$J_{4',5'}$ = 4 Hz, $C_{5'}H_2$), 4.05 (hex, 1, $J_{3',4'}$ = 2.5 Hz, $J_{4',5'}$ = 4 Hz, $C_{4'}H$), 4.54 (q, 1, $J_{1',2'}$ = 8.5 Hz, $J_{2',3'}$ = 6 Hz, $C_{2'}H$), 4.96 (q, 1, $J_{2',3'}$ = 6 Hz, $J_{3',4'}$ = 2.5 Hz, $C_{3'}H$), 5.88 (d, 1, $J_{5,6}$ = 8 Hz, C_5H), 6.37 (d, 1, $J_{1',2'}$ = 8.5 Hz, $C_{1'}H$), 7.61 (d, 1, $J_{5,6}$ = 8 Hz, C_6H), 9.81 (br s, 1, NH); mass spectrum (70 eV) m/e 506 (M^+), 379 (M − I), 319 (M − I − AcOH), 192 (M − I_2 − AcOH).

Anal. Calcd for $C_{11}H_{12}N_2O_5I_2$: C, 26.11; H, 2.39; N, 5.54. Found: C, 26.04; H, 2.43; N, 5.08.

3'-O-Acetyl-2',5'-dideoxyuridine (34d).—A solution of **34c** (85 mg) in 85% methanol (8 ml) containing sodium acetate (84 mg) was hydrogenated for 2 hr at 25° in the presence of 10% palladium on charcoal (32 mg). The mixture was then filtered through Celite, evaporated, and partitioned between ethyl acetate and very dilute aqueous sodium thiosulfate. Evaporation of the organic phase left 21 mg (50%) of **34d** as crystals, mp 182–183°. An analytical sample from chloroform–hexane had mp 185.5–186°: λ_{max}^{MeOH} 260 mμ (ε 10,200); ORD (MeOH) positive Cotton effect with a peak at 283 mμ (Φ +4400°), crossover at 273 mμ and a trough at 254 mμ (Φ −10,000°); nmr of the crude or recrystallized sample ($CDCl_3$) was very sharp with 1.42 ppm (d, 3, $J_{4',5'}$ = 6.5 Hz, $C_{5'}H_3$), 2.12 (s, 3, OAc), 2.16 (oct, 1, J_{gem} = 15 Hz, $J_{1',2'a}$ = 8 Hz, $J_{2'a,3'}$ = 6.5 Hz, $C_{2'a}H$), 2.54 (oct, 1, J_{gem} = 15 Hz, $J_{1',2'b}$ = 6 Hz, $J_{2'b,3'}$ = 3 Hz, $C_{2'b}H$), 4.23 (oct, 1, $J_{4',5'}$ = 6.5 Hz, $J_{3',4'}$ = 3 Hz, $C_{4'}H$), 4.88 (quint, 1, $J_{2'b,3'}$ = $J_{3',4}$ = 3 Hz, $J_{2'a,3'}$ = 6.5 Hz, $C_{3'}H$), 5.81 (d, 1, $J_{5,6}$ = 8 Hz, C_5H), 6.21 (q, 1, $J_{1',2'a}$ = 8 Hz, $J_{1',2'b}$ = 6 Hz, $C_{1'}H$), 7.46 (d, 1, $J_{5,6}$ = 8 Hz, C_6H).

Anal. Calcd for $C_{11}H_{14}N_2O_5$: C, 51.96; H, 5.55; N, 11.02. Found: C, 52.14; H, 5.96; N, 10.76.

Registry No.—**4a**, 25442-40-4; **4b**, 25442-42-6; **5a**, 14260-81-2; **5b**, 14259-59-7; **5c**, 25442-44-8; **7a**, 14260-87-8; **7b**, 14260-83-4; **9c**, 25383-77-1; **10c**, 25442-45-7; **17**, 25383-78-2; **19b**, 25442-46-0; **21**, 25383-79-3; **22**, 25383-80-6; **27**, 25383-81-7; **28a**, 25442-47-1; **28b**, 25383-82-8; **29**, 25442-48-2; **30**, 24514-27-0; **32**, 25442-49-3; **33a**, 25383-84-0; **34c**, 25383-85-1; **34d**, 25442-50-6; 3'-deoxy-3'-iodothymidine, 14260-82-3; methyltriphenoxyphosphonium iodide, 17579-99-6.

Synthesis of p-Aminobenzoyl Peptides[1a,b]

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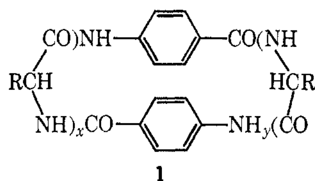
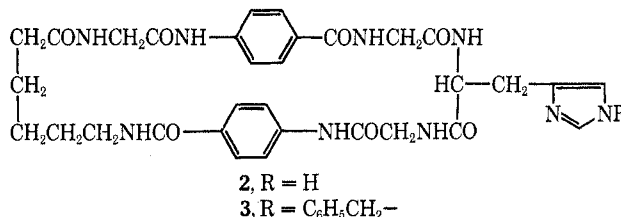
Cyclic peptides incorporating p-aminobenzoic acid are proposed as enzyme models. The p-aminobenzoyl residues may provide a relatively apolar cavity and substrate binding site, and the peptide bridges joining the p-aminobenzoyl residues allow the placement of functional side chains which can serve as a catalytic site. The synthesis of glycyl-p-aminobenzoylglycyl-*im*-benzyl-L-histidylglycyl-p-aminobenzoyl-ε-aminocaproic acid dihydrobromide (**4**) was carried out using the solid-phase method of peptide synthesis. Peptide **4** was cyclized using excess N,N'-dicyclohexylcarbodiimide in aqueous methanol to give *cyclo*-(glycyl-p-aminobenzoylglycyl-*im*-benzyl-L-histidylglycyl-p-aminobenzoyl-ε-aminocaproyl) (**3**). Peptide **3** was hydrogenated to give *cyclo*-(glycyl-p-aminobenzoylglycyl-L-histidylglycyl-p-aminobenzoyl-ε-aminocaproyl) (**2**), a simple example of the proposed class of peptides. The peptide was not sufficiently soluble in water to test its validity as an enzyme model. The saponification of p-aminobenzoyl peptide esters proceeds without major side reactions, contrary to reports in the literature. p-Aminobenzoyl peptides are cleaved by sodium in liquid ammonia.

The use of cyclic molecules as enzyme models has been explored in recent years. Synthetic cyclic peptides[2-5] and cycloamyloses[6-9] have been investigated. We propose molecules of the type **1** as enzyme models. The incorporation of p-aminobenzoyl residues



into a cyclic peptide provides a relatively apolar cavity that, in aqueous solution, might act as a substrate binding site. The peptide bridges between the p-aminobenzoyl residues allow the placement of functional side chains which can serve as a catalytic site. The preparation of p-aminobenzoyl peptides using conventional methods of peptide synthesis has received limited attention,[10-13] and the solid-phase method has not been used at all. In this communication we report the synthesis of the cyclic heptapeptide **2**, and also our investigation of two side reactions accompanying the synthesis of p-aminobenzoyl peptides.



The synthesis of **2** is outlined in Figure 1. The linear heptapeptide **4** was prepared by the solid-phase method of Merrifield,[14] starting with N-t-butyloxy-

(1) (a) This work was supported in part by grants from the U. S. Public Health Service (GM10591) and the National Science Foundation (GB6631). (b) This paper was reported in part at the 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969, Abstract ORGN 21. (c) Abstracted in part from a dissertation submitted by A. R. Mitchell to Indiana University in partial fulfillment of the requirements for the Ph.D. degree. Financial support from the U. S. Department of Health, Education, and Welfare is gratefully acknowledged. (d) To whom inquiries should be addressed. (e) Research Career Development Awardee of the U. S. Public Health Service.

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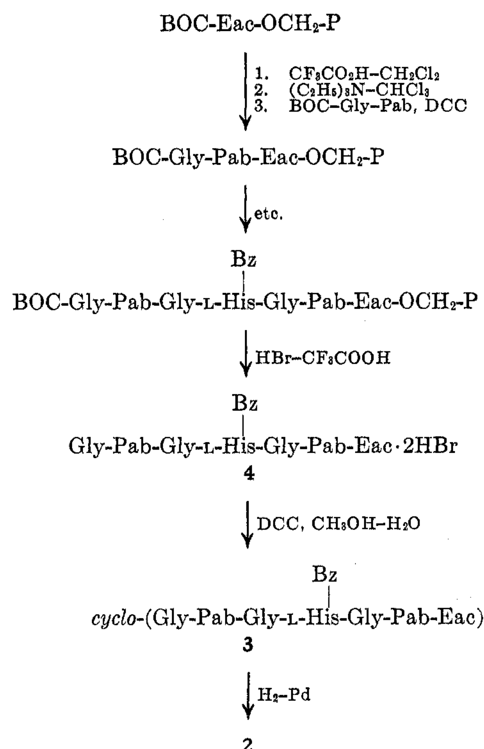

Figure 1.—Outline of synthesis of *cyclo*-(glycyl-*p*-aminobenzoylglycyl-L-histidylglycyl-*p*-aminobenzoyl-ε-aminocaproyl) (**2**); Eac, ε-aminocaproyl; Pab, *p*-aminobenzoyl; BOC–, *t*-butyloxycarbonyl–; Bz, benzyl; DCC, N,N′-dicyclohexylcarbodiimide; P, polystyrene–2% divinyl benzene copolymer.

carbonyl-ε-aminocaproyl resin.[15] The peptide chain was lengthened using 4 equiv of the *t*-butyloxycarbonyl derivatives of glycyl-*p*-aminobenzoic acid, *im*-benzyl-L-histidine, glycine, and glycyl-*p*-aminobenzoic acid, in that order. The dipeptide, *t*-butyloxycarbonylglycyl-*p*-aminobenzoic acid (**5**), was added as a unit because we were unable to prepare *t*-butyloxycarbonyl-*p*-aminobenzoic acid conveniently. Addition of the dipeptide as a unit also enabled us to monitor the coupling at this step with the amino acid analyzer, which we could not have done otherwise, since *p*-aminobenzoic acid does not give a positive ninhydrin test. Compound **5** was prepared from *t*-butyloxycarbonylglycine and *p*-aminobenzoic acid by the mixed anhydride procedure. All coupling reactions were carried out using N,N′-dicyclohexylcarbodiimide and were allowed to proceed for at least 15 hr. The linear heptapeptide was cleaved from the resin with hydrogen bromide in trifluoroacetic acid and purified by chromatography on Sephadex LH-20. The yield of purified **4** was 33%, based on ε-aminocaproyl resin. Peptide **4** was cyclized using a tenfold excess of N,N′-dicyclohexylcarbodiimide in aqueous methanol[16] to yield protected cyclic peptide **3** in 25% yield. Debenzylation of **3** by catalytic hydrogenolysis was monitored by thin layer chromatography. The slow disappearance of **3** and the appearance of a single ninhydrin-negative and Pauly-positive spot is evidence for the monomeric structure of **3** and **2**. Had **3** been a dimer containing two histidine residues, we should have observed during the hydrogenolysis an additional spot due to a monobenzyl derivative.

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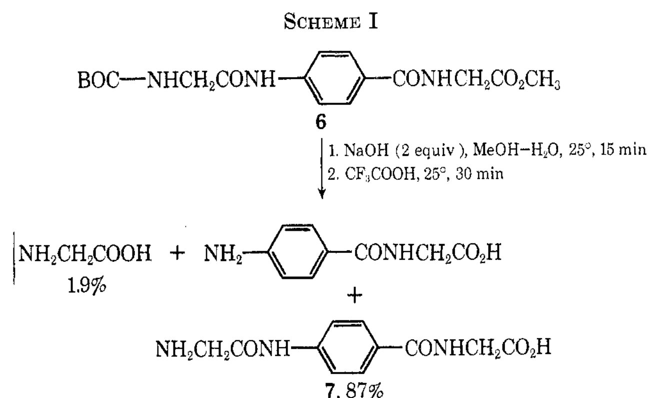
Determination of the molecular weight of **2** by X-ray diffraction also indicated a monomer, although the imperfect form of the crystals did not allow an accurate measurement (see Experimental Section). When peptide **4** was cyclized and hydrogenated without isolation of **3**, peptide **2** was obtained in 23% yield after chromatography. Titration of **2** in dimethyl sulfoxide–water (2:1) indicated a pK' of 5.8, compared with a value of 6.4 for imidazole in the same solvent.

The cyclic peptide **2** has a very low solubility (2×10^{-6} M) in neutral and basic aqueous solutions, which precludes a detailed study of its interaction with substrates. However, we were able to measure its activity in catalyzing the hydrolysis of an easily hydrolyzed substrate, 2,4-dinitrophenyl acetate. Table I summarizes the kinetic results. Peptide **2** is less active than unsubstituted imidazole in the catalysis of hydrolysis of 2,4-dinitrophenyl acetate, which is not surprising since molecular models indicate that the imidazole side chain of the peptide cannot interact easily with the substrate's carbonyl group when the substrate is in the cavity of the peptide ring. We are currently working on the synthesis of cyclic *o*-, *m*-, and *p*-aminobenzoyl peptides that are designed to be more water soluble than **2** and to have cooperatively interacting functional groups.

It has been claimed[12] that extensive peptide bond scission occurs during the basic hydrolysis of methyl, ethyl, and *p*-nitrophenyl esters of *p*-aminobenzoyl peptides. In order to evaluate this quantitatively, we synthesized N-*t*-butyloxycarbonylglycyl-*p*-aminobenzoylglycine methyl ester (**6**) and, after treating it with sodium hydroxide and removing the BOC group with trifluoroacetic acid, analyzed the products with an amino acid analyzer. The results are shown in Scheme I. Since no glycyl-*p*-aminobenzoic acid could be

SCHEME I



detected in the products, we assume that all the glycine arose from hydrolysis of the peptide bond preceding the *p*-aminobenzoic residue. When the saponification was allowed to run for 29 hr, glycine was formed in 8.4% yield and **7** in 92% yield. In a control experiment in which **6** was treated directly with trifluoroacetic acid at 25° for 30 min, no glycine was formed.

Subsequently, numerous hydrolytic reactions have been carried out on esters of other peptides containing *p*-aminobenzoyl residues, and good yields of the intact

TABLE I

HYDROLYSIS OF 2,4-DINITROPHENYL ACETATE (6×10^{-6} M) IN 0.01 M PHOSPHATE BUFFER (pH 7.19) AT 26° [a]

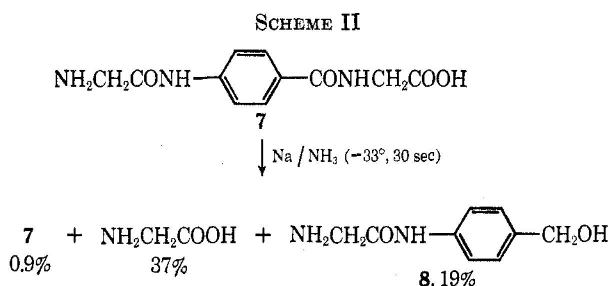
Catalyst	Molarity	$10^3 k_{obsd}$, min^{-1} (%)	Determinations	k_2, l./mol/min [b]
Buffer		3.58 ± 0.12 (3.4)	6	
2	1.0×10^{-6}	3.75 ± 0.02 (0.5)	3	170
2	2.0×10^{-6}	3.89 ± 0.02 (0.5)	3	155
Imidazole	1.0×10^{-6}	3.90 ± 0.05 (1.3)	3	320
Imidazole	2.0×10^{-6}	4.15 ± 0.12 (2.9)	3	300

[a] The solutions were 0.2% in acetonitrile and 0–0.15% in dimethylformamide. [b] $k_2 = (k_{obsd} - k_{solv})/c$ where k_{obsd} is the observed first-order rate constant measured in the presence of catalyst, k_{solv} is the constant in the absence of peptide or imidazole catalysts, and c is the molar concentration of catalyst.

peptide carboxylic acid have been obtained.[17] Compound **6** was prepared by coupling *t*-butyloxycarbonylglycine and *p*-aminobenzoylglycine methyl ester[18] using 1-ethyl-3-(N,N-dimethylaminopropyl)carbodiimide hydrochloride.[19]

The treatment of *p*-aminobenzoyl peptides with sodium in liquid ammonia, *e.g.*, to remove the iminobenzyl protecting group of histidine, results in extensive fragmentation of the peptide chain. Peptide **7** was almost completely destroyed under these conditions, and some of the products of the reaction were identified (Scheme II). Reductive cleavage of the *p*-aminobenzoyl

SCHEME II



residue was suggested by analogy to the cleavage of benzoyl amino acids by tetramethylammonium, generated at a mercury electrode,[20] but only partially accounts for the degradation of **7** by sodium in liquid ammonia. The product **8** was synthesized by coupling N-carbobenzyloxyglycine with *p*-aminobenzyl alcohol[21] using DCC, followed by catalytic hydrogenolysis of the carbobenzyloxy group.

The partial destruction of an N-*p*-aminobenzoyl derivative of lysine vasopressin by sodium in liquid ammonia was recently reported,[13] and the reductive cleavage of acylproline bonds by the same reagent has been observed.[22-24]

Experimental Section[25]

N-*t*-Butyloxycarbonylglycyl-*p*-aminobenzoic Acid (5).—A solution of N-*t*-butyloxycarbonylglycine[26] (14.0 g, 0.080 mol) and triethylamine (11.2 ml, 0.081 mol) in 200 ml of tetrahydrofuran was cooled to −15° in an ice–salt bath. Isobutyl chloroformate (10.2 ml, 0.078 mol) was added over a 10-min period. *p*-Aminobenzoic acid (11.0 g, 0.080 mol) in 50 ml of tetrahydrofuran was added. The reaction mixture was stirred in the ice bath for 1 hr, followed by stirring at room temperature for 10 hr. The mixture was evaporated *in vacuo* to a moist residue which was dissolved in 100 ml of 50% aqueous acetic acid. The solution was chilled and 600 ml of water was added with vigorous stirring. An off-white material (18.1 g) precipitated, mp 153–157°. The crude product was crystallized from tetrahydrofuran–petroleum ether (bp 30–60), yielding 10.4 g (44%) of a white powder, mp 167–169°, R_f (TCW) 0.50. Recrystallization from acetone gave an analytical sample, mp 170–172°.

Anal. Calcd for $C_{14}H_{18}N_2O_5$: C, 57.13; H, 6.16; N, 9.52. Found: C, 57.35; H, 6.44; N, 9.57.

N-*t*-Butyloxycarbonylglycyl-*p*-aminobenzoylglycine Methyl Ester (6).—A solution of N-*t*-butyloxycarbonylglycine (14.7 g, 0.084 mol) and *p*-aminobenzoylglycine methyl ester[18] (15.6 g 0.075 mol) in dichloromethane was cooled in an ice bath and 1-ethyl-3-(N,N-dimethylaminopropyl)carbodiimide hydrochloride[19] (15.0 g, 0.074 mol) was added. This was stirred at ice-bath temperature for 1 hr and overnight at room temperature. The resulting solution was washed with water, 1% sodium bicarbonate, 1% citric acid, and water. After the solution was dried over magnesium sulfate, the methylene chloride was removed *in vacuo* to give a white material which was crystallized from methanol–water (1:1) to give 24.0 g (87%) of white plates, 173–174°, R_f (CMA) 0.56. An analytical sample was recrystallized from ethyl acetate, mp 167.5–168°.

Anal. Calcd for $C_{17}H_{23}N_3O_6$: C, 55.90; H, 6.31; N, 11.50. Found: C, 56.29; H, 6.57; N, 11.40.

N-*t*-Butyloxycarbonylglycyl-*p*-aminobenzoylglycine.—Compound **6** (1.30 g, 0.0037 mol) was saponified for 30 min in a solution containing 5 ml of 1.5 N sodium hydroxide and 15 ml of methanol. The light yellow solution obtained was diluted with water (50 ml) and extracted with ethyl acetate. The ethyl acetate extract was discarded and the aqueous phase was acidified to pH 3.0 with citric acid, saturated with sodium chloride, and extracted with ethyl acetate. The ethyl acetate extract was washed with water, dried over magnesium sulfate, and evaporated *in vacuo* to give 1.00 g (96.1%) of a white crystalline compound: decomposing over 320°; R_f (BAWP) 0.59, R_f (PW) 0.56. The analytical sample was obtained through recrystallization from methanol–ethyl acetate–ether.

Anal. Calcd for $C_{16}H_{21}N_3O_6$: C, 54.69; H, 6.02; N, 12.01. Found: C, 54.73; H, 6.20; N, 12.18.

Glycyl-*p*-aminobenzoylglycine (7).—The above compound (0.20 g, 0.57 mmol) was treated with trifluoroacetic acid (2 ml) for 1 hr at 25°. The excess solvent was removed *in vacuo* and the resulting residue was triturated with ethyl acetate to give 200 mg of a white solid, melting and decomposing over 215°. The compound was crystallized from methanol–water–ethyl acetate (5:1:10) to yield the free tripeptide, melting with decomposition slowly over 215°, R_f (BAWP) 0.28.

(17) Experiments by Dr. Anand S. Dutta in this laboratory.

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(25) Melting points were taken in capillary tubes and are corrected. Elemental analyses were performed by Midwest Microlab, Indianapolis, Ind. The solvents used for thin layer chromatography on silica gel G were *n*-butyl alcohol–acetic acid–water (BAW) in a ratio of 4:1:1, *n*-butyl alcohol–acetic acid–water–pyridine (BAWP) in a ratio of 30:6:24:20, chloroform–methanol–acetic acid (CMA) in a ratio of 85:10:5, propanol–water (PW) in a ratio of 70:30, and tetrahydrofuran–cyclohexane–water (TCW) in a ratio of 93:7:5. A Technicon amino acid autoanalyzer was used for amino acid and peptide determinations. Amino acid hydrolyses were carried out in 6 N HCl in sealed evacuated tubes for 20 hr at 110° unless otherwise specified.

(26) E. Schnabel, *Justus Liebigs Ann. Chem.*, **702**, 188 (1967).

Anal. Calcd for $C_{11}H_{13}N_3O_4$: C, 52.58; H, 5.21; N, 16.72. Found: C, 52.35; H, 5.36; N, 16.46.

N-Carbobenzoxyglycyl-*p*-aminobenzyl Alcohol.—N-Carbobenzoxyglycine (2.09 g, 0.010 mol) and *p*-aminobenzyl alcohol[21] (1.23 g, 0.010 mol) were dissolved in 20 ml of tetrahydrofuran and cooled in an ice bath. N,N′-Dicyclohexylcarbodiimide (2.27 g, 0.011 mol) was added in 5 ml of tetrahydrofuran. The solution was stirred in the ice bath for 45 min and at room temperature overnight. The resulting suspension was filtered and the residue was washed well with tetrahydrofuran. The filtrate was evaporated *in vacuo* to yield a residue which was dissolved in 50 ml of hot methanol. Upon cooling 1.00 g (32%) of white crystals separated out of the solution: mp 160–162°; R_f (PW) 0.75, R_f (BAWP) 0.75. Recrystallization from ethyl acetate did not raise the melting point.

Anal. Calcd for $C_{17}H_{18}N_2O_4$: C, 64.95; H, 5.77; N, 8.91. Found: C, 64.80; H, 5.92; N, 8.69.

Glycyl-*p*-aminobenzyl Alcohol Hydrochloride (8).—The above compound (0.385 g, 0.001 mol) was dissolved in 150 ml of 95% ethanol containing 1 ml of 1 *N* HCl. Palladium (5%) on charcoal (0.100 g) was added and the suspension was hydrogenated at 46 psi for 1.5 hr. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The resulting residue was dissolved in hot methanol and ether was added until the solution became faintly turbid. White needles separated from the solution upon cooling. The material slowly decomposes above 280°: R_f (PW) 0.27, R_f (BAWP) 0.57.

Anal. Calcd for $C_9H_{13}N_2O_2Cl$: C, 49.89; H, 6.05; N, 12.93; Cl, 16.36. Found: C, 49.79; H, 6.35; N, 12.87; Cl, 16.49.

Reaction of 6 with Sodium Hydroxide.—A solution of 6 (0.130 g, 0.372 mmol) in 1.50 ml of methanol was treated with 0.50 ml of 1.50 *N* sodium hydroxide solution and the resulting solution was stirred at room temperature for 29 hr. At 0.25 hr and 29 hr, 0.050-ml aliquots were removed and treated with trifluoroacetic acid (10 ml) for 0.50 hr. The solutions were evaporated *in vacuo*. The resulting materials were dissolved in 1% HCl and applied to an amino acid analyzer previously calibrated with glycine, glycyl-*p*-aminobenzoic acid,[11] and compound 7. The presence of 0.008 mmol of glycine (1.9%) and 0.322 mmol of 7 (87%) was shown for the 0.25-hr sample. In the 29-hr sample, the release of 0.031 mmol of glycine (8.4%) and 0.340 mmol of 7 (92%) was indicated. No glycyl-*p*-aminobenzoic acid could be detected in either sample.

Reaction of 7 with Sodium in Liquid Ammonia.—A solution of 7 (0.106 g, 0.422 mmol) in approximately 20 ml of liquid ammonia (freshly distilled from sodium) was treated with freshly cut pieces of sodium until a blue color persisted for 30 sec. The color was discharged with ammonium bromide and the ammonia was allowed to evaporate at atmospheric pressure and then under reduced pressure. The resulting residue was dissolved in 58 ml of 50% aqueous acetic acid. An aliquot of this solution was placed on an amino acid analyzer previously calibrated with glycine, compound 7, and compound 8. The chromatogram indicated the release of 0.313 mmol of glycine (38% based on 0.844 mmol of glycine in 0.422 mmol of 7), 0.082 mmol of 8 (19%), and 0.004 mmol of 7 (0.9%).

N-*t*-Butyloxycarbonyl-ε-aminocaproyl Resin.—A solution of N-*t*-butyloxycarbonyl-ε-aminocaproic acid[15] (5.39 g, 23.3 mmol) and triethylamine (3.24 ml, 23.3 mmol) in 40 ml of ethanol was added to 10.00 g of chloromethylated polystyrene–2% divinyl benzene copolymer, 200–400 mesh (2.33 mmol of Cl/g). The mixture was stirred under reflux for 46 hr and filtered. The resin was washed thoroughly with ethanol and methanol. A sample of the dried resin was hydrolyzed in 1:1 dioxane–12 *N* HCl for 24 hr at 110°. Amino acid analysis showed the ε-aminocaproic acid content to be 0.620 mmol/g.

N-*t*-Butyloxycarbonylglycyl-*p*-aminobenzoylglycyl-*im*-benzyl-L-histidylglycyl-*p*-aminobenzoyl-ε-aminocaproyl Resin.—A portion of N-*t*-butyloxycarbonyl-ε-aminocaproyl resin (3.20 g, 2.20 mmol) was treated in the following manner for the incorporation of each residue: (1) washed (three 50-ml portions) with methylene chloride, (2) mixed with trifluoroacetic acid–methylene chloride (2:3) (50 ml) for 15 min, (3) washed (three 50-ml portions) with methylene chloride, (4) washed (three 50-ml portions) with ethanol, (5) washed (three 50-ml portions) with chloroform, (6) mixed with 40 ml of 10% triethylamine in chloroform for 10 min, (7) washed (three 50-ml portions) with chloroform, (8) washed (three 50-ml portions) with coupling solvent, (9) introduced 8.80 mmol of appropriate N-*t*-butyloxycarbonyl amino acid or peptide in 30–40 ml of coupling solvent and mixed for 10 min, (10) introduced 9.24 mmol of N,N′-dicyclohexylcarbodiimide in 10 ml of coupling solvent and let reaction proceed for 15 hr, (11) washed (three 50-ml portions) with coupling solvent, and (12) washed (three 50-ml portions) with ethanol.

The coupling solvents for the incorporation of N-*t*-butyloxycarbonylglycyl-*p*-aminobenzoic acid (5), N-*t*-butyloxycarbonyl-*im*-benzyl-L-histidine, and N-*t*-butyloxycarbonylglycine were tetrahydrofuran, dimethylformamide, and methylene chloride, respectively. After the first reaction cycle, the unreacted ε-aminocaproyl resin was acetylated with acetic anhydride (3 ml) and triethylamine (1 ml) in 20 ml of dimethylformamide. Three syntheses of protected heptapeptide resin were carried out, all starting with N-*t*-butyloxycarbonyl-ε-aminocaproyl resin (3.20 g, 2.20 mmol). Amino acid analyses were performed after each coupling step during the first synthesis of protected heptapeptide resin. The results are given in Table II.

TABLE II

Cooling step	Gly	Bz^{im}-His	Eac
1	0.80		1.0
2	0.72	0.80	1.0
3	1.62	0.78	1.0
4	2.05	0.76	1.0

Glycyl-*p*-aminobenzoylglycyl-*im*-benzyl-L-histidyl-glycyl-*p*-aminobenzoyl-ε-aminocaproic Acid Dihydrobromide (4).—The above protected heptapeptide resin was suspended in 40 ml of trifluoroacetic acid and a stream of hydrogen bromide (pretreated with 10% resorcinol in acetic acid) was passed through the suspension for 30 min with occasional shaking. The mixture was filtered and the resin was washed with trifluoroacetic acid (two 20-ml portions), trifluoroacetic acid–methylene chloride (1:1) (20 ml), and methylene chloride (two 25-ml portions). The pooled filtrates were evaporated *in vacuo* to yield a brown oil. The oil was taken up in methylene chloride which was removed *in vacuo*. The process was repeated and the resulting oil was triturated with ether to give a cream-colored powder. The material was washed well with ether and dried *in vacuo* to give 1.24 g of crude material displaying one major component and several minor components in BAW, PW, and BAWP. Amino acid analysis gave Gly:Bz^{im}-His:Eac (3.18:1.00:1.37). The other syntheses yielded 1.10 g (first synthesis) and 1.40 g (second synthesis) of crude heptapeptide dihydrobromide 4.

The crude material (1.16 g) was purified on a column (4.2 × 53 cm) of Sephadex LH-20 washed with methanol. Purified heptapeptide dihydrobromide (4) (0.566 g) was obtained in 33% yield (based on N-*t*-butyloxycarbonyl-ε-aminocaproyl resin): mp 162–164°; $[\alpha]^{26}D$ −7.50° (*c* 1, methanol); R_f (BAW) 0.09, R_f (PW) 0.31.

Anal. Calcd for $C_{39}H_{47}N_9O_8Br_2$: N, 13.56. Found: N, 13.68.

Amino acid analysis gave Gly:Bz^{im}-His:Eac (3.04:1.00:1.10).

***cyclo*-(Glycyl-*p*-aminobenzoylglycyl-*im*-benzyl-L-histidylglycyl-*p*-aminobenzoyl-ε-aminocaproyl) (3).**—Heptapeptide dihydrobromide 4 (0.156 g, 0.168 mmol) was dissolved in 80% aqueous methanol (168 ml). The solution was cooled to 0° and N,N′-dicyclohexylcarbodiimide (0.346 g, 1.68 mmol) was added in methanol (4 ml). The solution was stored at 5° for 2 days and at room temperature for 13 days. Acetic acid (2 ml) was added to destroy unreacted N,N′-dicyclohexylcarbodiimide and the solution was evaporated *in vacuo*. The resulting mixture was resolved on a column of Sephadex LH-20 (washed with methanol) to yield 0.031 g (25%) of a white material which began to brown at 245° and charred at 280–290°. Thin layer chromatograms of the material gave single components that were ninhydrin and Pauly negative and chlorine positive: R_f (BAW) 0.19, R_f (PW) 0.54, R_f (BAWP) 0.60.

Anal. Calcd for $C_{39}H_{43}N_9O_7 \cdot CH_3OH$: C, 61.44; H, 6.06; N, 16.12. Found: C, 61.66; H, 5.87; N, 16.36.

Amino acid analysis gave Gly:Bz^{im}-His:Eac (2.88:1.00:0.94).

***cyclo*-(Glycyl-*p*-aminobenzoylglycyl-L-histidylglycyl-*p*-aminobenzoyl-ε-aminocaproyl) (2) (Directly from 4).**—A solution of 4 (0.465 g, 0.500 mmol) in 80% aqueous methanol was cooled to 0° and N,N′-dicyclohexylcarbodiimide (1.03 g, 5.00 mmol) in methanol (20 ml) was added. The solution was stored at 5° for 3 days and at room temperature for 3 days. Acetic acid (6 ml) was added and the solution was evaporated to near dryness *in vacuo*. The wet residue was suspended in 50% aqueous acetic acid (50

ml) and shaken well. The suspension was filtered and the residue was washed with additional aqueous acetic acid (50 ml) and filtered. Palladium (5%) on charcoal (0.500 g) was added to the combined filtrates. The suspension was hydrogenated (40 psi) at room temperature for 66 hr. An aliquot taken at 66 hr gave a single Pauly-positive spot (R_f 0.41) on a thin layer plate developed with PW. The suspension was filtered and the filtrate was evaporated *in vacuo* to give 0.858 g of a foamy residue. A portion (0.644 g) of the residue was dissolved in 6 ml of butanol-water (6:1) and applied to a column (2.3 × 74 cm) of cellulose powder that had been washed with the same solvent mixture. Cyclic heptapeptide **2** was obtained as 0.057 g (23%) of a fine white material which charred at 279–282°. Thin layer chromatograms gave single components that were Pauly and chlorine positive and ninhydrin negative: R_f (BAW) 0.09, R_f (PW) 0.39, R_f (BAWP) 0.47.

Anal. Calcd for $C_{32}H_{37}N_9O_7 \cdot H_2O$: C, 56.71; H, 5.79; N, 18.60. Found: C, 56.42; H, 5.51; N, 18.51.

Amino acid analysis gave Gly:His:Eac (2.84:1.00:1.00).

Compound **2** has very limited solubility in water and is sparingly soluble in most organic solvents (methanol, pyridine, dimethyl sulfoxide, dimethylformamide) which precluded a molecular weight determination *via* osmometry. Compound **2** is soluble in acidic solvents (trifluoroacetic acid, formic acid, and 50% aqueous acetic acid.)

Compounds **2** and **3** were submitted for mass spectrometry. The samples were introduced using a direct probe. High-inlet temperatures (>290°) were required to volatilize the materials. No parent peaks were observed as the peptides decomposed under these conditions. The largest observable fragments were approximately 500 mass units.[27]

X-Ray Determinations of 2.[28]—Compound **2** (2.2 mg) was dissolved in 50% acetic acid (0.22 ml) and added to a flask containing approximately 30 ml of 0.01 *M* phosphate buffer (pH 7.19). The flask was sealed and upon several weeks' standing small clusters of very fine needles (barely visible without magnification) appeared. X-ray diffraction patterns were obtained with difficulty and the cell dimensions which were obtained from rotation and Weissenberg photographs are as follows: a = 9.32 ± 0.03 Å; b = 9.95 ± 0.03 Å; c = 36.57 ± 0.02 Å. The crystal system is orthorhombic, but the space group was not determined owing to difficulties in obtaining good films. As the crystals were small and badly formed, it was impossible to measure the density with any reasonable accuracy. Assuming four molecules per unit cell and a minimum density of 1.30 g/cc, the molecular weight would be 665 (theory 678). The crystals exhibited no obvious effects of drying out, so it is unlikely that they contain much solvent.

Kinetic Measurements.—The kinetic runs were performed with a Cary 14 recording spectrophotometer using a 10-cm silica cuvette. The reactions were carried out in 0.01 *M* phosphate buffer (pH 7.19) containing less than 0.4% organic solvents. Recrystallized imidazole was used for comparison with compound **2**. The cuvette was filled with 50 ml of buffer, with or without catalyst, and placed in a 26 ± 0.1° circulating bath for at least 15 min. The cuvette was placed in the cell compartment (thermostated at 26°) and balanced against air. The substrate, 2,4-dinitrophenyl acetate, was added in acetonitrile and the cuvette was gently agitated and returned to the cell compartment. The recording of a run began 60 sec after the addition of substrate. The appearance of 2,4-dinitrophenylate anion was measured at 360 mμ. All reactions were followed to greater than 90% completion. At the end of each run the pH was 7.19 ± .02. Infinity absorbances were taken at greater than ten half-lives. First-order rate plots were obtained for all reactions. The first-order rate constants were obtained by the method of Guggenheim.[29] The kinetic results are summarized in Table I.

Registry No.—**2**, 25383-41-9; **3**, 25533-69-1; **4**, 25442-38-0; **5**, 25442-39-1; **6**, 25383-67-9; **7**, 25383-68-0; **8**, 25383-69-1; N-*t*-butyloxycarbonylglycyl-*p*-aminobenzoylglycine, 25383-70-4; N-carbobenzoxyglycyl-*p*-aminobenzyl alcohol, 25383-71-5.

(27) The authors are indebted to Mr. Karl Kohler (Department of Chemistry, Indiana University, Bloomington, Ind.) and to Dr. William Hargrove (Eli Lilly and Co., Indianapolis, Ind.) for several attempts to obtain the molecular weights of compounds **2** and **3** by mass spectrometry.

(28) This work was performed by Dr. Jean Hamilton of this department.

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Selective Phosphorylation of the *cis*-2′,3′-Diol of Unprotected Ribonucleosides with Trimetaphosphate in Aqueous Solution

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Unprotected ribonucleosides are selectively phosphorylated at the *cis*-2′,3′-diol in high yield by trimetaphosphate at high pH. The reaction is used to prepare several ribonucleoside 2′(3′)-phosphates including α-cytidine 2′(3′)-phosphate.

Most methods of phosphorylating unprotected ribonucleosides with activated phosphates or with orthophosphate and a condensing agent yield mixtures of 2′-, 3′-, and 5′-monophosphates as well as di- and triphosphates.[1-4] The 5′-phosphate is usually the major monophosphate formed, atlhough the ratio of the products does depend on the nature of the reactants and solvent.[4] Holý and Smrt[5] have reported the synthesis of ribonucleoside 2′(3′)-phosphites from the unprotected ribonucleoside and triethyl phosphite and their oxidative cyclization to 2′,3′-cyclic phosphates, but there is no convenient method of directly phosphorylating the *cis*-2′,3′-diol of an unprotected ribonucleoside in good yield.

Feldman[6] has reported that sodium trimetaphosphate reacts with ethylene glycol at high pH to give β-hydroxyethyl phosphate. Sucrose yields sucrose phosphate under similar conditions, although the position of phosphorylation was not determined. Here we wish to report the synthesis of ribonucleoside and ribonucleotide 2′(3′)-phosphates by a modification of this reaction.

In a preliminary experiment adenosine (Ia) was treated with 10 mol equiv of sodium trimetaphosphate and 10 mol equiv of 1 *N* aqueous sodium hydroxide; there was a 63% conversion to adenosine 2′(3′)-phosphate (IIa) on standing overnight at room temperature. There was no further reaction after an additional day. When tri(tetramethylammonium) trimetaphosphate

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