Journal of Pharmaceutical Sciences

SEPTEMBER 1972
VOLUME 61 NUMBER 9

REVIEW ARTICLE

Synthesis of Cyclic Peptides

KENNETH D. KOPPLE

Keyphrases ☐ Peptides, cyclic—synthesis, homodetic and heterodetic, review ☐ Cyclization of peptides—homodetic and heterodetic, review ☐ Amino acids—synthesis of cyclic peptides, review ☐ Amide linkage—synthesis of homodetic cyclic peptides, review ☐ Disulfide linkage—synthesis of heterodetic cyclic peptides, review

The literature describing syntheses of cyclic peptides has steadily grown since the early 1950's, reflecting increasing interest in members of the class. Cyclic peptides are of interest because of the biological activity of some of them; the group includes hormones, antibiotics, fungal toxins, and, if peptide rings containing disulfide groups are included, also most proteins.

One source of pharmacological interest in cyclic oligopeptides is the fact that several of them have been shown to be resistant to enzymatic hydrolysis. In some cases [*e.g.*, the fungal toxins of *amanita* (1), the tyrocidins (2), and the cyclic portions of the polymyxins (3)], this is in part because of the presence of amino acid residues of unusual structure or of the unnatural D-configuration. Other cyclic peptides [*e.g.*, cyclo-(Gly-Lys-Gly-Lys-Gly)[1] (4) and antamanide (5)], which contain only normal residues, are also resistant to peptidases, probably because of conformational constraints resulting from the cyclic structure.

[1] The notation used for amino acids, amino acid derivatives, and peptides in this paper conforms with the 1971 recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature, as given in *J. Biol. Chem.*, 247, 977(1972). Other abbreviations used are given in Table I.

Additional interest in the potential of cyclic oligopeptides should also arise from the suggestion that the biological activity of a cyclic peptide will be retained in analogs of different sequence and configuration, provided the side chains are similarly arranged in space. This proposal was tested successfully for two peptide antibiotics by its authors (6, 7). Synthetic studies of cyclic peptides have, therefore, been undertaken for confirmation or test of suggested structures, for preparation of analogs of biologically active substances, and for commercial preparation in therapeutically useful cases.

Cyclic peptides are also prepared for study, by physical methods, of the forces that determine peptide and protein conformation. They have also appeared in a number of studies as models of enzyme active sites, although to date without important result. A recent review discussed interesting aspects of the conformations of naturally occurring and synthetic cyclic peptides (8).

Cyclic peptides are divided into two broad classes. Those in which the peptide backbone is formed by the usual amide linkages between amino acid residues are known as homodetic. Those in which the chain contains any other kind of linkage are called heterodetic. The most frequent nonamide backbone links in heterodetic peptides are disulfide bonds, which occur, for example, in the posterior pituitary hormones, oxytocin and vasopressin, and the ester function. Peptides containing ester groups in the main chain are known as depsipeptides; many interesting biologically active

Table I—Abbreviations Used in This Review

AA	An unspecified amino acid residue
Acm	Acetamidomethyl
Am^t	*tert*-Amyl
Apm	α-Aminopimelic acid
Asu	α-Aminosuberic acid
Boc	*tert*-Butoxycarbonyl
Bu^t	*tert*-Butyl
Bzl	Benzyl
Bzl(NO_2)	4-Nitrobenzyl
Dab	α,γ-Diaminobutyric acid
Dap	α,β-Diaminopropionic acid
DCCI	Dicyclohexylcarbodiimide
DMF	Dimethylformamide
Dnp	2,4-Dinitrophenyl
EDC	*N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide
HONSu	*N*-Hydroxysuccinimide
Hyiv	α-Hydroxyisovaleric acid
Lac	Lactic acid
MeVal	*N*-Methylvaline
Np	4-Nitrophenyl
Nps	*o*-Nitrophenylsulfenyl
Nva	Norvaline
Tos	*p*-Toluenesulfonyl
Z	Benzyloxycarbonyl
Z(OMe)	4-Methoxybenzyloxycarbonyl
Z(NO_2)	4-Nitrobenzyloxycarbonyl

substances of microbial origin are cyclic depsipeptides. Some cyclic depsipeptides have been of recent interest because of their ability to transport cations across membranes. The synthesis, occurrence, and action of all classes of cyclic peptides were reviewed in detail, through about 1963, by Schröder and Lübke (9). Abstracts of more recent literature appear in the admirable series of Specialist Periodical Reports published by The Chemical Society (10).

A problem in cyclic peptide synthesis is one of open-chain peptide elaboration, plus a cyclization (ring-formation) step. Current methods for the formation of linear open-chain peptides were reviewed recently (9–13), and these will not be directly touched upon here. This paper will review peptide cyclizations; first, the preparation of homodetic cyclic peptides by formation of a peptide bond and then the cyclization steps in the preparation of cyclic depsipeptides and cyclic peptide disulfides.

HOMODETIC CYCLIC PEPTIDES

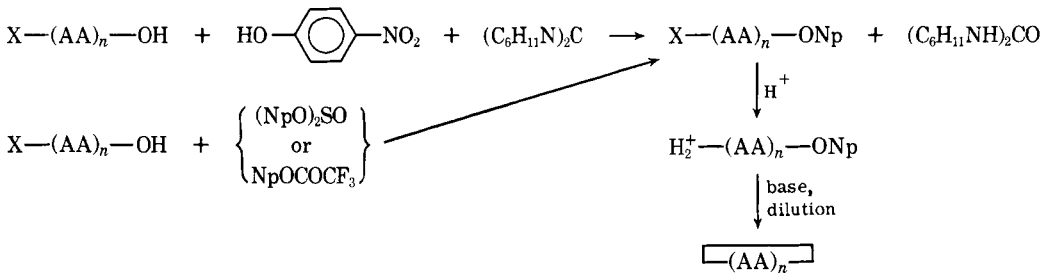
The formation of a peptide ring, like any other cyclization, requires the generation of mutually reactive chain ends and reaction of these ends under conditions favoring intramolecular processes. When a peptide bond is to be formed, these mutually reactive ends are commonly a free amino group, not reduced in nucleophilicity by protonation or substitution, and a carboxyl function activated to be susceptible to nucleophilic attack. Intramolecular reaction is favored by allowing the coupling of these ends to proceed at high dilutions (10^{-3}–10^{-4} *M*). To avoid intermolecular processes, the fully reactive peptide should only be generated at these low concentrations. Therefore, the carboxyl activation and cyclization steps ought to be separable.

The cyclization reaction itself may be an inherently improbable and slow process; for this reason, the activated carboxyl function ought not to undergo unimolecular or solvent-induced decomposition while awaiting approach of the terminal amino group. Since prolonged existence of the activated carboxyl group increases the likelihood of racemization of the activated residue, it is also desirable to devise a linear precursor with glycine or an *N*-substituted amino acid at the C-terminus, so that racemization will be impossible or unlikely.

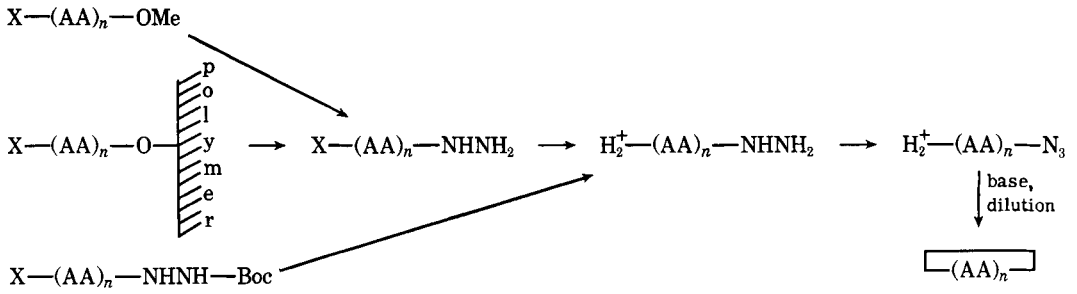
The requirements just stated are not absolute, and no single method is perfect in all of the respects mentioned. Among the methods that allow separate activation and cyclization, the active ester and azide procedures have been most widely employed in peptide cyclization. However, since it is generally simplest to obtain the linear precursor with blocking groups that are simultaneously removed from the *N*- and C-terminals, methods that operate on a terminally unblocked peptide are frequently used. The most common of these is the cyclization by means of *N,N'*-dialkylcarbodiimides.

Active Esters—The procedure using the active ester is shown in Scheme I. This method has the advantages that the activation and cyclization steps can be cleanly separated and that the activated intermediate is stable and can be used without decomposition even when cyclization is slow. Racemization may be a problem, although a proper choice of precursor may be able to eliminate it.

For conversion to the active ester, the open-chain precursor is selectively unblocked at the carboxyl end. Conversion is carried out commonly by reaction of the peptide acid with *p*-nitrophenyl sulfite (14) or *p*-nitrophenyl trifluoroacetate (15, 16). Reaction with *p*-nitrophenol and a carbodiimide may also be used (17). Frequently the ester is not purified but used directly as prepared. The *N*-terminal residue, usually *tert*-butyloxycarbonyl or a version of benzyloxycarbonyl, is then removed by acid cleavage, and the protonated ester is added to base in dilute solution. The solvent and base used for the cyclization must be free of acylable nucleophiles such as water, alcohol, or other



Scheme I—Peptide cyclization via an active ester. X = Z or Boc



Scheme II—Peptide cyclization via *the acyl azide. X is commonly Z or Boc*

amines. Cyclization is often carried out by adding the active ester hydrohalide to a large volume of pyridine held at 60–100°.

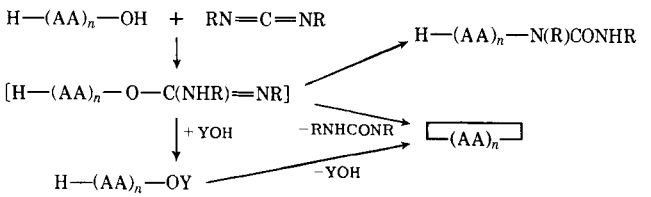
Preparation of nitrophenyl esters for cyclization has also been achieved by the "backing off" procedure, in which the active ester itself is, under controlled conditions, used as the carboxyl terminal blocking group in the preparation of the open-chain peptide (18, 19). Although it is used in the preparation of tripeptides, this is probably not always a satisfactory procedure when a long peptide chain is to be constructed.

Although most active ester peptide cyclizations to date have employed *p*-nitrophenyl esters, esters of *N*-hydroxysuccinimide or 2,4,5-trichlorophenol should be equally useful.

It has been reported that when the active ester is *p*-nitrothiophenyl and when the *N*-terminal blocking group is *o*-nitrophenylsulfenyl, cyclization is possible without a separate step to unblock the amino function. The Nps-nitrothiophenyl ester is treated with free nitrothiophenol, with or without catalytic imidazole, in pyridine (20).

Azide—Although the acyl azide function is not so thermally stable as the active esters, it has enjoyed considerable popularity as a method for peptide cyclization for two reasons. The first is its reputation for producing minimal racemization, so that it is the method of choice when cyclization cannot be carried out at glycine or proline. The second reason is the convenience of constructing a peptide chain from the methyl or ethyl ester of the C-terminal residue. The strategies of cyclic peptide synthesis *via* the azide are shown in Scheme II.

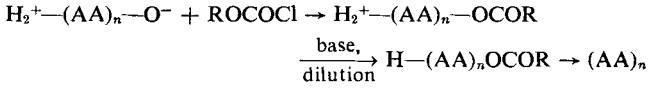
For azide cyclization the peptide chain has most commonly been elaborated from a C-terminal ester, which is hydrazinolyzed when the chain is complete. This approach is readily adopted to peptide synthesis using the Merrifield resin, in which case the completed chain is removed from the resin as the hydrazide on treatment with hydrazine (21). Alternatively, the chains may be elaborated from a C-terminal *tert*-butoxycarbonylhydrazide (22). The *N*-terminal amino and C-terminal hydrazide functions are simultaneously freed before diazotization of the hydrazide (23). However the hydrazide is prepared, diazotization occurs under acidic conditions. Coupling does not occur until base is added to free the terminal amino group. The protonated azide is added to base in a large volume of cold solvent (*e.g.*, water, dimethylformamide, or pyridine), so that again the fully reactive peptide is only generated in 10^{-3}–10^{-4} *M* solution. In most of the reported work, the azide was formed in aqueous acid, but recent experience indicates that diazotization with an alkyl nitrite in an organic solvent (24) system may give better results (25). When diazotization is carried out in an organic solvent, cyclization can be carried out in the complete absence of water, so competition by hydrolysis, at least, is ruled out.



Scheme III—Peptide cyclizations with the aid of dialkylcarbodiimides. Y = phenyl or succinimidyl

Direct Methods—Where there are functional side chains to be protected in the open-chain precursor of a cyclic peptide, it may be difficult to choose blocking groups so that the *N*- and C-terminals can be differentially unblocked. Therefore, direct cyclizations of peptides free at both ends have often been reported. The most commonly used reagents for this purpose have been the *N,N'*-dialkylcarbodiimides, *N,N'*-dicyclohexylcarbodiimide, and *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide. These reagents are very convenient to use, but the activation and cyclization steps cannot be separated. The activation step, being bimolecular, requires a high concentration of carbodiimide and peptide, while cyclization competes favorably with polymerization at a low concentration. In general, an excess of carbodiimide is used, with the peptide at a low concentration.

The carbodiimide method suffers from another disadvantage as well; when cyclization is slow, intramolecular rearrangement of active *O*-acylisourea to the inactive *N*-acylurea occurs. To reduce this latter hazard, a third component, capable of reaction with the *O*-acylisourea to form a thermally stable active ester, may be added. Cyclization reactions have been carried out using excess carbodiimide with phenol as the solvent (25, 26). Successes have also been reported using an excess of *N*-hydroxysuccinimide with excess carbodiimide in dichloromethane–dimethylformamide



R = ethyl or isobutyl

Scheme IV

Table II—Examples of Peptide Coupling to Form Homodetic Cyclic Peptides[a]

Blocked Precursor	Cyclization Method	Yield, %[b]	References
Four Residues in Ring			
Z-Phe-D-Val-Val-D-Phe-OH	Nitrophenyl ester	—	38
Z-Sar-Sar-Sar-Sar-OMe	2,4,5-Trichlorophenyl ester	40	39
Z-Gly-Sar-Gly-Sar-OMe		40	39
Z-β-Ala-Gly Tos-Dap-Gly-OBzl	*o*-Phenylene chlorophosphite	40	40
Five Residues in Ring (see Table IV)			
Z-Trp-Gly-Leu-Ala-D-Thr-OMe	Mixed anhydride	29	33
Z-Gly-Leu-Gly-Leu-Gly-OEt	Pyrophosphite	58	35
Z-D-Leu-Ile-Cys(Bzl)-Val-Cys(Bzl)-OBzl (and diastereomers)	DCCI/HONSu	30–50	41
Boc-Gly-Cys[CH(C$_6$H$_5$)$_2$]-Gly-Gly-Pro-OH	Nitrophenyl ester	68	42
Posterior Pituitary Analogs			
Am^{t}OCO-Tyr-Phe-Gln-Asn-Asu(OH)-Pro-Arg(Tos)-Gly-NH$_2$	Nitrophenyl ester	60	43,44
Boc-Tyr-Ile-Gln-Asn-Apm(Bu^t)-Pro-Leu-Gly-NH$_2$ (Apm = OH)	Nitrophenyl ester	45	45
Six Residues in Ring (see Table III)			
Z-(Gly$_n$, Ala$_{6-n}$)-OBu^t	Nitrophenyl ester	30–60[c]	23,46,47
Z-(Gly$_n$, Ala$_{6-n}$)-NHNH-Boc	Azide		
Z-(Gly$_n$, Ala$_{6-n}$)-OMe	Azide		
Z-Gly-His(Bzl)-Gly-Ala-Tyr-Gly-OBzl(NO$_2$)	DCCI	35	25
Z-Gly-His(Bzl)-Gly-Tyr-Ala-Gly-OBzl(NO$_2$)	DCCI	12	25
Z-Gly-Leu-Gly-Gly-Leu-Gly-OEt	Pyrophosphite	63	35
	Nitrophenyl ester	68	48
Z-D-Val-MeVal-D-Val-MeVal-D-Val-MeVal-OBu^t	DCCI	~15	49
Ferrichrome Series			
Boc-Gly-Nva(5-NO$_2$)-Nva(5-NO$_2$)-Nva(5-NO$_2$)-Gly-Gly-OMe	Nitrophenyl ester	77	50
Seven Residues in Ring			
Z(OMe)-Val-Orn(Z)-Leu-D-Phe-Pro-Gly-Gly-OMe	Nitrophenyl ester	50	51
Bacitracin Series			
HCO-Ile-Lys(Z)-D-Orn(Tos)-Ile-D-Phe-His(Bzl)-Asp(Bzl)-OMe	DCCI (Asp β → Lysε)	50	52
Evolidine			
Z-Ser-Phe-Leu-Pro-Val-Asn-Leu-OBu^t	Nitrophenyl ester	23	53
Polymyxin (Circulin, Colistin) Series			
R-Dab(Z)-Thr-D-Ser(Bzl)-Dab-Dab-D-Leu-OBu^t Boc-Thr-Dab(Z)-Dab(Z)-Thr(Z) (R = 6-Me-octanoyl)	DCCI (Leu → Thr)	—	54
Eight Residues in Ring			
Polymyxin Analogs			
HCO-D-Leu-Dab(Z)-Dab(Z)-Thr-Thr-Dab(Z)-Dab(Z)-Thr-OMe and related cases	Azide	45–60	55
Ten Residues in Ring			
Antamanide Series			
Boc-*xxx*-Pro-Pro-*yyy*-Phe-Phe-Pro-Pro-Phe-Phe-OMe (*xxx*: Ile, Leu, Gly, Ala, Val, Phe; *yyy*: Ala, Gly, Val, Phe)	DCCI/HONSu	45–50	29
	Mixed anhydride	20–30	29
Boc-Phe-Pro-Pro-Phe-Phe-Val-Pro-Pro-Aoa-Phe-OMe	Thiophenyl ester	40	56
Z-Phe-Phe-Pro-Pro-Phe-Phe-Val-Pro-Pro-Ala-OBu^t	DCCI/HONSu	37	28

Table II (*continued*)

Blocked Precursor	Cyclization Method	Yield, %[b]	References
Gramicidin S Series (*See Table IV*)			
Z(OMe)-D-Phe-Pro-Val-Orn(Z(NO$_2$))-Leu-D-Phe-Pro-Val-Orn(Z(NO$_2$))-Leu-O-resin	Azide	85	21
Boc-Val-Orn(Tos)-Leu-D-Phe-Pro-Val-Orn(Tos)-Leu-D-Phe-Gly-O-resin	DCCI/HONSu	32	27
Boc?-Val-Cys(Acm)-Leu-D-Phe-Pro-Val-Cys(Acm)-Leu-D-Phe-Pro-O-?	Nitrophenyl ester	—	57
Polymyxin Analogs			
Boc-Dab(R)-*a*Thr(Z)-Dab(Z)-Dab(Z)-D-Leu-Ile-Dab(Z)-Dab(Z)-OMe (R = 6-Me-octanoyl)	Azide	70	58
Tyrocidin Series			
Z(OMe)-*xxx*-D-*yyy*-Asn-Gln-*zzz*-Val-Orn(Z)-Leu-D-Phe-Pro-OH (*xxx*: Phe, Trp; *yyy*: Phe, Trp; *zzz*: Tyr, Phe)	Nitrophenyl ester	40–60	59–65
Bicyclic Peptide			
Phalloidine Series			
H—*a*4Hyp—Ala—NH—CH(CH$_2$-indole)—CH—CO—Nva—OMe	Mixed anhydride		
HO←Cys←D-Thr←Ala←Boc	Cys → *a*4Hyp	5.5	
	then		
	Nva → Ala	27	66
Twelve Residues in Ring			
H-(Val-D-Pro-D-Val-Pro)$_3$-OH	Woodward K	—	67

[a] All amino acid residues are L unless otherwise indicated. Abbreviations are defined in Table I. [b] Yields reported for cyclization step. [c] All diastereomers were prepared.

solutions (27–30). With phenol as a solvent, there is difficulty in removing traces of phenol from the peptide. On the other hand, there is a reaction between *N*-hydroxysuccinimide and carbodiimide, not involving the peptide, which consumes them both (31). Cyclization with the aid of carbodiimides is illustrated in Scheme III.

With coupling methods other than the carbodiimide, it is possible to separate the activation and cyclization steps. For example, the technique of carboxyl activation as a mixed carbonic anhydride, with the *N*-terminus protected by protonation (32), was recently revived (29, 33). Complete protonation of the amino group is ensured by the presence of pyridinium chloride buffer during formation of the mixed anhydride. Once the anhydride is formed, the activated peptide can be cyclized by addition to base in dilute solution (Scheme IV). This method may be less successful than the azide method for difficult cyclizations, because the active intermediate, the mixed anhydride, is less thermally stable.

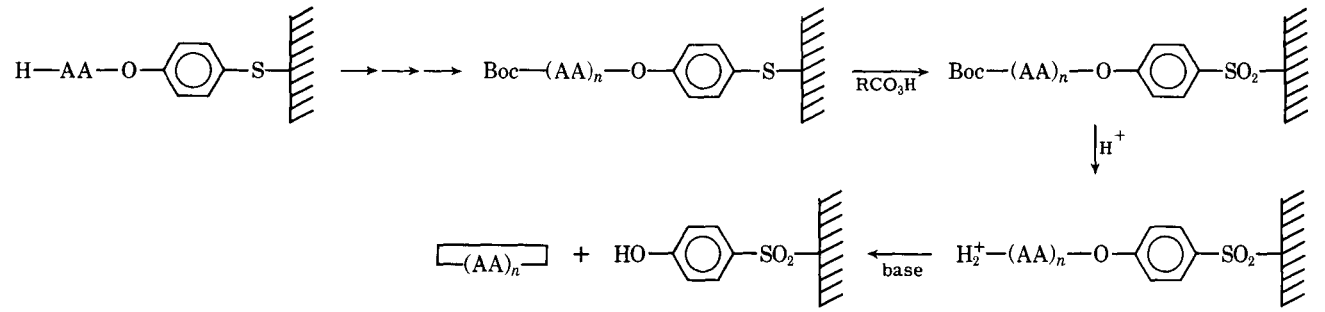
The activation and coupling steps can be similarly separated when a terminally unblocked open-chain peptide is cyclized with the aid of isoxazolium salts, such as Woodward reagent K (34).

Direct cyclization of unblocked open-chain peptides has also been achieved in a number of cases, using derivatives of pyrophosphorous acid in diethyl phosphite (35–37), although somewhat elevated temperatures (140°) are employed.

Examples—Table II lists a number of examples of peptide cyclizations through formation of a peptide bond in solution. The fully blocked open-chain precursors are shown, rather than the stage immediately before cyclization, to indicate the combinations of protective functions that were employed. However, the yields given are those reported for the cyclization step. This table is far from complete, but it should give an idea of the range of compounds that have been prepared. Generally, papers giving experimental details are cited. Additional examples are discussed in the next sections.

Cyclization on Polymeric Supports—Several groups have attempted to improve the ratio of cyclization to intermolecular condensation by isolating the linear precursor molecule on an insoluble substrate. In one technique, an *N*-terminally blocked peptide is attached to a polymeric support by an active ester linkage (68, 69). When the amino group is unmasked, cyclization releases the product from the resin. Cyclic tetraalanine has been prepared in this manner (70). In a variant of the same principle, a resin ester, used in a solid-phase build-up of the linear chain, is penultimately activated by oxidation, according to Scheme V. In this manner, cyclo-(Ala$_3$Gly) and cyclo-(Gly-Val-Ala-Phe-Ala-Gly) were prepared in yields of 40 and 50%, respectively, based on the amino acid initially esterified to the polymer (71).

The principle of dilution on a solid support has also been applied in the case of a peptide attached through



Scheme V—Cyclization from a solid support

a side chain, a cyclodecapeptide, an analog of gramicidin S, has been so prepared (72).

Steric and Conformational Influences on Peptide Cyclization—The rate of a cyclization reaction, thus its yield in competition with side reactions, is related to the probability of juxtaposition of the ends of the open-chain precursor. This probability increases with the configurational and conformational stability of the ring to be formed and decreases with increases in the loss of internal freedom that results from ring formation. The ease of formation of 3-, 5-, and 6-membered alicyclic rings relative to rings of other sizes, well documented in organic chemistry, is rationalized on this basis.

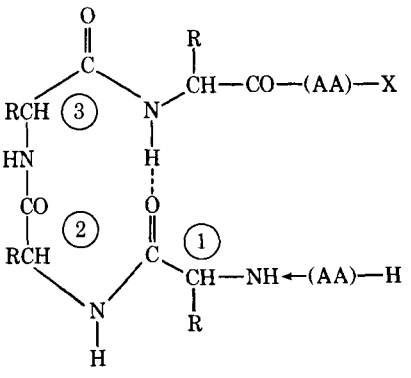
The conformational energy of a peptide ring contains the usual contributions from bond angle distortions, torsional preferences about single bonds, and van der Waals' forces between nonbonded atoms, plus important terms for dipolar interactions and hydrogen bonding. The losses of internal freedom that occur on cyclization of a linear peptide are predominantly in the loss of rotational freedom about the C^{α}—CO and N—C^{α} bonds of each residue, since rotation about the C′—N bond of an amide group is restricted by its electronic structure. Calculating the balance of all these factors with any confidence is not now possible. However, peptide rings exist with any number of residues, from two on up. There are restrictions, but an examination of peptide cyclizations to date suggests that the effects of internal strain are only obvious in rings of perhaps seven or fewer residues.

Rotation about the Peptide C—N Bond—Amide groups are generally planar, and a monosubstituted amide group is most stable in the *trans*-, planar configuration (73). Although the smallest peptide ring that can accommodate only *trans* peptide bonds is one containing five amino acid residues, smaller rings are known. Two-residue peptide cycles, commonly known as 2,5-dioxopiperazines or diketopiperazines, are well known and readily prepared. They form readily from unactivated dipeptide esters (74, 75) and can also be obtained directly from unblocked dipeptides (76) as well as by the methods already described for larger peptides. In diketopiperazines, both peptide bonds are necessarily *cis*, but cyclization is very facile. Rotation about only two single bonds must be fixed to bring the ends of a dipeptide chain together. The diketopiperazine ring itself is probably stabilized by a favorable relative orientation of the two amide dipoles.

Attempts to prepare cyclic tripeptides have only been successful when *N*-substituted amino acids, such as proline and sarcosine, are involved. For the ends of the linear tripeptide to meet, it is necessary that both peptide bonds in the initial chain be in the *cis*-configuration. This is only likely when they are *N*-substituted, since then the *cis*- and *trans*-configurations of the peptide bond are of comparable stability (73). The tripeptide nitrophenyl ester, H-Pro-Pro-Pro-ONp, cyclizes in 88% yield, whereas H-Pro-Pro-Gly-ONp gives *only* the corresponding cyclic dimer, a cyclic hexapeptide (77). Cyclo-(Pro-Pro-3Hyp) has also been prepared (78), as has cyclo-(Sar-Sar-Sar) (79).

A priori, cyclic tetrapeptides should have two *cis* and two *trans* peptide bonds in their stable configurations (80, 81); this has been established for two cases (one a cyclodepsipeptide) by X-ray crystallography (83, 84). For the ends of a tetrapeptide chain to meet, it is only necessary for one amide group to be in a *cis*-configuration; cyclic tetrapeptides have been prepared, without difficulty, with no *N*-substituted amino acid residues. Some cyclic tetrapeptide syntheses are indicated in Table II. It is conceivable that difficulties resulting from side-chain interactions might be encountered in cyclization of a tetrapeptide comprised of residues with large side chains, all of the same optical series.

Intrachain Amide–Amide Interactions—Intrachain hydrogen bonds, or dipolar interactions between amide groups, operating so as to stabilize an open chain in folded conformations approximating the cyclic structure, probably assist cyclization of larger peptides. An indication that this is so appears in the fact that attempts to prepare cyclic tripeptides from open-chain precursors containing *N*-unsubstituted amino acid residues consistently lead to cyclic hexapeptides. Poly-



Structure I—Type of peptide chain folding that may assist cyclization, the β-turn

Table III—Examples of Cyclodimerization of Tripeptides

Peptide	Yield, %	Reference
Gly-Leu-Gly-OH (*per* EDC)	23[a]	25
Gly-Leu-Gly-ONp	20	48
Gly-D,L-Phe-Gly-$OC_6H_5SO_2Me$	45[b]	89
Tyr-Gly-Gly-N_3	48	25
Gly-Pro-Gly-ONP	88,49	90,91
Gly-Gly-Pro-O-Dnp	73	90
Gly-Cys(Bzl)-Leu-N_3	12[c]	92
Gly-His-Ser-OH (*per* EDC methiodide)	~30	93
Pro-Ser-Gly-ONp	11	18

[a] Five percent of cyclo-(Gly-Leu-Gly)$_3$ was also isolated and identified (88). [b] Predominantly *meso*-product formed (22). [c] Yield is 16% when leucine residues are D.

mers are formed in good yields only at high (1 *M*) concentrations (85–87). Cyclization of the tripeptide is unlikely; thus the condensation of two tripeptide units, which are not sterically inhibited, can compete. Cyclization of the so-formed hexapeptide is probably facilitated by chain folding of the type indicated in Structure I. This 10-membered hydrogen-bonded ring has turned out to be a common feature in cyclic peptides containing five or more residues (8). It is commonly called a β-turn or β-loop.

Cyclodimerization has been applied to the syntheses of a number of cyclic hexapeptides that have C_2 symmetry. A set of representative examples is given in Table III. Cyclodimerization is, of course, only applicable to peptides of appropriate symmetry, and it is best used with thermally stable activated groups such as the active esters, although it has been successfully carried out using azides and carbodiimides. Where concentrations of 10^{-3}–10^{-4} *M* are used for cyclization of hexapeptides, cyclodimerization of tripeptides seems to be most effectively carried out in concentrations of the order of 0.1 *M*.

Cyclodimerization has also been used for synthesis of cyclic decapeptide analogs of the antibiotic gramicidin S, which also has C_2 symmetry. In the latter cases, a pentapeptide is used and cyclic pentapeptides are formed competitively. Table IV gives a series of examples of competitive cyclization and cyclodimerization of pentapeptides, taken from the work of one laboratory. Nitrophenyl esters were used, and cyclization was carried out at 3×10^{-3} *M*. The cyclic pentapeptides and decapeptides were separated by gel filtration.

R—CO—Thr—D-Val—Pro—Sar—MeVal

II

Effects of Side Chains—The influence of side chains on the probability of cyclization is not well understood because of the complexity and number of the interactions involved. In large peptide rings, it is likely that a conformation can always be found in which each amino acid residue is in a favorable position on a peptide conformational energy map; but in rings of five and six amino acid residues, certain indications of strain do appear. Conformational energy considerations indicate that the β-loop of the sort indicated in Structure I is more stable if the residues numbered 2 and 3 are of opposite configuration at the α-carbon atom, or if one of them is glycine (80, 105). In several clear cases in the literature, the yield of peptide cyclization was markedly changed by changing the configuration of one amino acid in the linear precursor. Such examples are given in Table V.

Since the cyclization reaction must compete with side reactions that do not require cyclic intermediate states, cyclization yield is a fair indication of the relative stability of cyclic and open conformations when the reactions compared are run identically. From the examples in Table V, it is clear that there is a preference for rings in which there is the sequence D-residue-L-residue, or its enantiomer. There is also an indication, from the cyclization of Gly-Tyr-Gly-Gly-His-Gly derivatives (Table V) and the cyclodimerization of Gly-D,L-Phe-Gly (Table III), that a roughly centrosymmetric cyclic hexapeptide backbone is preferred over one with approximately C_2 symmetry. This latter tentative conclusion is supported by the structure determined for cyclo-(Gly-Gly-Gly-Gly-D-Ala-D-Ala) by X-ray crystallography (106).

Specificity is also shown in cyclization of the cyclopentadepsipeptide moiety of actinomycin D (C_1) (II) at its ester link. Cyclization apparently fails if one of the residues Thr, Val, or Pro is inverted in configuration (110, 111).

Table IV—Effect of Sequence on Cyclization and Cyclodimerization Yields from a Series of Pentapeptide *p*-Nitrophenyl Esters

Sequence	Total Yield, %	Fraction, %		Reference
		Cyclopenta-	Cyclodeca-	
Val-Orn(δ-Z)-Leu-D-Phe-Pro	20	32	68	94
Gly	15	79	21	95
Sar	30	85	15	96
β-Ala	33	89	11	97
Gly-Pro	20	0	100	98
D-Ala	30	25	75	99
D-Leu	50	14	86	100
D-Val	25	12	88	100
Gly-D-Phe	35	59	41	101
Ala	25	43	57	101
Lys(ϵ-Z)-Leu	35	29	71	102
Dab(γ-Z)	45	30	70	102
Gly-Orn(δ-Z)	45	100	0	103
Ala	30	91	9	103
Leu	30	78	22	104
Gly-Lys(ϵ-Z)	—	100	0	94

Table V—Effects of Changes in Residue Configuration on Cyclization Yield

Peptide Cyclized	Yield, %	Reference
Gly-x-Leu-Leu-Gly-Gly-SNp	x=L 12 x=D 40	107
Gly-Cys(Bzl)-x-Leu-Gly-Cys(Bzl)-x-Leu-N_3	x=L 26 x=D 43	92
Orn(Z)-Orn(Z)-Orn(Z)-x-Ser-D-Ser-D-Ser-N_3	x=L 50 x=D 16	108
Gly-x-Tyr-Gly-Gly-His(Bzl)-Gly-OH (per EDC)	x=L 31 x=D,L 58	109
x-Leu-Dab(Z)-Dab(Z)-Thr-Dab(Z)-Dab(Z)-Thr-N_3	x=L 16 x=D 48	55

Additional information about the effects of side chains on cyclizations is contained in Table IV. The effect of the terminal residues on the monomer–dimer ratio is striking. Those cases in which the terminal residues are small (Gly, Sar, β-Ala, and Ala) yield high proportions of cyclic monomers. Cyclodimerization is favored when the C-terminal residue is the sterically constrained proline and the *N*-terminal residue has a large side chain, indicating difficulty in bringing the ends of the pentapeptide into reactive proximity and orientation. These results suggest that it is advisable to plan syntheses of smaller cyclic peptides so that at least one terminal residue in the chain to be cyclized is small. The many examples cited in Table II indicate that there is no such constraint on the cyclization of decapeptides.

It is certain that, at least for smaller cyclic peptides of a given sequence, the sequence chosen for the open precursor should influence the cyclization yield. The faint indications given by the data have yet to be systematically tested.

HETERODETIC CYCLIC PEPTIDES

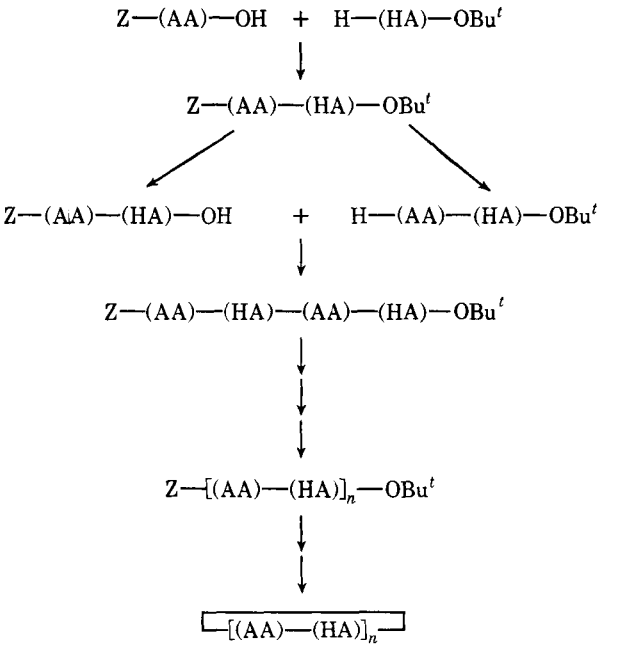
Cyclic Depsipeptides—The naturally occurring cyclodepsipeptides now known contain a wide range of structural features (10, 112). However, many of those on which synthetic attention has been concentrated, such as the enneatins, valinomycin, and their analogs, are relatively uncomplicated. These contain alternating α-hydroxy and amino acid residues and are built up by formation of amide bonds between depsipeptide fragments (Scheme VI).

Coupling of an *N*-blocked amino acid with a C-protected hydroxy acid is carried out by relatively energetic procedures. Mixed carbonic (113) or benzenesulfonic anhydrides (114, 115) have often been used. Carbonyldiimidazole has also been used as a coupling agent and, in at least one case, has proved superior to the sulfonic anhydride method (116). Dicyclohexylcarbodiimide, in the presence of pyridine, is also effective (117).

Coupling of the blocked aminoacyloxycarboxylic acid with an amino component has been carried out with carbodiimide (116), but most often the acid chloride, prepared with the aid of thionyl chloride or phosphorus pentachloride, is the activated carboxyl component (Scheme VI). Since a hydroxy acid is the C-terminal residue in this situation, there can be no azlactone formation, and the use of the acid chloride is apparently acceptable. [Some racemization of an α-acyloxyacyl chloride by the enolization mechanism (118) might be expected if excess base is used in the coupling step.]

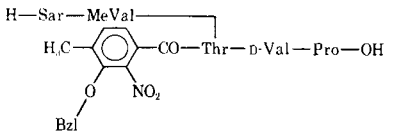
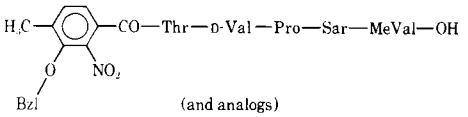
The cyclization process used for this class of cyclodepsipeptides has uniformly been to treat the terminally unblocked depsipeptide with thionyl chloride or phosphorus pentachloride and then to add the so-formed peptide acid chloride hydrochloride to a tertiary amine in benzene under conditions of high dilution. Cyclodepsipeptides of from 4 to 20 residues have been so prepared. Cyclooligomerization is occasionally observed (115, 117).

Of the cyclic depsipeptides of less regular structure, the cyclic pentapeptide portion of the actinomycins, which contains only one ester link, has been synthesized. This ring has been prepared by cyclization at a peptide bond (113) or at the ester bond. For cyclization at the ester link, a reagent formed from imidazole and over 2



Scheme VI—A method of preparation of cyclic depsipeptides containing alternating amino and hydroxy acid residues. HA = hydroxy acid

Table VI—Some Cyclodepsipeptide Syntheses

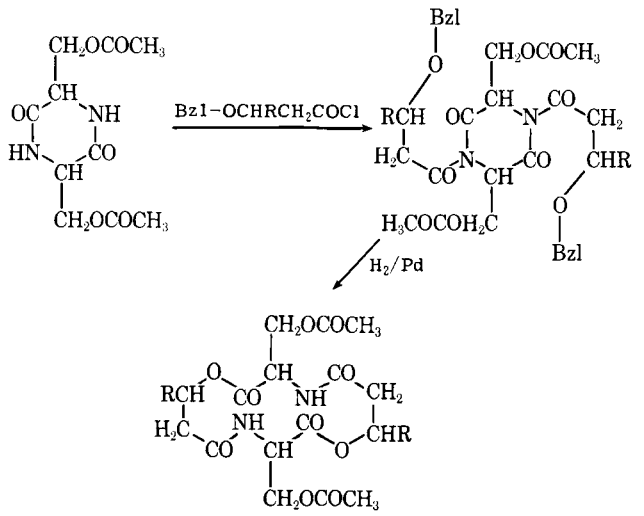
Precursor	Method	Yield, %	Reference
Actinomycin Series			
	Nitrophenyl ester	26	113
 (and analogs)	Acetylimidazole/ acetyl chloride	20–30	110,111,119,120
Enneatins and Analogs			
H-(MeVal-D-Hyiv)$_{2,3,4}$-OH and stereoisomers	Acid chloride	60–75	114,123,124,127
H-(MePhe-D-Hyiv)$_3$-OH	Acid chloride	25,60	125,126
H-(MeVal-D-Val)$_2$-MeVal-D-Hyiv-OH	Acid chloride	10	49
Valinomycin and Analogs			
H-(Val-D-Hyiv-D-Val-Lac)$_3$-OH	Acid chloride	50	116
H-(D-Val-Lac-Val-D-Hyiv)$_{1-4}$-OH diastereomers, analogs	Acid chloride	10–50	115,128
Serratomolide Analogs			
H-Ser-OCH$_2$CH$_2$CO-D-Ser-OCH$_2$CH$_2$COOH Bu^t Bu^t	Acid chloride	15	117
H-Ser-OCH$_2$CH$_2$COOH Bu^t	Dimerization of acid chloride	20	117

moles of acetyl chloride, the structure and action of which are not well established, has been used (109, 110, 119, 120). The synthetic problems in this series, as for most other cyclic depsipeptides, require the complete range of peptide synthetic tools.

A unique method of cyclodepsipeptide preparation, introduction of hydroxyacyl residues into an already cyclic amide, has been demonstrated in a number of cases (121) and applied to the synthesis of a derivative of the cyclotetradepsipeptide serratomolide (122) (Scheme VII).

Table VI lists some of the cyclodepsipeptide syntheses reported.

Cyclic Disulfides—The third class of cyclic peptides to which major synthetic attention has been given are



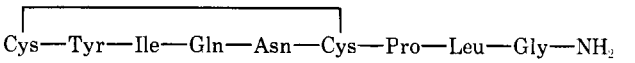
Scheme VII—Synthesis of diacetylserratomolide by hydroxyacyl interposition (122). R = n-C_7H_{15}

those in which rings are closed by one or more disulfide bonds. These include the posterior pituitary hormones, oxytocin and the vasopressins, of which more than 100 analogs have been prepared (129–131). These hormones contain a 20-atom ring of four amino acid residues between two cysteine residues joined by the disulfide link, *e.g.*, oxytocin (III).

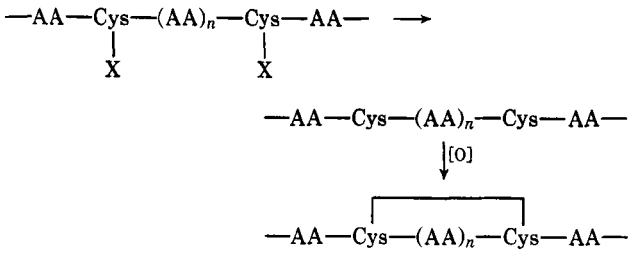
Disulfide links forming rings occur frequently, of course, in larger peptides and proteins. Insulin contains not only a six-residue disulfide ring but, in addition, a ring of 27 residues formed by the two disulfide bridges between the A and B chains. Synthesis of ribonuclease can also be considered the synthesis of a heterodetic cyclic peptide, since the final step involves formation of four disulfide bridges between eight cysteine residues of a single protein chain. Substances of this complexity are beyond the scope of this discussion.

The general practice in preparing cyclic disulfides has been to prepare a peptide chain, using blocked cysteine residues, and then to remove the *S*-protecting groups and oxidize (Scheme VIII).

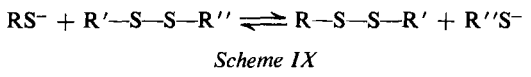
S-Benzyl has long been the protective group employed for the preparation of cysteine peptides. When it is used, sulfhydryl groups are freed for oxidation by treatment of the peptide with sodium in liquid ammonia; this procedure not only removes many other protective groups, circumscribing the overall synthetic strategy, but can also cause other decomposition of the peptide. For this reason, a variety of other sulfhydryl protecting groups have more recently been applied. These include trityl, benzhydryl, benzoyl, and carbobenzyloxy, as



III



Scheme VIII

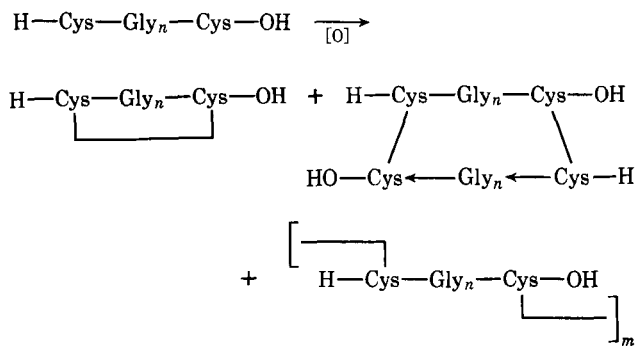


Scheme IX

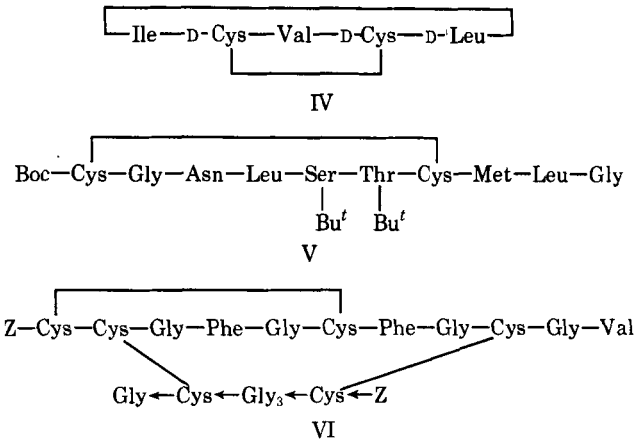
well as hemithioacetal, formation. The use and stability of these groups were described in several publications (132–134). Air, ferricyanide, iodine, and diiodoethane have been used for the oxidation process. The oxidation step is generally carried out in dilute (10^{-3} *M*) solution when cyclic products are described. Oxidizing agents and conditions are varied to optimize the yield of the desired cyclization product.

The procedure just described poses problems not encountered in cyclizations by peptide bond formation. The formation of a peptide bond is irreversible as usually carried out; so that in the absence of intolerable ring strain, conditions can generally be found under which cyclization will occur. However, a disulfide may be cleaved by sulfhydryl groups or other soft nucleophiles (Scheme IX). Because there is a likelihood of disulfide interchange *via* this process, unless the ring system formed is thermodynamically stable the desired disulfide may not be obtained in satisfactory yield.

The oxidation of peptides of the class Cys-Gly$_n$-Cys, where n = 0–6, has been investigated (135–137). Only where n = 4 or more is the cyclic monomer the dominant product. From the shorter chains, polymers and antiparallel dimers are chiefly formed, whether oxidation occurs in 1 or 0.1% solution (Scheme X). Although the cyclic dipeptide Cys-Cys can be prepared (138), the cyclic tripeptide (n = 1) was apparently not formed under the conditions of the study. However, a bicyclic peptide containing such a ring, malformin (IV), was synthesized (139). In this case, the disulfide ring is made favorable by the preexisting homodetic ring. Another bicyclic disulfide analogous to this, [2,7-cystine]-gramicidin S, has been reported (57).



Scheme X



IV

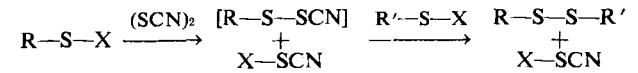
V

VI

Cyclic dimers are also formed in the reoxidation of reduced oxytocin (140) or vasopressin (141), indicating that a hexapeptide disulfide also may not be entirely free of strain. However, the preparation of a heptapeptide disulfide (V), part of the structure of human calcitonin, in 70% yield has been described (142).

Methods for selective coupling of pairs of cysteine sulfhydryls are necessary for preparation of peptides with more than one disulfide bond. Hiskey and his coworkers (143–145) have been exploiting the reactions of thiocyanogen and sulfenyl thiocyanates with mercaptans and certain blocked mercaptans (Scheme XI) with this in mind.

The differential sensitivity of trityl and benzhydryl thio ethers to thiocyanogen and sulfenyl thiocyanates was used to prepare, in a controlled manner, the bicyclic triscystine peptide (VI) (146).



Scheme XI—Sulfenyl thiocyanate method for forming disulfides. X = H, $(C_6H_5)_3C$—, $(C_6H_5)_2C$—, $(CH_3)_2CHCH_2OCH_2$—

REFERENCES

(1) T. Wieland, *Science*, **159**, 946(1968).

(2) R. D. Hotchkiss, *Advan. Enzymol.*, **4**, 153(1944).

(3) T. Suzuki, K. Hayashi, K. Fujikawa, and K. Tsukamoto, *J. Biochem. (Tokyo)*, **54**, 555(1963).

(4) G. W. Kenner and A. H. Laird, *Chem. Commun.*, **1965**, 305.

(5) T. Wieland, *Angew. Chem., Int. Ed.*, **7**, 204(1968).

(6) M. M. Shemyakin, Y. A. Ovchinnikov, V. T. Ivanov, and A. V. Evstratov, *Nature*, **213**, 412(1967).

(7) M. M. Shemyakin, Y. A. Ovchinnikov, V. T. Ivanov, and I. D. Ryabova, *Experientia*, **23**, 326(1967).

(8) C. H. Hassall and W. A. Thomas, *Chem. Brit.*, **7**, 145 (1971).

(9) E. Schröder and K. Lübke, "The Peptides," vols. I and II, E. Gross, Tr., Academic, New York, N. Y., 1965, 1966.

(10) "Specialist Periodical Reports, Amino Acids, Peptides and Proteins," vols. 1–3, G. T. Young, senior reporter, The Chemical Society, London, England, 1969–1971.

(11) A. Kapoor, *J. Pharm. Sci.*, **59**, 1(1970).

(12) M. Bodanszky and M. A. Ondetti, "Peptide Synthesis," Interscience, New York, N. Y., 1966.

(13) E. Wunsch, *Angew. Chem., Int. Ed.*, **10**, 786(1971).

(14) B. Iselin, W. Rittel, P. Sieber, and R. Schwyzer, *Helv. Chim. Acta*, **40**, 373(1957).

(15) S. Sakakibara and N. Inukai, *Bull. Chem. Soc. Japan*, **38**, 1979(1965).

(16) *Ibid.*, **39,** 1567(1966).
(17) M. Bodanszky and V. duVigneaud, *J. Amer. Chem. Soc.*, **81,** 5688(1959).
(18) D. A. Torchia, A. di Corato, S. C. K. Wong, C. M. Deber, and E. R. Blout, *ibid.*, **93,** 609(1971).
(19) D. A. Torchia, S. C. K. Wong, C. M. Deber, and E. R. Blout, *ibid.*, **93,** 616(1971).
(20) H. Faulstich, H. Trischmann, and T. Wieland, *Tetrahedron Lett.*, **1969,** 4131.
(21) M. Ohno, K. Kuromizu, H. Ogawa, and N. Izumiya, *J. Amer. Chem. Soc.*, **93,** 5251(1971).
(22) R. Schwyzer and T.-K. Aung, *Helv. Chim. Acta*, **45,** 859 (1962).
(23) V. T. Ivanov, V. V. Shilin, and Y. A. Ovchinnikov, *Zh. Obshch. Khim.*, **40,** 924(1970).
(24) R. H. Mazur and J. M. Schlatter, *J. Org. Chem.*, **29,** 3213 (1964).
(25) K. D. Kopple, A. Go, R. H. Logan, Jr., and J. Šavrda, *J. Amer. Chem. Soc.*, **94,** 973(1972).
(26) K. D. Kopple, M. Ohnishi, and A. Go, *Biochemistry*, **8,** 4087(1969).
(27) J. Halstrom and H. Klostermeyer, *Ann. Chem.*, **715,** 208 (1968).
(28) W. Konig and R. Geiger, *ibid.*, **727,** 125(1969).
(29) T. Wieland, L. Lapatsanis, J. Faesel, and W. Kunz, *ibid.*, **747,** 194(1971).
(30) T. Wieland, C. Birr, and A. vonDungen, *ibid.*, **747,** 207 (1971).
(31) H. Gross and L. Bilk, *Tetrahedron*, **24,** 6935(1968).
(32) R. A. Boissonnas and I. Schumann, *Helv. Chim. Acta*, **35,** 2229(1952).
(33) T. Wieland, J. Faesel, and H. Faulstich, *Ann. Chem.*, **713,** 201(1968).
(34) K. Blaha and J. Rudinger, *Collect. Czech. Chem. Commun.*, **30,** 3325(1965).
(35) A. W. Miller and P. W. G. Smith, *J. Chem. Soc., C*, **1967,** 2140.
(36) M. Rothe and F. Eissenbeiss, *Z. Naturforsch.*, **21b,** 814 (1966).
(37) M. Rothe, I. Rothe, H. Brunig, and K. Schwenke, *Angew. Chem.*, **71,** 700(1959).
(38) R. O. Studer, *Experientia*, **25,** 899(1969).
(39) J. Dale and K. Titlestad, *Chem. Commun.*, **1969,** 656.
(40) C. H. Hassall, D. G. Sanger, and B. K. Handa, *J. Chem. Soc., C*, **1971,** 2814.
(41) A. Schoberl, M. Rimpler, and E. Clauss, *Ann. Chem.*, **742,** 68(1970).
(42) R. Schwyzer, T.-K. Aung, M. Caviezel, and P. Moser, *Helv. Chim. Acta*, **53,** 15(1970).
(43) S. Sakakibara and S. Hase, *Bull. Chem. Soc. Japan*, **41,** 2816(1968).
(44) S. Hase, T. Morikawa, and S. Sakakibara, *Experientia*, **25,** 1239(1969).
(45) K. Jost and F. Sorm, *Collect. Czech. Chem. Commun.*, **36,** 2795(1971).
(46) V. T. Ivanov, V. V. Shilin, J. Biernat, and Y. A. Ovchinnikov, *Zh. Obshch. Khim.*, **41,** 2318(1971).
(47) H. Gerlach, Y. A. Ovchinnikov, and V. Prelog, *Helv. Chim. Acta*, **47,** 2294(1964).
(48) R. Schwyzer and B. Gorup, *ibid.*, **41,** 2199(1958).
(49) G. Losse and H. Raue, *Tetrahedron*, **25,** 2677(1969).
(50) W. Keller-Schierlein and B. Maurer, *Helv. Chim. Acta*, **52,** 603(1969).
(51) O. Abe, K. Kuromizu, M. Kondo, and N. Izumiya, *Bull. Chem. Soc. Japan*, **43,** 914(1970).
(52) Y. Ariyoshi, T. Shiba, and T. Kaneko, *ibid.*, **40,** 2648 (1967).
(53) R. O. Studer and W. Lergier, *Helv. Chim. Acta*, **48,** 460 (1965).
(54) *Ibid.*, **53,** 929(1970); see also R. O. Studer, W. Lergier, and K. Vogler, *Helv. Chim. Acta*, **49,** 974(1966), and earlier papers by these authors.
(55) E. M. S. Salem, N. V. Fedoseeva, and A. B. Silaev, *Zh. Obshch. Khim.*, **39,** 2541(1969); *ibid.*, **40,** 480, 655(170).
(56) T. Wieland, J. Faesel, and W. Konz, *Ann. Chem.*, **722,** 197(1969).
(57) U. Ludescher and R. Schwyzer, *Helv. Chim. Acta*, **54,** 1637(1971).
(58) H. Arold, *Ann. Chem.*, **731,** 152, 194(1970).
(59) M. Ohno, T. Kato, S. Makisumi, and N. Izumiya, *Bull. Chem. Soc. Japan*, **39,** 1738(1966).
(60) M. Ohno and N. Izumiya, *J. Amer. Chem. Soc.*, **88,** 376 (1967).
(61) K. Kuromizu and N. Izumiya, *Bull. Chem. Soc. Japan*, **43,** 2199, 2944(1970).
(62) K. Kuromizu and N. Izumiya, *Experientia*, **26,** 587(1970).
(63) K. Kuromizu and N. Izumiya, *Tetrahedron Lett.*, **1970,** 1471.
(64) N. Mitsuyasu, S. Matasura, M. Waki, M. Ohno, S. Makisumi, and N. Izumiya, *Bull. Soc. Chem. Japan*, **43,** 1829(1970).
(65) N. Mitsuyasu and N. Izumiya, *Experientia*, **26,** 476(1970).
(66) H. Fahrenholz, H. Faulstich, and T. Wieland, *Ann. Chem.*, **743,** 83(1971).
(67) B. F. Gisin and R. B. Merrifield, Abstract ORGN 69, Abstracts 163rd National Meeting, American Chemical Society, Boston, Mass., Apr. 1972.
(68) M. Fridkin, A. Patchornik, and E. Katchalski, *J. Amer. Chem. Soc.*, **90,** 2953(1968).
(69) M. Fridkin, A. Patchornik, and E. Katchalski, *Biochemistry*, **11,** 466(1972).
(70) M. Fridkin, A. Patchornik, and E. Katchalski, *J. Amer. Chem. Soc.*, **87,** 4646(1965).
(71) E. Flanagan and G. R. Marshall, *Tetrahedron Lett.*, **1970,** 2403.
(72) L. Y. Sklyarov and I. V. Shashikova, *Zh. Obshch. Khim*, **39,** 2778(1969).
(73) A. E. Tonelli, *J. Amer. Chem. Soc.*, **93,** 7153(1971) and references 2–8 cited therein.
(74) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," vol. 2, Wiley, New York, N. Y., 1961, pp. 782–804.
(75) D. E. Nitecki, B. Halpern, and J. W. Westley, *J. Org. Chem.*, **33,** 864(1968).
(76) K. D. Kopple and H. G. Ghazarian, *ibid.*, **33,** 862(1968).
(77) M. Rothe, K.-D. Steffen, and I. Rothe, *Angew. Chem., Int. Ed.*, **4,** 356(1965).
(78) C. M. Deber, D. A. Torchia, and E. R. Blout, *J. Amer. Chem. Soc.*, **93,** 4893(1971).
(79) J. Dale and K. Titlestad, *Chem. Commun.*, **1969,** 656.
(80) J. Dale, *Angew. Chem., Int. Ed.*, **5,** 1000(1966).
(81) J. Dale and K. Titlestad, *Chem. Commun.*, **1970,** 1403.
(83) P. Groth, *Acta Chem. Scand.*, **24,** 780(1970).
(84) J. Konnert and I. L. Karle, *J. Amer. Chem. Soc.*, **91,** 4888 (1969).
(85) D. F. DeTar, M. Gouge, W. Honsberg, and U. Honsberg, *ibid.*, **89,** 888(1967).
(86) D. F. DeTar and T. Vajda, *ibid.*, **89,** 998(1967).
(87) D. F. DeTar, F. F. Rogers, Jr., and H. Bach, *ibid.*, **89,** 3039(1967).
(88) K. D. Kopple and J. Šavrda, paper presented at 11th European Peptide Symposium, Vienna, Austria, Apr. 1971.
(89) R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, **41,** 2190 (1958).
(90) M. Rothe, K.-D. Steffen, and I. Rothe, *Angew. Chem., Int. Ed.*, **3,** 65(1964).
(91) R. Schwyzer, J. P. Carrion, B. Gorup, H. Nolting, and T.-K. Aung, *Helv. Chim. Acta*, **47,** 441(1964).
(92) K. Blaha, I. Frič, Z. Bezpalova, and O. Kaurov, *Collect. Czech. Chem. Commun.*, **35,** 3557(1970).
(93) J. C. Sheehan and D. N. McGregor, *J. Amer. Chem. Soc.*, **84,** 3000(1962).
(94) M. Waki and N. Izumiya, *ibid.*, **89,** 1278(1967).
(95) H. Aoyagi, T. Kato, M. Ohno, M. Kondo, M. Waki, S. Makisumi, and N. Izumiya, *Bull. Chem. Soc. Japan*, **38,** 2139 (1965).
(96) H. Aoyagi and N. Izumiya, *ibid.*, **39,** 1747(1966).
(97) S. Matsuura, M. Waki, S. Makisumi, and N. Izumiya, *ibid.*, **43,** 1197(1970).
(98) R. Nagata, M. Waki, M. Kondo, H. Aoyagi, T. Kato, S. Makisumi, and N. Izumiya, *ibid.*, **40,** 963(1967).
(99) S. Lee, R. Ohkawa, and N. Izumiya, *ibid.*, **44,** 158(1971).
(100) H. Aoyagi, T. Kato, M. Waki, O. Abe, R. Okawa, S. Makisumi, and N. Izumiya, *ibid.*, **42,** 782(1969).

(101) O. Abe and N. Izumiya, *ibid.*, **43,** 1202(1970).
(102) M. Waki, O. Abe, R. Okawa, T. Kato, S. Makisumi, and N. Izumiya, *ibid.*, **40,** 2904(1967).
(103) M. Kondo and N. Izumiya, *ibid.*, **40,** 1975(1967).
(104) *Ibid.*, **43,** 1850(1970).
(105) C. M. Venkatachalam, *Biopolymers*, **6,** 1425(1968).
(106) I. L. Karle, J. W. Gibson, and J. Karle, *J. Amer. Chem. Soc.*, **92,** 3755(1970).
(107) P. M. Hardy, G. W. Kenner, and R. C. Sheppard, *Tetrahedron*, **19,** 95(1963).
(108) A. M. El'Naggar and N. A. Poddubnaya, *Zh. Obshch. Khim.*, **38,** 450(1968).
(109) K. D. Kopple, R. R. Jarabak, and P. L. Bhatia, *Biochemistry*, **2,** 958(1968).
(110) H. Lackner, *Chem. Ber.*, **104,** 3653(1971).
(111) H. Brockmann and H. Lackner, *ibid.*, **101,** 1312(1968).
(112) A. Taylor, *Advan. Appl. Microbiol.*, **12,** 189(1970).
(113) J. Meienhofer, *J. Amer. Chem. Soc.*, **92,** 3771(1970).
(114) M. M. Shemyakin, Y. A. Ovchinnikov, V. T. Ivanov, and A. A. Kiryushkin, *Tetrahedron*, **19,** 581(1963).
(115) M. M. Shemyakin, E. I. Vinogradova, M. Y. Feigina, N. A. Aldanova, Y. B. Shvetsov, and L. A. Fonina, *Zh. Obshch. Khim.*, **36,** 1391(1966).
(116) B. F. Gisin, R. B. Merrifield, and D. C. Tosteson, *J. Amer. Chem. Soc.*, **91,** 2691(1969).
(117) C. H. Hassall, D. G. Sanger, and B. K. Handa, *J. Chem. Soc., C*, **1971,** 2814.
(118) D. S. Kemp and J. Rebek, Jr., *J. Amer. Chem. Soc.*, **92,** 5792(1970).
(119) H. Brockmann and H. Lackner, *Chem. Ber.*, **101,** 2231 (1968).
(120) H. Brockmann and F. Seela, *ibid.*, **104,** 2751(1971).
(121) M. M. Shemyakin, V. K. Antonov, A. M. Shkrob, V. I. Shchelokov, and Z. E. Agadzhanyan, *Tetrahedron*, **21,** 3537(1965).
(122) M. M. Shemyakin, Y. A. Ovchinnikov, V. K. Antonov, A. A. Kiryushkin, V. T. Ivanov, and A. M. Shkrob, *Tetrahedron Lett.*, **1964,** 47.
(123) P. A. Plattner, K. Vogler, R. O. Studer, P. Quitt, and W. Keller-Schierlein, *Helv. Chim. Acta*, **46,** 927(1963).
(124) M. M. Shemyakin, Y. A. Ovchinnikov, A. A. Kiryushkin, and V. T. Ivanov, *Tetrahedron Lett.*, **1963,** 885.
(125) Y. A. Ovchinnikov, V. T. Ivanov, and I. I. Mikhaleva, *ibid.*, **1971,** 159.
(126) R. W. Roeske, S. Isaac, L. K. Steinrauf, and T. King, *Fed. Proc.*, **30,** 1282 Abs. (1971).
(127) G. Losse and H. Raue, *Chem. Ber.*, **101,** 1532(1968).
(128) M. M. Shemyakin, N. A. Aldanova, E. I. Vinogradova, and M. Y. Feigina, *Tetrahedron Lett.*, **1963,** 1921.
(129) I. L. Schwartz and L. M. Livingston, *Vitam. Horm. (New York)*, **22,** 261(1964).
(130) "Handbook of Experimental Pharmacology," vol. 23, B. Berde, Ed., Springer, New York, N. Y., 1968.
(131) J. Rudinger, in "Drug Design," vol. II, E. J. Ariëns, Ed., Academic, New York, N. Y., 1971, pp. 319–420.
(132) K. Hammerstrom, W. Lunkenheimer, and H. Zahn, *Makromol. Chem.*, **133,** 41(1970).
(133) I. Photaki, *J. Amer. Chem. Soc.*, **88,** 2292(1966).
(134) R. G. Hiskey and J. T. Sparrow, *J. Org. Chem.*, **35,** 215 (1970).
(135) D. Jarvis, H. N. Rydon, and J. A. Schofield, *J. Chem. Soc.*, **1961,** 1752.
(136) D. G. Large, H. N. Rydon, and J. A. Schofield, *ibid.*, **1961,** 1749.
(137) G. S. Heaton, H. N. Rydon, and J. A. Schofield, *ibid.*, **1956,** 3157.
(138) R. Wade, M. Winitz, and J. P. Greenstein, *J. Amer. Chem. Soc.*, **78,** 373(1956).
(139) A. Schoberl, M. Rimpler, and E. Clauss, *Chem. Ber.*, **103,** 3159(1970).
(140) D. Yamashiro, D. B. Hope, and V. duVigneaud, *J. Amer. Chem. Soc.*, **90,** 3857(1968).
(141) A. V. Schally and J. F. Barrett, *ibid.*, **87,** 2497(1965).
(142) B. Kamber, H. Bruckner, B. Riniker, P. Sieber, and W. Rittel, *Helv. Chim. Acta*, **53,** 556(1970).
(143) R. G. Hiskey and R. L. Smith, *J. Amer. Chem. Soc.*, **90,** 2677(1968).
(144) R. G. Hiskey and B. F. Ward, Jr., *J. Org. Chem.*, **35,** 1118 (1970).
(145) R. G. Hiskey, G. W. Davis, M. E. Safdy, T. Invi, R. A. Upham, and W. C. Jones, Jr., *ibid.*, **35,** 12(1970).
(146) R. G. Hiskey, A. M. Thomas, R. L. Smith, and W. C. Jones, Jr., *J. Amer. Chem. Soc.*, **91,** 7525(1969).

ACKNOWLEDGMENTS AND ADDRESSES

Received from the *Department of Chemistry, Illinois Institute of Technology, Chicago, IL 60616*

Supported by National Institutes of Health Research Career Development Award GM 47357.