A Dozen Years of Retro-Inverso Peptidomimetics†

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Twelve years ago we published an Account in which we presented a stereochemical analysis of cyclic and linear retro-peptide systems.[1] We postulated that reversal of peptide bonds (isomeric replacement) accompanied by the appropriate configurational compensations may schematically result in a high degree of topochemical equivalence between the parent peptide and its retro-inverso analogs. In that conceptually oriented communication, we distinguished between a total retro-inverso modification, in which all the amide bonds are reversed, and a partially modified retro-inverso modification (PMRI modification), which involves the reversal of one or more amide bonds in the parent peptide (see Figure 1). The retro-inverso modification has since evolved into one of the most widely used peptidomimetic approaches for the design of novel bioactive molecules. In the present report, the application of partial retro-inverso structures to many families of biologically active peptides is presented. Furthermore, the basic chemistry and configurational stability of the *gem*-diaminoalkyl residues and 2-alkylmalonamides and the conformational aspects of retro-inverso modification will be discussed.

Biologically Relevant "Retro-Inverso" Peptidomimetics

At the present time there is rapid growth in the number of endogenous and exogenous biologically active peptides under investigation. Most of these peptides are short-lived molecules, degraded easily by enzymes, with little or no therapeutic use. To convert these molecules into useful drugs it is often necessary to transform the candidate compounds into peptidomimetics.

To date, the retro-inverso transformation remains an important pseudopeptidic modification undertaken by numerous bioorganic chemists. Since our last review

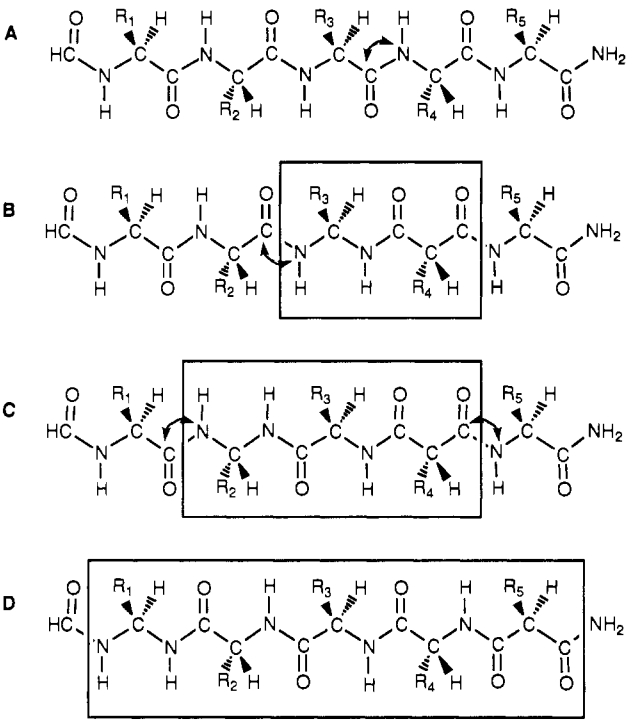

Figure 1. Schematic presentation of (A) an extended end-group-blocked pentapeptide, (B and C) its partial retro-inverso isomers, and (D) an end-group-modified retro-inverso analog. The reversal of a single peptide bond (B) (between residues with side chains R_3 and R_4) results in the formation of a *gem*-diaminoalkyl residue (containing R_3 alkyl) and a malonyl residue (containing R_4 alkyl). Reversal of two consecutive peptide bonds (C) (between residues with side chains R_2–R_4) results in addition to the *gem*-diaminoalkyl residue (containing R_2 alkyl) and the malonyl residue (containing R_4 alkyl) also in a D-amino acid residue incorporated in the reversed sense of direction (residue containing R_3 side chain). Reversal of all the peptide bonds (D) can be accomplished (without swapping amino and carboxy end groups) only in an end-group-blocked peptide.

of the early stages of this field, many PMRI analogs of peptides were synthesized and tested.[2-51] Table I

Michael Chorev graduated from The Hebrew University of Jerusalem, receiving a Ph.D. degree in 1976. After a postdoctoral fellowship at the University of California, San Diego, he joined the Faculty of Medicine at The Hebrew University of Jerusalem, School of Pharmacy, where he is now Associate Professor of Pharmaceutical Chemistry. His research interests have been directed toward structure–activity relationship studies of neuropeptides and calciotrophic peptide hormones, development of novel pseudopeptides, and cultivating approaches to transform a bioactive peptide into a nonpeptidic peptidomimetic structure based on topological criteria.

Murray Goodman was born in New York City and received his Ph.D. degree from the University of California, Berkeley. After completing postdoctoral research at the Massachusetts Institute of Technology and at Cambridge University, he joined the faculty of the Polytechnic Institute of Brooklyn. Since 1970 he has been Professor of Chemistry at the University of California, San Diego. His current research focuses on rational drug design, molecular modeling, peptide synthesis, and peptidomimetic structures for bioactive molecules.

† The following are the nonstandard abbreviations used in this Account: A_2Bu, α,γ-diaminobutyric acid; *c*[...], the enclosed sequence forms a cyclic structure; *g*Xaa, *gem*-diaminoalkyl analog of the indicated amino acid residue; HOBt, 1-hydroxybenzotriazole; HONSu, *N*-hydroxysuccinimide; Met(O), methionine sulfoxide; *m*Xaa, malonyl residue corresponding to the indicated amino acid residue; *r*Xaa, reversed residue of the indicated amino acid, namely, -COCHRNH-, from the conventional -NHCHRCO- direction.

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Table I. Summary List of Retro-Inverso Peptides Arranged According to Families of the Bioactive Peptides and the Corresponding References

family of bioactive peptides	ref
atrial natriuretic factor (ANF)	2
bombesin	3
bradykinin potentiating peptide (BPP)	4–7
cholecystokinin (CCK)	8–13
dermorphin	14–16
enkephalin	17–23
gastrin	24
glutathione	25
luteinizing hormone-releasing hormone (LHRH)	26
neurotensin	27
peptide sweetener	28–30
protein kinase C pseudosubstrate[a]	31
proteinase inhibitors	32–37
somatostatin[b]	38–42
substance P	43–48
thymopoietin	49
tuftsin	50–51

[a] End-blocked end-to-end retro-inverso peptide. [b] Analogs described in refs 41 and 42 are cyclo-retro-inverso isomers.

contains a list of the families of bioactive retro-inverso-modified peptides published to date. We cannot discuss all of the families shown but will deal with some important representative groups of biologically active molecules. The remarkable resistance of PMRI tuftsin analogs[51] to proteolytic degradation combined with retention of high biological activity following oral or intravenous administration provides a strong impetus for the continuing efforts on this modification.

Opioid Peptides, Substance P, and Enkephalinase Inhibitors. The partially modified retro-inverso analogs of enkephalin, modified at the C-terminus, were the very first examples of highly active analogs reported in this field of pseudopeptides.[22] These linear compounds were 2–15 times as active as methionine enkephalin and had a much longer duration of action, indicating that the retro-inverso linkages protect the peptide from hydrolysis and enzymatic attack. The modifications that followed included the Gly[3]-Phy[4]-Leu[5] sequence in either the linear[18,19] form or its sterically constrained 14-membered cyclic analog c[A_2bu[2],Leu[5]]enkephalin.[19–21] In vitro, both the linear analog, [*g*Gly[3],(*R*,*S*)-*m*Phe[4],Leu[5]]enkephalinamide, and

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the cyclic analog, c[D-A_2bu^2,gGly^3,(R,S)-mPhe^4,Leu^5]-enkephalin, had significantly reduced potencies at the guinea pig ileum (GPI) (<1% of Leu enkephalin).[19,21] Remarkable enhancement of potency and selectivity toward the μ-receptor was observed in c[D-Glu^2,gLeu^5]- and c[D-Glu^2,gPhe^4,D-mLeu^5]enkephalin analogs as compared to their corresponding parent cyclic peptides.[20] The separable isomers of c[D-A_2bu^2,gPhe^4,-(R,S)-mLeu^5]enkephalin were markedly different in potency and selectivity. The isomer containing the (S)-mLeu residue was 16-fold more active in the GPI assay than the isomer containing the (R)-mLeu, which led to about a 9-fold increase in selectivity toward the μ-receptor.[20]

For dermorphins, opioid heptapeptides isolated from the skin of South American frogs,[14,15] only the C-terminal partially modified retro-inverso analogs, H-Tyr-D-Ala-Phe-gGly-X, were found to be more active than the natural peptide in the GPI assay.[14] The X corresponds to either (S)-$COCH(CH_3)C_6H_5$ or adamantoyl.

Substance P (SP), the mammalian endogenous neuropeptide involved in sensory transmission, was extensively studied using retro-inverso modifications.[43,45-48] As in other systems, end-group-modified retro-inversion of SP(4–11) resulted in dramatic loss of potency (from ED_{50} = 1.4 nM to 4.8 μM).[47] The revesal of single peptide bonds in the fully active C-terminal SP-related hexapeptide [pGlu^6]SP(6–11) resulted in [pGlu^6,gPhe^8,mGly^9]SP(6–11). This analog maintained substantial activity in the GPI and parotid slice systems (22% and 15% of the parent peptide, respectively)[43] while [pGlu^6,gPhe^7,(R,S)-mPhe^8]SP(6–11) displayed only 7% of the potency of the parent peptide.[45] In addition, enhanced metabolic stability was observed for PMRI analogs of SP as compared to the parent peptide. For example, [pGlu^6,gPhe^8,mGly^9]SP(6–11) is completely stable to incubation for 1 h (at 37 °C) in the presence of either rat parotid and hypothalamic slices or pronase compared with about 75% degradation of the parent peptide in the slice systems and 90% degradation after 45 min in the presence of pronase.[48]

A different approach to the development of novel analgesics employing the retro-inverso modification focuses on the design of highly potent and selective inhibitors of enkephalinase. Thiorphan, N-[(R,S)-3-mercapto-2-benzylpropanoyl]glycine, is a highly potent inhibitor (K_i = 3.5 nM) of enkephalinase.[32] Thiorphan also inhibits angiotensin-converting enzyme (ACE) (K_i = 140 nM), a widely distributed metallodipeptidyl carboxypeptidase involved in the release of a potent hypertensive hormone from its nonactive precursor. Dramatic improvement in selectivity toward inhibition of enkephalinase was achieved by the synthesis of the retro-inverso thiorphan: (R,S)-$HSCH_2CH(CH_2C_6H_5)NHCOCH_2CO_2H$.[32] Retro-thiorphan retains its potency toward enkephalinase (K_i = 6 nM), but is about 40-fold less effective toward inhibition of ACE (IC_{50} > 10 000 nM).

Roques applied retro-inversion to kelatorphan, $HONHCOCH_2CH(CH_2C_6H_5)CO$-Ala-OH, a bidentate inhibitor which completely inhibits all zinc metallopeptidases involved in the in vitro degradation of enkephalin.[33] As was the case with retro-thiorphan, an analog of retro-kelatorphan, namely, $HONHCOCH_2CH(CH_2C_6H_5)NHCOCH(CH_3)CO_2H$, was as potent as kelatorphan but much more selective toward neutral endopeptidase 24–11. The retro-hydroxamate $HONHCOCH_2CH(CH_2C_6H_5)NHCOCH(CH_2C_6H_5)CH_2CO_2H$ is one of the most active inhibitors of the three enkephalin-degrading enzymes.[34]

Somatostatin. In an attempt to extend the very short in vivo half-life (plasma $t_{1/2}$ = 2.3 min) of somatostatin, a potent inhibitor of hormones such as glucagon, growth hormone, insulin, and gastrin, the retro-inverso modification was applied to the putative cleavage sites, namely, residues 6–11.[40] The extended partially modified retro-inverso analog [Ala^4,gPhe^6,D-rPhe^7,D-rTrp^8,D-rLys^9,D-rThr^{10},(R,S)-mPhe^{11}]-somatostatin was devoid of any biological activity.[40] Introducing retro-inversion at single peptide bonds led to analogs [gPhe^7,(R,S)-mTrp^8]- and [gTrp^8,(R,S)-mLys^9]somatostatin.[38] Only the former analog maintained significant activity (12% and 75% of the in vitro potency of somatostatin for the fast or slow eluting components on reversed-phase high-pressure liquid chromatography (RP-HPLC), respectively). The lack of activity of PMRI analogs containing gTrp^8-mLys^9 was attributed to the role of these residues as i + 1 and i + 2 residues, respectively, of a type II′ β-turn which is part of the putative bioactive conformation.

Retro-inverso modifications of cyclic hexa- and octapeptides related to somatostatin were carried out.[41,42] For example, the retro-inverso compound c[D-N-MePhe-D-Thr-D-Lys-L-Trp-D-Phe-D-Ala] exhibits 25% of the potency of the parent cyclic hexapeptide c[N-Me-Ala-Phe-D-Trp-Lys-Thr-Phe].[42] The retro-inverso octapeptide H-D-Thr-c[D-Cys-D-Thr-D-Lys-Trp-D-Phe-D-Cys]-Phe-NH_2 retained very low in vitro activity (less than 0.001%) but significant in vivo activity (about 50%) compared to the parent peptide H-D-Phe-c[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr(ol).[41]

Partial retro-inverso modifications introduced into the cyclic hexapeptide related to somatostatin, c[Pro^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}-Phe^{11}] (the numbering of residues relates to the corresponding positions in the parent somatostatin) resulted in a series of analogs containing gSar^6-(R,S)-mPhe^7, gVal^{10}-(R,S)-mPhe^{11}, and gPhe^{11}-(R,S)-mAla^6 which belong to the so-called bridging domain (residues 7–10).[39] The analog c[gSar^6-(S)-mPhe^7-D-Trp-Lys-Thr-Phe] was active (about 50% of the potency in inhibiting the release of growth hormone assigned for the most closely related cyclic hexapeptide, c[Pro-Phe-D-Trp-Lys-Thr-Phe]) while the R isomer was inactive.[39] The configurational stability of the mPhe^7 residue demonstrated the dramatic differences in potency as a function of chirality of the malonyl residue leading to changes affecting conformations essential for bioactivity.[52]

Gastrin and Cholecystokinin. The C-terminal end-group-modified partial retro-inverso analogs of tetragastrin were found to be potent antagonists of gastrin activity.[24] Substitution of Asp-Phe-NH_2 in Boc-Trp-Met-Asp-Phe-NH_2 with either gAsp-$CO(CH_2)_2C_6H_5$ or gAsp-(R,S)-mPhe-NH_2 or extending the modification by substituting Met-Asp-Phe-NH_2 with gLeu-D-rAsp-(R,S)-mPhe-NH_2 led to potent in vivo activities in rats (0.15–0.3 μmol/kg). In each of these examples, partial

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retro-inverso modification of the Asp-Phe peptide bond allowed an efficient dissociation of efficacy and receptor affinity.

A series of PMRI CCK-8 related analogs was studied by Martinez and co-workers.[8-11] Reversal of Nle[28]-Gly[29], a major site of cleavage by a thiol protease, in either Boc[Nle[28,31]]CCK-8 or Boc[Nle[28],*N*-Me-Nle[31]]CCK-8 maintained affinities to the CNS CCK receptor (K_i in the subnanomolar range), but resulted in a substantial loss of affinities to the peripheral CCK receptor and very low potencies in the functional in vitro assays.[10,12] The N-methylated PMRI analog was very resistant to degradation by rat brain homogenate and ≈400-fold more selective toward the central CCK receptor.[11]

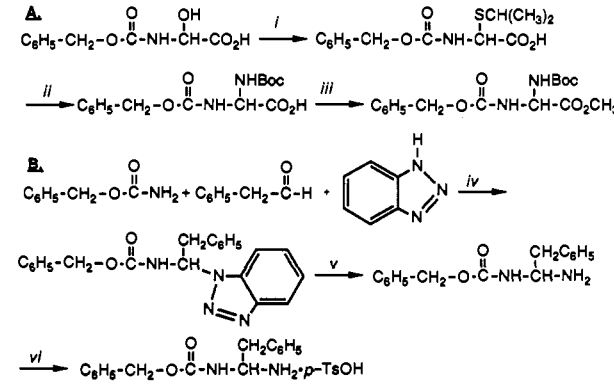
Advances in Chemistry

Methodology in Solution. Much research has concentrated on the optimization of synthetic routes to prepare the non-amino acid residues required for the retro-inverso modifications. Early synthetic preparations of *gem*-diaminoalkyl derivatives utilized the classical Curtius rearrangement.[53-55] These early methods produced isocyanates which were trapped by alcohols to produce urethane-type protecting groups.[54] In the Goldschmidt and Wick type reaction[55] the isocyanate reacts with either an excess of malonic acid or N-protected amino acid to produce the retro-inverso peptide bond surrogate and the pseudopeptidic unit.[26]

We have carried out comparative studies on the different approaches to the Curtius rearrangement and studied conditions to optimize the yield of the N,N'-diacylated *gem*-diaminoalkyl residue.[53-56] The most frequently employed procedure in the synthesis of *gem*-diaminoalkyl derivatives subjects N-protected peptidyl or aminoacyl carboxamide derivatives to the Hofmann-type rearrangement using the mild oxidizing reagent iodobenzene bis(trifluoroacetate) (IBTFA).[57,58] This route leads to high yields of mono-N-acylated optically pure *gem*-diaminoalkyl trifluoroacetates[59] which can be coupled to a malonic acid derivative, thereby extending the pseudopeptide toward the C-terminus.[60] Treatment of a malonylaminoacyl amide moiety with IBTFA will yield the pseudopeptidic unit directly: the (*gem*-diaminoalkyl)malonyl fragment. The non-peptidyl residues are linked together in a form to be extended toward the N-terminus.

The use of IBTFA in the oxidative rearrangement of carboxamides containing Gln, Asn,[61] and oxidizable amino acid residues such as tyrosine, tryptophan, and methionine is not suitable. Instead, appropriately protected derivatives such as Tyr(O-tBu), N^{in}-For-Trp, and Met(O) must be used to avoid side reactions.[16,60,62] Treatment of *N*-[(2-methyl-2-nitrophenoxy)propionyl]-*O*-*tert*-butylthreonine amide [MNP-Thr(tBu)-NH_2] with IBTFA led to the removal of the *tert*-butyl ether and formation of the corresponding 2-oxazolidone.[63] To avoid this problem a new procedure was developed in which Curtius rearrangement of the MNP-Thr(tBu)-N_3 resulted in the isocyanate, which was trapped in situ by thiophenol. Mild alkaline hydrolysis of the phenylthiocarbonyl protecting group yielded the anticipated MNP-*g*Thr(tBu)H.[63] The latter procedure provides the means to transform Thr and Ile, both containing a chiral β-carbon, to the topochemically equivalent *gem*-diaminoalkyl residue.

Scheme I[a]



[a] (i) $(CH_3)_2CHSH$, H_2SO_4 (cat.), AcOH; (ii) $(CH_3)_3COCONH_2$, Hg^{2+}/THF; (iii) CH_3I, K_2CO_3/DMF; (iv) *p*-TsOH (cat.)/$C_6H_5CH_3$; (v) K_2CO_3, NH_3/MeOH; (vi) *p*-TsOH/ether.

Comparison of different coupling methods to accomplish amide bond formation between a monoacylated *gem*-diaminoalkyl and a monoamidated malonic acid established HOBt- or HONSu-mediated DCC coupling to be superior to the mixed-anhydride method.[53,59] The lower yields found in the mixed-anhydride coupling resulted from the condensation between the alcohol (which arises from the chloroformate) and the malonic acid residue.[59]

Another synthetic approach to prepare a differentially protected α-aminoglycine involves amidoalkylation of 2-propanethiol, followed by Hg^{2+}-mediated replacement of the isopropylthio group by a *tert*-butyl carbamate yielding *N*-(benzyloxycarbonyl)-*N'*-(*tert*-butyloxycarbonyl)-α,α-diaminoacetic acid in 70% overall yield (see Scheme I, part A).[64] Katritzky and co-workers[65] showed that the Mannich reaction of various amides, aldehydes, and benzotriazole yields an intermediate (70%) which upon reaction with ammonia results in racemic monoacylaminals of the general structure R'CONHCHR''NH_2.[65] Using either N-acylated or N-protected amino acid amides in this synthetic procedure leads to the corresponding N-protected or N-acylated aminoacylaminal (see Scheme I, part B).

In addition to the direct reaction of isocyanates with an excess of malonic acid, indirect routes to provide

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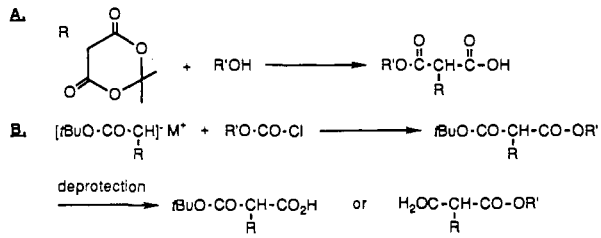
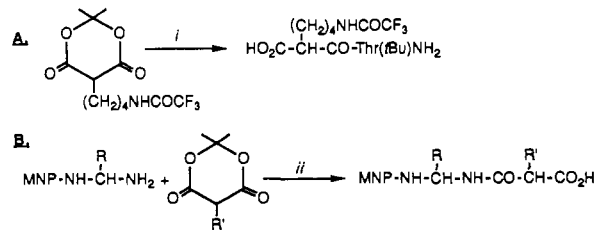
(65) Katritzky, A. R.; Urogdi, L.; Mayence, A. *J. Org. Chem.* **1990**, *55*, 2206-2214.

Scheme II



Scheme III[a]



[a] (i) H-Thr(tBu)NH_2, BTMSA; (ii) BTMSA.

intermediates for the incorporation of the malonyl moiety into the pseudopeptidic structure were developed (see Scheme II). Monoalkyl malonates were obtained by partial hydrolysis of homodialkyl malonates and alcoholysis of Meldrum's acids (see Scheme II, part A).[43] Acylation at the α-carbon of the enolate salt of *tert*-butyl acetate by either methyl or benzyl haloformate yields heterodialkyl malonates which upon deprotection give the monoalkyl malonates (see Scheme II, part B).

Rigo and co-workers[66] prepared malonic acid monoamides from monosubstituted Meldrum's acids and amines in the presence of *O,N*-bis(trimethylsilyl) acetamide (BTMSA). Recently, this method was applied by Verdini and colleagues toward the preparation of a PMRI tuftsin analog (see Scheme III, part A).[50,51] In the presence of BTMSA the keto–enol equilibrium of monosubstituted Meldrum's acids shifts toward the reactive keto form. Substituted Meldrum's acid derivatives were shown to N-acylate monoacylaminals, amino acid derivatives, and peptides directly (see Scheme III, part B).

Solid-Phase Methodology. Verdini and his colleagues were the first to employ solid-phase methodology in the synthesis of PMRI peptide analogs.[44] They utilized their approach for the synthesis of [pGlu^6, *g*Phe^8,*m*Gly^9]substance P(6–11). The coupling was carried out on a polydimethylacrylamide resin utilizing $N\alpha$-Fmoc protection and symmetrical anhydrides for activation. The pseudopeptide unit was constructed through fragment condensation of a malonyl-D-amino acid amide to the growing resin-bound peptide. The oxidative Hofmann-type rearrangement employing IBTFA was carried out directly on the N-terminal carboxamide function.[44] In a similar manner [*g*Lys^6,-(*R,S*)-*m*Phe^7,Ala^8]bradykinin potentiating peptide $(BPP)_{9a}$ was prepared.[4]

Two new synthetic manipulations have been introduced by Verdini and co-workers to avoid side reactions caused by the presence of residues such as Trp, Tyr, Cys, Met, Asn, and Gln in the resin-bound malonyl-containing pseudopeptide precursors and to directly anchor *N*-acyl protected retro-inverso pseudopeptides to polyamide resins.[63,67] The *N*-[(2-methyl-2-nitrophenoxy)propionyl]aminoacyl amides (MNP-NHCHR-$CONH_2$) were found to be suitable precursors for the preparation of stable monoacyl *gem*-diamines via IBTFA treatment. These MNP-$NHCHRNH_2$·TFA salts can be coupled to monoester monoacid malonates or allowed to react with Meldrum's acid derivatives following in situ trimethylsilylation with BTMSA (see Scheme III) to yield the *N*-MNP protected retro-inverso pseudodipeptides. These units could then be used as synthons for the extension of the N-terminus of a resin-bound peptide. The removal of the amino terminal MNP protecting group can be accomplished by a mild treatment with DMF solution of $SnCl_2 \cdot H_2O$ at room temperature. Difficulties associated with anchoring of malonyl residues, such as *N*-MNP-*g*Ile-(*R,S*)-*m*Leu-OH, to a 4-alkoxybenzyl alcohol linker on the polyamide resin were overcome by activation with *N*-methylimidazole and bis(trichloromethyl)carbonate.[27]

Configurational Aspects of the Retro-Inverso Modifications

The *gem*-diaminoalkyl residues are derived from amino acid precursors and are generally configurationally stable.[59] However, deprotection of *N,N'*-bis-(benzyloxycarbonyl)-5-aminopyrrolidin-2-one by heterogeneous catalytic hydrogenation resulted in the racemization of the *gem*-diaminoalkyl residue.[68] Racemization was avoided by employing a catalytic transfer hydrogenation procedure, reconfirming that the configurational integrity of the asymmetric Cα of the amino acid residue is maintained during transformation to the corresponding N,N'-bis(acylated) *gem*-diaminoalkyl derivative.[59]

The synthesis of the optically pure pseudopeptides containing alkylmalonamide residues requires the separation of diastereomers and assignment of the absolute configuration at the malonyl center. Configurational lability of the bis(amidated) malonyl residue during the synthetic manipulation prevents separation of the diastereomeric mixture of the final PMRI analogs. Once configurational stability of the malonyl residue is established, resolution, configurational assignment, and biological characterization of each diastereomer become feasible.

Reversed-phase HPLC has been used to separate the diastereomers and to monitor the kinetics of racemization of the asymmetric bis(amidated) malonyl residue under a variety of experimental conditions.[18,19,38,45,60,69,70] The half-lives reported for the isomerization of the diastereomers of Tyr-D-Ala-*g*Gly-(*R,S*)-*m*Phe-Leu-NH_2 in ammonium acetate buffer at pH 7 and 34 °C are 6.2 and 8.9 h for the slow- and fast-eluting components in the RP-HPLC, respectively, leading to about a 65:35 equilibrium mixture of diastereomers.[18] In contrast, under similar conditions,

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(67) Gazerro, L.; Pinori, M.; Verdini, A. S. In *Innovation and perspectives in solid phase synthesis*; Epton, R., Ed.; SPCC (UK) Ltd: Birmingham, 1990; pp 403–412.

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diastereomers of the cyclic enkephalin analog, H-Tyr-*c*[D-A_2bu-*g*Gly-(*R*,*S*)-*m*Phe-Leu], were configurationally stable even after 2 days.[19] In the case of [pGlu^6,*g*Phe^7,(*R*,*S*)-*m*Phe^8]substance P(6-11), fast equilibration of the separated diastereomers was observed at 40 °C with half-lives of 45 and 56 min for the slow- and fast-moving components in the RP-HPLC, respectively.[45] Thus, although both the linear and the cyclic analogs incorporate the same malonyl residue (2-benzylmalonyl), they differ markedly in their configurational stability.

In several cases, configurational assignment was achieved by converting the malonyl amide group into the corresponding amino acid residue. For example, the phenylalanylvaline dipeptides prepared from the RP-HPLC resolved epimers of 2-benzylmalonamidyl-L-valine by treatment with IBTFA were compared with authentic dipeptides obtained through standard coupling procedures.[70] A similar approach was undertaken to assign absolute configurations of PMRI analogs of a bradykinin potentiating peptide 5a (BPP_{5a})[5] and retrothiorphan derivatives.[69]

Two tentative indicators of configurational content of the retro-inverso unit are suggested: (1) The retention time (on RP-HPLC) of diastereomers in which the hydrophobic substituents on asymmetric carbons are in the "syn" orientation, as in D,L or L,D dipeptides, are longer than for the "anti" diastereomers (L,L or D,D).[19,71] (2) The deshielding effect of the side-chain methyl group (e.g. Ala) when it occurs adjacent to a benzyl side chain (e.g. Phe) is greater when the α-carbons carrying these side chains are of opposite absolute configurations.[69,72]

Configurational assignments in the cases of PMRI somatostatins[39] and cyclic PMRI enkephalins[73] were based on resistance to proteolytic degradation and ^{1}H NMR. For example, the chemical shift of *m*Leu γ-CH in H-Tyr-*c*[D-A_2bu-Gly-*g*Phe-(*R*,*S*)-*m*Leu] was found to be 1.04 and 1.37 ppm for the (*R*)- and (*S*)-*m*Leu, respectively.[73]

Recently, it was suggested that a general unambiguous assignment of absolute configuration for malonyl residues in PMRI analogs could be achieved by measuring the sequential NOE between NH_i and $C\alpha H_{i-1}$ and the NOE and coupling constants between vicinal protons H–N–Cα–H.[74] This approach was successfully utilized to assign the absolute configuration of the *m*Leu residue in the superactive and nonactive diastereomers of the cyclic PMRI enkephalin analog H-Tyr-*c*[D-A_2bu-Gly-*g*Phe-(*R*,*S*)-*m*Leu] (see Figure 2).[74]

Since isomerization occurs under both neutral and basic conditions, it is likely that a general acid–base catalysis involves a coplanar enol structure. Steric hindrance that prevents the coplanar arrangement or blocks effective hydration may enhance the configurational stability.[45]

Conformational Aspects of the Retro-Inverso Modifications

The original predictions of conformational properties of retro-inverso peptides were established through

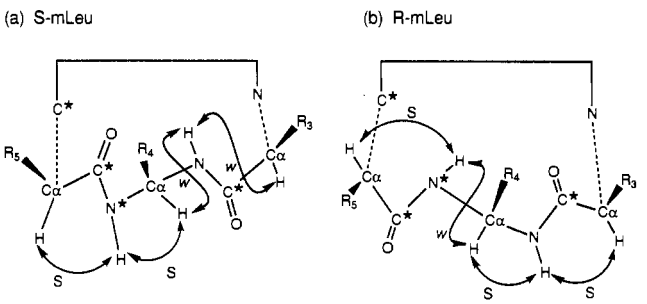

Figure 2. Assignment of absolute configuration of 2-substituted malonyl residue in cyclic dermorphin analogs (a) Tyr-*c*[D-A_2bu-Phe-*g*Phe-(*S*)-*m*Leu] and (b) Tyr-*c*[D-A_2bu-Phe-*g*Phe-(*R*)-*m*Leu]. Analysis of NOEs around the Phe-*g*Phe-*m*Leu fragment characteristic of the stereochemical disposition of the malonyl Cα-H in the *m*Leu residue relative to adjacent backbone protons was performed. The NOEs observed for atomic groupings are shown in the figures: S, strong; W, weak. The vicinal coupling constants in the *g*Phe residue were J_{NH-CH} (*g*Phe NH-α) 8.63 and 6.47 Hz and J_{NH-CH} (*g*Phe N*H-α) 7.21 and 7.52 Hz for a and b, respectively.

either empirical force field calculations[75] or ab initio studies[76] of basic model compound units. These studies predicted that contrary to the C_7^{eq} low energy conformation preferred by amino acid derivatives, the *gem*-diaminoalkyl and 2-alkylmalonyl residues have their lowest energy minima in the "α-helical regions". X-ray analysis revealed that the observed conformations of model compounds such as tBuCO-*g*Val-COtBu and Ac-*g*Gly-Ac[77,78] correspond to a saddle point on the energy map.[76] The crystal structure of MeNH-*m*Val-NHMe[79] is very similar to the global minimal a_R conformation calculated by Stern and co-workers.[76] Recently Osguthorpe and co-workers studied the structural implications of the conformational preferences of the *gem*-diaminoalkyl and 2-alkylmalonyl residues on different secondary structures.[80] When *g*Ala and *m*Ala are in the $i + 1$ and $i + 2$ positions, respectively, the lowest energy of the global minima of the isolated residues allows the formation of a type II β-turn.[80] The reversal of a single peptide bond in an α-helical array results in the disruption of the hydrogen-bonding network. In this case, additional destabilization arises from the repulsive interactions between two proximal carbonyls and two proximal NH groups.[80] Reversal of every second amide bond allows the construction of fully hydrogen bonded parallel and antiparallel β-sheets.[80]

Analysis by X-ray diffraction of model structures such as MeNH-*m*Gly-Gly-NHMe and tBuCO-*g*Ala-*m*Gly-NHiPr reveals that reversal of the amide bond results in an extended structure in which the amide bond is almost trans-planar and is involved only in intermolecular hydrogen bonds.[81,82] The conformations of *g*Ala

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(81) El Masdouri, L.; Aubry, A.; Gomez, E. J.; Vitoux, B.; Marraud, M. *J. Chim. Physique* **1988**, *85*, 583–588.
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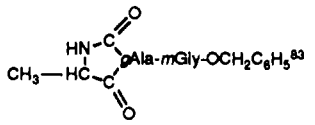
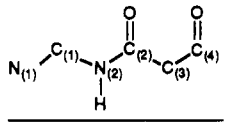
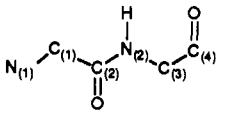
Table II. Bond Lengths and Bond Angles for the Retro-Inverso Unit in

(A) Boc-*g*Ala-*m*Gly-NHiPr[82]

(B) 





	A	B	A* [a]
	Bond Lengths (Å)		
N(1)–C(1)	1.448	1.487	1.459
C(1)–N(2)	1.456	1.430	1.524[b]
N(2)–C(2)	1.337	1.341	1.348
C(2)–C(3)	1.506	1.489	1.439[c]
C(3)–C(4)	1.510	1.542	1.508
	Bond Angles (°)		
N(1)–C(1)–N(2)	111.0	110.3	110.6[d]
C(1)–N(2)–C(2)	123.6	123.1	114.9[e]
N(2)–C(2)–C(3)	115.0	115.3	121.6[f]
C(2)–C(3)–C(4)	113.9	111.5	115.6[g]

[a] A* is the parent peptide of (A). [b] $C_{(1)}$–$C_{(2)}$. [c] $N_{(2)}$–$C_{(3)}$. [d] $N_{(1)}$–$C_{(1)}$–$C_{(2)}$. [e] $C_{(1)}$–$C_{(2)}$–$N_{(2)}$. [f] $C_{(2)}$–$N_{(2)}$–$C_{(3)}$. [g] $N_{(2)}$–$C_{(3)}$–$C_{(4)}$.

and *m*Gly residues in the crystal are 2.5 and 5 kcal/mol, respectively, above the minimum calculated by Stern and associates.[75] Table II lists the bond length, bond angles, and dihedral angles of the retro-inverso units as determined by X-ray analysis of two single-crystal structures.[82,83]

The conformational properties of the end-group-blocked dipeptide are different from those of the corresponding PMRI isomers.[82] The β-turn characteristic of the parent peptide is disrupted upon reversal of amide bonds at either the C- or N-terminus. However, reversal of the central amide bond does not affect the propensity for a β-turn. In dichloromethane, a very weakly hydrogen bonding solvent, $Me_2NHCOCH$-(R)CO-Gly-$NHCH_3$ forms 9-membered hydrogen-bonded rings which have a higher degree of linearity of the hydrogen bond than either the 6- or 7-membered hydrogen-bonded rings.[84] Further studies on a variety of model systems must be undertaken to understand in detail the effect of retro-inverso modification on conformation.[85]

PMRI structures, very often, closely mimic the structural features of the corresponding parent peptides. The PMRI dipeptide sweetener *N*-(L-aspartyl)-*N'*-(2,2,5,5-tetramethylcyclopentanylcarbonyl)-(*R*)-1,1-diaminoethane and its L-*S*-isomer, which are diacylaminals, were reported to be as sweet as their corresponding Asp-D-alanine amide analog. The minimum-energy conformations (calculated using flexible geometry approximations) and the NMR studies, in DMSO-d_6, of the L-*S* and L-*R* PMRI dipeptide sweeteners suggest close resemblance of the former and very high similarity of the latter to the parent L,D-dipeptide amide sweetener.[28] In the solid state, X-ray analysis of a cocrystal of L-*S* and L-*R* PMRI dipeptide sweeteners indicates an overall topological resemblance to the "L-shaped" postulated bioactive conformation found in solution and by computer simulations.[86] Only minor rotations around two backbone torsion angles are needed to transform the solid-state structures into the conformation found in solution.

Conformational analysis of constrained cyclic PMRI analogs derived from H-Tyr-*c*(D-A_2bu-Gly-Phe-Leu),[20,21] a highly potent μ-selective 14-membered cyclic enkephalin analog,[87,88] allowed further evaluation of the conformational changes induced by the retro-inverso modification.[73,89] Molecular dynamics and energy minimization studies indicate that the frequency of C_6- or C_8-hydrogen-bonded rings in the cyclic PMRI enkephalin analogs is smaller than in the parent cyclic peptide.[89] For example, energy minimization suggests that only two out of the six minimum energy conformations of H-Tyr-*c*(D-A_2bu-*g*Gly-D-*m*Phe-Leu) contain a C_6-hydrogen-bonded ring formed between CO^{I} and NH^{II} in the modified portion, -NH^{I}-CH_2NH^{II}-CO^{II}-CH-($CH_2C_6H_5$)-CO^{I}-.[89] Conformational analysis by ^{1}H NMR of the same enkephalin analog could not confirm the presence of any conformation containing either C_6- or C_8-hydrogen-bonded rings.[73] Molecular dynamics simulations suggest no significant differences in the relative mobility and the correlated movements of pairs of dihedral angles (ψ_i,φ_{i+1}) of the *gem*-diaminoalkyl and 2-alkylmalonyl residues as compared to normal amino acids. These studies reveal that retro-inverso residues in cyclic PMRI peptides adopt α_L and α_R conformations[53] as opposed to C_7^{eq} and C_7^{ax} conformations typical for amino acid residues in their lowest energy minima.[75,76]

The difference in the biological activity of epimeric cyclic PMRI enkephalins is attributed to the different orientation of the side chain of their malonyl residues of opposite configurations. For example, side-chain orientation of the *m*Leu residue in H-Tyr-*c*[D-A_2bu-Gly-*g*Phe-(*R*,*S*)-*m*Leu] renders the *S* isomer 17-fold more potent than the *R* isomer in the electrically induced GPI assay.[20] The (*S*)- and (*R*)-*m*Leu residues topologically resemble the L- and D-Leu, respectively.[89] Furthermore, ^{1}H NMR studies in DMSO-d_6 of isomers of H-Tyr-*c*[D-A_2bu-*g*Gly-(*R*,*S*)-*m*Phe-Leu], a weakly potent enkaphalin analog, reveal that the position of the *m*Phe phenyl ring relative to the rest of the molecule is very different in both diastereomers.[73] Only one C_7-hydrogen-bonded ring was found in the preferred conformation of each of the cyclic PMRI enkephalin analogs, Tyr-*c*[D-Glu-Gly-Phe-*g*Leu], Tyr-*c*[D-A_2bu-Gly-*g*Phe-(*R*,*S*)-*m*Leu], and Tyr-*c*[D-A_2bu-*g*Gly-(*R*,*S*)-*m*Phe-Leu], as compared to two C_7 structures in the parent cyclic enkephalin analog H-Tyr-*c*[D-A_2bu-Gly-Phe-Leu] (i.e., $Gly^3CO\cdots HN\text{-}Leu^5$ and $A_2bu^2CO\cdots HN\gamma\text{-}A_2bu^2$).[90]

Only one isomer in each pair of diastereomeric cyclic PMRI dermorphin analogs, Tyr-*c*[D-A_2bu-Phe-*g*Phe-(*R* and *S*)-*m*Leu] and Tyr-*c*[D-Glu-Phe-*g*Phe-(D and

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L)-*r*Leu], exhibits cis–trans isomerization of the amide bond in Phe-*g*Phe.[91] This isomerization is attributed to the effect of the chirality of the residue in the fifth position.

In a similar manner, conformational analyses employing NMR and molecular dynamics studies were carried out on cyclic PMRI somatostatin analogs derived from the highly potent cyclic hexapeptide *c*[Pro^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}-Phe^{11}].[89] In this hexapeptide, the tetrapeptide Phe^7-D-Trp^8-Lys^9-Thr^{10} is considered to be the biologically important sequence forming a type II′ β-turn. The Phe^{11}-Pro^6 acts as a bridging region locking the tetrapeptide in the proper orientations.[92] The retro-inverso modifications were carried out within the "bridging region".[39] Partial retro-inverso modifications at either end of the tetrapeptide, Phe^7-D-Trp-Lys-Thr^{10}, or at the "bridging region" did not disrupt the type II′ β-turn. Studies of conformation–activity relationships in this series provided further support that close spatial proximity between the Lys and D-Trp side chains is not a sufficient condition from biological activity. Therefore, it was concluded that in the "bioactive conformation", the "bridging region" must involve a cis peptide bond in order to maintain the type II′ β-turn. The structural framework provides constraints that bring together the side chains of D-Trp^8 and Phe^7 residues and hold them in close proximity to the Lys side chain.[52]

Recently, Roques and co-workers reported that close topochemical resemblance exists between a peptidomimetic structure and its corresponding retro-inverso analog.[32] Thiorphan– and retro-thiorphan–thermolysin complexes were compared by modeling studies[93] and by X-ray analysis[94] (see Figure 3). The very similar disposition of thiorphan and retro-thiorphan in the active site resulted in equivalent hydrogen bond interactions of Arg^{203} and Asn^{112} with either the amide or the reversed-amide groups.

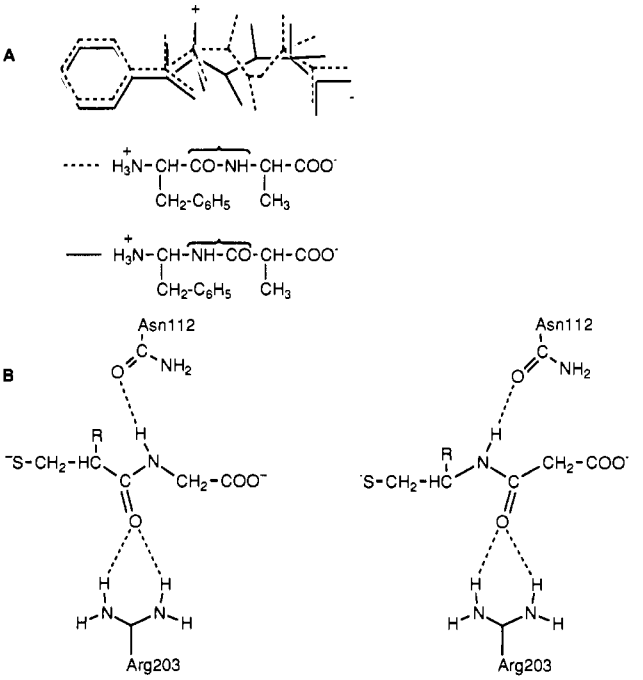

Figure 3. Structural comparisons between dipeptide inhibitor of enkephalinase and its retro-inverso analog and between thiorphan and retro-thiorphan. (A) Topological analogy achieved by superposition of H-Phe-Ala-OH and its retro-inverso analog showing the equivalence in the spatial disposition of the hydrogens and carbonyl oxygens of the amide and retro-amide bonds. (B) Schematic illustration showing the close similarity among the hydrogen bond lengths in the enzyme–inhibitor complexes formed between thiorphan or retro-thiorphan and thermolysin.

Conclusions

This Account describes the biological activities, synthesis, and conformational analysis of molecules incorporating the retro-inverso peptide bond modification. Retro-inverso bonds are the most closely related isosteric replacement for the original peptide bond. This modification maintains the major characteristics of the peptide backbone while enabling (or providing) a substantial departure from the native structure. Therefore, the potential role of partially modified retro-inverso (PMRI) analogs in future peptide-based drug design will be to provide a modification which maintains the polarity and rigidity of a peptidic backbone and contributes its unique conformational preferences and topochemical consequences. Extensive conformational analysis of many constrained retro-inverso analogs of bioactive peptides will significantly contribute to the rational design of peptidomimetic drugs. Most of the PMRI analogs studied displayed not only stability toward enzymatic degradation and bioavailability but also improved potency and selectivity. As such, the retro-inverso modifications of bioactive molecules remain a major challenge for bioorganic chemists. It is clear from this Account that many additional bioactive peptides will be modified using retro-inverso structures.

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