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On the Concept of Linear Modified Retro-Peptide Structures

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The biological activities of peptides are most often dependent upon primary and secondary structure. It has been shown that retro-isomers of certain cyclic peptides and cyclic depsipeptides, i.e., cyclic peptides in which ester bonds replace some of the amide linkages of the ring, maintain biological activity even though the direction of the amide bonds (defined as that from the carbonyl carbon atom to the nitrogen atom) linking the residues has been reversed. In these cases the biological activity must result from the absolute three-dimensional orientation of the side chains and not from the backbone structure. It is most attractive to extend this topochemical approach to linear biologically active peptides. Since linear peptides have end groups and are nonpalindromic, i.e., not giving the same structure in the forward and reverse direction, reversal of a peptide sequence leads to an isomer which is not topochemically related to its parent analogue.

In this Account we present a stereochemical analysis of retro-isomers of cyclic and linear systems. We consider the end group manipulations for the linear compounds and also the numerous modifications used to solve the problems associated with the end groups. A list of the stereochemical terms we will use is given in Table I.

Table I
Summary List of Stereochemical Terms

cycloenantiomer: an isomer derived by reflection in a plane which results in the same distribution of the chiral centers and reversed peptide bonds
cyclodiastereoisomer: an isomer of the same distribution of chiral centers and opposite ring directions but not a mirror image of the parent peptide
cycloisomer: a structural isomer of a cyclic compound
cycloretro-enantiomer: a cycloisomer in which the sequence is reversed and each residue is inverted
cyclic meso-isomer: a stereoisomer which contains an element of symmetry, leading to superimposable enantiomers
retro-isomer: an isomer in which the direction of the sequence is reversed compared with the parent peptide
retro-inverso-isomer: an isomer of a linear peptide in which the direction of the sequence is reversed and the chirality of each amino acid residue is inverted; there can be no end-group complementarity
end group modified retro-inverso-isomer: a retro-inverso-isomer in which end groups were modified to obtain close complementarity with the parent peptide
partially modified retro-inverso-isomer: an isomer of a linear peptide in which only part of the sequence was reversed and the chirality of the amino acid residues in the reversed portion is inverted

Cyclic Peptides

Before discussing linear peptides, it is appropriate to briefly consider the topochemical approach developed[1-4] for and applied[5-11] to cyclic structures such as cyclic peptides and cyclic depsipeptides. In an elegant treatment, Prelog[2] analyzed the stereochemistry of cyclic isomers of the general formula $(-C^*HRCONH-)_{2n}$. Figure 1 contains a schematic representation in which examples of various possible stereoisomers are shown, where $n = 5$ and the number of R chiral centers equals the number of S centers. Prelog recognized that in addition to the meso isomers there are the enantiomeric pairs which are mirror-image isomers of each other. A distinction is made in Figure 1 between two enantiomeric types: the enantiomers,

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(1) M. M. Shemyakin, Yu. A. Ovchinnikov, and V. T. Ivanov, *Angew. Chem., Int. Ed. Engl.*, 8, 492 (1969).
(2) V. Prelog and H. Gerlach, *Helv. Chim. Acta*, **47**, 2288 (1964).
(3) H. Gerlach, J. A. Ovchinnikov, and V. Prelog, *Helv. Chim. Acta*, **47**, 2294 (1964).
(4) H. Gerlach, G. Haas, and V. Prelog, *Helv. Chim. Acta*, **49**, 603 (1966).
(5) M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, and A. V. Evstratov, *Nature (London)*, **213**, 412 (1967).
(6) M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, and I. D. Ryabova, *Experientia*, **23**, 326 (1967).
(7) Y. Chen-su, K. Blaha, and J. Rudinger, *Collect. Czech. Chem. Commun.*, **29**, 2633 (1964).
(8) L. Mladenova-Orlinova, K. Blaha, and J. Rudinger, *Collect. Czech. Chem. Commun.*, **32**, 4070 (1967).
(9) K. Blaha, I. Fric, and J. Rudinger, *Collect. Czech. Chem. Commun.*, **34**, 3497 (1969).
(10) T. Wieland, B. Penke, and C. Birr, *Justus Liebigs Ann. Chem.*, **759**, 71 (1972).
(11) L. G. Snezhkova, E. N. Shepel, I. D. Ryabova, A. I. Miroshnikov, V. T. Ivanov, and Yu. A. Ovchinnikov, *Bioorg. Khim.*, **1**, 347 (1975); *Chem. Abstr.*, **83**, 193686y (1975).

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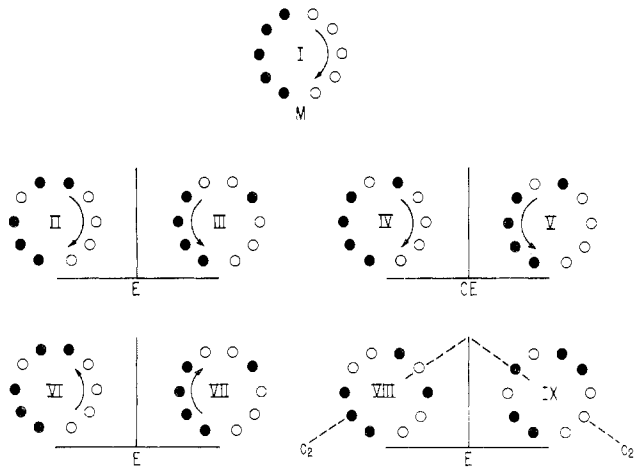


Figure 1. Representative cyclostereoisomers (7 out of total 26) composed of five pairs of enantiomeric chiral centers. ● = *R*, ○ = *S* configuration; M, meso; E, enantiomer; CE, cycloenantiomer. The arrow represents amide bond direction, i.e., carbonyl followed by amide nitrogen.

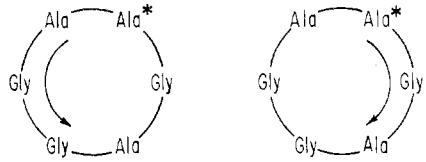


Figure 2. Cycloisomers–constitutional isomers. The same arbitrary starting point is denoted in both structures by an asterisk. The arrow indicates amide bonds direction in the ring.

i.e., isomers II and III, VI and VII, VIII and IX, and the cycloenantiomers, i.e., isomers IV and V. In the former, reflection in the plane results in molecules with a new distribution of the chiral centers and reversed peptide bond direction. In the latter, reflection in the plane results in molecules with the same distribution of chiral centers and reversed direction of peptide bonds. Prelog also introduced a new term for nonenantiomeric pairs, i.e., cyclodiastereoisomers, which describes the stereochemical interrelations among such compounds as II and VI and III and VII in Figure 1. These diastereoisomers have the same distribution of chiral centers and opposite ring directions. Only pairs of enantiomers which lack a c_2 axis of symmetry can lead to cyclodiastereoisomeric pairs.

Prelog and Ovchinnikov[3] studied the spectroscopic and optical properties of a number of cycloenantiomers such as *cyclo*-hexaalanyl (c-LDLLDD and c-LDDLLD) and *cyclo*-(L-Ala-Gly-D-Ala)$_2$. Rudinger and his coworkers studied various cyclostereoisomers with the sequence *cyclo*-(Gly-Phe-Leu-Gly-Phe-Leu).[7-9]

The introduction of different amino acids into cyclic structures without maintaining a symmetrical distribution of the various amino acids in the ring results in structural isomers[4] called cycloisomers which are not cyclostereoisomers.[2,3] The distinction between the stereoisomers and structural isomers becomes obvious when the sequence of amino acids in the two structures of Figure 2 are compared. Different structural sequences are encountered by starting from the same residue but proceeding in opposite directions along the ring (Figure 2). Thus, these structures are not stereoisomers but simply structural isomers.

The first attempt to apply a topochemical approach to cyclic peptides was carried out by Shemyakin, Ovchinnikov, and their co-workers who studied biologically active cyclic depsipeptides. Synthetic *enantio*-enniatin A and B displayed absolutely the same biological activity as the natural compound.[1,5] These biologically active antibiotics possess an unusually high degree of symmetry which allows absolute topochemical complementarity with their enantiomers.

The Russian group[6] defined a series of retro structures which belong to the class of cycloisomers proposed by Prelog.[4] In Figure 3 we see a schematic representation of the transformation of a hetero-cyclo-all-L-tripeptide (A) to its retro structures. Reversal of the amide bonds in the ring isomer A results in its retroisomer B, which is not a cyclostereoisomer of the parent peptide A but only a cycloisomer (according to Prelog's nomenclature[4]). Isomers A and B fail to fulfill the basic requirement of cyclostereoisomerism, i.e., forming the same sequence of residues starting with the same residue but proceeding along the ring in opposite directions. The mirror image of isomer B is its enantiomer C whose relation to the parent peptide A was defined by Shemyakin and Ovchinnikov as retro-enantiomeric.[6] In Prelog's terms compounds B and C are cycloisomers of compound A. He would describe the unique topochemical relationship between isomers A and C as follows: both are equivalent in the spatial arrangement of the various side chains but differ in the chirality of each center and the direction of the amide bonds. Rather than use the term retro-enantiomer, Wieland[10] employed the term "retro-all-D-cyclopeptide" to describe the same topological relationship shown by compounds A and C in Figure 3.

The Russian group[6,11] synthesized *retro-enantio*-[Gly^5,Gly^{10}]gramicidin S whose antiobiotic activity was the same as that of [Gly^5,Gly^{10}]gramicidin S. Wieland[10] prepared *retro-enantio*-[D-Tyr^6]antamanid which ex-

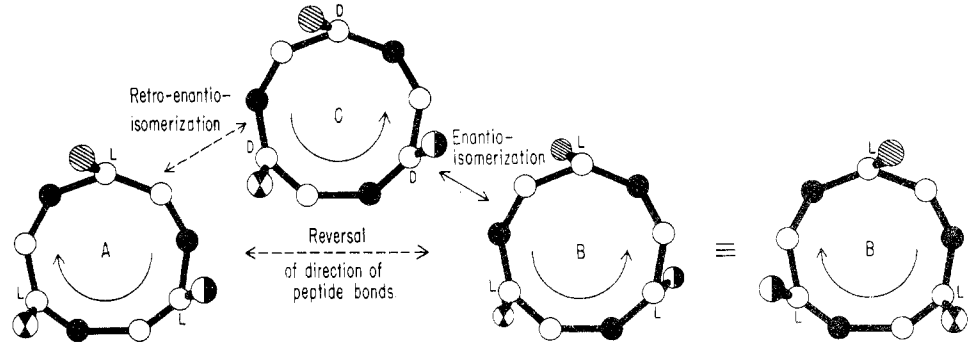


Figure 3. Schematic representation of the cycloisomers of a hetero-cyclo-all-L-tripeptide, A; B, retro-isomer; C, retro-enantiomer (darkened spheres in the backbone represent nitrogen atoms); → amide bond direction, i.e., carbonyl followed by amide nitogen.

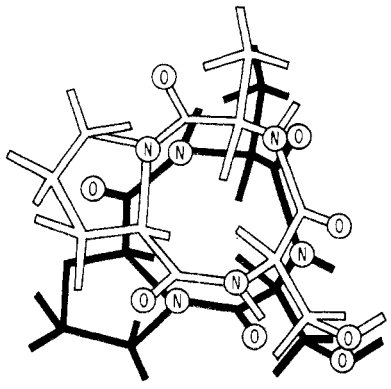


Figure 4. Topological difference between a proline-containing cyclic peptide and its retro-enantiomer.

hibited substantial antiphalliodine-like activity. Shemyakin chose [Gly^5,Gly^{10}]gramicidin S for the retro-enantio transformation studies in order to remove the proline residues in the parent cyclic peptide.[1] He anticipated the lack of spatial coincidence of the proline rings when a proline-containing cyclopeptide is superimposed on its retro-enantiomer, as shown in Figure 4. This effect arises from the proline side chain which must form a ring incorporating the backbone nitrogen atom. In the case of the retro-enantiomer, this nitrogen atom has changed positions with the carbonyl carbon atom leading to an offset of the position of the pyrrolidine ring. Such spatial imperfections in complementary topochemical structure might cause a less effective interaction of a peptide with its biological receptor. It is interesting to point out that the antitoxic activity of *retro-enantio*-[D-Tyr^6]antamanid is almost the same as that of the parent peptide,[10] although it is not topochemically equivalent to the parent peptide because of the two pairs of proline residues in the ring. Clearly, activity in this case does not depend upon exact topochemical equivalence. From these results it can be readily seen that the topochemical approach is a promising tool for studying structure–activity relationships of cyclic depsipeptides and cyclic peptides.

Linear Peptides

Linear peptides contain end groups. Thus, one should not expect structural equivalence by simply reversing a peptide sequence. In order to achieve a topochemically related isomerization, it is necessary to maintain absolute side-chain orientation and to solve the end group problems. The different straightforward stereoisomers and structural isomers of a linear peptide are shown in Figure 5. Structures 1 and 2 are enantiomers, as are 3 and 4. The relation of structure 1 to 3 is that of a retro-isomer, i.e., the direction of the amide bonds is reversed but the chirality of the amino acids in the sequence is retained, which results in noncomplementary side-chain topology. The same relationship exists between structures 2 and 4. A different topochemical relationship holds for pairs 1 and 4 and 2 and 3. In these pairs the backbone direction is reversed but the chirality of each residue is inverted, which results in maintenance of the side-chain topology. Reversal of the end groups creates a major problem in structural complementarity for these linear peptides. Note that residues α and δ in structure 1 are at the amino and carboxyl end of the chain respectively, while the positions of the same residues are reversed in structure 4.

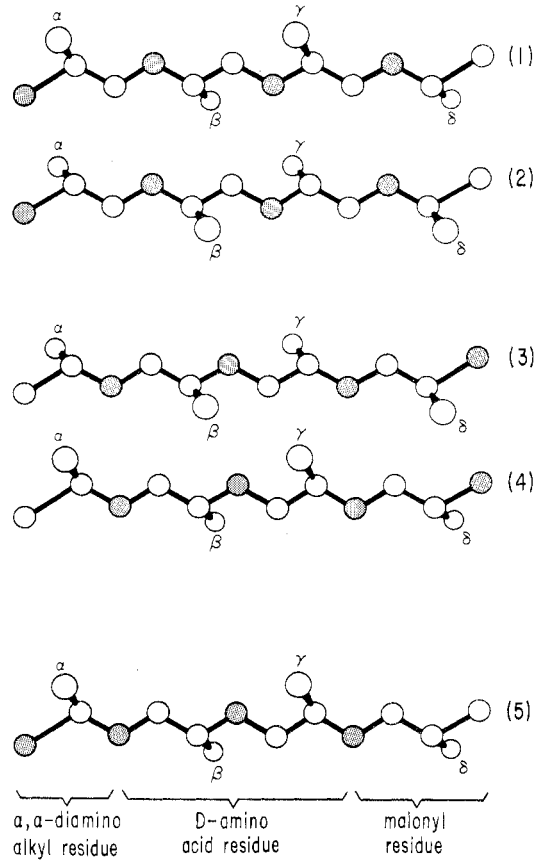


Figure 5. Isomers of linear parent (all-L) peptide (1): (2) enantiomer, (3) retro-isomer (all-L), (4) retro-all-D-isomer, (5) end group modified retro-all-D-isomer. ○ ≡ C, ○ ■ N; α, β, γ, δ represent various side-chain residues.

Many enantiomers of biologically active peptides[12–16] have been examined and found to be totally devoid of biological activity. An exception appears to be *enantio*-α-MSH-(5–9)-pentapeptide which was reported to possess some antagonistic hormonal activity.[17] In like manner retro-peptides such as structure 3 in Figure 5 where the sequence of amino acid residues is reversed were examined for biological activity. Bradykinin and its retro-isomer were studied.[18,19] *retro*-Bradykinin showed no kinin activity, which may be related to the presence of proline residues in the sequence; cf. discussion of gramicidin S above.

Structure 5 represents a general approach to solving the end group problem. An α,α-diaminoalkyl residue (*gem*-diaminoalkyl) and a C-2 substituted malonic acid residue replace the N-terminal and C-terminal amino acids respectively of the retro-all-D-isomer structure (4).

Retro-Inverso-Peptides

To obtain structure 4 from structure 1 in Figure 5 it is necessary to reverse the direction of the peptide bond and to invert the chirality of each chiral center. We

(12) E. Schröder, K. Lübke, and R. Hempel, *Experientia*, **21**, 70 (1965).
(13) J. M. Stewart and D. W. Woolley, *Nature (London)*, **206**, 619 (1965).
(14) G. Flouret and V. du Vigneaud, *J. Am. Chem. Soc.*, **87**, 3775 (1965).
(15) K. Vogler, R. O. Studer, W. Lergier, and P. Lanz, *Helv. Chim. Acta*, **48**, 1407 (1965).
(16) J. S. Morley, H. J. Tracy, and R. A. Gregory, *Nature (London)*, **207**, 1356 (1965).
(17) K. Hano, M. Koida, K. Kubo, and H. Yajima, *Biochim. Biophys. Acta*, **90**, 201 (1964).
(18) M. Bodanszky, M. A. Ondetti, and J. T. Sheehan, *Ann. N.Y. Acad. Sci.*, **104**, 24 (1963).
(19) K. Vogler, P. Lanz, and W. Lergier, *Helv. Chim. Acta*, **45**, 561 (1962).

Table II
Parent and End Group Modified Retro-Inverso-Peptides (a-g)

mode of variation	parent peptide A	parent peptide B	end group modified retro-inverso-peptide A'	end group modified retro-inverso-peptide B'	ref
(a) N- and C- protected	RCONH-	-CONHR'	RNHCO-	-NHCOR'	1
(b) Desamino-, decarboxy-[a]	H-	-H	H-	-H	26
(c) desamino-, malonamyl residue	H-	$-CONH_2$	H-	$-CONH_2$	24
(d) malonyl residue	H_2N-	-COOH	HCOO-	-COOH	27
(e) desamino-, malonyl residue	H-	-COOH	H-	-COOH	27
(f) *gem*-diaminoalkyl, malonyl ester residues	H_2N-	-COOR	H_2N-	-COOR	28
(g) N-acylated-*gem*-diaminoalkyl, malonamyl residues	RCONH-	$-CONH_2$	RCONH-	$-CONH_2$	29

[a] In variation b the peptide chain is shortened by the two amide end residues, the side chains of which form the resulting amino and carboxyl terminal protecting groups. Thus, variation b is conceptually a special case of variation a.

suggest that the general term retro-inverso-peptides clearly shows the relationships of structure 4 to structure 1. If a parent structure (1) contains all-L residues, then it is proper to call the corresponding structure (4) a retro-all-D-peptide. Retro-inverso-peptides such as retro-all-D-bradykinin,[20,21] retro-all-D-α-MSH-(5-9)-pentapeptide,[22] and retro-all-D-tuftsin[23,24] have been synthesized without regard to the end group reversal. None of these retro-all-D-peptides showed significant biological activity. In the case of retro-all-D-bradykinin the rationale for not treating the end-group problem was that arginine residues are found at both ends.[19] The lack of activity of the retro-all-D-bradykinin may occur because the carboxyl function is essential for activity in bradykinin itself.[25]

Shemyakin et al.[1] recognized that the principles he and his associates had developed for cyclic structures cannot be applied to linear peptides with free amino and free carboxyl end groups. Therefore, they prepared peptide derivatives with altered end group structures. End groups of any type introduce an intrinsic structural nonequivalence between a parent and a retro-peptide. The most that can be achieved is to create a close resemblance between end groups in a spatial sense. The term retro-enantio-peptide implies too much structural equivalence and should not be used for linear systems. The term retro-inverso-peptide does not imply such a close stereochemical relationship and should be used even in cases where end groups have been modified.

End Group Problem

A number of approaches can be designed to treat the end group problem. Three types of structural modifications (see Table II) have been considered: variations a–b—peptide derivatives with protected or eliminated end groups; variations c–g—peptides with modified carboxyl and amino end residues; variation h—partially modified retro-inverso-peptides.

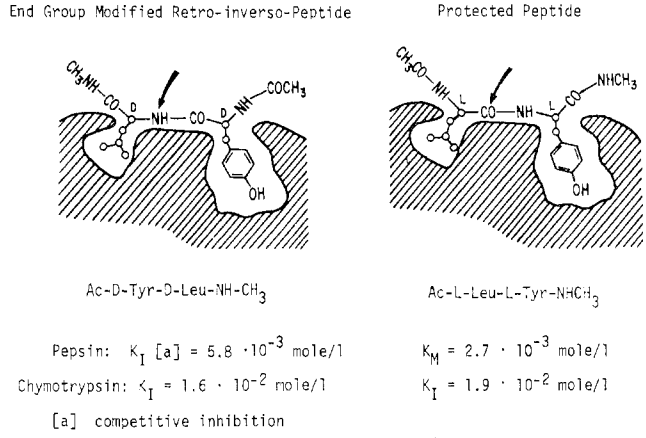


Figure 6. Schematic representation of a specific substrate–proteolytic enzyme binding site interaction.[1]

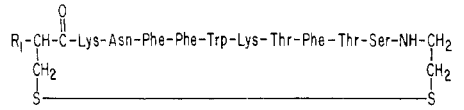
Table II includes examples of modifications in which the end groups are either blocked, eliminated, or retained.

An example of variation a was studied by Shemyakin and his group using Ac-L-Leu-L-Tyr-$NHCH_3$, a substrate for pepsin and chymotrypsin.[1] The end group modified retro-inverso-peptide, in this case the end group modified retro-all-D-isomer, Ac-D-Tyr-D-Leu-$NHCH_3$, of the parent dipeptide was found to be an effective competitive inhibitor of pepsin with an inhibition constant (K_I) very close to the Michaelis constant (K_M) for the substrate. Both have the same effectiveness on inhibition of catalytic hydrolysis of *p*-nitrophenyl pivalate by chymotrypsin (see Figure 6). Because of similarities of spatial distribution (i.e., topology) of the side chains and of the electron densities of the end group modified retro-all-D-analogue and the parent peptide, the binding process should not be greatly perturbed.

Modifications of somatostatin studied by Immer et al.[26] may be classified under variation b. We consider somatostatin to be a valid example of a linear peptide, since ring closure does not involve the backbone and is accomplished by bridging two side chains via a di-

(20) K. Vogler and P. Lanz in "Hypotensive Peptides", E. G. Erdos, N. Back, and F. Sicuteri, Eds., Springer, Berlin, 1966, p 14.
(21) K. Vogler, P. Lanz, W. Lergier, and W. Haefely, *Helv. Chim. Acta*, **49**, 390 (1966).
(22) D. Chung and C. H. Li, *Biochim. Biophys. Acta*, **136**, 570 (1967).
(23) V. A. Najjar, German Patent 2124003 (Cl. C07c, A61k), June 22, 1972; U.S. Appl. 98890, Dec 16, 1970; *Chem. Abstr.*, **77**, 118186h (1972).
(24) C. F. Hayward and J. S. Morley on "Peptides 1974, Proceedings of the 13th European Peptide Symposium", Y. Wolman Ed., Wiley, New York, and Israel Universities Press, Jerusalem, 1975, p 287.
(25) W. D. Johnson, H. D. Law, and R. O. Studer, *Experientia*, **25**, 573 (1969).
(26) H. U. Immer, N. A. Abraham, V. R. Nelson, W. T. Robinson, and K. Sestanj in "Peptides 1976, Proceedings of the 14th European Peptide Symposium", A. Loffet, Ed., Editions de l'Universite de Bruxelles, Brussels, 1976, p 471.

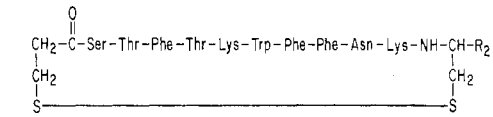
sulfide linkage. [Desamino[1],decarboxy[14]]somatostatin and des[Ala,[1]Gly[2],desamino[3],decarboxy[14]]somatostatin, structures **1** and **2**, respectively, were both found to have



$\underset{\sim}{1}$ $R_1 = CH_3CH_2-CO-Gly-NH$

$\underset{\sim}{2}$ $R_1 = H$

$\underset{\sim}{1}$ and $\underset{\sim}{2}$ all L-amino acids



$\underset{\sim}{3}$ $R_2 = CO-Gly-NH-CH_2CH_3$

$\underset{\sim}{4}$ $R_2 = H$

$\underset{\sim}{3}$ and $\underset{\sim}{4}$ all D-amino acids

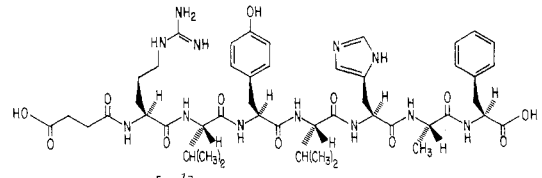
the same releasing activity as somatostatin for growth hormone. The former was also found to inhibit cyclic AMP production induced by prostaglandin E_2. None of the corresponding end group modified retro-all-D-analogues of variation b (structures **3** and **4**) was found to have somatostatin-like activity.

The structural changes introduced into end group modified retro-inverso-isomers of variations a and b are substantial. With reference to somatostatin analogue **1**, glycine is acylated by a propionyl residue (for desaminoalanine) and serine acylates the 2-thioethylamine residue (for decarboxycysteine). In the corresponding end group modified retro-all-D-analogue (structure **3**) glycine acylates an ethylamine residue (in place of decarboxyalanine), while serine is acylated by the 2-thiopropionyl residue (in place of desaminocysteine). These changes are much more substantial than those involved in the transformation to which cyclic peptides are subjected in order to obtain the retro-enantio-isomers.

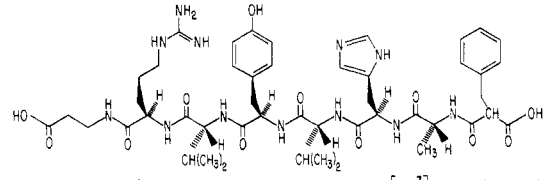
Variations c–g include structures in which modified carboxyl and/or amino end residues are introduced into the retro-inverso-analogues. The modified carboxyl terminal residue is obtained by replacing the original C-terminal amino acid by the appropriate C-2 substituted malonic acid derivative, as shown for variations c–g. The modified N-terminal residue is introduced by replacing the original N-terminal amino acid by the appropriate *gem*-diaminoalkyl residue, as shown by variations f–g.

The introduction of the malonic or malonamic acid residues as the modified C-terminal groups was suggested by Rudinger.[30] The first application of this was in the synthesis of the end group modified retro-all-D-analogue of desamino-gastrin C-terminal tetrapeptide amide by Morley,[24] i.e., D-2-benzylmalonamyl-D-Asp-D-Met-tryptamide. This analogue, which is an example of variation c, was found to be biologically inactive.[24]

Paiva et al.[27] utilized aspects of the approach shown in variations d and e for the preparation of end group modified retro-all-D-angiotensin analogues. Since retro-analogues of angiotensin and its analogues contain proline, they must include a topochemical imperfection. Paiva resolved this problem by replacing the propyl residue in some analogues by alanyl or β-alanyl residues, e.g., structures **5** and **6**. Only end group modified re-



$\underset{\sim}{5}$ [Ala[7]]-desamino-angiotensin

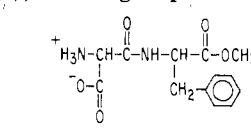


$\underset{\sim}{6}$ End group modified retro-inverso-[Ala[7]]-desamino-angiotensin

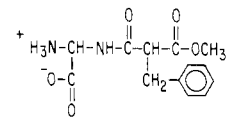
tro-all-D-isomers of angiotensin analogues, where proline has been replaced, possess substantial biological activity.

The chemistry involved in the preparation of C-2 substituted malonic and malonamic acid derivatives is well established.[31] Resolution of the racemic C-2 substituted malonic acid monoamide by formation of diastereoisomeric salts was first carried out for (*RS*)-2-isopropylmalonamic acid by Fischer and Brauns.[32] In the case of end group modified retro-all-D-angiotensin analogues (above), no resolution of diastereoisomeric mixtures was undertaken.[27] The observed biological activity was assumed to arise from only one isomer out of the two (*RS*)-2-benzylmalonamic acid derivatives present in the mixture.

Incorporation of a modified amino terminal residue such as an α,α-diaminoalkyl residue provides the only method for retention of the amino-terminal end group of the parent peptide after transformation into an end group modified retro-inverso-peptide structure. Variations f and g in Table II indicate the structures of end group modified retro-inverso-peptides in which an acylated α,α-diaminoalkyl residue is included. In connection with our work on dipeptide sweetners we have successfully synthesized synthesized (*RS*)-*N*-[(*RS*)-2-benzylmalonyl methyl ester]-α-aminoglycine (**8**), an end group modified retro-inverso-analogue of the



$\underset{\sim}{7}$



$\underset{\sim}{8}$

parent sweet dipeptide, (*RS*)-α-aminomalonyl-L-phenylalanine methyl ester (**7**).[28] This rather unstable topochemical analogue was not sweet.

(27) G. Goissis, V. L. A. Nouailhetas, and A. C. M. Paiva, *J. Med. Chem.*, **19**, 1287 (1976).

(28) M. Chorev, C. G. Willson and M. Goodman in "Peptides: Proceedings of the 5th American Peptide Symposium", M. Goodman and J. Meienhofer, Eds., Wiley, New York, 1977, p 572.

(29) C. G. Willson, M. Goodman, J. Rivier, and W. Vale in ref 28, p 579.

(30) J. Rudinger in "Drug Design", Vol. 2, E. J. Ariens, Ed., Academic Press, New York–London, 1971, p 319.

(31) A. C. Cope, H. L. Holmes, and H. O. House, *Org. React.*, **9**, 107 (1957).

(32) E. Fischer and F. Brauns, *Sitzungsber. Preuss. Akad. Wiss., Phys.-Math. Kl.*, 714 (1914).

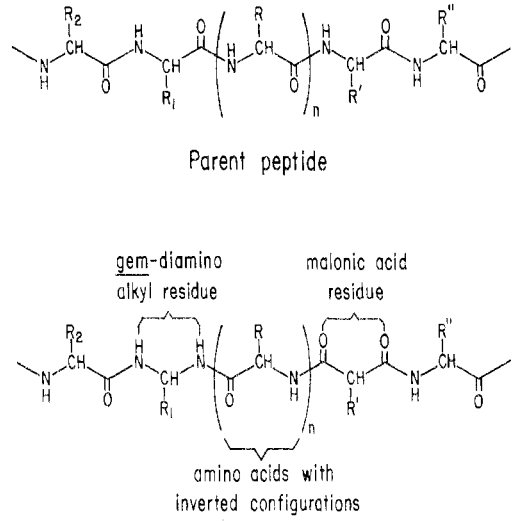
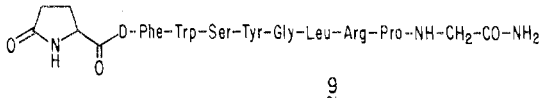


Figure 7. Partially modified retro-inverso-peptide analogue (h) of a parent peptide. R_2 and R″ amino acid residues from the original sequence, R_1 and R′ amino acid residues replaced by *gem*-diaminoalkyl and suitably C-2 substituted malonyl residues, respectively. *R*-Amino acid residues (in parentheses) in the original sequence have inverted configuration in structure h.

We have extended our studies to the area of the hypothalamic hormone releasing factors, e.g., the luteinizing hormone releasing factor (LRF). We prepared the end group modified retro-inverso-peptide (**10**) of [D-Phe^2]-LRF (**9**) which corresponds to variation g in which the terminal amino group is acylated.[29] The [D-Phe^2]-LRF (**9**) is known to be a competitive inhibitor



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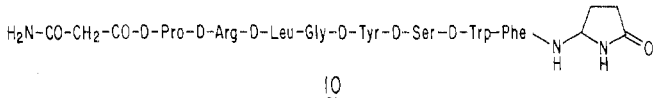

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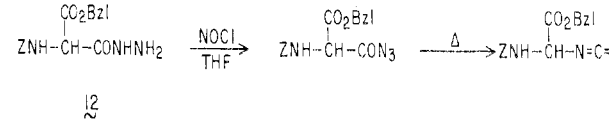
of LRF.[33] The corresponding end group modified retro-inverso-analogue **10** was devoid of either agonistic or antagonistic activity. In the latter end group modified retro-inverso-analogue of LRF the proline residue is acylated by a malonamyl residue functioning as the modified carboxylic end terminus. As with bradykinin and angiotensin, topological noncomplementarity exists because of the proline residue. This unresolved "proline problem" might be the basis for lack of activity of the end group modified retro-inverso-LRF analogues we have prepared.[29]

A substantial part of our work in this field involves partially modified retro-inverso-peptide analogues. This general approach (variation h) is outlined in Figure 7 in which the end group modified retro-inverso sequence is incorporated in the middle of a peptide sequence. The modification commences with an α,α-diaminoalkyl residue and ends with a malonic acid residue. The number of amino acid residues with inverted configurations between two modified residues can be varied

(33) R. Rees, T. Foell, S-Y. Chai, and N. Grant, *J. Med. Chem.*, **17**, 1016 (1974).

Scheme I



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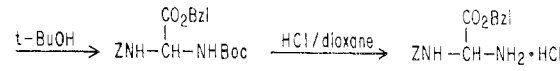

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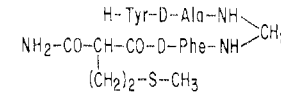
widely. Work on partially modified *retro-inverso*-Met-enkephalin amide (**11**) and LRF are in progress in



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our laboratory. An example of partially modified *retro-inverso*-[D-Ala^2]-Met-enkephalin amide is *N*-(L-tyrosyl-D-alanyl)-*N*′-[(*RS*)-2-(*S*-methylthioethylene)-malonamyl-D-phenylalanyl]diaminomethane (**11**).[34]

The chemistry involved in the conversion of N-acylated amino acids into the corresponding diacylaminated aldehydes was discovered by Bergmann and Zervas.[35] That was the basis of their method for sequencing peptides or proteins (the so-called "carbobenzoxy degradation").

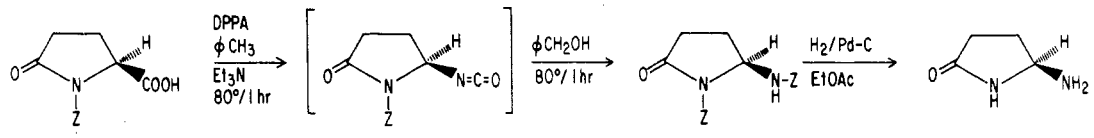
We have modified the Curtius rearrangement used in the above-mentioned "carbobenzoxy degradation" by introducing nitrosyl chloride as the reagent of choice.[36] An example of this synthetic route we have used for the synthesis of *N*-(benzyloxycarbonyl)-(*RS*)-α-aminoglycine benzyl ester (**13**) from N^{α}-(benzyloxycarbonyl)-(*RS*)-α-aminoalonylhydrazide benzyl ester (**12**) is outlined in Scheme I.[28]

In the approach we devised for the end group modified retro-inverso-LRF analogues such as compound **10**, the *p*Glu residue was converted to the corresponding α,α-diamino compound, i.e., (*S*)-2-amino-5-pyrrolidone (**14**).[29] In this reaction, we followed another modification of the Curtius rearrangement utilizing diphenylphosphoryl azide (DPPA),[37] as presented in Scheme II.

A derivative of the above-mentioned reagent, bis-(*p*-nitrophenyl)phosphoryl azide, was used by Parham and Loudon[38] in solid-phase peptide sequencing, a modification of the original "carbobenzoxy degradation".[35] Another contribution from these workers involves a Hoffman rearrangement of a peptide amide using iodobenzene diacetate to give the corresponding α,α-diamino residue.[38] All these reactions involve rearrangements which are considered to occur with a high degree of configurational retention. Our general synthetic approach to the synthesis of end group modified and partially modified retro-inverso-peptide

(34) M. Chorev and M. Goodman, unpublished results.
(35) M. Bergmann and L. Zervas, *J. Biol. Chem.*, **113**, 341 (1936).
(36) J. Honzl and J. Rudinger, *Collect. Czech. Chem. Commun.*, **26**, 2333 (1961).
(37) T. Shioiri, K. Ninomyia, and S. Yamada, *J. Am. Chem. Soc.*, **94**, 6203 (1972).
(38) M. E. Parham and G. M. Loudon, *Biochem. Biophys. Res. Commun.*, **80**, 1 (1978); **80**, 7 (1978).

Scheme II



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analogues was published recently.[39]

Concluding Remarks

Although the utilization of the topological approach to the synthesis of analogues of biologically active peptides is not new, the application of the conceptual approach to linear peptides has not been presented previously in a systematic manner. We have tried to place the terminology on a sound footing. We believe that the new term, "end group modified retro-inverso-peptide", defines precisely the types of transformations to which a linear peptide is subjected. We clearly stress that the modified peptides retain a full topological relationship to the parent peptide. Our studies on partially modified retro-inverso-peptides is a novel approach to the preparation of modified peptides through which we hope to assess the relative importance of side-chain vs. backbone structure for biological activity. Enhanced resistance to biodegradation processes might be another benefit of this type of structural modification.

Since this field is in its early stages, more examples of suitable analogues of biologically active peptides are needed to have a better understanding of the scope and limitations of the approach. Spectroscopic and theoretical conformational analyses will provide insights into the effects of such modifications on conformational preferences. With this information we should be better able to choose appropriate analogues of biologically active peptides to be synthesized and studied.

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(39) M. Chorev, C. G. Willson, and M. Goodman, *J. Am. Chem. Soc.*, **99**, 8075 (1977).

Interactions in Aqueous Solution

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The behavior of aqueous solutions of small and large molecules is influenced considerably by the nature of the solvent itself.[1-6] Thus, water plays a very important role in determining the properties of colloidal and macromolecular systems and, in particular, the manner in which proteins acquire their native structure and then interact with other small and large molecules.[7] In the absence of water, the interactions between the various functional groups of a polypeptide chain can be described in terms of empirical potential energy functions that have been parameterized with crystal-structure and gas-phase data on small molecules.[8] However, since protein folding occurs in water, the final conformation is influenced strongly by the solvent. Therefore, it is necessary to understand the nature of the interactions between water and the functional groups of proteins.

Harold A. Scheraga was born in Brooklyn, New York. He attended the City College of New York, where he received his B.S. degree, and went on to graduate work at Duke University, receiving the Ph.D. in 1946, and, in 1961, a Sc.D. (Hon). Following postdoctoral work at Harvard Medical School, he joined the faculty at Cornell University, where he is Todd Professor of Chemistry. His research interests are in the physical chemistry of proteins and other macromolecules, chemistry of blood clotting, and structure of water and dilute aqueous solutions. This Account is based on Professor Scheraga's Award address for the 1978 ACS Award in Colloid or Surface Chemistry sponsored by Kendall Co.

Several different types of approaches have been taken to investigate such interactions in aqueous solutions.[9] Initially, these involved the formulation of suitable models and their treatment by statistical mechanical methods. The cluster model of Nemethy and Scheraga,[10-13] with its improvements by Hagler et al.,[14] Lentz

(1) W. Kauzmann, *Adv. Protein Chem.*, **14**, 1 (1959).
(2) D. Eisenberg and W. Kauzmann, "The Structure and Properties of Water", Oxford University Press, Oxford, 1969.
(3) R. A. Horne, "Water and Aqueous Solutions", Wiley-Interscience, New York, N.Y., 1972.
(4) F. Franks, "Water, A Comprehensive Treatise", Plenum Press, New York, N.Y.: Vol. 1, 1972; Vol. 2, 1973; Vol. 3, 1973; Vol. 4, 1975; Vol. 5, 1975.
(5) W. A. P. Luck, "Structure of Water and Aqueous Solutions", Verlag Chemie, Weinheim, Germany, 1974.
(6) A. Ben-Naim, "Water and Aqueous Solutions", Plenum Press, New York, N.Y., 1974.
(7) G. Nemethy and H. A. Scheraga, *Q. Rev. Biophys.*, **10**, 239 (1977).
(8) F. A. Momany, R. F. McGuire, A. W. Burgess, and H. A. Scheraga, *J. Phys. Chem.*, **79**, 2361 (1975).
(9) H. A. Scheraga, *Ann. N.Y. Acad. Sci.*, **303**, 2 (1977).
(10) G. Nemethy and H. A. Scheraga, *J. Chem. Phys.*, **36**, 3382 (1962).
(11) G. Nemethy and H. A. Scheraga, *J. Chem. Phys.*, **36**, 3401 (1962).
(12) G. Nemethy and H. A. Scheraga, *J. Phys. Chem.*, **66**, 1773 (1962).
(13) G. Nemethy and H. A. Scheraga, *J. Chem. Phys.*, **41**, 680 (1964).
(14) A. T. Hagler, H. A. Scheraga, and G. Nemethy, *J. Phys. Chem.*, **76**, 3229 (1972).

0001-4842/79/0112-0007$01.00/0