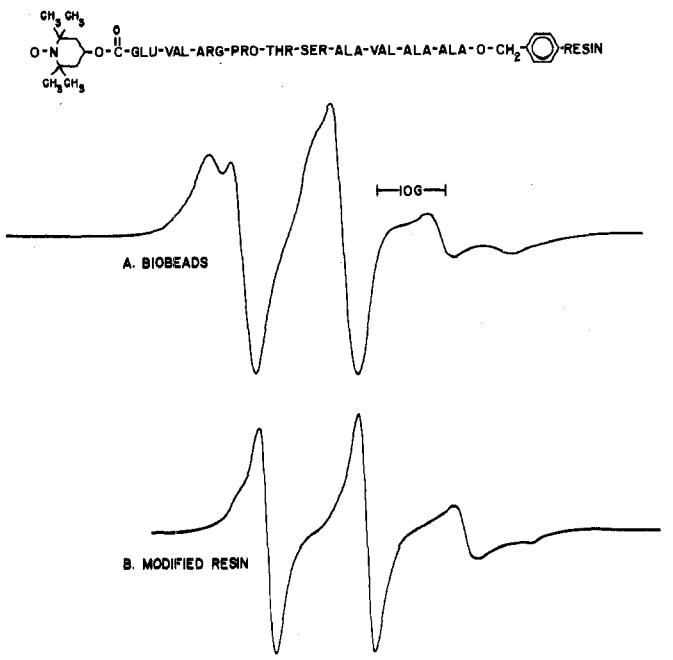


Figure 2. Electron paramagnetic resonance spectrum of 1-oxido-2,2,6,6-tetramethylpiperidinyloxycarbonyl peptide resins in methylene chloride: curve A, peptide-Bio-Beads; curve B, peptide-modified resin.

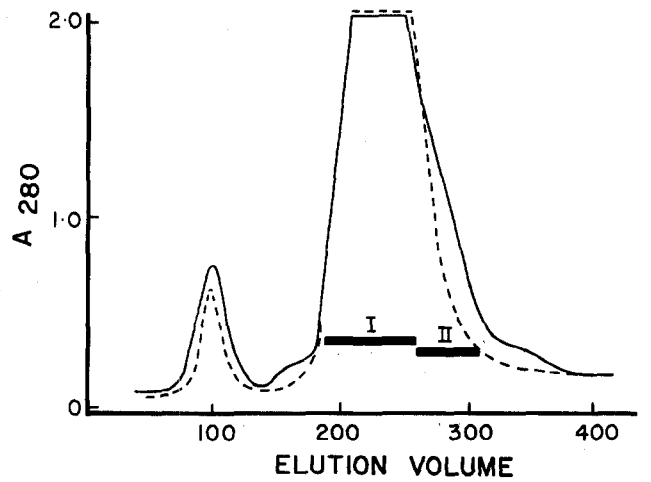


Figure 3. Elution profile of the HF cleaved products on Bio-Gel P-10 in 0.1 M ammonium bicarbonate: —, Bio-Beads product; - -, modified resin product.

in 0.1 M ammonium bicarbonate gave a symmetrical peak (Figure 3) which analyzed correctly for the 19-residue fragment (Table I). The total yield of peptide was 38.7% based on 0.13 mmol of alanine per gram of starting resin in contrast to 31.7% yield using the commercial resin loaded at 0.25 mmol/g. Further chromatography on AG 1X2 ion exchange resin permitted a 70% recovery of pure peptide for an overall yield of 27.1% with the modified resin, as contrasted to a 27.6% recovery (8.7% overall yield) of material from the synthesis on the unmodified resin (Figure 4 and Table II).

Based on these results and those obtained from the synthesis of similar peptides, this new resin appears to offer these two important advantages: (1) higher overall yields of hydrogen fluoride cleavable peptide and (2) peptides of greater homogeneity. It seems plausible that the increased yields result from reduced peptide–resin interactions of the growing peptide chains thereby freeing the amino terminus for coupling and reducing premature termination.

Table I. Amino Acid Analysis of Peptide Fractions from Bio-Gel P-10

Amino Acid	Improved Resin [a]		Biobeads [b]		Theoretical
	I	II	I	II	
Aspartic Acid	2.03	0.92	2.14	2.14	2
Threonine	0.96	0.64	0.96	0.85	1
Serine [c]	1.80	1.04	1.83	1.70	2
Glutamic Acid	1.99	1.08	2.11	1.94	2
Proline	1.90	1.29	2.11	2.19	2
Alanine	3.00	3.00	3.00	3.00	3
Valine	2.05	1.51	2.26	2.14	2
Leucine	0.99	0.42	1.01	1.02	1
Phenylalanine	2.06	0.64	2.05	1.61	2
Arginine	1.09	0.72	1.04	1.13	1
Tryptophan [d]	-	-	-	-	1

[a] Amino acid analysis of Fractions I and II from Fig. 3. Modified Resin Product (---).
[b] Amino acid analysis of Fractions I and II from Fig. 3. Bio Beads Product (—).
[c] Uncorrected for destruction
[d] Not quantitated, but present in amino acid hydrolysis profile.

Table II. Amino Acid Analysis of Peptide Isolated from AG 1X2

	Improved Resin	Bio Beads
Aspartic Acid	2.09	1.96
Threonine	0.99	0.94
Serine [a]	1.76	1.74
Glutamic Acid	2.01	2.04
Proline	2.06	2.03
Alanine	3.00	3.00
Valine	1.97	2.07
Leucine	1.00	1.03
Phenylalanine	2.00	2.05
Arginine	0.98	0.97

[a] Uncorrected for destruction

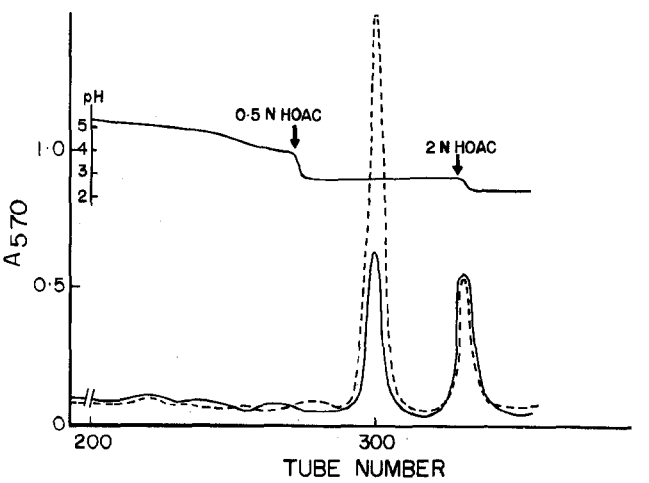


Figure 4. Elution profile on AG 1X2 of fraction I from Bio-Gel P-10: —, Bio-Beads product; - -, modified resin product. The column was eluted in a stepwise manner according to Hirs.[12] The peptide eluted with 0.5 N HOAc.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Microanalyses were performed by Micro-Tech Laboratories, Skokie, Ill. Infrared spectra were recorded on a Beckman Acculab IV. Boc-amino acids were purchased from Peninsula Labs, Beckman, and Bachem and checked by TLC in two solvent systems before use. Other chemicals were of reagent quality.

Phthalimidomethylpolystyrene (Resin I). Chloromethylpolystyrene cross-linked with 1% divinylbenzene (20 g Bio-Beads, 0.75

mequiv Cl^-/g) was suspended in 150 ml of dry distilled dimethylformamide (DMF) and 2.77 g of potassium phthalimide was added. The mixture was stirred at 50 °C for 18 h, after which the resin was washed three times each with DMF, methanol, water, and methanol. After drying in vacuo overnight, an active chloride determination[11] indicated <0.05 mequiv Cl^-/g. The ir spectrum in a KBr disk showed phthalimide bands at 1710 and 1775 cm^{-1}.

Aminomethylpolystyrene (Resin II). Resin I (20 g) was treated overnight with 1.5 ml of hydrazine hydrate in refluxing ethanol. The resin was filtered from the hot ethanol and washed three times with ethanol, 5% aqueous KOH, water, and ethanol. After drying overnight in a vacuum desiccator, the resin gave a strong fluorescamine test by the procedure of Felix and Jimenez[8] and the ir bands at 1710 and 1775 cm^{-1} were absent.

(*p*-Bromomethylphenyl)acetamido-11-hendecanamido-11-hendecanamidomethylpolystyrene (Resin V). Resin II (5 g) was transferred to the shaker vessel of a Schwarz/Mann peptide synthesizer and washed with 30 ml of the following reagents: CH_2Cl_2 (5×), *t*-BuOH (3×), CH_2Cl_2 (5×), 5% *i*-Pr_2EtN in CH_2Cl_2 (2×), CH_2Cl_2 (5×), *t*-BuOH (3×), and CH_2Cl_2 (5×). Each washing was for 1 min. The second treatment with *i*-Pr_2EtN was for 5 min. The Boc-11-aminohendecanoic acid (4 mmol in 10 ml of CH_2Cl_2) was added and shaken with the resin for 1 min, and 15 ml (2 mmol) of DCCI–CH_2Cl_2 solution was then added and the coupling was allowed to proceed for 1 h. The resin was then washed with 30 ml of the following reagents: 10% EtOH–CH_2Cl_2 (3×), CH_2Cl_2 (3×), *t*-BuOH (3×), CH_2Cl_2 (5×), 5% *i*-Pr_2EtN–CH_2Cl_2 (2×), and CH_2Cl_2 (5×). The coupling sequence was repeated as above and the resin washed in the same manner. A resin aliquot was removed at this point and treated with fluorescamine. Acetylation was carried out by washing the resin with 30 ml of 10% Ac_2O–py and then treating for 10 min with 30 ml of this reagent. The resin was then washed five times with CH_2Cl_2 to complete the program.

A second residue of Boc-11-aminohendecanoic acid was added after the following deblocking program: 25% TFA–CH_2Cl_2 (2×), CH_2Cl_2 (3×), *t*-BuOH (3×), CH_2Cl_2 (5×), 25% TFA–CH_2Cl_2 (2×). The second TFA treatment in each case was for 10 min. The washings, neutralizations, and couplings were performed as described above. A sample was likewise removed before acetylation for testing with fluorescamine. The (*p*-bromomethylphenyl)acetic acid was coupled according to the above program except the acid was dissolved in DMF and the coupling done in DMF–CH_2Cl_2. The acetylation step was omitted. The resin was analyzed for active bromide by the procedure described by Stewart and Young.[11] Resins were obtained that contained from 0.13 to 0.17 mequiv of bromide/g.

Boc Alanine-Resin V. According to the method of Gisin,[9] resin V (5 g) was treated in DMF at 50 °C with dry cesium Boc-alaninate prepared from 1.89 g of Boc-alanine and 1 equiv of CsOH in MeOH. The resin was washed three times with the following solvents: DMF, MeOH, H_2O, MeOH, and CH_2Cl_2. The degree of amino acid substitution was determined by amino acid analysis after HF cleavage and/or hydrolysis with 12 N HCl–propionic acid (1:1 v/v) at 135 °C for 2 h. The resin was found to contain 0.130 mmol of alanine/g. An active halide determination indicated <0.05 mequiv X^-/g. The resin was placed in the shaker vessel and washed with 30 ml of the following: CH_2Cl_2 (5×), *t*-BuOH (3×), CH_2Cl_2 (5×). The resin was acetylated by the procedure described in the preparation of resin V.

Synthesis of Phe-Ser-Glu-Phe-Trp-Asp-Leu-Asp-Pro-Glu-Val-Arg-Pro-Thr-Ser-Ala-Val-Ala-Ala. The amino acids were coupled using the same program employed in the preparation of resin V except that after the coupling of Boc-Trp, 0.5% ethanedithiol was added to the TFA deblocking reagent and 0.25% to the CH_2Cl_2 washes following the deblocking. Boc-Thr and Boc-Val were coupled using 8 mmol of protected amino acid and 4 mmol of DCCI–CH_2Cl_2 instead of the 4 mmol of amino acid and 2 mmol of DCCI used in the other couplings. The following side-chain protecting groups were employed: benzyl esters for Asp, and Glu, benzyl ethers for Thr and Ser, and G-tosyl for Arg.

HF Cleavage Procedure. The peptide resin (500 mg) was stirred in the reaction vessel of a Toho Kasei hydrogen fluoride apparatus for 30 min with 2 ml of anisole and 0.5 ml of ethanedithiol. Anhydrous hydrogen fluoride (10 ml) was condensed into the evacuated reaction vessel which had been precooled with liquid nitrogen. After warming to 0 °C, the resin was stirred for 30 min at 0 °C and the hydrogen fluoride evaporated at 0 °C with a vacuum pump protected with a train of CaO, liquid N_2, KOH traps. The resin was transferred to a sintered glass funnel with anhydrous ether and the peptide extracted with trifluoroacetic acid. The TFA was removed in vacuo at 15 °C. The peptide was solubilized in 7.5 ml of 1 M Tris and the pH adjusted to 8. This solution was applied to a 2.5 × 100 cm column of Bio-Gel P-10 equilibrated with 0.1 M ammonium bicarbonate, pH 8.0. The peptide containing fractions (Figure 3) were lyophilized and an aliquot of the solubilized peptide hydrolyzed for amino acid analysis (Table I). The yield was calculated on the basis of the original load of alanine. The peptide was further purified by chromatography[12] on AG 1X2 (Figure 4). The peptide was eluted with 0.5 N acetic acid. Recoveries were determined by amino acid analysis of an aliquot and by the absorbance at 280 nm (Table II). The peptide appeared as a single spot on cellulose F plates developed with 1-butanol–pyridine–glacial acetic acid–water (30:20:6:24) and visualized with ninhydrin.

Boc-11-aminohendecanoic Acid (Method A). The amino acid (20.13 g, Aldrich) was suspended in 250 ml of Me_2SO and 25 ml of Et_3N was added followed by 15 ml of Boc azide. The reaction mixture was stirred for several days until the amino acid had dissolved. In several preparations, it was necessary to add an additional 5 ml of Boc-N_3. After diluting with water and adjusting the pH to 10, the excess Boc-N_3 was extracted with diethyl ether. The aqueous phase was carefully acidified to pH 3.5 with cooling. The precipitate of Boc-11-aminohendecanoic acid was extracted with ethyl acetate. The ethyl acetate was dried over $MgSO_4$ and evaporated to give a residue which crystallized from hot hexane-benzene, yield 18.0 g (60%), mp 67–68 °C.

Anal. Calcd for $C_{16}H_{31}NO_4$: C, 63.75; H, 10.37; N, 4.65. Found: C, 63.41; H, 10.46; N, 4.59.

Method B. 11-Aminohendecanoic acid (20.13 g) was suspended in 200 ml of 1.5 N NaOH solution and warmed to 40 °C until the acid had dissolved. At 40 °C, Boc azide (15 ml) was added slowly during which time the temperature increased to 55 °C. After standing for 12 h at 50 °C, the reaction mixture was diluted with 1 l. of H_2O and the pH adjusted to 3.5 with cooling. The precipitated product was extracted with ethyl acetate. The organic layer was washed with H_2O, dried with $MgSO_4$, and evaporated. The solid residue (29.45 g) was crystallized from hexane–benzene, yield 23.4 g (78%), mp 67–68 °C.

***p*-Bromomethylphenylacetic Acid.** A solution of 27.5 g of tolylacetic acid and 11 ml of Br_2 in 400 ml of CCl_4 was brought to a reflux. The reaction mixture was illuminated with a 150-W tungsten lamp until HBr evolution had begun. After refluxing overnight, the reaction mixture was cooled and the precipitated product filtered off and washed with CCl_4. This solution was again heated at reflux temperature overnight, and the cooling and filtration repeated. In this manner, 22.3 g (63% yield) of pure product could be obtained after crystallization from benzene–hexane, mp 178–179 °C (lit.[13] mp 177–179 °C).

***p*-Nitrophenyl 1-Oxido-2,2,6,6-tetramethylpiperidinyl Carbonate.** A cold solution of 1-oxido-2,2,6,6-tetramethylpiperidinol (1.74 g) and 1.55 ml of triethylamine in 25 ml of dry ethyl acetate was treated with a solution of 2.02 g of *p*-nitrophenyl chloroformate in 25 ml of ethyl acetate. After standing at 5 °C overnight, the reaction mixture was poured over ice water and more ethyl acetate added. After washing well with H_2O, the ethyl acetate was dried with $MgSO_4$ and evaporated. The red product was crystallized from hexane–ether, yield 2.02 g (60%), mp 114–116 °C.

Anal. Calcd for $C_{16}H_{22}N_2O_6$: C, 56.96; H, 6.28; N, 8.31. Found: C, 56.85; H, 6.39, N, 8.22.

Labeling of Peptide Resins. Approximately 10 mg of deblocked and neutralized peptide resin was treated for 2 h with 200 μl of a 1% solution (w/v) of the above carbonate in CH_2Cl_2. The resin was washed with CH_2Cl_2, 10% HOAc–CH_2Cl_2, CH_2Cl_2, 5% *i*-Pr_2EtN–CH_2Cl_2, CH_2Cl_2, EtOH, and CH_2Cl_2 and suspended in CH_2Cl_2 before recording the electron paramagnetic resonance spectrum with a Varian E-12 spectrometer.

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Registry No.—Phe-Ser-Glu-Phe-Trp-Asp-Leu-Asp-Pro-Glu-Val-Arg-Pro-Thr-Ser-Ala-Val-Ala-Ala, 49777-12-0; *t*-Boc-11-aminohendecanoic acid, 10436-25-6; 11-aminohendecanoic acid, 2432-99-7; *t*-Boc azide, 1070-19-5; *p*-nitrophenyl 1-oxido-2,2,6,6-

tetramethylpiperidinyl carbonate, 58229-35-9; 1-oxido-2,2,6,6-tetramethylpiperidinol, 58229-36-0; p-nitrophenyl chloroformate, 7693-46-1.

References and Notes

(1) R. B. Merrifield, *Adv. Enzymol.*, **32**, 221 (1969).
(2) W. S. Hancock, G. R. Marshall, and P. R. Vagelos, *J. Biol. Chem.*, **248**, 2424 (1973).
(3) J. T. Sparrow, A. M. Gotto, Jr., and J. D. Morrisett, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 2124 (1973).
(4) H. B. Brewer, Jr., R. Shulman, P. Herbert, R. Ronan, and K. Wehrly, *J. Biol. Chem.*, **249**, 4975 (1974).
(5) E. Bayer, E. Breitmaier, G. Jung, and W. Paar, *Hoppe-Seyler's Z. Physiol. Chem.*, **352**, 759 (1971).
(6) G. W. Treagear in "Chemistry and Biology of Peptides", J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1972, p 175.
(7) W. B. Jakoby and M. Wilchek, *Methods Enzymol.*, **34** (1974).
(8) A. M. Felix and M. H. Jimenez, *Anal. Biochem.*, **52**, 377 (1973).
(9) B. F. Gisin, *Helv. Chim. Acta*, **56**, 1476 (1973).
(10) L. J. Berliner, "Spin Labeling: Theory and Applications", Academic Press, New York, N.Y., 1975.
(11) J. M. Stewart and J. D. Young, "Solid Phase Peptide Synthesis", W. H. Freeman, San Francisco, Calif., 1969, p 27.
(12) C. H. W. Hirs, *Methods Enzymol.*, **11**, 361 (1967).
(13) L. Chanffe, L. S. Andrews, and R. M. Keefer, *J. Org. Chem.*, **31**, 3758 (1966).

Synthesis of Specifically Deuterated *S*-Benzylcysteines and of Oxytocin and Related Diastereomers Deuterated in the Half-Cystine Positions[1,2]

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S-Benzylcysteine derivatives specifically deuterated at the α carbon only, the β carbon only, and at both the α and β carbons have been synthesized. These labeled compounds have been enzymatically resolved and the enantiomers and reacemates have been converted to the *N-tert*-butyloxycarbonyl derivatives. The deuterium labels were not exchanged under the conditions of the syntheses. Condensation of the sodium salt of diethyl α-acetamidomalonate with benzyl chloromethyl sulfide followed by hydrolysis with DCl afforded *S*-benzyl-DL-[α-$^2H_1$]cysteine. Acetylation followed by treatment with hog renal acylase separated the stereoisomers. A Mannich reaction with [$^2H_2$]methylene diacetate, diethyl α-acetamidomalonate, and dimethylamine followed by quaternization of the amino nitrogen with methyl iodide gave diethyl α-acetamido-α-dimethylamino[$^2H_2$]methylmalonate methiodide (**15**). Treatment of **15** with sodium benzylmercaptide gave diethyl α-acetamido-α-benzylthio[$^2H_2$]methylmalonate, which was hydrolyzed with HCl to yield *S*-benzyl-DL-[β,β-$^2H_2$]cysteine or with DCl to afford *S*-benzyl-DL-[α,β,β-$^2H_3$]cysteine. These compounds were resolved as before. The preparation of *S*-benzyl-DL-[α,β,β-$^2H_3$]cysteine required an efficient source of ethanol-*d*. This deuterated solvent was prepared in quantitative yield in 2 h from tetraethoxysilane, D_2O, and a catalytic amount of thionyl chloride. The protected deuterated amino acids were used in the preparation of several oxytocin analogues in which the specific deuteration appears in either the 1-hemicystine or the 6-hemicystine residues.

Specific deuterium labels in amino acids, peptides, and proteins are very useful for studying the chemical, biological, and especially the physical properties of these molecules. If the label is introduced at nonexchangeable positions, it provides a durable marker which does not significantly alter the properties of the molecule.[3-13]

The usefulness of such labels has been demonstrated for proteins in studies of structure and folding.[14-19] Unambiguous proton[8-11,20-22] and carbon-13[12,13,23] nuclear magnetic resonance assignments for many peptides have been made using specifically deuterated derivatives. The development of deuteron magnetic resonance spectroscopy has opened the way for a direct study of the microdynamical behavior of specific segments of the neurohypophyseal peptide hormones and their binding to the neurophysins, their biological carrier proteins.[24,25] These studies have begun to provide insights into the conformational aspects of these biologically active compounds, giving more direct evidence for structure–activity relationships than has been available from amino acid substitutions, which are more likely to perturb the structure of the molecule.[26-29]

In order to perform these experiments it is necessary to prepare the appropriate specifically deuterium labeled amino acid or amino acid derivative by a synthetic route which maintains the integrity of the deuterium label throughout the synthesis.

We report here the total synthesis of *S*-benzylcysteine deuterated specifically in the α position only, the β positions only, and in both the α and β positions. The specifically deuterated cysteine derivatives were also resolved into their enantiomeric pairs without loss of the label, and one was used for the synthesis of the specifically labeled oxytocin [6-hemi[β,β-$^2H_2$]cystine]oxytocin (**2**). The racemic compounds were used to incorporate specifically deuterated cysteine into the half-cystine positions of the oxytocin derivatives [6-hemi-DL-[α-$^2H_1$]cystine]oxytocin (**3**), [1-hemi-DL-[β,β-$^2H_2$]cystine]oxytocin (**4**), and [1-hemi-DL-[α,β,β-$^2H_3$]cystine]oxytocin (**5**) and the diastereomeric pairs were separated by partition chromatography on Sephadex G-25.[31,32,45]

H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2
1 2 3 4 5 6 7 8 9
1

The synthesis of each of the partially deuterated *S*-benzylcysteine derivatives was accomplished using different approaches. The *S*-benzyl-DL-[α-$^2H_1$]cysteine (**6**) was made by displacement of chloride from benzyl chloromethyl sulfide by sodium diethyl acetamidomalonate followed by hydrolysis of the adduct with 11 N DCl in D_2O (Scheme I).

The *S*-benzyl-DL-[β,β-$^2H_2$]cysteine (**7**) and *S*-benzyl-DL-[α,β,β-$^2H_3$]cysteine (**8**) were made using a modification of the methods of Cornelius[30] and Atkinson, et al.[33] This approach involved making the Mannich adduct of diethyl acetamidomalonate, formaldehyde, or its equivalent, and