detected by their UV absorbance at 254 nm using an ISCO UA-5 monitor. A Varian XL-100 and a Varian CFT-20 spectrometer provided the proton magnetic resonance spectra (^{1}H NMR); chemical shifts are reported in parts per million downfield from tetramethylsilane as internal standard. UV spectra were recorded with a Varian Super Scan 3 spectrometer. XAD-2 resin (Rohm and Haas) was washed with acetone, CH_3OH, and H_2O before use. HMPA was stored over 4A molecular sieves; $SOCl_2$ was used as received from Aldrich; 2′-deoxyadenosine (Calbiochem) was dried at 70 °C in a vacuum oven overnight to remove 1 mol of H_2O, when anhydrous material was required.

9-(3,5-Dichloro-2,3,5-trideoxy-β-D-*threo*-pentofuranosyl)adenine (1). Addition of 2′-deoxyadenosine monohydrate (3.82 g, 14.2 mmol) to a stirred solution of $SOCl_2$ (6.2 mL, 86 mmol) in 40 mL of HMPA produced a red solution and generated a moderate amount of heat, which was readily controlled with an ice bath (on other occasions the addition was made to an already-chilled solution; no effect was observed on yield or product). The reaction mixture was stirred at room temperature overnight and poured onto 200 mL of ice. The resulting turbid suspension was made strongly basic with NH_4OH; this produced a clear yellow solution which was decanted from a small amount of black residue. Upon standing for several hours at 0 °C, the solution deposited a tan solid which was collected and dried, giving 2.2 g (57%) of 1. The filtrate was shown by LC to be a roughly equimolar mixture of adenine and 1. The crude product was dissolved in 1 N HCl, treated with charcoal, and precipitated by addition of 1 N NaOH to a pH of 7.5. This gave 2.17 g of white powder, pure by TLC and LC. As previously reported,[2] melting point varied with rate of heating: at 2 °C/min, mp 161–162 °C dec; UV λ_{max} (pH 1) 257 nm (ε 16 000); UV λ_{max} (pH 13) 259 nm (ε 18 500); ^{1}H NMR (80 MHz) (Me_2SO-d_6) 3.2 (m, 2, $C_{2'}$H), 3.92 (d, 2, $C_{5'}$H), 4.45 (m, 1, $C_{4'}$H), 4.90 (m, 1, $C_{3'}$H), 6.35 (dd, 1, $C_{1'}$H), 7.29 (s, 2, NH_2), 8.13 (s, 1, C_2H or C_8H), 8.24 (s, 1, C_2H or C_8H). Anal. Calcd for $C_{10}H_{11}N_5Cl_2O$: C, 41.68; H, 3.84; N, 24.30; Cl, 24.61. Found: C, 41.46; H, 3.62; N, 24.27; Cl, 24.49.

Bis(3′-*O*-5′-chloro-2′,5′-dideoxyadenosine) Sulfoxide (2). Addition of anhydrous 2′-deoxyadenosine (3.0 g, 11.9 mmol) to a stirred solution of $SOCl_2$ (1.5 mL, 21 mmol) in 30 mL of HMPA produced a clear red solution. This was stirred overnight at room temperature and diluted with 150 mL of ice water, and the resulting solution was extracted four times with 200-mL portions of $CHCl_3$. Adjustment of the pH of the aqueous phase to 7 with NH_4OH produced an immediate precipitate, which was cooled to 0 °C and collected to give 2.18 g (62%) of **2**. The product was further purified by re-precipitation from 1 N HCl by addition of 1 N NaOH. The resulting white solid gave a single peak on LC with a retention time more than twice that of **1**: mp 172–176 °C dec; UVλ_{max} (pH 1) 257 nm (ε 27 000), UV λ_{max} (pH 13) 260 nm (ε 26 000); ^{1}H NMR (80 MHz) (Me_2SO-d_6) δ 2.75–3.25 (m, 2, $C_{2'}$H), 3.90 (m, 2, $C_{5'}$H), 4.30 (m, 1, $C_{4'}$H), 5.40 (m, 1, $C_{3'}$H), 6.35 (t, 1, $C_{1'}$H), 7.25 (s, 2, NH_2), 8.05 (s, 1, C_2H or C_8H), 8.25 (s, 1, C_2H or C_8H). Anal. Calcd for $C_{20}H_{22}N_{10}Cl_2O_5S$·1.5$H_2O$: C, 39.22; H, 4.11; N, 22.86; Cl, 11.58; S, 5.24. Found: C, 38.90; H, 3.91; N, 22.89; Cl, 12.23; S, 5.35 (Karl Fisher water determination confirms sesquihydrate).

5′-Chloro-2′,5′-dideoxyadenosine (3). Method 1. A suspension of unpurified **2**, prepared as above from 12.0 g (47.6 mmol) of anhydrous 2′-deoxyadenosine, was stirred overnight in 200 mL of CH_3OH and 45 mL of concentrated NH_4OH at room temperature. The resulting clear solution was concentrated in vacuo and the residue was recrystallized from 50 mL of H_2O to give 9.0 g (68% overall) of **3** as white crystals. Product was a single spot on TLC, lower than 1: mp 120 °C softens, 162–168 °C dec; $[M]_D^{20}$ −60.5° (*c* 1.02, DMF); UV λ_{max} (pH 1) 257 nm (ε 14 600); UV λ_{max} (pH 13) 260 nm (ε 15 200); ^{1}H NMR (100 MHz) (Me_2SO-d_6) δ 2.4 (m, 1, $C_{2'\alpha}$H), 2.95 (m, 1, $C_{2'\beta}$H), 3.9 (m, 2, $C_{5'}$H), 4.05 (m, 1, $C_{4'}$H), 4.52 (m, 1, $C_{3'}$H), 5.54 (d, 1, $C_{3'}$OH), 6.45 (t, 1, $C_{1'}$H), 7.28 (s, 2, NH_2), 8.16 (s, 1, C_2H or C_8H), 8.33 (s, 1, C_2H or C_8H). Anal. Calcd for $C_{10}H_{12}N_5ClO_2$·0.5H_2O; C, 43.09; H, 4.70; N, 25.13; Cl, 12.72. Found: C, 43.20; H, 4.48; N, 25.19; Cl, 12.92.

Method 2. Addition of 1.0 mL (10 mmol) of CCl_4 to a solution of anhydrous 2′-deoxyadenosine (0.54 g, 2.2 mmol) and triphenylphosphine (1.05 g, 4.0 mmol) in 5 mL of HMPA produced a white precipitate after 45 min of stirring. Reaction was continued overnight and terminated by addition of 5 mL of CH_3OH. The resulting clear solution was poured into 200 mL of ether at 0 °C. A white precipitate formed, which was collected, washed well with ether, and dissolved in a small volume of H_2O and the pH adjusted to 7 with 1 N NaOH, producing a small amount of gummy precipitate (several spots by TLC, including 1). The supernatant was chromatographed on a 2.5 × 20 cm column of XAD-2 resin. Elution with H_2O removed salts, 10% ethanol eluted a small amount of 2′-deoxyadenosine, and 30% ethanol (300 mL) eluted **3**, which was isolated by concentrating the eluent to dryness, followed by precipitation from ethanol with ether: yield 0.20 g (32%), identical with the previous preparation by LC (0.1% 2′-deoxyadenosine present) and ^{1}H NMR; mp 137 °C softens, 169–171 °C dec; $[M]_D^{20}$ 64.2° (*c* 1.02, DMF); UV λ_{max} (pH 1) 257 nm; UV λ_{max} (pH 11) 260 nm. Anal. Calcd for $C_{10}H_{12}N_5ClO_2$·0.5EtOH: C, 45.13; H, 5.17; N, 23.92; Cl, 12.11. Found: C, 45.32; H, 4.93; N, 23.97; Cl, 11.61 [ethanolate not removed by drying at 50 °C (0.2 mm)].

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Registry No. 1, 63162-55-0; 2, 71001-16-6; 3, 57274-14-3; 2-deoxyadenosine, 958-09-8.

Practical Synthesis of Cyclic Peptides, with an Example of Dependence of Cyclization Yield upon Linear Sequence

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A convenient general procedure for the synthesis of cyclic peptides is reported. The linear intermediates, obtained via solid-phase synthesis, are cyclized by the coupling agent diphenylphosphoryl azide (DPPA). A simple procedure allows rapid isolation of the cyclic products. Peptides of varying ring size have been prepared in amounts of up to 50 g. A significant variation of cyclization yield with sequence has been observed in the conversion of three different linear peptides to the same cyclic product. Several related linear sequences have also been cyclized, and the results are discussed.

Naturally occurring and synthetic cyclic peptides have been subject to intensive study in recent years.[1] Their unique role in the binding and transport of cations and the diversity of their biological effects make them of in-

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Table I. Cyclic Peptides Prepared

structure (*cyclo*-)[a]	method[b]	yield,[c] %	mol wt[d]	$[\alpha]^{22}_D$,[e] deg	mp, °C
(D-Phe-Pro)$_3$ (1)	A	66	732	−75 (1.1, C)	233-234
(Azt-D-Phe)$_3$ (2)	B	31	690	−69 (0.2, M)	240-242
(D-Phe-Pro)(Phe-Pro)$_2$(3)	A	50	732	+2.1 (0.9, M)	*i*
(D-Pro-Phe)$_3$	A	46	732	+73 (1.0, C)	233-234
(D-Ala-Pro)$_3$	A	44	504	−33 (0.7, 2M:1W)	212-213.5
(D-Ala-Pro)$_2$(D-Phe-Pro)	A	40	580	−56 (1.0, M)	*i*
(Sar-Ala)$_3$[f]	A	69	426	−97 (0.9, M)	*i*
(Sar-Phe)$_3$	A	51	654	−48 (1.1, M)	*i*
(Sar-Leu)$_3$	A	48	552	−68 (1.0, M)	*i*
(*N*-Me-Ala-D-Phe)$_3$	B	32	696	−165 (1.0, M)	282.5-284
(D-Ala-Ala)$_3$	A	50[g]	426	−0.3 (0.9, TFA)	>340 dec[g]
(D-Phe-Pro)$_2$	A	18[h]	488	+48 (0.9, DMF)	257-258
(D-Ala-Pro)$_2$(β-Ala-Pro)	A	54	504	−7.3 (1.0, M)	334-335
(D-Phe-D-Ala-Pro)$_2$	A	43	630	+67 (1.0, M)	*i*

[a] Formulas as written conform to the sequence of the linear precursor. [b] See Scheme I. [c] Overall (isolated) peptide yield based on resin-bound precursor. [d] Molecular ion peak in the mass spectrum. [e] Solvent: M = MeOH; W = H_2O; C = $CHCl_3$. [f] Purified via the crystalline NaSCN complex from $CHCl_3$: mp 327-328 °C dec. Anal. Calcd for 3:2 NaSCN/peptide ratio: S, 8.78. Found: S, 8.3. [g] Lit. [Ivanov, V. J.; Shilin, V. V.; Ovchinnikov, Y. A. *J. Gen. Chem. USSR (Engl. Transl.)* **1970**, *40*, 902] yield 30%; mp 330 °C dec. [h] *cyclo*-(D-Phe-Pro)$_4$ also isolated in 6% yield. [i] Amorphous.

terest to biologists and chemists. Consequently, the development of reliable and convenient methods for the synthesis of cyclic peptides has been a continuing concern of peptide chemists.[2]

We now report a convenient general procedure for the synthesis of cyclic peptides. This procedure has several attractive features: (1) reasonable overall yields; (2) ready adaptability to large-scale work; (3) rapidity of individual operations; (4) ease of isolation; and (5) minimal racemization at the carboxyl-terminal residue.

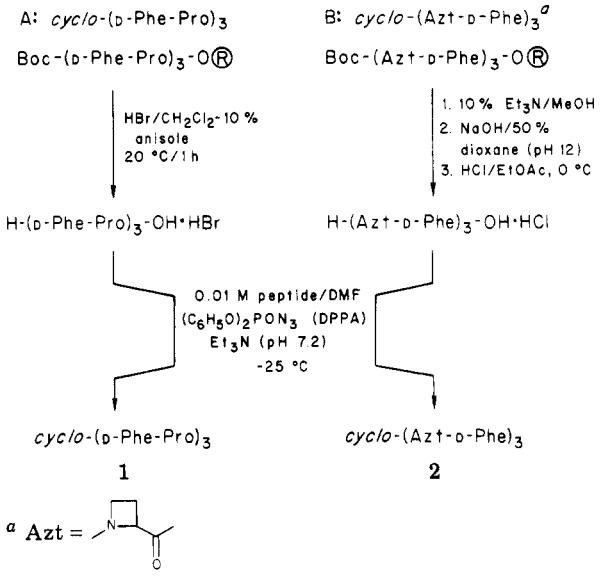
The process starts with synthesis of the linear peptide, using the Merrifield solid-phase technique. Release of the peptide from the resin is accomplished with HBr in methylene chloride. The linear intermediate is cyclized, as obtained from the resin, without purification. Ring formation is effected at low temperature by the coupling agent diphenylphosphoryl azide (DPPA).[3] Triethylamine is added prior to the DPPA to free the amino group and is periodically added throughout the reaction to maintain approximate neutrality. Upon completion of the reaction, as monitored by TLC (3–4 days), all charged species remaining in the reaction mixture are removed by adsorption onto mixed strong acid–base ion-exchange resin.

Although the use of DPPA as a coupling reagent does not constitute a new chemical procedure, our overall process is dependable and highly practical for preparing a wide variety of cyclic peptides on a moderate-to-large scale. A number of other approaches to cyclization[2,4] have been examined in these laboratories. In our experience, the method we describe has been most consistently successful, particularly when large quantities of product have been desired.

Our methodology is illustrated by preparation of the hexapeptide *cyclo*-(D-phenylalanyl-L-prolyl)$_3$ (1) as outlined in Scheme IA. As much as 50 g of this peptide in a single run has been prepared with this procedure.

For the preparation of peptides sensitive to the action of HBr, cleavage of the resin-bound precursor can be

Scheme I



accomplished by an alternative route (Scheme IB). Illustrative of this approach is the preparation of the HBr-labile hexapeptide *cyclo*-(L-azetidinyl-2-carbonyl-D-phenylalanyl)$_3$ (2).

Some examples of peptides prepared by these procedures are summarized in Table I. A number of the peptides were crystallized simply by removal of the DMF in vacuo and addition of a suitable solvent. A few cyclic products were purified via the crystalline sodium thiocyanate complex.[5] The samples were judged to be homogeneous on the basis of TLC data, sharp melting points, confirmatory ^{1}H NMR spectra, and amino acid analyses. For each product, a strong molecular ion peak in the mass spectrum and lack of free amine, as demonstrated by TLC, were accepted as evidence for assignment of structure as cyclic monomer. All noncrystalline samples were subjected to the additional criterion of Sephadex G-25 gel filtration in 50% acetic acid. Elution volumes were compared with reference to the linear precursor to hexapeptide 1, H-(D-Phe-Pro)$_3$-OH, and some of our crystalline cyclic products. Gel filtration thus readily enabled us to distinguish between monomeric and oligomeric peptides and

(1) For reviews of the chemistry and biological properties of cyclic peptides, see: (a) Wieland, T.; Birr, C. *MTP Int. Rev. Sci.: Org. Chem., Ser. Two* **1976**, *6*, 183-218. (b) Ovchinnikov, Yu. A.; Ivanov, V. T. *Tetrahedron* **1975**, *31*, 2177.

(2) For a comprehensive review of synthetic methods for cyclic peptides, see: Kopple, K. D. *J. Pharm. Sci.* **1972**, *61*, 1345.

(3) Shioiri, T.; Ninomiya, K.; Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 6203. See also: Kopple, K. D.; Schamper, T. J.; Go, A. *J. Am. Chem. Soc.* **1974**, *96*, 2597.

(4) (a) Bycroft, B. W.; Wels, C. M. *Spec. Period. Rep.: Amino-Acids, Peptides, Proteins*, **1977**, *8*, 320–1. Bycroft, B. W. *Ibid.* **1976**, *7*, 332–5.

(5) Madison, V.; Deber, C. M.; Blout, E. R. *J. Am. Chem. Soc.* **1977**, *99*, 4788. See also: Deber, C. M.; Blout, E. R. *Isr. J. Chem.* **1974**, *12*, 15.

Table II. Variation of Cyclization Yield with Sequence

sequence	yield of cyclic,[a] %	peptide balance,[b] %	method of purif[c]	$[\alpha]^{22}_D$,[d] deg
D-Phe-Pro-Phe-Pro Phe-Pro (3a)	57 (3)	78	P	−64 (0.9, M)
Phe-Pro-D-Phe-Pro-Phe-Pro (3b)	2 (3)	73	P	
Phe-Pro-Phe-Pro-D-Phe-Pro (3c)	1 (3)	61	P	
Phe-Pro-Phe-Pro-Phe-Pro (4)	2	57	P/G	−108 (0.6, M)
D-Ala-Pro-Phe-Pro-Phe-Pro (5)	76	78	P	−15 (0.9, M)
Ala-Pro-Phe-Pro-D-Phe-Pro (6)	3	82	P/G	−86 (0.8, M)

[a] Isolated yield of monomer based on resin-bound precursor (method A). [b] Total percentage recovery of peptidal components. [c] P = precipitation, P/G = precipitation + gel filtration. [d] See Table I, footnote *e*.

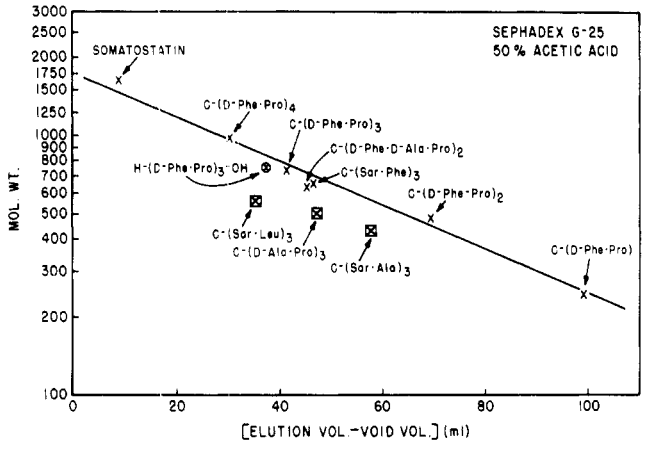
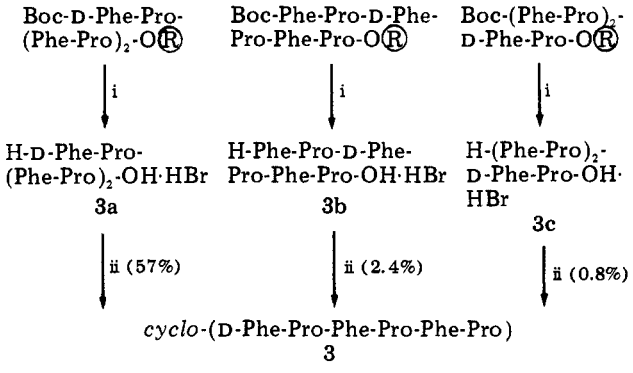


Figure 1. Gel filtration of cyclic peptides: logarithm of molecular weight as a function of the elution volume (Sephadex G-25 Super-Fine/50% acetic acid; 113.5 cm × 2.0 cm i.d.).

Scheme II. Approach to Cyclic Peptide 3 via Three Precursors[a]



[a] i, HBr/CH_2Cl_2-10% anisole, 20 °C, 1 h; ii, 0.01 M peptide/DMF, DPPA, Et_3N (pH 7.5), −25 °C/3 days, 0 °C/4 days.

ensured, in addition, that the entire sample was being evaluated and not just a minor component volatile in the mass spectrometer.

As shown in Figure 1, there exists a linear relationship between the logarithm of the molecular weight and the elution volume on gel filtration when 50% acetic acid is used as eluant.

We have also determined that there is an important relationship between the degree of adsorption and the elution volume, roughly in proportion to the number of phenylalanyl residues in the molecule. In particular, the three peptides lacking aromatic residues are eluted earlier than expected on the basis of the elution behavior of the peptides containing phenylalanine and, in fact, define a second line on the graph. We have encountered even more striking adsorption effects on Sephadex gel at lower concentrations of acetic acid.

The extent of racemization with DPPA was assessed by examining the cyclization of H-(sarcosyl-L-alanyl)$_3$-OH. Upon TLC of the crude reaction mixture under conditions where 1% of the diastereoisomeric cyclic product *cyclo*-(sarcosyl-L-alanyl)$_2$(sarcosyl-D-alanyl) would have been evident, we failed to detect any of this byproduct.[6] Moreover, we considered the possibility that the linear diastereoisomer resulting from racemization, H-(sarcosyl-L-alanyl)$_2$(sarcosyl-D-alanyl)-OH, could yield products other than the above cyclic monomer. However, analysis of the total reaction mixture by the method of Manning and Moore[7] showed 0.9% of D-alanine, which represents the maximum amount of racemization that could have taken place during the reaction process.

Yamada and co-workers[8] have also reported low levels of racemization during peptide couplings with DPPA. In addition, a recent study[9] has shown that DPPA leads to about the same low amount of racemization as that observed in azide couplings.

During the course of this work we also encountered an example of considerable variation in cyclization yield with respect to sequence of linear precursor. Upon cyclization of three diastereoisomeric precursors of the cyclic peptide **3** differing from one another in the position of the D-phenylalanyl residue (see Scheme II), only from the D-Phe[1] hexapeptide (**3a**) did we isolate a significant amount of cyclic monomer; from sequences **3b** and **3c**, we merely obtained unidentified peptidal byproducts. These results are analogous to those of recently reported work on dependence of the yield of a decapeptide and a tetrapeptide upon the sequence of different linear precursors.[10]

Examination of CPK models of the three linear sequences **3a–c** has indicated no obvious hindrance to cyclization in any of these hexapeptides; nor have we seen any distinctions between the three peptides that might explain our results.

(6) Application of our cyclization technique to the peptide H-(sarcosyl-D-alanyl)(sarcosyl-L-alanyl)$_2$-OH, obtained from resin-bound precursor, provided a sample of *cyclo*-(sarcosyl-L-alanyl)$_2$(sarcosyl-D-alanyl) for comparison with *cyclo*-(sarcosyl-L-alanyl)$_3$. Upon TLC analysis of the cyclization reaction of H-(sarcosyl-L-alanyl)$_3$-OH we saw none of the racemized cyclic byproduct. After dilution by 1:100 we could still detect *cyclo*-(sarcosyl-L-alanyl)$_3$, thus confirming that we would have detected as little as 1% racemization.

(7) Manning, J. M.; Moore, S. *J. Biol. Chem.* **1968**, *243*, 5591.
(8) Shioiri, T.; Yamada, S. *Chem. Pharm. Bull.* **1974**, *22*, 849.
(9) Kitada, C.; Fujino, M. *Chem. Pharm. Bull.* **1978**, *26*, 585.
(10) (a) Rothe and Kreiss (Rothe, M.; Kreiss, W. "Peptides 1976, Proceedings of the 14th European Peptide Symposium, Wepion, Belgium", Elsevier: Amsterdam, 1976; pp 71–8) note a dependence of cyclization yield of antamanide with respect to two discrete linear decapeptide precursors; they attribute this result to marked differences in conformation of the linear sequences, inferred from differing elution behavior on gel filtration (both are identical in molecular weight). (b) Wieland (Wieland, T., paper presented at the 15th European Peptide Symposium, Gdansk, Poland, 1978) reported isolation of only traces of cyclic monomer from linear precursor H-Ala-Pro-Phe-Pro-OH but a 54% yield of the cyclic tetrapeptide from precursor H-Pro-Phe-Pro-Ala-OH.

Thus, in an attempt to define elements critical for successful cyclization, we altered certain structural features in linear peptides **3a–c**. By examining the cyclization of the all-L diastereoisomer H-(Phe-Pro)$_3$-OH (**4**) (see Table II), we could assess the role of the D-phenylalanyl residue. The isolation of *cyclo*-(Phe-Pro)$_3$ in only 2% yield shows that the presence of the D-phenylalanyl residue in the N-terminal position is crucial for a high yield.

We further considered that the aromatic properties of the D-Phe^1 residue of peptide **3a** might contribute to exceptionally facile cyclization. Thus, D-Phe^1 was replaced with D-Ala^1 (see structure **5**). However, the comparable yield of *cyclo*-(D-Ala-Pro-Phe-Pro-Phe-Pro) from sequence **5** relative to that of *cyclo*-(D-Phe-Pro-Phe-Pro-Phe-Pro) (**3**) from sequence **3a** indicated the negligible influence of aromatic nuclei and constitutes further evidence for the importance of configuration at the N terminus. This result, together with the results of cyclization of sequences **3c** and **6** (see below), suggests that the presence of a substituent such as phenyl at the β position of an alanyl residue may even have an inhibiting effect on cyclization.

We had, in fact, considered that steric bulk might be the overriding factor in certain cases; so the N-terminal phenylalanyl residue (Phe^1) in the linear peptide **3c** was replaced with the alanyl (Ala^1) residue (see structure **6**). The low yields of cyclic monomer from both precursors **6** and **3c** demonstrated that the orientation of the N-terminal side chain, along with the consequent orientation of the amino group, and not the size of this side chain, is a significant factor governing the success or failure of a cyclization.

We have also shown that the DPPA-mediated coupling of Boc-L-proline with a mixture of D- and L-phenylalanine methyl esters is only marginally selective in favor of the D enantiomer. This finding indicates that cyclization of the open-chain precursors of cyclic peptide **3** is controlled by factors within the peptide chain and not properties of the amino acid residues undergoing coupling.

On the basis of these observations, we propose that the best linear precursor of a cyclic hexapeptide containing a D amino acid residue is the sequence with the D residue at the amino terminus.

Experimental Section[11]

Capillary melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Thin-layer chromatograms were developed on silica gel (Quantum Industries, Q-1 plates), and components were visualized either by *tert*-butyl hypochlorite–KI reagents[12] or by ninhydrin reagent. Systems used in TLC were as follows: EtOAc–HOAc–H_2O (upper layer), 14:2:10 (A); EtOAc–pyridine–HOAc–H_2O, 10:5:1:3 (B); $CHCl_3$–CH_3OH–H_2O, 85:15:1.5 (C); EtOAc–pyridine–HOAc–H_2O, 5:5:1:3 (D); EtOAc–HOAc–isooctane–H_2O (upper layer), 10:2:4:10 (E); EtOAc–HOAc–isooctane–H_2O (upper layer), 12:2:2:10 (F); EtOAc–pyridine–HOAc–H_2O, 30:5:1:1 (G); EtOAc–*n*-BuOH–HOAc–H_2O, 1:1:1:1 (H); $CHCl_3$–CH_3OH–H_2O, 50:40:10 (I).

Unless otherwise noted, amino acids were all of the L configuration. The Boc amino acids used were obtained from commercial sources, except for Boc-*N*-Me-alanine, which was synthesized from Boc-alanine via an established procedure,[13] and Boc-azetidine-2-carboxylic acid, which was made by reaction of *tert*-butyl 2,4,5-trichlorophenyl carbonate with commercially available azetidine-2-carboxylic acid (Aldrich).

Synthesis of Cyclic Peptides. Method A (Scheme I): *cyclo*-(D-Phe-Pro)$_3$ (1). A sample of 61.8 g of Boc-(D-Phe-Pro)$_3$-O®,[14] equivalent to 32.9 mmol of hexapeptide on the basis of amino acid analysis, was suspended in a mixture of 400 mL of CH_2Cl_2 and 40 mL of anisole. Anhydrous HBr gas was bubbled through the stirred mixture at 0 °C until the saturation point (about 5–10 min) and then at 15–20 °C for 60 min. Concentration of the mixture at reduced pressure afforded a thick slurry, to which 1000 mL of ether was added, and the solids were collected by filtration, washed with ether, and dried in vacuo.

The peptide/resin mixture was thoroughly extracted with a total of 4 L of dry, degassed DMF in several portions, and the pH was adjusted to 7.2 (estimated by spotting moistened narrow-range pHydrion indicator paper) with triethylamine. The solution was cooled to −25 °C, and 8.6 mL (equivalent to 40 mmol) of DPPA (Aldrich) was added. The solution was allowed to stand at −20 °C for 2 days and at 2 °C for 2 days, while pH 7.0–7.5 was maintained by periodic addition of triethylamine.

About 500 mL of H_2O and 450 mL of Bio-Rad AG 501-X8 (D) (mixed-bed) resin were added, followed by stirring for 6 h.[15] The resin was separated by filtration, and after removal of solvent in vacuo the cyclic peptide crystallized upon addition of 200 mL of *n*-butyl alcohol. The solid was collected by filtration, washed with two portions of ether, and dried in vacuo to give 10.6 g (42%) of crystalline peptide, mp 233–234.5 °C. Concentration of the filtrate and crystallization from warm *n*-butyl alcohol yielded another 5.9 g (24%) of product, mp 233–235 °C.

Cyclic product could also be isolated by crystallization from $CHCl_3$/ether: mp 233–234 °C; TLC R_f 0.37 (A), 0.59 (B), 0.68 (C); amino acid analysis, $Phe_{3.04}Pro_{2.96}$; mass spectrum (70 eV) m/e 732; ^{1}H NMR $\delta^{Me_4Si}_{CDCl_3}$ (90 MHz) 8.82 (d, J = 8 Hz, NH), 7.30 (s, C_6H_5), 4.85 (m, Phe $^\alpha$CH), 4.47 (d, J = 7 Hz, Pro $^\alpha$CH).

Anal. Calcd for $C_{42}H_{48}N_6O_6$: C, 68.83; H, 6.60; N, 11.47. Found: C, 68.8; H, 6.8; N, 11.0.

Method A was applied to the compounds so designated in Table I, with minimal modification except for crystallization conditions.

Method B (Scheme I): *cyclo*-(Azt-D-Phe)$_3$ (2). A sample of 1.54 g of Boc-(Azt-D-Phe)$_3$-O R ,[14] equivalent to 1.0 mmol of peptide on the basis of starting Boc-D-Phe-O R , , was suspended in 25 mL of methanol with 2.8 mL of triethylamine for 24 h. The resin was separated by filtration and the filtrate was evaporated at reduced pressure to give an oily residue, which was freed of excess triethylamine by twice flushing/evaporation, using methanol; TLC R_f 0.37 (E).

The crude methyl ester in 30 mL of dioxane–H_2O was treated with 2.5 N NaOH to maintain pH ≥12 for 5.5 h. The pH was adjusted to 6 by addition of 0.3 M H_2SO_4, the reaction mixture was evaporated in vacuo, and the product was partitioned with EtOAc–0.3 M H_2SO_4. The organic portion was washed with 50% saturated brine, and evaporation of solvent, followed by addition of ether/low-boiling petroleum ether, gave 669 mg (83%) of white solid: TLC R_f 0.22 (F), 0.38 (G).

A solution of this peptide (660 mg) in 80 mL of EtOAc at 0 °C was treated with gaseous HCl until saturation was attained (ca. 5 min), then stirred an additional 10 min, maintaining a slow flow of HCl, and finally purged with N_2 for about 1 h; addition of 100 mL of ether and then 100 mL of low-boiling petroleum ether afforded white solid, which was isolated by filtration. This sample was then dissolved in 120 mL of DMF, the pH was adjusted to 7.5 with triethylamine, and at −25 °C 0.18 mL of DPPA was added. The pH was kept at 7.5 for 2 days at −25 °C and for 4 days at 0 °C. The workup procedure was exactly as described in method A. The cyclic product **2** was obtained by precipitation from ether/low boiling petroleum ether and isolated by filtration: yield 276 mg (31% based on resin-bound peptide);[16] TLC R_f 0.56 (G), 0.52 (F); amino acid analysis, $Phe_{3.00}$ (Azt unstable to acidic hydrolysis conditions); mass spectrum (70 eV) m/e 690; ^{1}H NMR $\delta^{Me_4Si}_{CDCl_3}$ 9.22 (d, J = 7 Hz, NH), 7.32 (s, C_6H_5).

An analytical sample was obtained after gel filtration in 50% acetic acid (Sephadex G-25) by crystallization from $CHCl_3$/ether: mp 240–242 °C dec.

(11) Abbreviations: DPPA, diphenylphosphoryl azide; DMF, dimethylformamide; Azt, azetidine-2-carbonyl.
(12) Schwartz, D. P.; Pallansch, M. J. *Anal. Chem.* **1958**, *30*, 219.
(13) Cheung, S. T.; Benoiton, N. L. *Can. J. Chem.* **1977**, *55*, 906.
(14) Prepared from 2% cross-linked chloromethylated polystyrene resin (Lab Systems) according to standard Merrifield technique. Cf.: Stewart, J. M.; Young, J. D. "Solid Phase Peptide Synthesis", W. H. Freeman: San Francisco, Calif., 1968.
(15) This treatment sequesters the ionic byproducts of the reaction.
(16) Yield corrected for ca. 15% of an impurity by TLC (F).

Anal. Calcd for $C_{39}H_{42}N_6O_6$: C, 67.81; H, 6.13; N, 11.89. Found: C, 67.6; H, 6.4; N, 11.9.

Method B was applied to *cyclo*-(*N*-Me-Ala-D-Phe)$_3$ shown in Table I, with minor modification.

Sequence Dependence of Cyclization Yield. The linear peptides shown in Table II were prepared by solid-phase synthesis and subjected to cyclization, using DPPA (method A). In each case disappearance of starting linear peptide was monitored by TLC, and cyclic monomer was isolated by successive precipitation by using solvents of decreasing polarity (EtOAc → ether → low-boiling petroleum ether) to recover peptidal components. The presence or absence of monomeric peptide in each isolate was determined by TLC. Isolates containing cyclic monomer were subjected to gel filtration on Sephadex G-25 in 50% acetic acid to assess molecular weight homogeneity and to separate and purify monomeric product. The peptide balance reflects the amount of peptidal material recovered, including cyclic monomer (see Table II).

Application of the DPPA Procedure to Coupling of Boc-proline with 2 Equiv of DL-Phenylalanine Methyl Ester. A mixture of 108 mg (0.5 mmol) of Boc-proline and 228 mg (1.0 mmol) of DL-phenylalanine methyl ester HCl salt in 50 mL of DMF was cooled to 0 °C. Triethylamine was added until the pH was 7.5 (as determined by spotting moistened pHydrion paper) followed by 0.11 mL of DPPA. The mixture was stirred for a total of 41 h at 0 °C, the pH being adjusted to 7.5 after 24 h. The reaction mixture was treated with 10 mL of H_2O and then with ca. 20 mL of AG 501-X8 (D) mixed-bed resin, with stirring for 4 h. The resin was removed by filtration, and TLC of the filtrate indicated that >98% of the excess DL-phenylalanine methyl ester had been removed by the resin treatment. The reaction solution was made up to 100 mL with DMF, and aliquots were analyzed, after acid hydrolysis, by the procedure of Manning and Moore.[7] The data showed 50% conversion to the diastereoisomeric dipeptides Boc-Pro-D-Phe-OMe and Boc-Pro-Phe-OMe, with a D-Phe/Phe ratio of 56:44.

A second reaction using 108 mg (0.5 mmol) of Boc-proline and 228 mg (1.0 mmol) of D-phenylalanine methyl ester HCl salt was carried out under exactly the same conditions to be sure that neither the reaction conditions nor the workup procedure was causing racemization. Analysis of aliquots by the procedure of Manning and Moore,[7] after acid hydrolysis, showed 61% conversion to the dipeptide Boc-Pro-D-Phe-OMe, of enantiomeric purity ≥97%.

The results show that coupling of DL-phenylalanine methyl ester is only slightly selective in favor of the D isomer.

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Registry No. 1, 68319-17-5; 2, 69541-41-9; 3, 71048-71-0; **3a**, 71001-82-6; **3b**, 71001-83-7; **3c**, 71001-84-8; 4, 71001-85-9; 5, 71001-86-0; 6, 71001-87-1; (D-Pro-Phe)$_3$, 71048-72-1; (D-Ala-Pro)$_3$, 71001-88-2; (D-Ala-Pro)$_2$(D-Phe-Pro), 71001-89-3; (Sar-Ala)$_3$, 71001-90-6; (Sar-Phe)$_3$, 71001-91-7; (Sar-Leu)$_3$, 71001-92-8; (*N*-Me-Ala-D-Phe)$_3$, 71001-93-9; (D-Ala-Ala)$_3$, 27454-49-5; (D-Phe-Pro)$_2$, 71001-94-0; (D-Ala-Pro)$_2$(β-Ala-Pro), 71001-95-1; (D-Phe-D-Ala-Pro)$_2$, 71048-73-2; H-(D-Phe-Pro)$_3$OH·HBr, 68370-22-9; H-(Azt-D-Phe)$_3$OH·HCl, 69541-40-8; H-(D-Phe-Pro)(Phe-Pro)$_2$OH·HBr, 71001-96-2; H-(D-Pro-Phe)$_3$OH·HBr, 71001-97-3; H-(D-Ala-Pro)$_3$OH·HBr, 71001-98-4; H-(D-Ala-Pro)$_2$(D-Phe-Pro)OH·HBr, 71001-99-5; H-(Sar-Ala)OH·HBr, 71002-00-1; H-(Sar-Phe)$_3$OH·HBr, 71002-01-2; H-(Sar-Leu)$_3$OH·HBr, 71002-02-3; H-(Me-Ala-D-Phe)$_3$OH·HCl, 71002-03-4; H-(D-Ala-Ala)$_3$OH·HBr, 71002-04-5; H-(D-Phe-Pro)$_2$OH·HBr, 71002-05-6; H-(D-Ala-Pro)$_2$(β-Ala-Pro)OH·HBr, 71002-06-7; H-(D-Phe-D-Ala-Pro)$_2$OH·HBr, 71002-07-8; *cyclo*-(D-Phe-Pro)$_4$, 71002-08-9; Boc-azetidine-2-carboxylic acid, 51077-14-6; *tert*-butyl 2,4,5-trichlorophenyl carbonate, 16965-08-5; azetidine-2-carboxylic acid, 2517-04-6.

Norsteroids. 11. Reaction of Various Steroid Bromohydrins with Silver Oxide

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Reaction of 2α-bromo-5α-cholestan-3β-ol with silver oxide gave *A*-nor-5α-cholestane-2-carboxaldehyde (**2a**). Similar treatment of 6α-bromo-5α-cholestane-3β,7β- and -3β,7α-diol, **4** and **6**, gave 3β-hydroxy-*B*-nor-5α-cholestane-6-carboxaldehyde (**5**), but 6β-bromo-5α-cholestane-3β,7β-diol (**7**) gave 3β-hydroxy-5α-cholestan-7-one (**8**) with no rearrangement. 5α-Bromocholestane-3β,6β-diol (**9**) gave mainly 5β,6β-oxidocholestan-3β-ol 3-acetate (**10**) plus a small amount of Westphalen's diol **11**. Finally, 11α,23ζ-dibromo-5α,22a-spirostane-3β,12β-diol 3-acetate (**12**) gave the *C*-noraldehyde **14**. These results show that bromohydrins undergo ring contraction with silver oxide to the noraldehyde if the bromine atom is equatorial but give an oxide or ketone if the bromine atom is axial.

In a previous publication,[1] the reaction of 2α-bromo-5α-cholestan-3β-ol (**1**) with ethanolic silver nitrate was shown to give the diethyl acetal of *A*-nor-5α-cholestane-2-carboxaldehyde (**2a**). This report outlines additional studies which demonstrate that the silver ion induced ring contraction of sterioidal bromohydrins is both stereospecific and highly dependent on the conformation of the bromine atom. Application of the reaction to the synthesis of both B- and C-ring-contracted steroids is also disclosed.

Although the original study of the silver nitrate induced rearrangement of **1** was believed to be stereospecific, firm evidence on this point could not be obtained, due to formation of a 7:3 mixture of the known methyl *A*-nor-5α-cholestane-2α- and 2β-carboxylates from the initial acetal product (Scheme I). In particular, the acid-catalyzed liberation of the intermediate noraldehyde was considered as one cause of the resulting mixture of isomeric esters. Thus a sequence was examined that would avoid isomerization during transformation of the bromohydrin to the *A*-norester. Reaction of 2α-bromo-5α-cholestan-3β-ol (**1**) with silver oxide in hexane resulted in a direct

(1) H. R. Nace and G. A. Crosby, *J. Org. Chem.*, **33**, 834 (1968).

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