Partially Modified Retro-Inverso Peptides: Development, Synthesis, and Conformational Behavior

Matthew David Fletcher* and Malcolm M. Campbell

School of Chemistry, University of Bath, Bath, BA2 7AY, United Kingdom

Received August 20, 1997 (Revised Manuscript Received January 2, 1998)

Contents

Matthew D. Fletcher was born in Chipping Norton, United Kingdom, in 1970. He studied Natural Sciences at St. John's College, Cambridge, obtaining his B.A. in 1991. In 1996 he received his Ph.D. from the University of Bath, where he was supervised by Malcolm Campbell. Following a postdoctoral position with Chris Willis at the University of Bristol, he is now a postdoctoral fellow, working with Chris Moody at the University of Exeter. His research interests include peptidomimetics, combinatorial chemistry, organofluorine chemistry, chemo-enzymatic synthesis, and heterocyclic chemistry. Apart from chemistry, he is a Methodist Local Preacher, and supporter of Aston Villa F.C.

Malcolm M. Campbell was educated in Glasgow, his Ph.D. training being in terpene chemistry. He was appointed to Heriot-Watt University in Scotland and then to the Chair of Organic Chemistry in the University of Bath in 1980. He is currently Vice-Chair of the European Federation of Medicinal Chemistry. His research portfolio includes the shikimate pathway, β-lactams, steroids, organosulfur chemstry, organophosphorus chemistry, peptidomimetics, and molecular dynamics.

I. Introduction

A. Peptides

Peptides occur throughout nature in a wide range of roles. They act as extracellular messengers—hormones, neurotransmitters, and neuromodulators—in plants and animals and thus influence such vital functions as metabolism, immune defense, respiration, and reproduction. They carry out intracellular functions, for example the antioxidant and transport tripeptide glutathione. Indeed, they are essential to virtually every biochemical process. Peptides are also implicated in the appearance or maintenance of various diseases, for example the plaques associated with Alzheimer's disease.[1] Peptides find other structural applications, for example as the cross-links in the peptidoglycan cell walls of bacteria; but the structural domain is more properly the realm of polypeptides and proteins. More esoteric roles for peptides include mushroom toxins, components of snake venoms, and antifreeze in fish.

This broad spectrum of activity has attracted much attention to peptides from bioorganic, medicinal, and polymer chemists. Much of the effort of these scien-

* Corresponding author: Department of Chemistry, University of Exeter, Stocker Road, Exeter, EX4 4QD, United Kingdom.

S0009-2665(97)00468-8 CCC: $30.00
Published on Web 03/14/1998

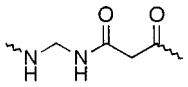


Figure 1. −Glyψ(NHCO)Gly−.

tists has focused upon the manipulation of peptide properties through peptide modification.

B. Scope

This review describes the development of a particular family of modified peptides, partially modified retro-inverso (PMRI) peptides, and their place within the wider realm of peptide modifications. The various methods of PMRI peptide solution and solid-phase synthesis are described, and all the applicable methods of *gem*-diaminoalkyl and C-2-substituted malonyl residue synthesis and coupling are compared. Literature describing methodological advances is cited (as opposed to that applying previously developed methodology). Significant examples of biologically active PMRI peptides are given. In the final section generally applicable theoretical and experimental conformational studies of PMRI peptides are described.

C. Notes on Nomenclature

IUPAC nomenclature and symbolism for peptides and peptide analogues is used throughout.[2] The ψ (Greek psi) notation for amide bond surrogates indicates that the amide bond between the two residues is replaced by the unit in brackets following the ψ, for example −Glyψ(NHCO)Gly− represents a glycylglycyl segment wherein the peptide bond is reversed (Figure 1).

For PMRI peptides the *g*/*m*/*r* prefix system is also used in the literature: *g*Xaa symbolizes the *gem*-diaminoalkyl analogue of the indicated amino acid residue, *m*Xaa symbolizes the malonyl residue corresponding to the indicated amino acid residue, and *r*Xaa symbolizes the reversed amino acid residue, that is −COCHRNH− rather than the conventional −NHCHRCO− direction. Thus, −*g*Gly-*m*Gly− corresponds to the structure shown in Figure 1. This system of nomenclature is only used herein to refer to PMRI peptides where the ψ notation is inappropriate, for example to refer to isolated residues.

The following terms are used in this review with the given meaning.

- Backbone: a homomeric, homodetic peptide "backbone" has three repeating units, the amide nitrogen, α-carbon, and amide carbonyl, see Figure 10a. R is referred to as the side chain. The backbone dihedral angles (ϕ_i and ψ_i), describe the conformation of the backbone at residue i.[3] Furthermore, in this review the corresponding dihedral angles of both *gem*-diamino residues and malonyl residues are labeled (ϕ,ψ), but in other literature the labels (ϕ,ϕ') and (ψ,ψ') or (ϕ_1,ϕ_2) and (ψ_1,ψ_2) respectively, are used.
- Cycloretro-enantiomer: an isomer of a cyclic compound in which the sequence is reversed and each residue is inverted.
- End group modified retro-inverso isomer/peptide: a retro-inverso isomer in which the peptide chain end groups are altered to better correspond with those of the parent peptide.
- Modified peptide: a peptide with some of its peptide functional groups replaced by other groups, but which retains properties analogous to the original peptide.
- Partially modified retro-inverso (PMRI) peptide: an isomer of a linear peptide in which some of the peptide bonds are reversed and the chirality of the amino acids in the reversed section is inverted.
- Peptidomimetic: a molecule with some or all peptide functional groups replaced by other groups, yet which exhibits properties analogous to a peptide.
- Pseudopeptide: a peptide analogue with a backbone modification.
- Retro-inverso peptide: an isomer of a linear peptide in which the direction of the amino acid sequence is reversed and the chirality of each amino acid is inverted.
- Retro-isomer: an isomer (of a peptide) in which the direction of the amino acid sequence is reversed.
- Surrogate: an unnatural replacement for a natural entity.

The terms cis and trans are used in this review to describe the configuration of amide bonds in accordance with their general use in peptide and protein chemistry, that is the amide bond depicted in Figure 1 is described as being in the trans configuration. Formally, the amide bond depicted in Figure 1 would be described as the s-cis (or *Z*) form.

II. Why Modify?

The desire to use peptides as pharmaceuticals is the major incentive for modification. The pharmacological properties of most peptides preclude their use as drugs. The mammalian body presents many barriers to the entry of macromolecules and thus peptides fall foul of poor absorption, because they do not readily pass across biological membranes; there is swift metabolism by proteolytic enzymes; and there is rapid excretion through the liver and kidneys.[4−6] These barriers result in peptides suffering from low bioavailability and short biological half-lives. Specificity is also a problem: peptide receptors can be widely distributed in an organism and their stimulation results in a variety of desired and undesired effects, especially when the peptide is conformationally flexible and hence able to interact with alternative receptors.[5]

The aim of peptide modification is to determine the structure−activity relationships of endogenous peptides and to produce analogues that can overcome the barriers and problems described above, while retaining selected activity (i.e., specific receptor agonists). Conversely, receptor antagonists and enzyme inhibitors are also desirable targets attainable through peptide modification.[5,7,8] The realization of these goals is aided by the simultaneous program of discovery and development of peptidomimetics from leads other than the endogenous peptides whose action is of interest.

Various definitions of peptidomimetics or peptide mimetics (the terms are used interchangeably) exist in the literature.

• Wiley and Rich: "Chemical structures designed to convert the information contained in peptides into small nonpeptide structures."[9]

• Giannis and Kolter: "A compound that, as a ligand of a receptor, can imitate or block the biological effect of a peptide at the receptor level."[5]

• Moore: "A molecule that mimics the biological activity of a peptide but...no longer contains any peptide bonds..."[10]

• Morgan and Gainor: "Structures which serve as appropriate substitutes for peptides in interactions with receptors and enzymes."[11]

• Gante: "[A] chemical 'Trojan horse'... A substance having a secondary structure as well as other structural features analogous to that of the original peptide, which allows it to displace the original peptide from receptors or enzymes. As a result the effects of the original peptide are inhibited (antagonist, inhibitor) or duplicated (agonist)."[7]

• Kemp: "Elements which mimic the structure of natural peptide components."[12]

Olson et al. do not offer a formal definition, but their comments are informative:

> The term...'peptidomimetics' [has been] utilized ...to describe compounds discovered through a variety of research strategies. Indeed, even compounds identified by random screening and subsequently optimized through structural modification have been termed peptidomimetics if the initial lead was found in an assay in which the natural ligand is a peptide or protein. The field of enzyme inhibitors uses peptide mimetics terminology for the replacements of segments of peptide-based substrates and inhibitors... The broad use of the term 'peptide mimetics' is unavoidable, but the advocates of rational design do not favor its use to describe compounds found by screening.[13]

Veber recently coined the term "peptide limetic" (a contraction of ligand mimetic) to distinguish between rationally derived peptidomimetics and compounds (discovered by screening) so divergent from peptides "that specific connections are no longer recognizable" but which exhibit activity in endogenous peptide assays.[14] This term finds sporadic use in the literature, with its own twists of definition.[9]

The rather broad definition of a peptidomimetic conformed to here is given above (section I.C). Accordingly, a modified peptide may also be described as a peptidomimetic, but a peptidomimetic is not necessarily a modified peptide.

These definitions and comments bring to light the importance of the screening of natural products (and other compound collections) in the discovery and development of peptidomimetics.[5,9,11,15] Indeed the recent ascendance of peptide libraries is founded predominantly on their promise of leads for peptidomimetics,[16−19] and attention is now directed upon the development of peptidomimetic libraries, which offer further molecular diversity.[20−27] Compounds unearthed by screening are particularly valuable in the search for antagonistic peptidomimetics and enzyme inhibitors.[5,9,11,15]

There are sources of peptidomimetics other than screening and modification of an endogenous peptide. A different approach is that of Dougall et al. in which peptidomimetics are developed from the complimentarity determining region of monoclonal antibodies raised against receptors of interest.[28] This antibody approach and Kemp's distinct definition of peptidomimetics[12] reveal motives for modification that are less firmly in the medicinal chemistry camp: the study of molecular recognition[28] and investigations into protein folding.[29] (See also the studies of Dado and Gellman on small molecules, for example section VI.C.3.e.ii.)

III. The Range of Peptide Modifications and Peptidomimetics

As might be expected from the variety of definitions, the range of peptidomimetics is wide, and different reviewers adopt different modes of classification. Thus peptidomimetics may be classified according to origin,[9,11] activity,[7,9,10,30,31] secondary structure,[32−38] topographical considerations,[39] that part of the endogenous peptide which is modified,[5,7] or which peptide torsion angles are constrained.[33] Modification can be made at any point in a peptide and modifications can be applied in any combination:[40,41] amino acids can be deleted, or added/replaced;[42] short-[43] or long-range cyclizations[44] may be applied; backbone peptide bonds can be replaced with surrogates;[41,45] or the backbone may be replaced altogether by a novel scaffold.[7,15] An overview of modifications, and combinations thereof, used in the development of therapeutic peptides, is provided by Dutta, in his review of the design of peptide-based drugs.[46] Rational, general strategies for the use of modifications are under development: both Hruby[47−49] and Marshall[50] have recently published their approaches for the use of modifications (with the emphasis on conformationally constrained and chimeric, novel amino acids) to produce selective, biologically active, peptide, and peptidomimetic ligands.

The subject of this review is a particular peptide bond modification/peptidomimetic concept, therefore an attempt to tidily classify the world of peptidomimetics is beyond its scope (the interested reader is referred to the reviews already cited and the annual Chemical Society specialist reports[51]), and further discussion is restricted to peptide bond surrogates in general, and the retro-inverso modification in particular.

IV. Peptide Bond Surrogates

Peptide bond surrogates are amide look-alikes used to replace backbone peptide bonds. Generally these surrogates are isosteric and/or isoelectronic with the peptide bond and, indeed, are often referred to as isosteres.[52] However, the isosteric and/or isoelectronic character of the surrogates is not always obvious, nor even necessary for biological activity.

Peptide bond surrogates are used to investigate the role and function of backbone peptide bonds and to modify the properties of the parent peptides. In his peptide backbone modifications review,[41] Spatola

raised these questions of interest (with particular reference to peptide hormone analogues):

(1) Is there a functional role for the peptide bond itself? Or does the backbone merely serve to orient and align the essential side chain residues?

(2) To what extent are the alignment, bond lengths, and stereochemistry of the peptide backbone critical for the resulting biological function?

(3) How does the modification of the peptide backbone affect the resistance (as measured by biological half-lives) toward enzymatic degradation?

(4) To what extent are rigidity and flexibility of peptides manifested in the backbone and can these elements be exploited in designing more potent peptide analogues, or conversely, peptide antagonists? What are the related consequences of introducing peptide backbone modifications in terms of their altered electronic and stereochemical properties, hydrophilicity and hydrophobicity, and their effects on adsorption, transport, and the ability to penetrate the blood−brain barrier?

Partial answers to these questions emerge:

(1) Work on peptidomimetics in general suggests that in the context of peptide hormone−receptor binding, the peptide backbone largely serves to position the essential side chains[15,41] (hence the advent of scaffold mimetics[7,15] and Hruby's topographical design approach[47,48]). In contrast, with protease inhibitors a nonscissile peptide bond surrogate (especially one that can mimic the transition state of amide bond hydrolysis) at the substrate cleavage site is a key approach to activity,[7,8,41,45] and enzyme−substrate/inhibitor binding involves hydrogen bonding to the substrate/inhibitor backbone peptide bonds.[15]

(2)/(4) Most peptide bond surrogates do not greatly restrict global peptide conformation, but their different influences on the conformational preference of adjoining residues, and their capacity to form hydrogen bonded secondary structures, are much studied and frequently important.

(3) Well-placed peptide bond surrogates do indeed increase the biological half-lives of their parent peptides: nearly all peptide bond surrogates, with the notable exception of the ester and thioester surrogates, are more stable to enzymatic hydrolysis than the natural peptide bond.[41,53]

(4) Alterations of electronic properties introduced by peptide bond surrogates can significantly affect the transport properties of their parent peptides.[54,55]

Thus the role of peptide bond surrogates is to increase bioavailability and (often subtly) to influence the conformation of the parent peptide, and, in the case of protease inhibitors, to interact directly with the target enzyme.

In addition, those peptide bond surrogates that mimic the transition state of amide bond hydrolysis, or conversely the aminolysis of an amino acid, find use in haptens for the generation of catalytic antibodies.[56−58] Up until now, success in this field has been largely restricted to the phosphorus-based surrogates (see Table 1),[56,57] for example in haptens used to raise antibodies that catalyze peptide bond formation.[59−61]

A few comparisons of peptide bond surrogates appear in the literature. Spatola compared peptide bond surrogates with respect to enzyme stability, hydrophobicity, conformational influence, etc.[41] Fauchère and Thurieau, to aid rational drug design, reviewed the in vivo proteolytic degradation of endogenous peptides and the stabilizing influence of (backbone) modifications used in (potentially) therapeutically useful modified peptides.[53] Aubry and coworkers reviewed the influence of a selection of peptide bond surrogates on the stability of β-turns, as determined by experimental conformational studies on small model peptides.[62−64] Fincham et al. compared 11 peptide bond surrogates by modeling physiochemical properties (dimensions, volume, lipophilicity, and hydrogen-bonding capability) and biologically in the specific context of two cholecystokinin (CCK)-B ligands.[65] Most of their analogues displayed lower binding affinity than the parent ligands, but no correlation with the modeled properties was determined, suggesting that factors such as conformation are more important in this case. Nonetheless the study constitutes progress toward rational peptide bond surrogate selection.[65]

Significant surrogates are presented in Table 1, with leading references.

V. Retro-Inverso Peptides

The peptide bond provides the peptide backbone with a "sense of direction". Thus peptides and proteins are inherently nonpalindromic. The convention for the construction of peptide names and sequence representation proceeds from the amino terminus (written on the left) to the carboxy terminus (written on the right).[2] Therefore the direction of the peptide bond is defined as that from the carbonyl carbon atom to the adjoining nitrogen atom.

Hence it is possible to envisage the retro-isomer of a peptide, that is an isomer in which the direction of the amino acid sequence is reversed;[66] see Figure 2, parts a and d, for a cyclic example, and **1** and **4** (Figure 3) for a linear example.

A. Cyclic Peptides

This concept of retro-isomers was recognized early in the study of the biological activity of peptides and was applied to cyclic peptides and cyclic depsipeptides. (For an overview of this early work see ref 66.) Shemyakin, Ovchinnikov, and co-workers,[67,68] and later Wieland et al.,[69] further recognized that reversal of the residue sequence of the enantiomer of a cyclic (depsi)peptide (or conversely, enantiomerization of the retro-isomer) maintains topochemical complimentarity between the parent cyclic (depsi)peptide and its isomer.[66] The resultant isomer is called a cycloretro-enantiomer (or, for the isomer of an all-L-cyclopeptide, a retro-all-D-cyclopeptide[69]) and is defined as an isomer of a cyclic compound in which the sequence is reversed and each residue is inverted[66] (Figure 2).

It is important to note that (1) the cycloretro-enantiomer is not the same isomer as the enantiomer of a cyclic compound, cf. Figure 2, parts c and b; and

Table 1. Peptide Bond Surrogates

name	symbol	comments
N-substituted	ψ(CONR)	R = Me: site-selective *N*-methylation of peptides during SPS[a] R = Me, NH_2 or OH: conformational implications[63,b]
ester (depsipeptide)	$\psi(CO_2)$	implications for turn formation: conformational study[c]
ketomethylene	$\psi(COCH_2)$	synthetic methodology for the core units: $R^1COCH_2CHR^2CO_2R^3$; R^1 = alkyl, heterocycle, or ZNHCHBn; R^2 = H or alkyl; R^3 = H, alkyl, Pro-OMe, or NHCHMeBn[d]
reduced or methyleneamino	$\psi(CH_2NH)$	SPS methodology using 2,4-dimethoxybenzyl protection[e]
thioamide	ψ(CSNH)	compatibility with reverse turn: conformational study[f]
phosphinate (n = 1)/ phosphonate (n = 2) (and their esters)	$\psi[PO_{n+1}R(CH_2)_{2-n}]$	R = H; n = 1, 2: SPS methodologies for the incorporation of Phe$\psi(PO_2HCH_2)$Gly,[g] Phe$\psi(PO_3H)$Gly,[h] and Xaa$\psi(PO_3H)$Leu[i]
phosphonamidate (and phosphonamidate ester)	$\psi(PO_2RNH)$	R = Me or H: HIV−1 protease inhibitor[j]
retro	ψ(NHCO)	reviews[66,85,86]
alkene (*trans*)	ψ(*E*-CR=CH)	synthetic methodology for Xaaψ(*E*-CH=CH)Yaa (R = H)[k] and Alaψ(*E*-CR=CH) Xaa (R = H or Me)[l] via S_N2' attack of organocuprates on alkenylaziridines
	ψ(*E*-CMe=CMe)	β-hairpin promoter[m]
fluoroalkene	ψ(*Z*-CF=CH)	assessment of binding of tripeptide inhibitors to thermolysin[n]
carba or dimethylene	$\psi(CH_2CH_2)$	synthesis, conformational study, and SAR of a substance P NK-1 agonist[o]
thioether	$\psi(CH_2S)$	review [including mention of sulfoxide variant, $\psi(CH_2SO)$][p]
hydroxyethylene	ψ[CH(OH)CH_2]	synthetic methodology for the lactone derivative leading to Xaaψ[CH(OH)CH_2]Phe, from α-amino ketones[q]
dihydroxyethylene	ψ[CH(OH)CH(OH)]	synthetic methodology for the lactone corresponding to PG- Leuψ[CH(OH)CH(OH)]Ala, from *N*-protected leucinal[r]
methyleneoxy	$\psi(CH_2O)$	conformational implications[s] synthesis and biological activity of substance P and Leu enkephalinamide analogues[t]
tetrazole	$\psi(CN_4)$	*cis* amide bond surrogate: synthesis and solution conformation of an active, cyclic somatostatin analogue[u]
cyanomethyleneamino	ψ[CH(CN)NH]	synthesis of a CCK-B anatagonist[v]
retrothioamide	ψ(NHCS)	synthetic methodology for Xaaψ(NHCS)Yaa via endothionation of Xaaψ(NHCO)Yaa, and subsequent elongation[108]
retroreduced	$\psi(NHCH_2)$	synthetic methodology for Ac-Pheψ(NHCO)Phe$\psi(NHCH_2)$-(*R*,*S*)-Val-OR[w]
sulfonamido	$\psi(SO_2NH)$	synthesis of the glutathione disulfide analogue, [Glu(-$\psi(SO_2NH)$Cys-Gly)]$_2$[x]
sulfinamido (n = 1)/sulfonamido (n = 2)	$\psi(CHRSO_nNH)$	synthetic methodology for Gly$\psi(CHRSO_nNH)$Xaa (R = Me or Bn) and Yaa$\psi(CH_2SO_nNH)$Zaa via α- and β-substituted sulfinyl chlorides respectively, and subsequent elongation[y]
retrosulfonamide	$\psi(NHSO_2)$	synthesis and crystal structure of Boc-Pro-Leu$\psi(NHSO_2)$Gly-NH_2[z]

[a] Miller, S. C.; Scanlan, T. *J. Am. Chem. Soc.* **1997**, *119*, 2301−2302. Yang, L.; Chiu, K. *Tetrahedron Lett.* **1997**, *38*, 7307−7310. [b] Dupont, V.; Lecoq, A.; Mangeot, J.-P.; Aubry, A.; Bousssard, G.; Marraud, M. *J. Am. Chem. Soc.* **1993**, *115*, 8898−8906. [c] Gallo, E. A.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 9774−9788. [d] Hoffman, R. V.; Kim, H.-O. *Tetrahedron Lett.* **1992**, *33*, 3579−3582. Hoffman, R. V.; Kim, H.-O. *J. Org. Chem.* **1995**, *60*, 5107−5113. [e] Sasaki, Y.; Abe, J. *Chem. Pharm. Bull.* **1997**, *45*, 13−17. [f] Sherman, D. B.; Spatola, A. F. *J. Am. Chem. Soc.* **1990**, *112*, 433−441. [g] Campagne, J.-M.; Coste, J.; Guillou, L.; Heitz, A.; Jouin, P. *Tetrahedron Lett.* **1993**, *34*, 4181−4184. [h] Campagne, J.-M.; Coste, J.; Jouin, P. *Tetrahedron Lett.* **1995**, *36*, 2079−2082. [i] Campbell, D. A.; Bermak, J. C. *J. Am. Chem. Soc.* **1994**, *116*, 6039−6040. [j] McLeod, D. A.; Brinkworth, R. I.; Ashley, J. A.; Janda, K. D.; Wirsching, P. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 653−658. [k] Fujii, N.; Nakai, K.; Tamamura, H.; Otaka, A.; Mimura, N.; Miwa, Y.; Taga, T.; Yamamoto, Y.; Ibuka, T. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1359−1371. [l] Wipf, P.; Henninger, T. C. *J. Org. Chem.* **1997**, *62*, 1586−1587. [m] Gardner, R. R.; Liang, G.-B.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, *117*, 3280−3281. [n] Bartlett, P. A.; Otake, A. *J. Org. Chem.* **1995**, *60*, 3107−3111. [o] Lavielle, S.; Chassaing, G.; Brunissen, A.; Rodriguez, M.; Martinez, J.; Convert, O.; Carruette, A.; Garret, C.; Petitet, F.; Saffroy, M.; Torrens, Y.; Beaujouan, J.-C.; Glowinski, J. *Int. J. Pept. Protein Res.* **1993**, *42*, 270−277. [p] Spatola, A. F. *Methods Neurosc.* **1993**, *13*, 19−42. [q] Lagu, B. R.; Liotta, D. C. *Tetrahedron Lett.* **1994**, *35*, 547−550. [r] Rehders, F.; Hoppe, D. *Synthesis* **1992**, 859−864. Rehders, F.; Hoppe, D. *Synthesis* **1992**, 865−870. [s] Villeneuve, G.; DiMaio, J.; Drouin, M.; Michel, A. G. *J. Chem. Soc., Perkin Trans. 2* **1994**, 1631−1640. [t] Roubini, E.; Laufer, R.; Gilon, C.; Selinger, Z.; Roques, B. P.; Chorev, M. *J. Med. Chem.* **1991**, *34*, 2430−2438. [u] Beusen, D. D.; Zabrocki, J.; Slomczynska, U.; Head, R. D.; Kao, J. L.-F.; Marshall, G. R. *Biopolymers* **1995**, *36*, 181−200. [v] Herrero, S.; Suárez-Gea, M. L.; González-Muñiz, R.; García-López, M. T.; Herranz, R.; Ballaz, S.; Barber, A.; Fortuño, A.; Del Río, J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 855−860. [w] Retroreduced pseudopeptides are intrinsically unstable unless the surrogate is preceded by a retro amide bond. Campbell, M. M.; Ross, B. C.; Semple, G. *Tetrahedron Lett.* **1989**, *30*, 6749−6752. [x] Sulfonamido (and sulfinamido, ψ(SONH)) pseudopeptides are intrinsically unstable when preceded by −NHCHR− (i.e., when the surrogate is inserted in the peptide backbone), hence the advent of the subsequent entries. Luisi, G.; Calcagni, A.; Pinnen, F. *Tetrahedron Lett.* **1993**, *34*, 2391−2392. [y] Moree, W. J.; van Gent, L. C.; van der Marel, G. A.; Liskamp, R. M. J. *Tetrahedron* **1993**, *49*, 1133−1150. Moree, W. J.; van der Marel, G. A.; Liskamp, R. J. *J. Org. Chem.* **1995**, *60*, 5157−5169. [z] Pagani Zecchini, G.; Paglialunga Paradisi, M.; Torrini, I.; Lucente, G.; Gavuzzo, E.; Mazza, F.; Pochetti, G. *Tetrahedron Lett.* **1991**, *32*, 6779−6782.

(2) there are (many) other cycloisomers of cyclic peptides. (See ref 66 for further discussion.)

Shemyakin, Ovchinnikov, and co-workers applied this concept to [Gly^5,Gly^{10}]gramicidin S, the cyclo-retro-enantiomer of which displayed undiminished antimicrobial activity.[67,68,70]

B. Linear Peptides

The extension of the retro-enantiomer concept to linear peptides was hampered by their possession of end groups,[66] which are obviously absent in cyclic peptides. Thus reversion of sequence and enanti-

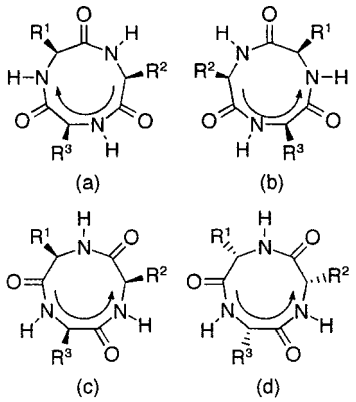


Figure 2. *cyclo*(-Xaa-Yaa-Zaa-) and some of its cycloisomers: (a) *cyclo*(-Xaa-Yaa-Zaa-); (b) the enantiomer, *cyclo*(-D-Xaa-D-Yaa-D-Zaa-); (c) the cycloretro-enantiomer, *cyclo*(-D-Zaa-D-Yaa-D-Xaa-); and (d) the retro-isomer, *cyclo*(-Zaa-Yaa-Xaa-); where R^1 is the side chain of the amino acid Xaa, etc.

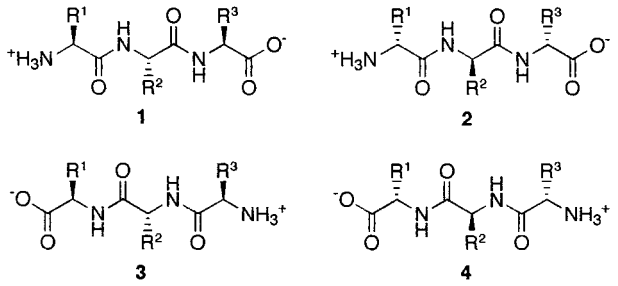


Figure 3. Xaa-Yaa-Zaa, **1**; the enantiomer, D-Xaa-D-Yaa-D-Zaa, **2**; the retro-inverso peptide, D-Zaa-D-Yaa-D-Xaa, **3**, obtained from **2** by reversal of sequence, or from **4** by enantiomerization; and the retro-isomer, Zaa-Yaa-Xaa, **4**.

omerization of a linear peptide produces an isomer topochemically complimentary to the parent peptide along the chain length, but with noncomplimentary end groups (Figure 3).[66]

The term retro-inverso peptides (or retro-inverso pseudopeptides, the "pseudo" being tautologous in this context) is used to describe the relationship between peptides **1** and **3**, (and that between **2** and **4**) because **3** is obtained from **1** (and vice versa) by reversal of the direction of the amino acid sequence and inversion of each C^{α} stereogenic center.[66] Note that for threonine and isoleucine, which have C^{β} stereogenic centers, enantiomerization results in topochemical noncomplimentarity with the parent peptide at C^{β}. This is often ignored in retro-inverso studies, but may be overcome by incorporation of the appropriate D-*allo*-amino acid, or by suitable choice of synthetic method for *g*Xaa residues (see section VI.A.2.b). The term retro-inverso peptide is preferable to retro-enantio peptide in this linear peptide context, because the latter implies too much structural equivalence between the parent peptide, **1**, and the isomer, **3**.[66]

Although Shemyakinin and co-workers synthesized an effective retro-inverso pepsin inhibitor without complimentary end groups,[68] the noncomplimentarity of end groups (termed the "end group problem") was recognized by the pioneers of the retro-inverso concept as the probable cause of the inactivity of various other retro-inverso peptide hormones.[66,68] However,

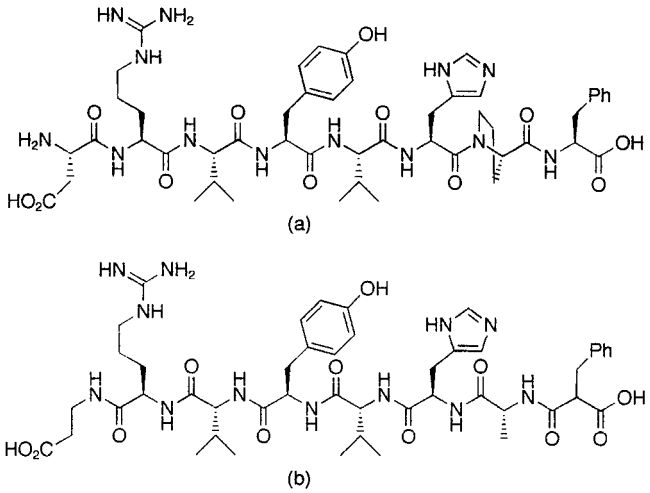


Figure 4. (a) [Val^5]angiotensin II and (b) end group modified retro-inverso[Val^5,Ala^7]desaminoangiotensin II.

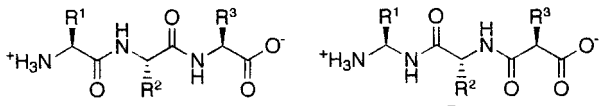


Figure 5. Xaa-Yaa-Zaa, **1**; and the end group modified retro-inverso isomer, Xaaψ(NHCO)Yaaψ(NHCO)Zaa, **5**.

the inactivity of those retro-inverso peptide hormones containing proline, for example bradykinin, could equally well be due to the lack of spatial coincidence between the proline rings of, for example bradykinin and retro-inverso bradykinin (see also Figure 4). This topological difference was dubbed the "proline problem", and arises because the proline side chain is cyclized onto the proline backbone nitrogen, which changes position with the carbonyl carbon in the retro-inverso isomer.[66,68,71]

1. End Group Modification

These set backs prompted the development of end group modifications, designed to improve complimentarity between the parent peptide and its retro-inverso isomer at the chain termini. Various modifications were employed with differing degrees of success (as judged by the retention of biological activity by the end group modified retro-inverso peptides).[66,68] Goissis et al. synthesized the first biologically active end group modified retro-inverso peptide hormones, two angiotensin analogues, which demonstrated that the (C-2-substituted) malonyl (or malonamyl) residue suggested by Rudinger[72] was suitable for carboxy terminal modification (Figure 4).[73]

Hayward and Morley first proposed (and incorporated) the *gem*-diaminoalkyl residue as an amino terminus modification.[74] They replaced a pyroglutamyl residue with a 5-oxo-2-pyrrolidinylamino residue in a model pseudodipeptide, **56** (see Scheme 20),[74] but did not follow up this work. As shown in Figure 5, the incorporation of an amino terminal *gem*-diaminoalkyl residue and a carboxy terminal (C-2-substituted) malonyl residue results in good structural complimentarity between the parent peptide, **1**, and its end group modified retro-inverso isomer, **5**.

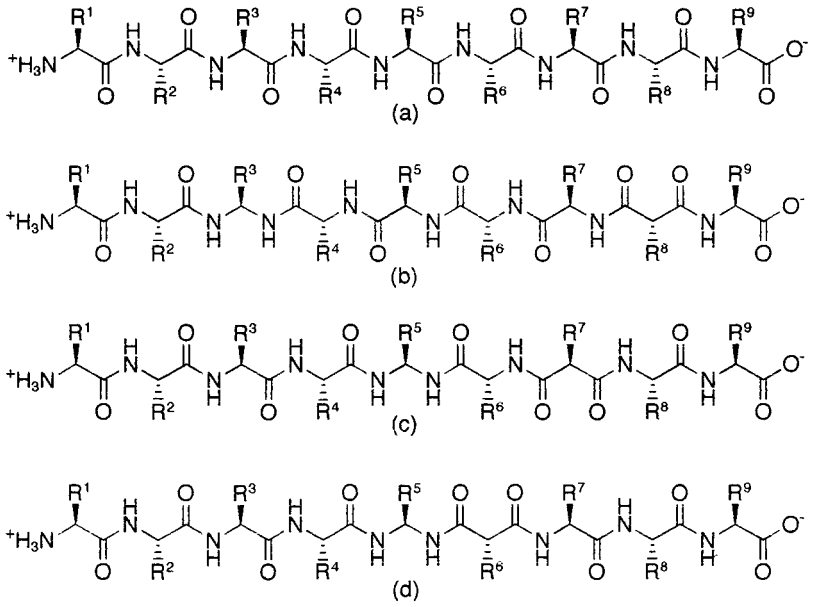


Figure 6. (a) The parent peptide and its PMRI isomers, (b) five bonds reversed, (c) two bonds reversed, and (d) one bond reversed. Parts b and c are examples of structures that are more conveniently represented using the *g*/*m*/*r* system of symbols, rather than the ψ system.

Chorev and Goodman took up the retro-inverso concept and, through the general synthetic methodology they established, are responsible for the widespread adoption of the *gem*-diaminoalkyl residue as an amino terminus modification.[66,75,76]

VI. Partially Modified Retro-Inverso Peptides: The Retro Peptide Bond as a True Peptide Bond Surrogate

gem-Diaminoalkyl and C-2-substituted malonyl residues are not restricted to terminal incorporation: their incorporation within the peptide sequence generates a new class of peptide isomers. Hayward and Morley first introduced this concept,[74] but it was Chorev and Goodman who first implemented it and who termed the resultant isomers partially modified retro-inverso (PMRI) peptides.[66] Such isomers consist of the parent peptide sequence with two (or more) internal residues replaced by *gem*-diaminoalkyl and C-2-substituted malonyl residues, and with the intervening amino acids having inverted configuration and reversed sense of direction; that is an end group modified retro-inverso sequence is incorporated within a peptide sequence (Figure 6).[66,74] (Note that an end group modified retro-inverso isomer, such as that depicted in Figure 5, is simply a special example of a PMRI peptide with *all* the amide bonds reversed and amino acids inverted. Therefore all subsequent discussion of PMRI peptides also applies to this special case). The application of the *gem*-diaminoalkyl and C-2-substituted malonyl residues in this context thus makes the retro peptide bond into a true peptide bond surrogate, in that any *one* peptide bond can be replaced (Figure 6d).

However, the PMRI peptide concept transcends that of the peptide bond surrogate, for, unlike any other peptide bond surrogate, a contiguous sequence of retro peptide bonds remains completely peptidic in character with the nonpeptidic *gem*-diamino and malonyl residues being present only at each end of the modified sequence (Figure 6, parts b and c).

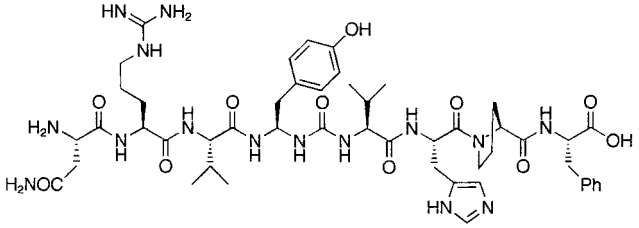


Figure 7. [Asn[1],aza-α′-homoTyr[4],Val[5]]angiotensin II,[79] *cf.* Figure 4a.

At about the same time as the PMRI peptide concept emerged, Ancans and co-workers developed a related mode of peptide modification involving the incorporation of "aza-α′-homoamino acids".[77,78] The net effect of this modification is to add an amino group between C^{α} and C′ of an α-amino acid residue thus generating a *gem*-diaminoalkyl residue and an adjacent ureide linkage in the peptide backbone.[77,79,80] Various angiotensin analogues incorporating aza-α′-homoamino acids of natural and inverted configuration have been synthesized, for example Figure 7.[77−79,81−84]

Chorev and Goodman exhaustively reviewed the field of PMRI peptides in 1993[85] and further reviewed developments in enantio-, retro-, and retro-inverso peptides and proteins in 1995.[86] Subsequent discussion here is largely restricted to PMRI peptides, taking pertinent examples from the literature, with the focus on synthesis and conformation.

A. Synthetic Methodology

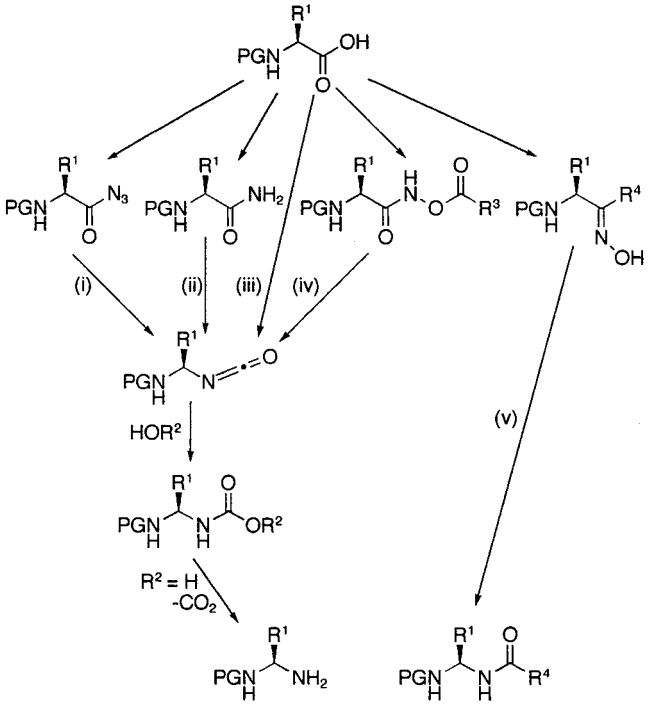
The principal concern of PMRI peptide synthesis is the construction and coupling of the *gem*-diaminoalkyl and C-2-substituted malonyl residues. Assembly of the remainder of the PMRI peptide chain involves the protection and coupling of L- or D-α-amino acids, which is the stuff of standard peptide

chemistry.[87,88] As with standard peptide chemistry the methodology is readily divided into two categories: that carried out (1) in solution, and (2) on a solid support (SPS); there being considerable common ground between the two.

1. Solution-Phase Methodology

a. *gem*-Diaminoalkyl Derivatives. *i. Rearrangements.* Most of the pioneering syntheses of *gem*-diaminoalkyl derivatives for PMRI peptides used a rearrangement step to obtain the *gem* relationship of the amino groups. Rearrangements remain the methods used most commonly for this manipulation. In theory, any of the rearrangements depicted in Scheme 1 could be employed, but in practice only the

Scheme 1. Rearrangements Leading to *gem*-Diamino Derivatives[a]



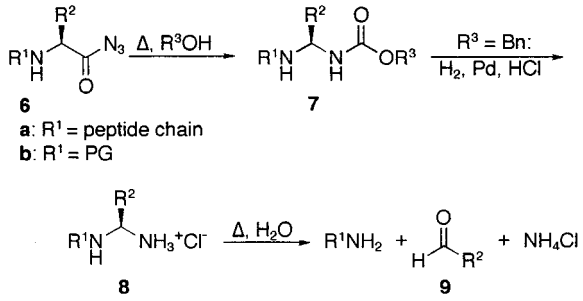
[a] (i) Curtius: Δ. (ii) Hofmann: NaOBr. (iii) Schmidt: HN_3. (iv) Lossen: OH^-. (v) Beckmann: PCl_5.

Curtius and Hofmann rearrangements (in various forms) are utilized in this context.

During all these rearrangements the migrating group retains its configuration; thus an L-α-amino acid as starting material yields the topographically complimentary *gem*-diaminoalkyl derivative.

The Curtius rearrangement was employed by Chorev, Goodman, and co-workers in their first preparations of PMRI peptides.[66,75,76,89] Their approach to the *gem*-diaminoalkyl residues, **7** and **8**, was based on the Bergmann and Zervas stepwise degradation method ("carbobenzoxy degradation") for polypeptide sequencing (Scheme 2).[90] But, rather than degrade the *gem*-diaminoalkyl derivatives, **7** and **8**, to the corresponding aldehydes, **9**, for identification, Chorev, Goodman, and co-workers utilized them in their PMRI peptides.[66,76,89] As indicated in Scheme 2, they applied this methodology to both protected peptides, **6a**, leaving the *gem*-diaminoalkyl group attached to the peptide chain (**7a**) and to *N*-protected amino

Scheme 2. Carbobenzoxy Degradation Approach to *gem*-Diaminoalkyl Derivatives



acids, **6b**, leading to isolated, protected *gem*-diaminoalkyl derivatives (**7b**).[66,76,89] This latter synthesis obviously requires an additional deprotection and coupling step over the former, to assemble the PMRI peptide.

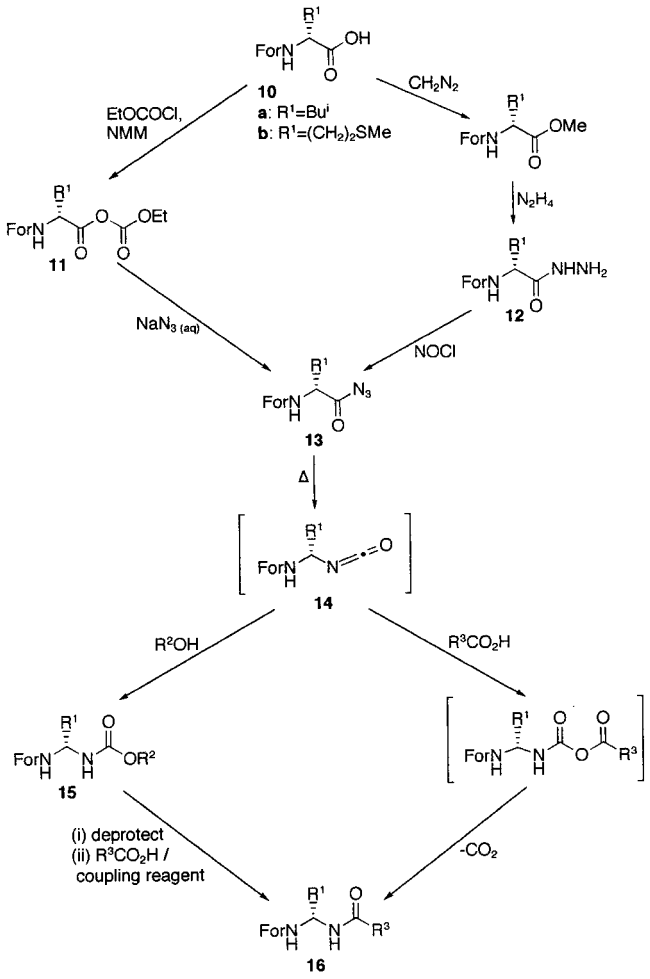
Of course, this approach raises its own questions: (1) how best to synthesize the required α-aminoacyl azides, **6**; (2) which are the best protecting groups for the *gem*-diaminoalkyl derivatives, **7**; (3) which is the best coupling procedure to employ with the *gem*-diaminoalkyl derivatives, **8**; and (4) is it better to synthesize free or peptide bound *gem*-diaminoalkyl derivatives, that is **7** and **8 a** vs **b**?

Chorev, Goodman, and co-workers performed limited comparative studies on the use of the Curtius rearrangement, to begin to answer these questions.[76,91,92] Since acyl azides have been heavily utilized in peptide chemistry as acylating agents[87,93] (which probably explains their immediate popularity for PMRI peptide synthesis), a number of syntheses of α-aminoacyl azides, **6**, exist.[87,93] Chorev and Goodman compared two methods of *N*-formylaminoacyl azide, **13**, preparation: nitrosylation of an *N*-formylaminoacyl hydrazide, **12**, and formation of a mixed-anhydride, **11**, followed by nucleophilic displacement by sodium azide (Scheme 3).[91] Curtius rearrangement of the α-aminoacyl azides, **13**, yielded the isocyanates, **14**, which were trapped with benzyl or *tert*-butyl alcohol to yield the orthogonally protected *gem*-diaminoalkyl residues, **15**, or reacted with a carboxylic acid derivative to yield a PMRI peptidic unit, **16**, directly (the so-called "Goldschmidt and Wick type reaction", after the chemists who employed a closely related procedure for peptide synthesis[94,95]).[91]

Chorev and Goodman concluded that the mixed-anhydride procedure results in better yields of the desired *gem*-diaminoalkyl derivatives, **15**, than the nitrosylation method.[91] Moutevelis-Minakakis and Photaki[96] used the *tert*-butyl nitrite nitrosylation method (untested by Chorev and Goodman) and obtained yields of orthogonally protected *gem*-diaminoalkyl derivatives comparable with those of Chorev and Goodman's nitrosyl chloride method[91] (11−45%); therefore, in this context, nitrosylation is generally inferior to the mixed-anhydride method of α-aminoacyl azide preparation.

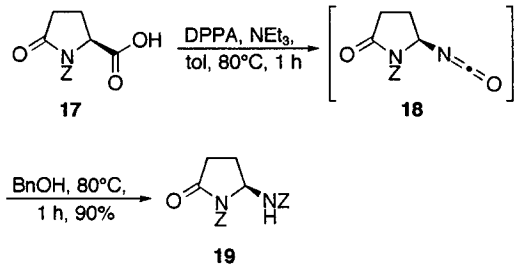
The other popular method for the synthesis of α-aminoacyl azides en route to PMRI peptides uses the reagent diphenylphosphoryl azide (DPPA).[97,98] Reaction of DPPA with an *N*-protected α-amino acid

Scheme 3. Chorev and Goodman's Comparative Curtius Rearrangements[a]



[a] Yields: **15a** (R^2 = Bn), 66 and 77%; **15b** (R^2 = Bu^t), 37 and 66%; for the NOCl and NaN_3 routes respectively (from **10**). R^3 = **a** Z-Phe or **b** Boc-Phe.[91]

Scheme 4. An Example of the Use of DPPA for the Synthesis of the Protected *gem*-Diaminoalkyl Derivative Z-*g*Glp-Z, 19[66,99]



(e.g., **17**) or peptide yields the corresponding acyl azide. Under the usual reaction conditions the acyl azide undergoes the Curtius rearrangement to yield the corresponding isocyanate (e.g., **18**), which is trapped with an appropriate alcohol (either in situ or added subsequently) to furnish the desired *gem*-diaminoalkyl derivative (e.g., **19**) (Scheme 4).[66,96,99]

DPPA may be used quite generally for the synthesis of *gem*-diaminoalkyl derivatives, without the need for extraordinary side chain protection.[100] However, a "one-pot" Goldschmidt and Wick type reaction is, of course, not possible without ensuring complete consumption of the DPPA prior to addition of the second carboxylic acid.

No direct comparison of the DPPA method with other approaches to α-aminoacyl azide synthesis has been undertaken, nor have variants of this reagent[101] found widespread application in PMRI peptide synthesis (but see refs 102 and 103).

The literature contains other classical and more modern routes to acyl azides;[104−107] these have been but little used in PMRI peptide synthesis. The only example of the use of TMS azide to synthesize an acyl azide en route to a PMRI peptide is Fincham et al.'s synthesis of CCK-B ligands.[65] They synthesized 2-Adoc-Trp-N_3 by reaction of the amino acid mixed-anhydride and TMS azide. Curtius rearrangement and trapping of the isocyanate with *p*-nitrobenzyl alcohol/DABCO furnished racemic 2-Adoc-(*R,S*)-*g*Trp-OBn(*p*-NO_2) in 42% yield.[65] They offered no explanation for the racemization.

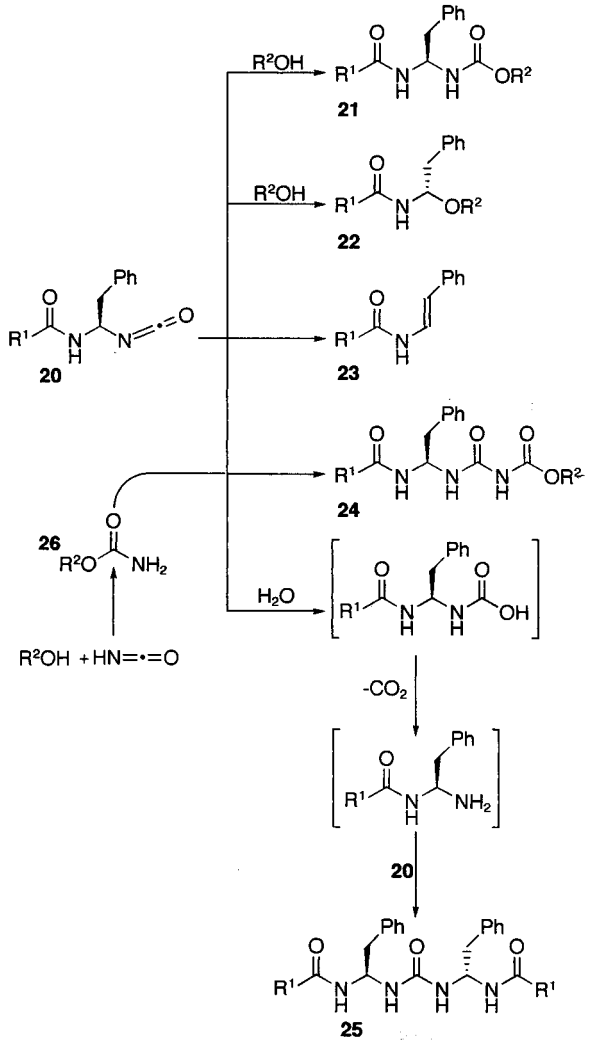
The criteria against which to judge protecting groups for the *gem*-diaminoalkyl derivatives are, as with any protecting groups, the efficacy of protection, indicated by the prevention of decomposition and side reactions (including racemization), and their ease of incorporation and removal.

Chorev, Goodman, and MacDonald studied side reactions during the synthesis of protected *gem*-diaminoalkyl derivatives obtained by the Curtius rearrangement of acetyl, Boc, or Z-protected phenylalanyl azide, trapping the intermediate isocyanate in situ with methanol or benzyl alcohol.[92] They obtained the desired *gem*-diaminoalkyl derivatives, **21**, plus numerous byproducts, in a ratio that depended upon the nature of the phenylalanyl azide *N*-protecting group, and the nature and molar excess of the alcohol.[92] They rationalized the formation of the byproducts as shown in Scheme 5; the yields are shown in Table 2.

With regard to the choice of protecting group for *gem*-diaminoalkyl derivatives, they concluded that acetyl is better than Z, which is better than Boc, on the basis of maximization of product yield and concurrent minimization of the yield of byproducts.[92] They reasoned that acetyl protection resulted in fewer byproducts than carbamate protection due to a reduced tendency of isocyanate **20c** to undergo the heterolytic or displacement reactions.[92] Although acetyl is not a practical amino protecting group, they argued that it is a suitable model for a peptide chain, and therefore the results they obtained suggest that, in answer to question 4 above, it is better to synthesize peptidyl *gem*-diaminoalkyl derivatives.[92] Loudon and co-workers studied the hydrolysis of *gem*-diaminoalkyl derivatives. Their results, which are summarized in section VI.A.1.a.vi, support this assertion.

DeBons and Loudon and Moutevelis-Minakakis and Photaki both obtained urea byproducts, corresponding to **25**, in their studies of *gem*-diaminoalkyl derivative synthesis.[96,102] Both argued that the water required for urea formation is generated in situ by the dehydration of *tert*-butyl alcohol by the intermediate isocyanate.[96,102] This provides a basis to disfavor *tert*-butyl alcohol as an isocyanate trap. Chorev, Goodman, and Willson counseled against Boc

Scheme 5. Formation of Byproducts during the Synthesis of Bis-Protected 1,1-Diamino-2-phenylethane, 21[a]



[a] The 1-amino-1-alkoxy-2-phenylethane byproduct **22** arises from cyanate displacement by the alcohol, whereas the styrene byproduct **23** arises by elimination of cyanate. Reaction of displaced cyanate and alcohol produces protected ammonia **26** which adds to the isocyanate **20** to yield the allophanate byproduct **24**. Traces of water present in the reaction mixture lead to the formation of the urea byproduct **25**.[92] No racemization was observed.

deprotection of *gem*-diaminoalkyl derivatives when hydrogenation of a Z group is possible instead, but offered no rationale.[76] Campbell and co-workers demonstrated that Boc and Z are equally effective protecting groups during the Goldschmidt and Wick type synthesis of PMRI dipeptides, but that subsequent Boc deprotection by conventional acid hydrolysis results in some decomposition.[108,109] So, we may conclude from these results that Z is generally to be favored over Boc for the protection of *gem*-diaminoalkyl compounds.

Fmoc and 2-nitrophenylsulfenyl are also acceptable protecting groups for *gem*-diaminoalkyl compounds, and provide additional, orthogonal dimensions;[109−111] no comparison of these with the other protecting groups discussed has been undertaken.

With regard to the coupling of *gem*-diaminoalkyl derivatives to carboxylic acids (question 3 above), no

Table 2. Yields and Conditions for Scheme 5

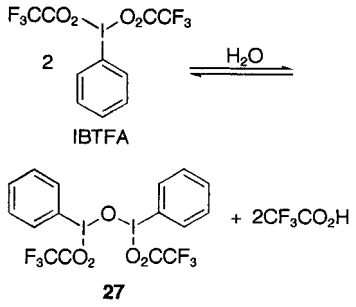
			yield,[b] %				
isocyanate	R^1	alcohol, no. of equiv[a]	**21**	**22**	**23**	**24**	**25**
20a	BnO	MeOH, 20	49	9	6	5	1
		MeOH, 2	65	nr	nr	2	nr
20b	Bu^{t}O	MeOH, 20	29	44	nr	12	nr
		MeOH, 2	45	nr	nr	4	nr
		BnOH, 10	18	18	nr	20	nr
		BnOH, 2	31	nr	nr	18	nr
20c	Me	MeOH, 10	78	2	nr	nr	nr

[a] Chorev, Goodman, and MacDonald rationalized the increase in the yield of byproducts on increasing the molar excess of alcohol (a finding contrary to their original expectation) by reasoning that increasing the alcohol content leads to an increasingly polar reaction mixture (the solvent being toluene) which relatively favors the heterolytic or displacement reactions. A control experiment proved that the desired product, **21b** (R^2 = Me), is stable under the reaction conditions.[92] [b] Isolated yields relative to starting *N*-protected phenylalanine.

comprehensive comparison of the vast array of available methods has been undertaken (in theory, any peptide coupling procedure[87,112] is applicable). Due to the danger of *gem*-diaminoalkyl derivative decomposition (see section VI.A.1.a.vi), a rapid coupling procedure is essential; however, most peptide-coupling procedures currently in use meet this requirement. The method used almost exclusively by researchers engaged in PMRI peptide synthesis is the *N*-hydroxybenzotriazole (HOBt)- or *N*-hydroxysuccinimide (HOSu)-catalyzed carbodiimide (usually DCC) procedure. The BOP reagent has also been successfully employed.[113−115] Although catalyzed carbodiimide mediated couplings give good results, activated malonates have a lower coupling efficiency than normal amino acids.[110] Thus yields tend to be lower, and reaction times longer than those usually employed for peptides. In addition, couplings to *gem*-diaminoalkyl derivatives tend to be sluggish per se.[110] The reduced coupling efficiency of both modified residues is presumably electronic in origin.[116] Therefore, a wise strategy for the assembly of a PMRI peptide avoids fragment couplings (i.e., the coupling of partial sequences, as opposed to individual residues) to *gem*-diaminoalkyl derivatives, if possible.[110] For further discussion of considerations for malonyl residue activation see section VI.A.1.b.i.

An important advantage of the Goldschmidt and Wick type synthesis of PMRI peptides is that it reduces the necessary number of couplings involving *gem*-diamino residues (see Scheme 3). Chorev and Goodman, in their aforementioned comparison of Curtius rearrangements,[91] found that the yield of the Goldschmidt and Wick type reaction varied with the carboxylic acid employed (e.g., R^3CO_2H, Scheme 3). *N*-Protected phenylalanine (1.5 equiv) gave a reduced yield of the PMRI dipeptide (**16a**, 35%; **16b**, 6%; from **10**, Scheme 3), but malonic acid (3 equiv) gave an increased yield of PMRI tripeptide Boc-Phe-Alaψ(NHCO)Gly (47% from Boc-Phe-Ala) (changes relative to the corresponding multistep deprotection and coupling procedures: **16a**, 66%; **16b**, 22%; from **10**; and Boc-Phe-Alaψ(NHCO)Gly, 11% from Boc-Phe-Ala).[91]

Scheme 6. Dimerization of IBTFA



A Goldschmidt and Wick type reaction, monitored by IR spectroscopy, using a malonate monoester, was successfully used by Campbell and co-workers to synthesize PMRI dipeptides, which are not readily prepared by acylation of monoprotected *gem*-diaminoalkyl compounds because of the tendency of monoprotected *gem*-diaminoalkyl compounds to decompose when the protecting group is a carbamate (see section VI.A.1.a.vi but *cf.* section VI.A.2.b).[108,109] However, a variant of this procedure employing DPPA to generate the intermediate acyl azide provided a very poor yield (<5%) of the desired PMRI dipeptide.[109]

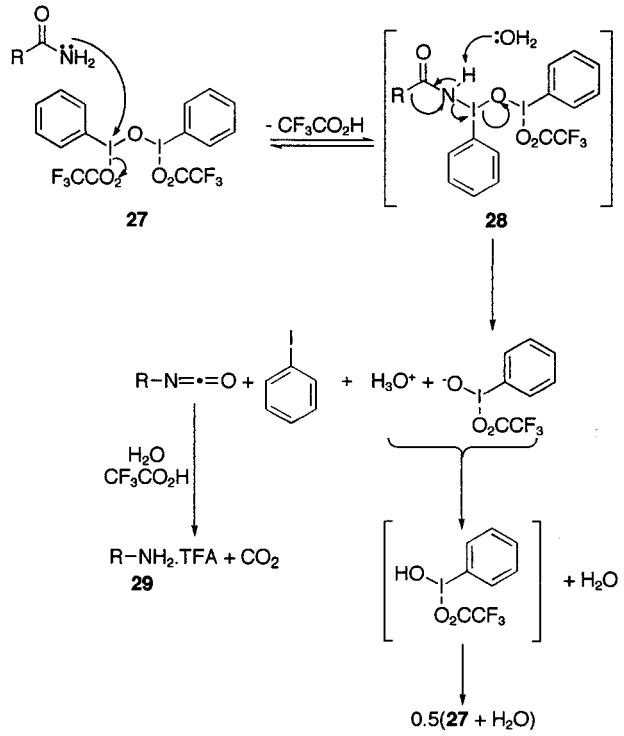
Thus the Curtius rearrangement, with suitable precautions, provides ready access to *gem*-diaminoalkyl compounds appropriate for PMRI peptide synthesis and, in combination with the Goldschmidt and Wick type reaction is a valuable method for the direct synthesis of PMRI peptides.

The Hofmann rearrangement is extensively employed for the synthesis of PMRI peptides, using exclusively the mild oxidant iodobenzene bis(trifluoroacetate) (IBTFA),[117−119] other conditions being too harsh. IBTFA effects the Hofmann rearrangement on primary amides, under acidic or neutral conditions (giving an amine trifluoroacetate salt, **29**, and carbon dioxide), but does not affect secondary or tertiary amides.[118] Therefore IBTFA may be employed to synthesize monoprotected *gem*-diaminoalkyl derivatives from *N*-protected α-amino acid primary amides or peptidyl *gem*-diaminoalkyl derivatives from peptidyl primary amides (which may be hydrolyzed for *C*-terminal sequential degradation,[120] *cf.* Scheme 2).[110,118,121] However, monocarbamate-protected *gem*-diaminoalkyl derivatives decompose under the reaction conditions.[110] Therefore, the requirement to favor the synthesis of peptidyl *gem*-diaminoalkyl derivatives encountered with the Curtius rearrangement (in answer to question 4, above) is absolute in the case of the IBTFA-mediated Hofmann rearrangement, unless special protection is used (see section VI.A.2.b).

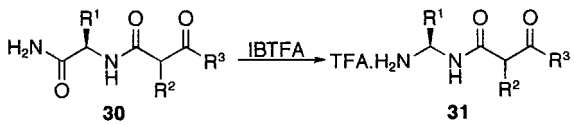
The mechanism of the IBTFA-mediated Hofmann rearrangement is complex. Boutin and Loudon performed a mechanistic study (using hexanoamide as substrate),[119] the results of which are in disagreement with the earlier study of Swaminathan and Venkatasubramanian of the mechanism of the reaction of the similar reagent iodobenzene diacetate with aromatic amides.[122] Boutin and Loudon demonstrated that IBTFA forms a dimer, **27**, under the reaction conditions (water/acetonitrile (50:50)) (Scheme 6).[119]

It is not clear, however, whether the dimer, **27**, or/and the IBTFA monomer is the reactive species;

Scheme 7. The Mechanism of the IBTFA-Mediated Hofmann Rearrangement[119]



Scheme 8. Direct Synthesis of a PMRI Dipeptide Unit 31 Using IBTFA[a]



[a] R^3 = peptide chain or OR^4; R^4 = alkyl, Bn.

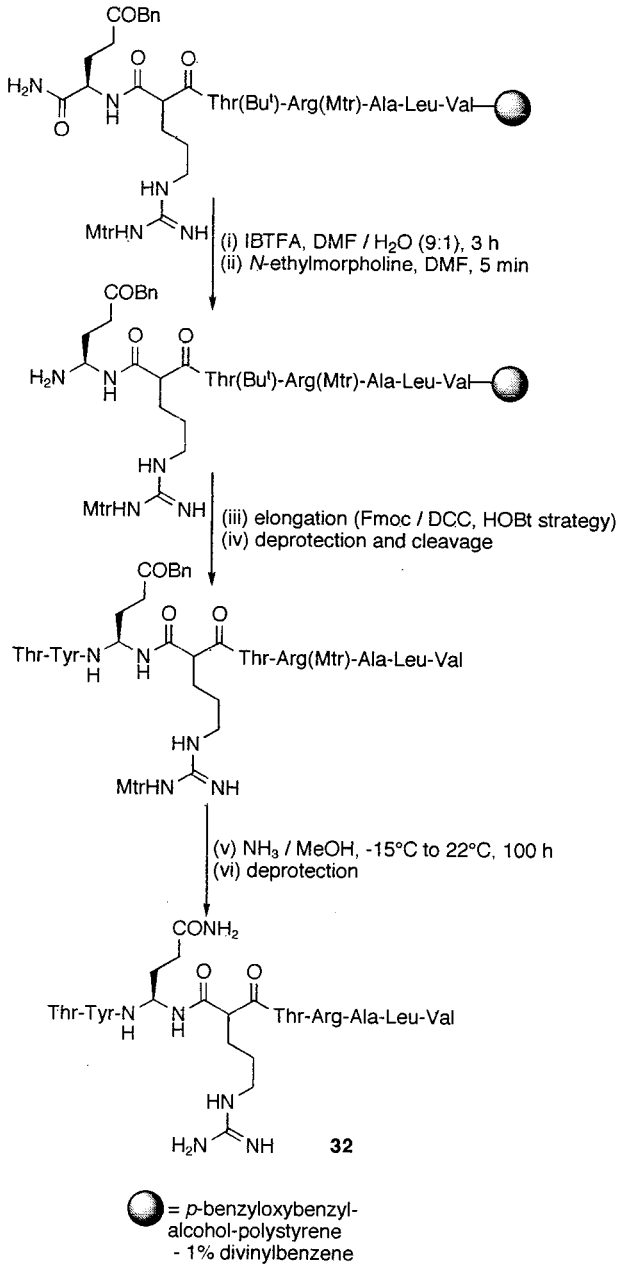
Scheme 7 shows the mechanism with the dimer, **27**, as the reactive species.[119] The rate-determining step is the rearrangement of the amide−IBTFA dimer complex, **28**.[119]

As this mechanism suggests, the reaction proceeds with complete retention of configuration of the migrating group.[118,121]

The considerations discussed above (question 3) with respect to coupling procedures, also apply to the IBTFA procedure, although no Goldschmidt and Wick type reaction is known to use IBTFA because the reaction conditions do not permit trapping, rather than hydrolysis, of the intermediate isocyanate. However, a conceptually analogous, direct synthesis of a PMRI dipeptide unit, **31**, is possible by the reaction of IBTFA with a malonylaminoacyl amide, **30** (Scheme 8).[85,110]

Special considerations are necessary for the synthesis of Gln- or Asn-containing PMRI peptides using IBTFA. The side chains of these amino acids contain primary amides, which undergo the Hofmann rearrangement under the action of IBTFA.[120] Dürr, Goodman, and Jung circumvented this problem by carrying out the IBTFA-mediated Hofmann rearrangement on a protected Glu residue, which was subsequently converted to the required Gln by treatment with ammonia (Scheme 9).[123] Their approach is generally applicable for the synthesis of PMRI

Scheme 9. Synthesis of the PMRI Killer-Cell Epitope of Influenza Nucleoprotein, 32[123]

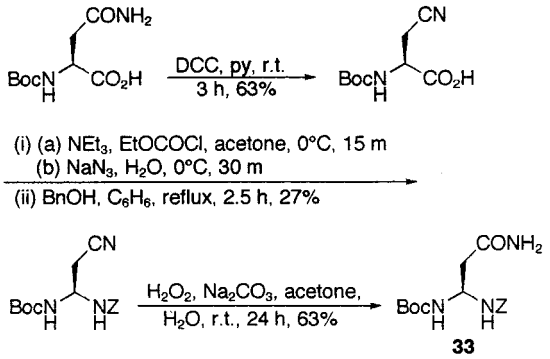


peptides containing Asn and/or Gln, with appropriate protecting group manipulation.

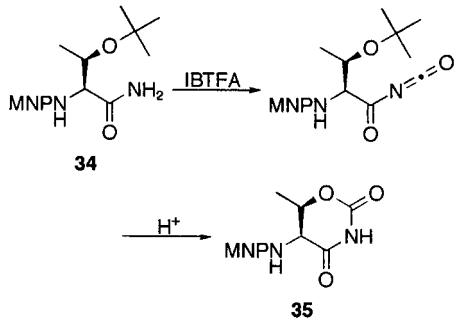
The problems associated with the synthesis of *g*Asn are not confined to IBTFA. Cushman et al. were unable to produce satisfactorily protected *g*Asn from Z-Asn via the Curtius rearrangement using either the mixed-anhydride or DPPA method of acyl azide synthesis.[113] They apportioned blame on the side chain amide group of Asn, which they therefore "protected" as a nitrile during synthesis (Scheme 10).[113]

The oxidation-sensitive amino acids, tyrosine, tryptophan, and methionine also require special attention if they are to be exposed to IBTFA. Their side chains may be effectively protected with Bu^t, For, and as Met(O), respectively.[85,110] IBTFA oxidizes cysteine to cystine and cleaves or oxidizes the usual cysteine protecting groups: no way around this problem exists, other than strategic assembly.[124]

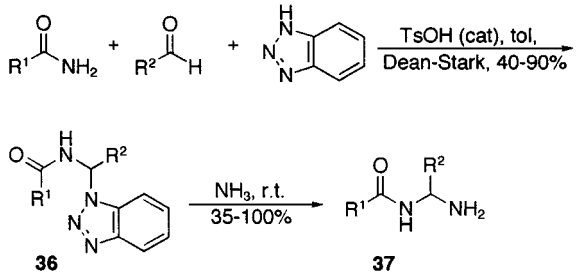
Scheme 10. Synthesis of Boc-*g*Asn-Z, 33[113]



Scheme 11. Acid-Catalyzed Deprotection of MNP-Thr(Bu^t)-NH$_2$, 34, during IBTFA Treatment[125]



Scheme 12. Mannich Reaction Leading to *gem*-Diaminoalkyl Compounds[a]



[a] In all cases small quantities of the benzotriazol-2-yl isomer of the intermediate **36** were also produced.

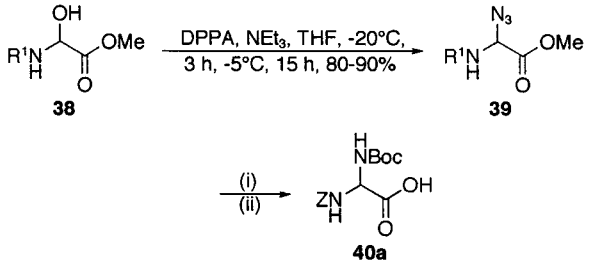
No problems have been reported with any of the other coded amino acid residues, except for one case of *tert*-butyl ether cleavage and subsequent 2-oxazolidone, **35**, formation with MNP-Thr(Bu^t)-NH$_2$, **34** (Scheme 11).[125]

However, IBTFA does oxidatively degrade free (unprotected) α-amino acids.[118]

The IBTFA-mediated Hofmann rearrangement is now the most frequently employed method of *gem*-diaminoalkyl derivative synthesis, with the above-mentioned provisos. It is certainly the method of choice for the synthesis of peptidyl *gem*-diaminoalkyl derivatives.

ii. The Mannich Reaction. Katritzky et al. synthesized racemic, monoprotected *gem*-diaminoalkyl compounds, **37**, via the Mannich reaction of an amide, aldehyde, and benzotriazole (Bt) to generate the (isolated) intermediate adduct, **36**, and subsequent reaction with ammonia (Scheme 12).[126] Use of *N*-protected α-amino acid amides as the amide component gave peptidyl *gem*-diaminoalkyl derivatives.[126] This procedure suffers from the drawback

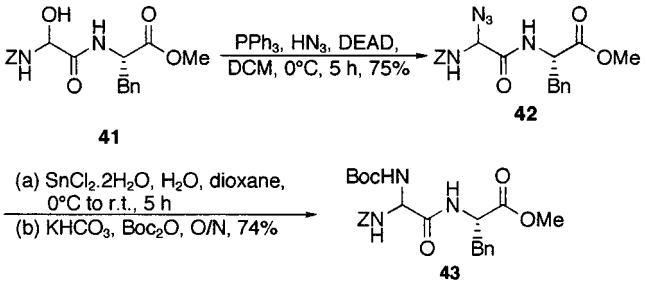
Scheme 13. Preparation of α-Boc-amino-Z-glycine, 40a[a]



38 39 40a

[a] (i) R^1 = Z, Lindlar cat., H_2, Boc_2O, MeOH, room temperature, 5 h, 56%; R^1 = Boc, (a) Pd/C, H_2, MeOH, AcOH, room temperature, 2 h, (b) ZCl, NEt_3, DCM, 0 °C to room temperature, 15 h, 60%; (ii) NaOH, H_2O, dioxane, room temperature, 94%.

Scheme 14. Preparation of α-Boc-amino-Z-glycylphenylalanine Methyl Ester 43[127]



41 42 43

of producing both epimers (at the *gem*-diamino carbon) of the product, **37**. Diastereoselective generation of the intermediate, **36**, was observed in some cases when an amino acid amide was employed. However, the ammonia displacement of Bt proceeded with complete racemization, presumably via an S_N1 mechanism. The peptidyl *gem*-diaminoalkyl compounds, **37**, were readily resolved during purification to provide diastereomerically pure products.[126] This method expands the range of easily accessible *gem*-diaminoalkyl derivatives beyond those derived from α-amino acids.

*iii. Syntheses of Orthogonally Protected α-Aminoglycine, **40**, by the Displacement of α-Heteroatoms.* Schmidt et al. have developed methodology for the conversion of the protected α-hydroxyglycines, **38**, to the orthogonally protected α-aminoglycine, **40a**, via the corresponding α-azidoglycine derivatives, **39**.[127] DPPA or a Mitsunobu reaction with hydrazoic acid introduced the α-azido group, the latter method being favored for dipeptides, for example **41** (Schemes 13 and 14).[127]

The diastereomeric dipeptides, **42**, were separable by MPLC.[127]

Bock et al. synthesized an orthogonally protected α-aminoglycine, **40a**, from α-hydroxy-Z-glycine, **44**.[128] Amidoalkylation of 2-propanethiol and subsequent mercuric ion mediated displacement of sulfur with *tert*-butyl carbamate yielded racemic α-Boc-amino-Z-glycine, **40a** (Scheme 15).[128] Standard protection, deprotection and coupling steps facilitated elaboration at either amino group.[128]

Both Bock et al.[128] and Schmidt et al.[127] synthesized their α-hydroxyglycine derivatives by the reaction of glyoxylic acid, **45**, and the appropriate carbamate, as

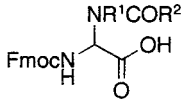
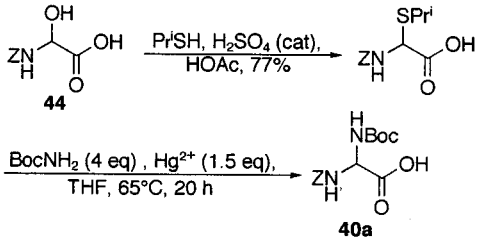


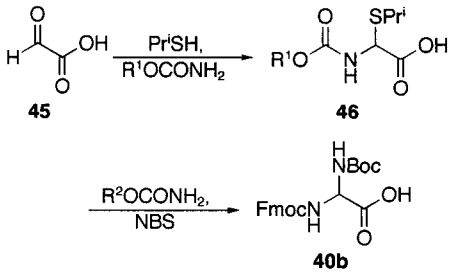
Figure 8. The betidamino acid concept. For Fmoc-betidalanine (α-For-amino-Fmoc-glycine) $R^1 = R^2 = H$.

Scheme 15. Synthesis of α-Boc-amino-Z-glycine, 40a[128]



44 40a

Scheme 16. Synthesis of α-Boc-amino-Fmoc-glycine, 40b[111,a]



45 46 40b

[a] R^1 = Bu^t, R^2 = 9-fluorenylmethyl, or vice versa.

previously described by Zoller and Ben-Ishai[129] and Losse and Strobel.[130]

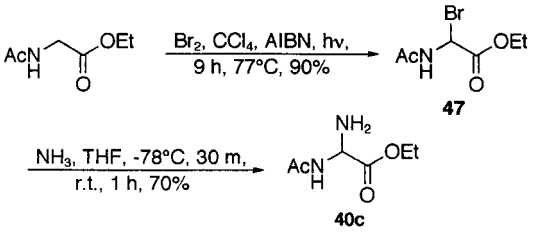
Qasmi et al. synthesized α-Boc-amino-Fmoc-glycine, **40b**, also via an α-thioglycine derivative. A Mannich-type reaction of a carbamate, 2-propanethiol, and glyoxylic acid hydrate, **45**, furnished the intermediate **46**, which yielded racemic α-Boc-amino-Fmoc-glycine, **40b**, upon NBS-mediated displacement of sulfur (Scheme 16).[111] α-Boc-amino-Fmoc-glycine, **40b**, was used to incorporate an α-amino-glycine residue into an octapeptide using SPS.[111]

Rivier and co-workers propose the use of a family of α-aminoglycine derivatives as amino acid mimics.[131] Their concept differs from partial retro-inverso modification in that the peptide backbone is unmodified but the amino acid side chains feature an (*N*-substituted) amide bond in place of the natural C^β (see Figure 8). Rivier and co-workers call their modified monomers betidamino acids and synthesized representative examples using a method similar to that of Qasmi et al., described above.[131,132] Furthermore they calculated Ramachandran maps for a series of betidamino acids and synthesized, by solid-phase methods, betidamino acid containing gonadotrophin-releasing hormone antagonists and somatostatin analogues, which retained biological activity.[131,133]

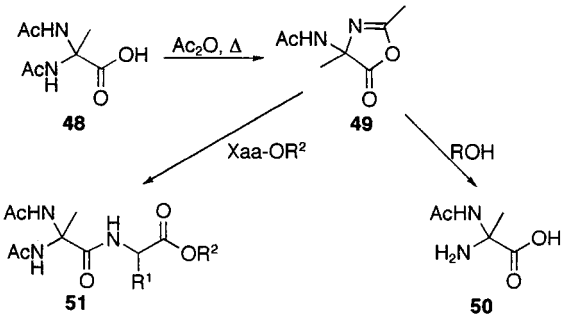
Kohn et al. synthesized the racemic α-aminoglycine derivative, **40c**, from the corresponding α-bromoglycine derivative, **47**,[134] prepared using the protocol of Kober and Steglich[135] (Scheme 17).

No α-aminoglycine residues have been introduced into PMRI peptides despite the availability of suitable derivatives by these routes.

Scheme 17. Preparation of α-Acetylaminoglycine Ethyl Ester, 40c[134,135]

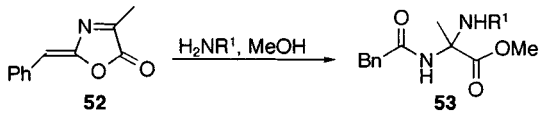


Scheme 18. Synthesis of α-Acetylaminoalanine Derivatives[136−138,a]



[a] R^1 = H, Bn; R^2 = H, Et.

Scheme 19. Synthesis of α-Phenylacetylaminoalanine Derivatives[139,140,a]



[a] R^1 = H, $CHR^2CO_2R^3$; R^2 = h, Pr^i, Ph; R^3 = H, Me.

iv. Via Oxazolones. Bergmann and Grafe synthesized α,α-diacetaminopropionic acid, **48**, by condensing pyruvic acid and acetamide or acetonitrile.[136] They converted α,α-diacetaminopropionic acid (**48**) to the corresponding 5(4*H*)-oxazolone (azlactone) (**49**) which yields α-amino-α-acetaminopropionic acid, **50**, and its peptide derivatives, **51**, on treatment with alcohols and α-amino acids respectively (Scheme 18).[137,138]

Romeo and co-workers synthesized similar α,α-diaminopropionic acid derivatives, **53**, via 2-benzylidene-4-methyl-5(2*H*)-oxazolone, **52** (Scheme 19).[139,140]

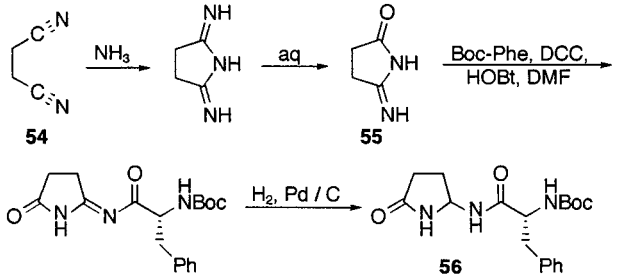
gem-Diaminoalkyl compounds synthesized in this fashion (which do not correspond to coded α-amino acids) have not been utilized in PMRI peptides.

v. From Nitriles. Hayward and Morley's synthesis of the first *gem*-diaminoalkyl residue containing peptide, **56**, started from succinodinitrile, (**54**).[74] They prepared 5-iminopyrrolidin-2-one (**55**) by cold aqueous hydrolysis of the product from succinodinitrile (**54**) and ammonia; iminoacylation and subsequent hydrogenation yielded a (separable) mixture of the desired pseudodipeptide diastereomers, **56** (Scheme 20).[74]

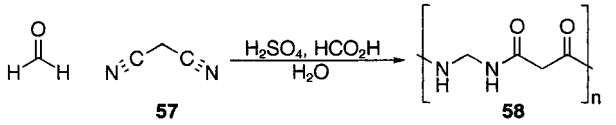
Although theoretically general, no method based on the reduction of acylamidines, such as **55**, has since found use in PMRI peptide synthesis.

Finally, although not generally applicable to the synthesis of PMRI peptides, Puiggalí and Muñoz-Guerra's synthesis of nylon 1,3 {i.e., [Glyψ(NHCO)-Gly]$_n$} (**58**) from malononitrile (**57**) and formaldehyde using Magat's formic acid/sulfuric acid catalyzed polymerization,[141] is worthy of note (Scheme 21).[142]

Scheme 20. Synthesis of (*R,S*)-*g*Glp-*r*Phe-Boc, 56[74]



Scheme 21. Synthesis of Nylon 1,3[142]

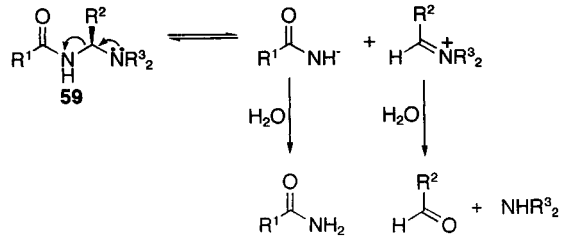


The literature contains other methods of *gem*-diamino derivative synthesis, but the resultant derivatives are unsuitable for incorporation into PMRI peptides.

vi. Decomposition. Several research groups have studied the decomposition of *gem*-diaminoalkyl moieties.[143,144] However only Loudon and co-workers, following on from their work on carboxy-terminal peptide degradation, studied the mechanism of hydrolysis of monoprotected *gem*-diaminoalkyl compounds of the variety encountered in PMRI peptides.[103,120]

Under basic conditions the mechanism of hydrolysis determined by Loudon and co-workers is as shown in Scheme 22, and the observed rates of hydrolysis

Scheme 22. Hydrolysis of *gem*-Diaminoalkyl Compounds under Basic Conditions[a]



[a] **a**: R^1 = Me, R^2 = Pr^i, R^3 = H. **b**: R^1 = $MeOCH_2$, R^2 = Pr^i, R^3 = H. **c**: R^1 = Bu^tO, R^2 = Pr^i, R^3 = H. **d**: R^1 = Me, R^2 = Pr^i, R^3 = Me.[103]

were in the order **59d** > **59b** > **59a**, due to imine stabilization in **59d** and a better leaving group in **59b** than in **59a**.[103]

Under acidic conditions the mechanism shown in Scheme 23 operates, with a possible transition state illustrated in Figure 9.[103]

The study of *gem*-diaminoalkyl compound **59c** was limited due to its instability: under acidic conditions,

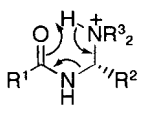
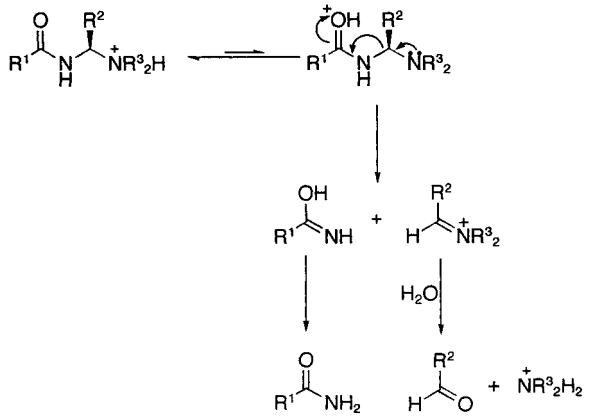


Figure 9. Possible transition state.[103]

Scheme 23. Hydrolysis of *gem*-Diaminoalkyl Compounds under Acidic Conditions[103,a]



[a] R^1, R^2, R^3 as Scheme 22.

at 50 °C, it was hydrolyzed 9.2 times faster than **59a**.[103] This rate acceleration is due to the better leaving group in **59c** overcoming its lower basicity.[103]

These mechanisms suggest that *gem*-diaminoalkyl compounds **59** with R^2 = H should be more stable than those with R^2 = alkyl.[103] The results enable an estimate of 10−50 h for the biological half-life of **59a**,[103] and reiterate the advantage of synthesizing peptidyl rather than monocarbamate-protected *gem*-diaminoalkyl derivatives.[103] Loudon and co-workers also measured the pK_a of $^+$H*g*Gly-Ac as 7.85 (26 °C), a value in close accord with that predicted by comparison with other amines.[103]

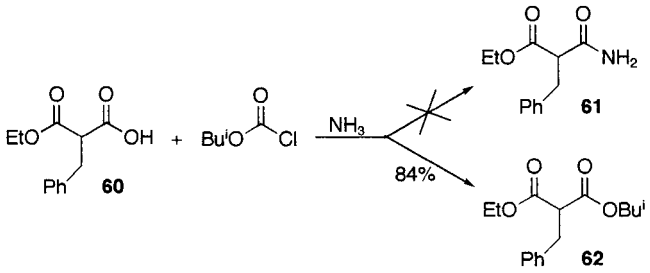
Overall the stability of monoacyl *gem*-diaminoalkyl compounds is much the same at mildly acidic and mildly basic pH, and is greater than one might expect.[103] These mechanisms explain this stability: under basic conditions the leaving group (an amide anion) is poor, whereas under acidic conditions an unfavorable equilibrium must be overcome.[103]

b. C-2-Substituted Malonyl Derivatives. *i. Specific Considerations for Malonyl Residue Activation.* Considerations for the coupling and direct incorporation of malonyl derivatives are discussed above (section VI.A.1.a.i). As mentioned there, the method used almost exclusively for malonyl residue activation during PMRI peptide synthesis is the HOBt- or HOSu-catalyzed carbodiimide procedure, with BOP also proving useful.

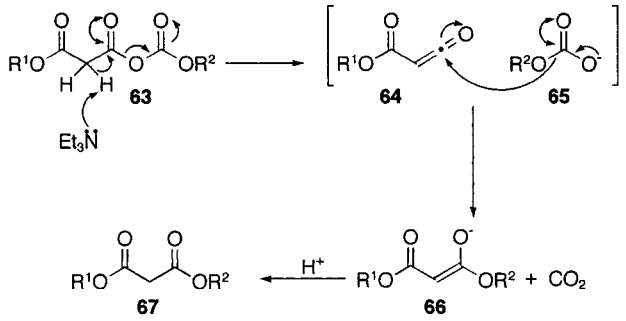
The only standard peptide coupling procedure found to be unsatisfactory for malonyl residues is the carbonate mixed-anhydride method. Goodman and co-workers discovered that the carbonate mixed-anhydride coupling of monoethyl 2-benzylmalonate (**60**) to ammonia, yielded only ethyl isobutyl 2-benzylmalonate (**62**) and none of the expected amide, **61** (Scheme 24).[110]

The same side reaction was observed by Kametani et al. while attempting to convert a malonate monoester to the corresponding diazo ketone via a mixed carbonic anhydride.[145] Indeed, Gutman and Boltanski advocate such a procedure for the synthesis of malonate esters.[146,147] The mechanism proposed by Gutman and Boltanski for this reaction is depicted in Scheme 25: after generation of the mixed-anhydride, **63**, deprotonation by base results in

Scheme 24. A Failed Mixed-Anhydride Coupling[110]



Scheme 25. The Mechanism of Ester Formation from Malonate/Carbonate Mixed Anhydrides[146]



elimination of the monoalkyl carbonate, **65**, and the formation of a ketene, **64**. These two products then interact and the alkoxide residue of **65** is transferred to the ketene, **64**, affording the enolate, **66**, and carbon dioxide. Finally protonation yields the dialkyl malonate, **67**.[146]

There have been some reports of successful mixed-anhydride couplings of malonyl residues and α-amino acids or peptides. Carmona and Juliano synthesized EtO-(*R,S*)-*m*Trp-D-Val-OMe via a "mixed-anhydride procedure"; however scant details of the procedure were divulged.[148] In particular it is unclear what type of mixed-anhydride was employed.[87,149] Mixed anhydrides of malonic and carboxylic acids (e.g., acetic or pivalic acid) react with alcohols and amines to produce the expected malonate esters and amides in moderate to excellent yields.[150,151] Angelastro et al. successfully coupled Mor-*m*Val and Val-Pro-Val-C_2F_5 by activating the malonyl residue through mixed-anhydride formation with isobutyl chloroformate.[152] They made no mention of the ester, Mor-*m*Val-OBu^i, which might be expected in the light of the results discussed above. The results of Carmona and Juliano and Angelastro et al. notwithstanding, it is prudent to avoid carbonate mixed-anhydride procedures for the coupling of malonyl residues to amino acids or *gem*-diamino residues.

The symmetrical anhydride procedure has not been applied for the activation of malonyl residues during PMRI peptide synthesis. Presumably this is due not only to the waste of 1 equiv of the malonyl residue, but also to the problems associated with the preparation of malonic anhydrides, that is efforts to form them tend to result in ketenes (cf., Scheme 25). Nevertheless, such ketene species could act as acylating agents (e.g., carbon suboxide has been successfully coupled with amines).[153,154] Monomeric malonic anhydride may be synthesized by ozonolysis of diketene, whereas usual dehydration methods

yield carbon suboxide.[155] C-2-substituted, polymeric malonic anhydrides may be synthesized via their monochlorides.[156] The anhydride of monoethyl malonate, prepared using DCC, has been employed (without isolation) for couplings outside the PMRI peptide field, but the yields were only moderate.[157,158]

Some of the theoretical advantages of mixed-anhydride couplings (i.e., easily removable byproducts) may be obtained by using reagents that are carbonic acid derivatives wherein both leaving groups are auxiliary nucleophiles: any side reaction analogous to that depicted in Scheme 25 is then immaterial for it merely generates an activated coupling species. Such reagents are yet to find widespread use in PMRI peptide synthesis (but see section VI.A.2.c). Nevertheless, carbonyldiimidazole has been used to couple monoesters of malonic acid with amines (albeit in poor yields),[157] and we have used the reagent *N,N′*-disuccinimidyl carbonate, developed by Ogura et al., to prepare the HOSu active ester of a PMRI dipeptide in good yield.[109,159]

In contrast to malonic anhydrides, monochlorides of malonic acids are readily accessible by conventional methods and couple to α-amino acids and peptides with varying efficiency (e.g., see refs 152, 160, and 161). In common with malonic anhydrides and α-amino acid chlorides, monochlorides of malonic acids are seldom employed in (PMRI) peptide synthesis, probably due to concerns about "overactivation".[87]

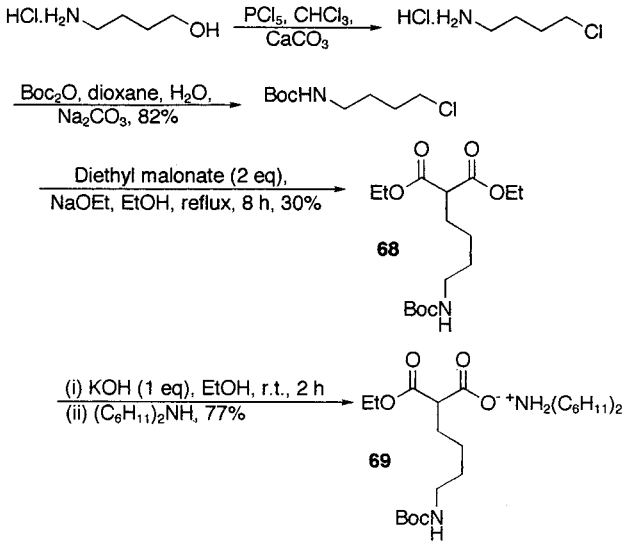
The only other previously unmentioned procedure that has been used to couple a malonyl residue to an α-amino acid employs DPPA as the coupling reagent.[162]

ii. Alkylation and Partial Hydrolysis of Malonic Acid Diesters. Here, and in the following sections, we consider the preparation of C-2-substituted malonyl derivatives appropriate for incorporation into PMRI peptides. The derivatives required are substituted at C-2 with an amino acid side chain and are monoprotected, for example **69**.

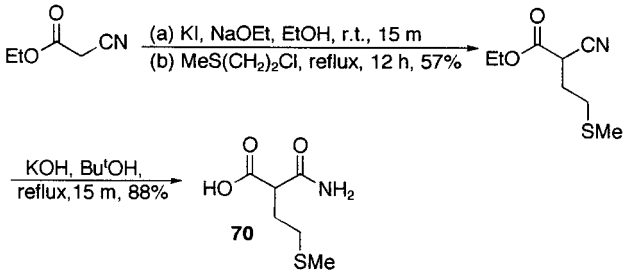
The classical method of alkylation and partial hydrolysis of malonic acid diesters provides access to many appropriate C-2-substituted malonyl derivatives, for example those corresponding to Phe, Lys, Trp, Leu, Ala, His and Met.[95,110] Deprotonation of malonic acid diesters with sodium ethoxide inhibits dialkylation by most electrophiles (because the monoalkyl derivative, for example **68**, is less acidic than ethanol).[163] Partial saponification of the resultant C-2-substituted diesters yields the corresponding monoesters: for example Scheme 26.

iii. Alkylation and Hydrolysis of Cyanoacetates. C-2-substituted malonamic acids, suitable only for carboxy terminal incorporation, may be synthesized by alkylation and hydrolysis of cyanoacetates; however, dialkylation is a more serious problem here, due to the substrates' increased acidity.[163] For example Cushman et al. synthesized *m*Met-NH_2, **70**, for incorporation into end group modified bombesin *C*-terminal nonapeptide (Scheme 27).[113] The product, **70**, existed in equilibrium with both enol tautomers, but this did not prevent subsequent peptide coupling using standard methods.[113]

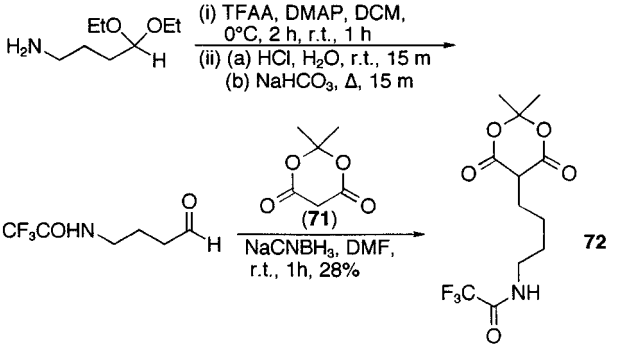
Scheme 26. Synthesis of (*R,S*)-*m*Lys(Boc)-OEt, 69, for Incorporation into PMRI Somatostatin, by Alkylation of Diethyl Malonate[164]



Scheme 27. Synthesis of *m*Met-NH_2, 70[113]



Scheme 28. Synthesis of the Meldrum's Acid Derivative Corresponding to *m*Lys(TFA), for Incorporation into PMRI Tuftsin[166]

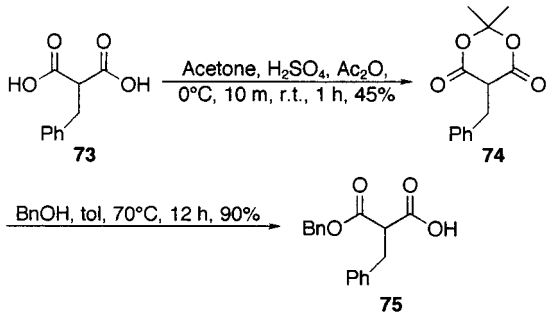


iv. From Meldrum's Acid. Suitable side chains may be introduced at C-5 of Meldrum's acid (**71**) by (1) reductive alkylation, or (2) a two-step process via an alkylidene or a cyclopropyl derivative.[124,165]

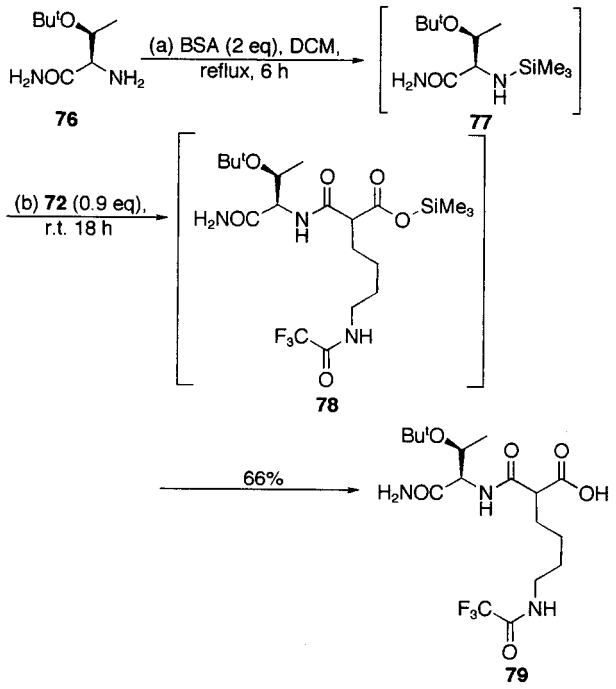
Knoevenagel reaction of Meldrum's acid, **71**, with aldehydes (or some ketones) and reduction (in situ with borane−dimethylamine complex, borane−trimethylamine complex, or sodium cyanoborohydride; subsequently with sodium borohydride) yields mono-C-5-substituted Meldrum's acids, for example Scheme 28.[115,166−168] Subsequent alcoholysis yields C-2-substituted malonic acid monoesters.

Indeed, this alcoholysis is sufficiently convenient that Chorev, Goodman, and co-workers prepared the Meldrum's acid derivative of 2-benzylmalonic acid

Scheme 29. Synthesis of Monobenzyl 2-Benzyl Malonate by Alcoholysis of the Corresponding Meldrum's Acid Derivative[169]



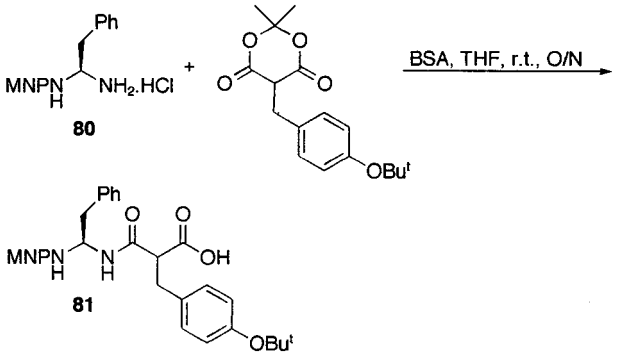
Scheme 30. Synthesis of H_2N-D-*r*Thr(Bu^t)-*m*Lys(TFA), 79[166]



(**73**) to obtain the corresponding malonic monoester, **75**, by ring opening with benzyl alcohol (Scheme 29).[169]

Alternatively, mono-C-5-substituted Meldrum's acids may be ring opened with silylated amines.[170] Silylation prevents the amine acting as a base and deprotonating the Meldrum's acid derivative, which thus remains in the keto form appropriate for the ring opening reaction.[166] The reaction mechanism is complex and probably involves a cyclic transition state which utilizes a vacant silicon 3d-orbital.[170,171] Verdini and co-workers used this approach to ring open Meldrum's acid derivatives with amino acids or monoprotected *gem*-diaminoalkyl compounds which they silylated, in the same pot, with *O*,*N*-bis(trimethylsilyl)acetamide (BSA).[124,166,172,173] Thus they prepared H_2N-D-*r*Thr(Bu^t)-*m*Lys(TFA), **79**, Scheme 30, which they subsequently incorporated into PMRI tuftsin.[166] The synthesis depicted in Scheme 30 was a one-pot reaction and the intermediates, **77** and **78**, were not isolated or characterized.[166] It is distinctly possible that D-Thr(Bu^t)-NH_2, **76**, was bis-silylated under the reaction conditions (i.e., as **77** but with a second TMS group on the amide nitrogen).[170,172] The

Scheme 31. Synthesis of MNP-Pheψ(NHCO)(*R*,*S*)-Tyr(Bu^t), 81, for Use in SPS[124]



silylated intermediate **78** was hydrolyzed during workup. Note that C^β of −D-Thr− was not inverted during this procedure.

Verdini and co-workers also used this method to prepare the PMRI dipeptide MNP-Pheψ(NHCO)-(*R*,*S*)-Tyr(Bu^t) (**81**, Scheme 31), having demonstrated that trimethylsilylation does not adversely affect the stability of MNP-*g*Phe·HCl, **80**.[124] Excess BSA was used, and no base was necessary.[124,172,174] Again, the silylated species was not isolated or characterized.

Dal Pozzo and co-workers, in their synthesis of a PMRI analogue, **83**, of the growth factor Gly-His-Lys, compared BSA-mediated amidative ring opening with alcoholysis and subsequent coupling of the Meldrum's acid C-5 derivatives **82a** and **82b**, respectively (Scheme 32).[175] Both routes gave similar yields (31% and 24%, respectively).[175]

Nucleophilic addition to Meldrum's acid Knoevenagel products, **84**, permits access to C-5 derivatives, **85**, suitable for elaboration as above (Scheme 33).[124,165]

Alternatively a C-5 cyclopropyl derivative, **86**, may be prepared and similarly attacked with nucleophiles (Scheme 34).[165,176]

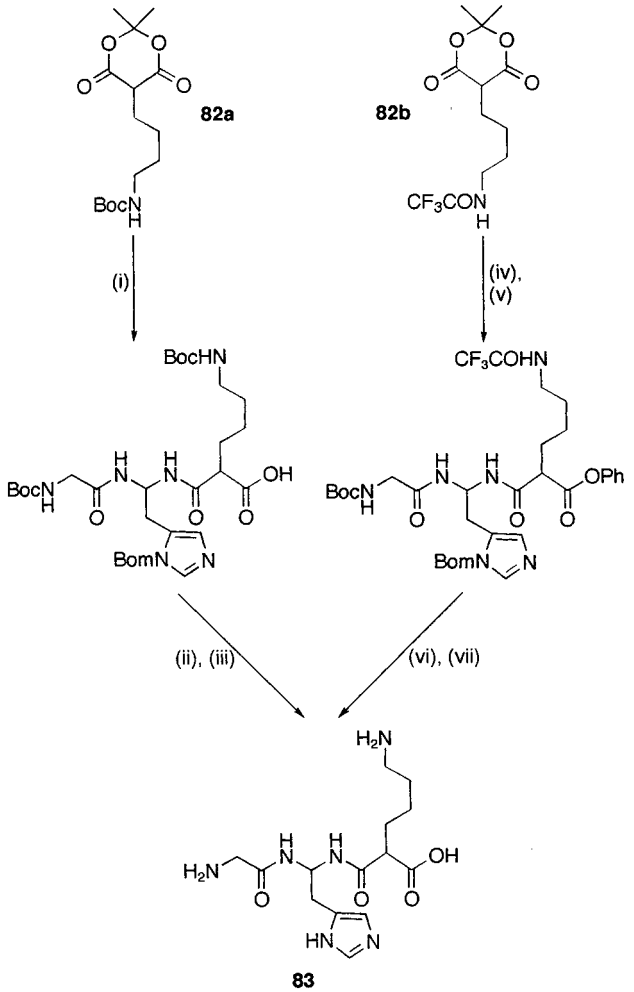
v. Alkylation of PMRI Dipeptides. Campbell and co-workers found that the yield of Goldschmidt and Wick type reactions using C-2-substituted monomalonates was lower than when using C-2-unsubstituted monomalonates.[108] Thus they introduced the desired side chains (i.e., the malonate C-2 substituents) by specific alkylation of the PMRI dipeptides PG-Xaaψ(NHCO)Gly-OR^2, **87** (for example Scheme 35).[108] Note that this is a special case of the alkylation of malonic acid esters, discussed in section VI.A.1.b.ii.

vi. Acylation of Carboxylates. Acylation of the enolate of a *tert*-butyl carboxylate, **88**, with an alkyl or benzyl chloroformate, **89**, yields an orthogonally protected, C-2-substituted malonate, **90** (Scheme 36).[85]

Similarly, monoesters of malonic acid, **92**, may be synthesized by the acylation of a carboxylic acid enolate, **91**, with a carbonate or (preferably) a chloroformate (Scheme 37).[178]

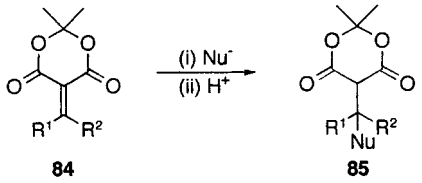
Krapcho and co-workers obtained good yields of C-2-disubstituted malonate monoesters but only moderate yields of un- and monosubstituted malonate monoesters using this method.[178] The lower yields

Scheme 32. Alternative Routes to Gly-Hisψ(NHCO)(*R*,*S*)-Lys, 83[a]



[a] (i) Boc-Gly-*g*His(Bom), BSA, DCM, reflux, 6 h, then **82a**, room temperature, O/N. (ii) TFA, room temperature, 20 m, 79%. (iii) Thioanisole, TFA, TMSOTf, 0 °C, 30 min, 53%. (iv) PhOH, 110 °C, 2.5 h + 15 min under reduced pressure, 74%. (v) Boc-Gly-*g*His(Bom), DCC, HOBt, py, DCM, 0 °C, 5 min, room temperature, 3 h, 63%. (vi) NaOH, H_2O, MeOH. (vii) NaI, TMSCl, MeCN, 80°C, 6 h, 51%.[175]

Scheme 33. Synthesis of *m*Xaa Precursors

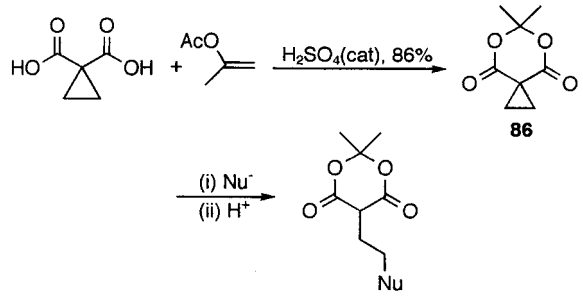


of un- and monosubstituted malonate monoesters are caused by abstraction of the C-2 proton of the initially formed monoester malonate salt by the carboxylic acid enolate, **91**.[178]

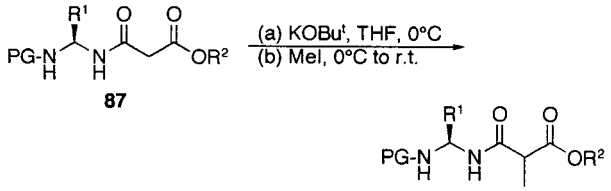
Conversely, Krapcho and co-workers and Reiffers et al. synthesized monoesters of malonic acid, **92**, in good yield by the carboxylation of carboxylate ester enolates with carbon dioxide.[178,179] Again the yields improve as C-2 substitution is increased, enolate self-condensation being a major side reaction in less substituted cases.[179]

Krapcho and co-workers have also synthesized malonamic acids, **93**, by the analogous reaction of a

Scheme 34. Synthesis of *m*Xaa Precursors via Meldrum's Acid C-5 Cyclopropyl Derivative[165,176]

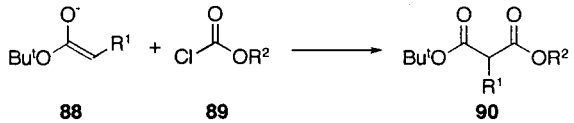


Scheme 35. Alkylation of PMRI Dipeptides Containing −*m*Gly− to Produce −*m*Ala−[108,177,a]



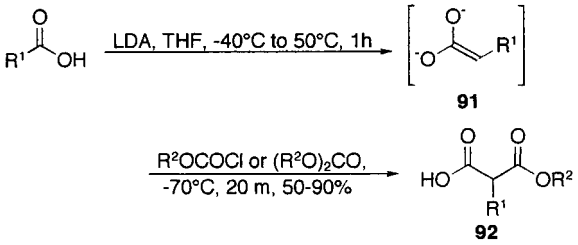
[a] PG = Ac, Boc or Z; R^1 = amino acid side chain; and R^2 = Et or Ph.

Scheme 36. Synthesis of Bu^{t}O-*m*Xaa-OR^2, 90[a]



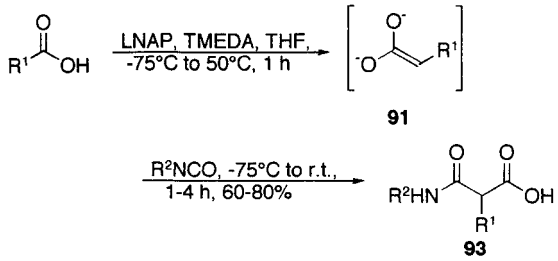
[a] R^1 = suitable amino acid side chain, R^2 = alkyl or benzyl.

Scheme 37. Synthesis of Monoesters of Malonic Acids by α-Carbalkoxylations of Carboxylic Acids[178,a]



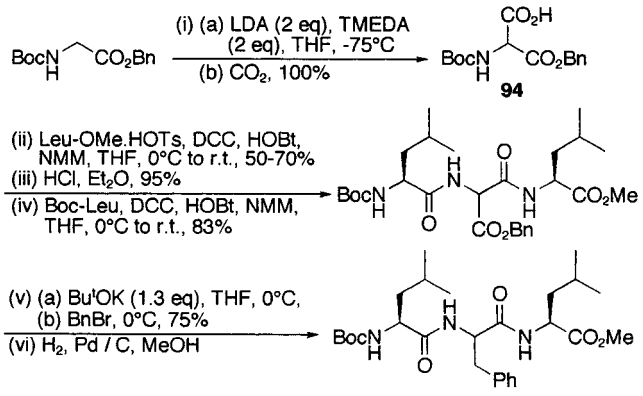
[a] R^2 = Me or Et.

Scheme 38. Synthesis of Malonamic Acids by α-Amidation of Carboxylic Acids with Isocyanates[180]



carboxylic acid enolate, **91**, with an isocyante (Scheme 38).[180] In this case lithium naphthalenide, rather than LDA, was the base of choice for carboxylic acid enolate, **91**, generation because its conjugate acid (naphthalene) is insufficiently nucleophilic to undergo side reactions with the isocyante (in contrast to diisopropylamine).[180]

Scheme 39. Peptide *C*-Alkylation Facilitated by an Aminomalonate Moiety[181]



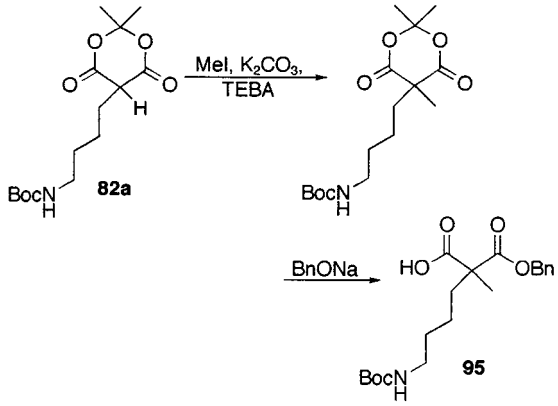
Bossler et al. applied a carboxylation strategy to introduce a malonyl moiety into a peptide in order to facilitate specific *C*-alkylation (for example Scheme 39).[181]

This method necessitated the synthesis of C-2 malonyl derivative **94**, which does not, however, correspond to a coded amino acid when viewed from the PMRI peptide perspective. This approach illustrates the simplicity of malonate alkylation (sections ii and v above).

vii. Protection of the Acidic C-2 Hydrogen of Malonate Esters. An alkoxycarbonyl group,[182] an allyl group,[183] or an alkylidene group[184] may be employed as protection for the C-2 hydrogen of malonates, the alkylidene group is also suitable for cyanoacetates. Use of these protecting groups permits the synthesis of classically challenging C-2-substituted malonate esters (such as those with halogen-containing side chains, which are useful synthetic intermediates)[182−184] and may facilitate further development of the methods described in sections VI.A.1.b.ii, iii, and v. The deprotection protocols are, for the alkoxycarbonyl group, monodecarboxylation using lithium diisopropylamide or boron trichloride;[182] deallylation using (η^2-propene)-titanium(II)bis(isopropoxide), generated in situ from isopropylmagnesium chloride or bromide and titanium(IV) isopropoxide;[183] and sequential ozonolysis and alcohololysis for the alkylidene group.[184] Given that the C-2 hydrogen is implicated in the failure of the mixed-anhydride coupling method for malonates (see Scheme 25), it is possible that judicious employment of C-2 protection may increase the yields and range of useful malonate couplings, for Gutman and Boltanski demonstrated that disubstituted malonates do not form esters on reaction with chloroformates.[146]

viii. Configurational Lability: C-2 Fluorinated and Bis-alkylated Malonyl Derivatives. None of the above syntheses of C-2-substituted malonyl derivatives are stereoselective: all yield both epimers at C-2. However, this lack of selectivity is not a serious consideration because the C-2 malonyl position is configurationally labile during synthesis.[85] C-2-substituted malonamic acids are more configurationally stable than C-2-substituted malonic acids; therefore, the configurational stability of malonyl residues tends to increase upon their incorporation into PMRI peptides; however, other factors such as the surrounding amino acid sequence also influence the configurational stability.[113] Many PMRI peptide epimers (at the C-2 malonyl position) have been separated by RPHPLC and the half-lives for the epimerization measured.[85] The half-lives vary from minutes to days, with cyclic PMRI peptides generally displaying greater stability.[85] Since the epimerization occurs (with general acid−base catalysis) via a coplanar enol structure, the extra stability of cyclic PMRI peptides can be understood in terms of steric hindrance (which blocks the deprotonation) and/or conformational strain (which disfavors the coplanar arrangement).[85,185] Because the epimers of PMRI analogues of peptides often display significantly different biological activities, and, indeed, only one can exhibit full topochemical complimentarity with the parent peptide, the assignment of configuration is important. Several methods of configurational assignment (which utilize RPHPLC retention times, degradation, or NMR spectroscopy) have been developed.[85] However, it must be borne in mind that, particularly in the case of epimers with short half-lives, separation and assignment may be futile, for subsequent epimerization under assay conditions is a possibility, leading to blurred bioactivity results.[185]

Scheme 40. Preparation of 2-Me-*m*Lys(Boc)-OBn, 95[186]



Further derivatization of the C-2 malonyl position overcomes the configurational lability.

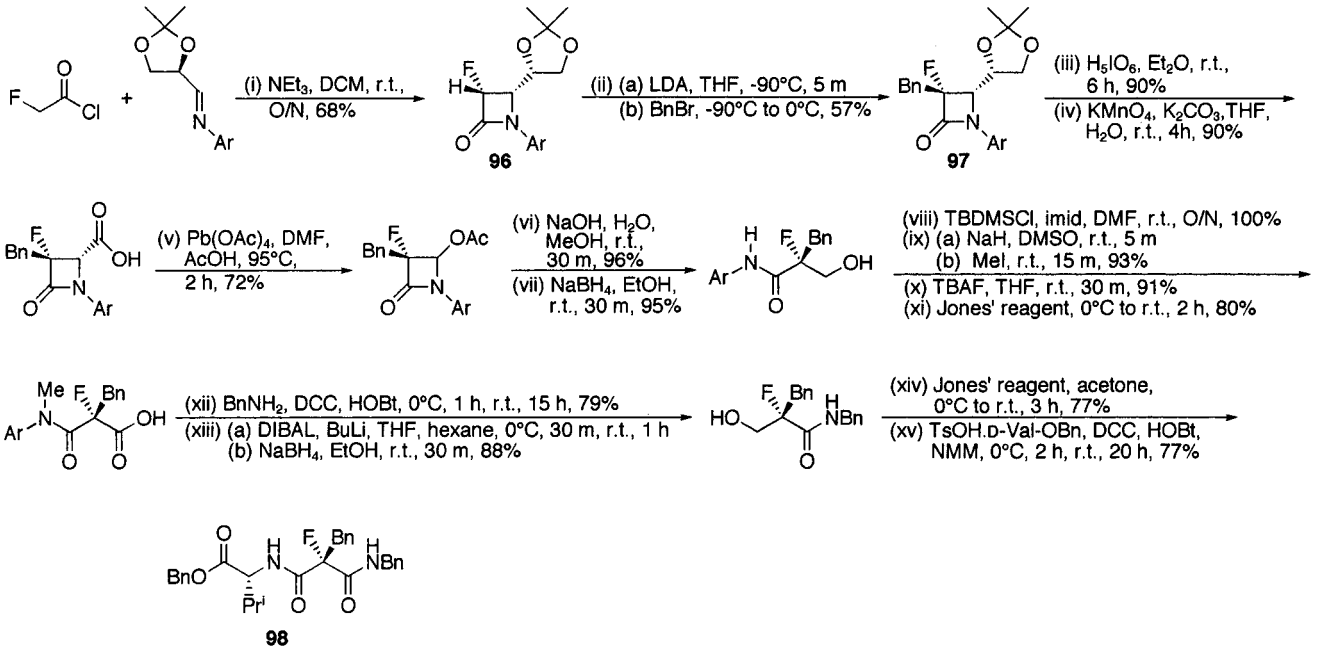
Dal Pozzo and co-workers prepared a racemic 2-methyl-2-alkyl malonyl residue (Scheme 40).[186,187] They incorporated the racemate into a PMRI growth factor, analogous to **83**, and separated the diastereomers by HPLC.[186] The resultant PMRI growth factor retained biological activity.[186,187]

Welch and co-workers replaced the labile malonyl C-2 proton with fluorine, resulting in an optically pure and configurationally stable 2-fluoro-2-benzyl malonyl residue which was incorporated into a (proposed) HIV-1 protease inhibitor **98**.[188] The synthesis was somewhat tortuous (Scheme 41); this idea has yet to be adopted by other researchers, but may prove to be very useful since a wide range of 3-fluoro β-lactams, analogous to **97**, are available by stereoselective alkylation or aldol reaction of **96**.[189]

2. Solid-Phase Methodology

Special considerations for SPS of PMRI peptides essentially concern the compatibility of the usual

Scheme 41. Synthesis of a PMRI Peptide Containing a Configurationally Stable Fluoromalonyl Residue (−*mF*Phe−)[188,189,a]



[a] Ar = *p*-methoxy-phenyl.

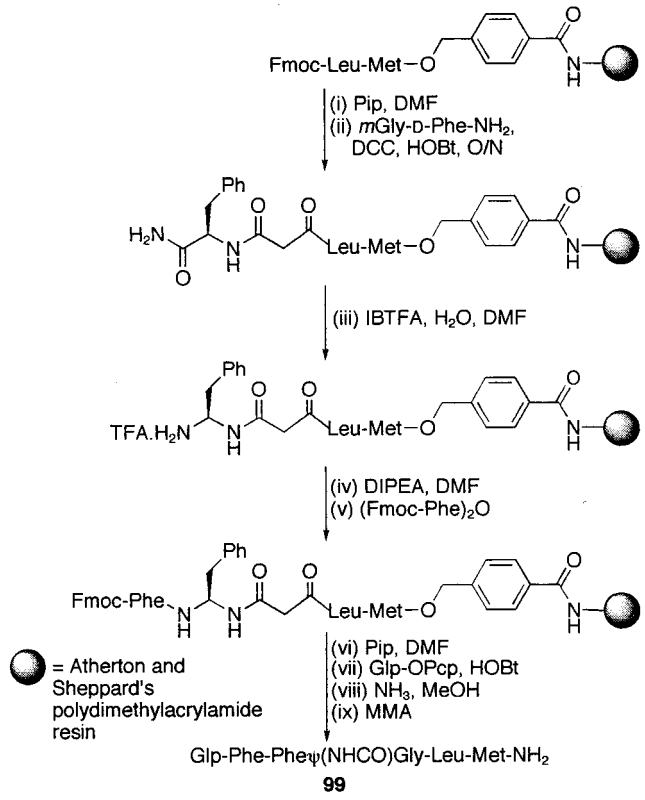
solution-phase methodology with the solid support. In this section significant examples of PMRI peptide SPS are considered, which demonstrate the compatibility (or otherwise) of the solution-phase methodology with the resin-bound conditions, and which introduce new or modified methodology into the SPS arena.

a. IBTFA. Verdini and co-workers performed the first PMRI peptide SPS[190] and have been the major contributors to the development of PMRI peptide SPS. Their pioneering synthesis of the PMRI substance P analogue, **99**, demonstrated the compatibility of IBTFA with the solid support (Scheme 42) (see also Scheme 9). Noteworthy points are as follows: (1) The incorporation of malonyl-D-phenylalanine amide (step ii) required an unusually long reaction time and resulted in a ~20% loss of peptide, probably due to Leu-Met diketopiperazine formation.[190] This is a symptom of the reduced coupling efficiency of malonates, mentioned above (section VI.A.1.a.i). (2) The direct, on resin, synthesis of the PMRI dipeptide unit used IBTFA (step iii, Scheme 42, also see Scheme 8). (3) The final treatment with *N*-methylmercaptoacetamide (MMA) was necessary to reverse the effect of IBTFA on −Met−, that is to reduce methionine sulfoxide back to −Met−.[190]

Verdini and co-workers used a similar procedure to synthesize the PMRI bradykinin potentiating peptide analogue, Glp-Trp-Pro-Arg-Pro-Lysψ(NHCO)-(*R*,*S*)-Phe-Ala-Pro, which exhibited in vivo activity and enhanced resistance toward cleavage by angiotensin converting enzyme (ACE) in vitro.[191] However, to avoid substantial Ala-Pro diketopiperazine formation during malonyl coupling, the malonyl residue was incorporated as Ala-(*R*,*S*)-*m*Phe-D-Lys-(Boc)-NH_2.[191]

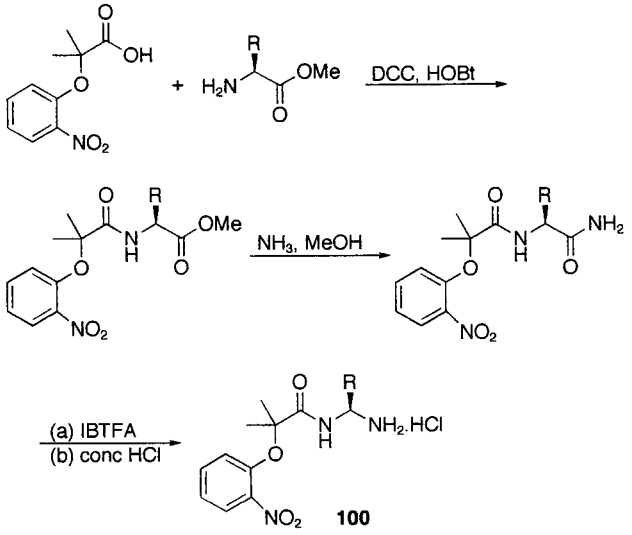
None of the other methods of *gem*-diaminoalkyl compound synthesis, successful in solution, have been tried on resin in SPS, presumably due to synthetic complications.

Scheme 42. SPS of a PMRI Substance P Analogue[190]



b. The (2-Methyl-2-*o*-nitrophenoxy)propionyl (MNP) Group. Although the treatment of a resin bound malonyl-D-amino acid amide with IBTFA, described above (section VI.A.2.a), is a useful method, it suffers from the previously discussed residue specific problems inherent to IBTFA (section

Scheme 43. Synthesis of MNP-*g*Xaa·HCl, 100[124]



VI.A.1.a.i), plus the rather high expense of D-amino acids. It is therefore desirable (and may be essential) to avoid treatment of a growing, resin bound peptide chain containing Asn, Gln, Met, Cys, Tyr or Trp, with IBTFA if side reactions and/or extra synthetic manipulations are to be avoided. This objective is readily achieved by incorporation of preformed PMRI dipeptides, PG-Xaaψ(NHCO)Yaa (or the appropriate *gem*-diaminoalkyl residue containing fragment for the synthesis of PMRI peptides with a consecutive sequence of reversed bonds). Therefore, suitably protected PMRI dipeptides are required. Difficulties in synthesizing carbamate-protected PMRI dipeptides are discussed above (section VI.A.1.a.i). Boc-protected PMRI dipeptides are accessible[108] and compatible with Merrifield SPS, but have not been utilized. We have synthesized Fmoc-protected PMRI dipeptides (from the corresponding Boc compounds) and employed them in SPS with qualified success.[109]

Verdini and co-workers found that mono-Fmoc-, trifluoroacetyl-, or diphenylphosphinoyl-*gem*-diaminoalkanes were insufficiently stable to be useful for PMRI dipeptide synthesis.[124] They therefore searched for a new protecting group and discovered that the MNP group is appropriate.

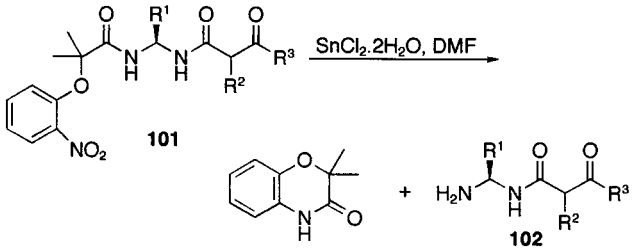
MNP-protected *gem*-diaminoalkanes may be synthesized as shown in Scheme 43.

The resultant mono MNP-*gem*-diaminoalkanes are stable for prolonged periods as their hydrochlorides, **100**.[124] When deprotonated they are sufficiently stable to be acylated (DCC/HOBt) by monoesters of (C-2-substituted) malonic acids, and saponified to furnish the desired PMRI dipeptides.[124] Alternatively, in situ trimethylsilylation with BSA facilitates acylation by Meldrum's acids, as described above (section VI.A.1.b.iv, Scheme 31).

After incorporation of the MNP-PMRI dipeptide into the resin-bound peptide, the MNP group is removed by treatment with tin(II) chloride in DMF, conditions compatible with Atherton and Sheppard's SPS protocol (using either acid or base labile linkers), leaving the resin-bound PMRI peptide, **102**, ready for further elaboration (Scheme 44).

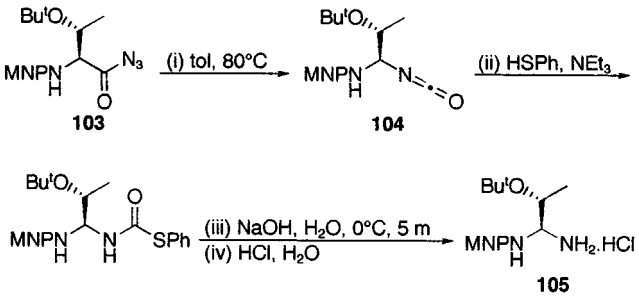
As shown in Scheme 11, Verdini and co-workers encountered a problem when they tried this approach

Scheme 44. Deprotection of MNP-Xaaψ(NHCO)Yaa-R^3, 101[124,a]



[a] R^3 = resin-bound peptide chain.

Scheme 45. Synthesis of MNP-*g*Thr(Bu^t)·HCl, 105, Avoiding 2-Oxazolidone Formation (cf. Scheme 11)[125]



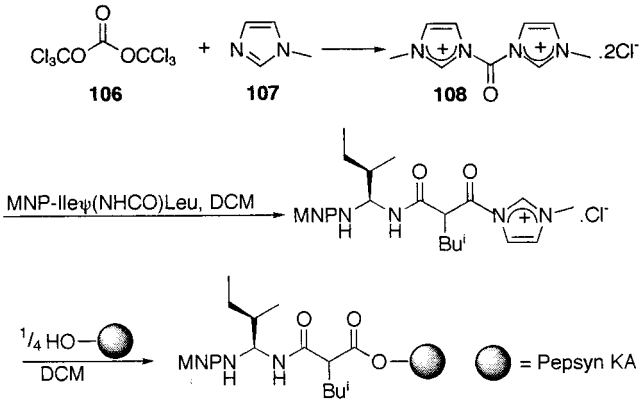
to MNP-*g*Thr(Bu^t)·HCl (**105**). However, the desired compound was obtained from the acyl azide, **103**, using thiophenol to trap the intermediate isocyanate, **104** (Scheme 45).[125]

This procedure has another advantage over the IBTFA method in that the resultant −*g*Thr− derivative, **105**, is topochemically equivalent to −Thr−. (When −*g*Thr− is obtained from −D-Thr-NH$_2$ by the action of IBTFA (cf. Scheme 30) then its C$^\beta$ stereochemistry is nonequivalent.) The same topochemical considerations favor this procedure for -*g*Ile-.[85]

Verdini and co-workers experienced a different problem with MNP-*g*Ile− during the SPS of the PMRI neurotensin(8−13) analogue Arg-Arg-Pro-Tyr-Ileψ(NHCO)(*R,S*)-Leu.[192] Steric hindrance from the Ile side chain caused incomplete MNP cleavage under standard deprotection conditions and, consequently, byproduct formation. A longer deprotection time diminished the problem.[192]

c. Anchorage. When preparing a PMRI peptide with a carboxy terminal malonyl residue by SPS, it is necessary to attach ("anchor") the malonyl residue to the solid support (via a linker). Therefore an ester linkage (if the target pseudopeptide has a free carboxy terminus) between the malonyl residue and the resin must be made. Under these conditions the reduced coupling efficiency of malonates (see section VI.A.1.a.i) poses a significant challenge: Verdini and co-workers obtained yields of less than 15% when anchoring MNP-Ileψ(NHCO)(*R,S*)-Leu to the Kieselguhr supported hydroxymethyl polyamide resin Pepsyn KA (using DCC/HOBt or BOP, with or without DMAP).[192] Furthermore, model esterifications of MNP-Ileψ(NHCO)(*R,S*)-Leu with *p*-methoxybenzyl alcohol also gave poor yields.[192] Their solution to this problem was the in situ generation of the active coupling reagent carbonyl bis(*N*-methylimidazolium) dichloride (**108**) from triphosgene (**106**) and *N*-meth-

Scheme 46. Anchoring MNP-Ileψ(NHCO)(*R,S*)-Leu Using Bis(*N*-methylimidazolium) Dichloride[192]



ylimidazole (**107**) which gave anchorage yields of 50−70% (Scheme 46).[192] Subsequent elongation (Fmoc strategy) and cleavage−deprotection yielded the desired, agonistic, PMRI neurotensin analogues.[192]

In our SPS of a PMRI peptide with a carboxy terminal malonyl residue we used the PAL-PEG-PS support, which anchors the (PMRI) peptide through an amide bond and ultimately yields a (PMRI) peptide amide.[109,193]

B. Biologically Active PMRI Peptides

Many biologically relevant PMRI (and end group modified retro-inverso) peptides have been synthesized and tested; most are hormone analogues, but there are some examples of protease inhibitors and sweeteners. Chorev and Goodman reviewed the majority of these examples.[85,86] In this section we briefly cover the significant bioactive PMRI peptide milestones, and recent, promising developments.

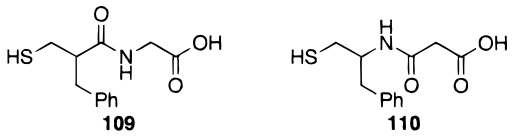
1. Enkephalin

Chorev, Goodman, and co-workers synthesized the first highly bioactive PMRI peptides, the enkephalin analogues: Tyr-D-Ala-Gly-Pheψ(NHCO)Leu-NH_2, Tyr-D-Ala-Gly-Pheψ(NHCO)Met-NH_2, Tyr-D-Ala-Gly-*g*Phe-D-*r*Leu-For, and Tyr-D-Ala-Gly-*g*Phe-D-*r*Met-For.[89] All four analogues displayed higher activity than Met-enkephalin in an in vitro test, and prolonged duration of action (in vitro and in vivo), presumably because the modifications protect the PMRI peptides from enzymatic degradation.[89] The latter two analogues were the more active of the four.[89]

Further work on PMRI enkephalins has met with more qualified success.[85]

2. Protease Inhibition

By applying the retro-inverso concept to thiorphan (**109**), Roques and co-workers achieved complete differentiation of its inhibitory activity.[194] Thus, whereas thiorphan (**109**) is a highly potent inhibitor of both enkephalinase (K_i = 3.5 nM) and a less potent inhibitor of ACE (K_i = 140 nM), "retro-thiorphan" (**110**) inhibits enkephalinase with great selectivity (K_i = 6 nM vs IC_{50} (against ACE) > 10 000 nM).[194]



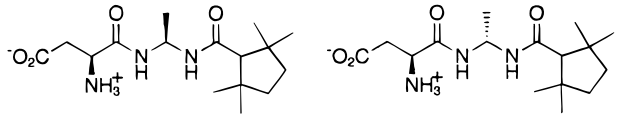
Retro-thiorphan (**110**) and thiorphan (**109**) similarly affected analgesia (due to protection of endogenous enkephalins) in in vivo studies.[194] Crystal structures of (*S*)-thiorphan (**109**) and (*R*)-retro-thiorphan (**110**) bound to thermolysin (which shares many active site residues with enkephalinase) show that the two inhibitors utilize very similar interactions with the enzyme (including the hydrogen bonding to the amide linkage).[195]

Roques and co-workers applied the same approach, and further modifications to the zinc metallopeptidase inhibitor kelatorphan, to similar effect.[196,197]

Carmona and Juliano used the retro-inverso modification to investigate the differentiation of ACE inhibition and potentiation of bradykinin responses by small peptides. Bz-Pheψ(NHCO)Gly-Trp, for example, competitively inhibited ACE (albeit with a higher K_i than its parent peptide) and did not potentiate a contractile response to bradykinin, in vitro.[148]

3. Sweeteners

As part of their ongoing investigation of the structure−taste relationship of peptides and peptidomimetics, Goodman and co-workers synthesized the retro-inverso peptide sweeteners (based on the Asp-D-Ala-NHR motif), **111** and **112**, which are ~800 times sweeter then sucrose.[198−202]



4. Gastrin Antagonism

Martinez and co-workers produced potent gastrin antagonists by partial retro-inverso modification of its carboxy terminal tetrapeptide, Trp-Met-Asp-Phe-NH_2.[203] The analogue Boc-Trp-Leu-*g*Asp-$CO(CH_2)_2$-Ph displayed in vivo gastrin antagonism (and no agonism) with enhanced duration of action.[203]

5. CCK

Following on from their work on gastrin, Martinez and co-workers applied the PMRI approach to CCK-7 and 8, (Asp-)Tyr(SO_3^-)-Met-Gly-Trp-Met-Asp-Phe-NH_2.[114] Roques and co-workers also synthesized PMRI CCK-7 analogues.[204] Both groups reversed the Met^{28}−Gly^{29} bond (a major cleavage site), replaced both Met^{28} and Met^{31} with Nle, and used amino terminal Boc protection (the latter two modifications being precedented[205]). All of these PMRI CCK-7 and -8 analogues exhibited affinity to the CNS CCK receptor, much lower affinity to the peripheral CCK receptor, and low functional potency. Martinez and co-workers' analogue with highest affinity to the CNS receptor, Boc-Asp-Tyr(SO_3^-)-Nleψ(NHCO)Gly-Trp-Nle-Asp-Phe-NH_2, displayed lower affinity than Boc-

[Nle[28,31]]CCK-7,[114] whereas Roques and co-workers' analogue, Boc-Tyr(SO_3^-)-Nleψ(NHCO)Gly-Trp-(*N*-Me)Nle-Asp-Phe-NH_2, displayed higher affinity and high resistance to proteolysis.[204]

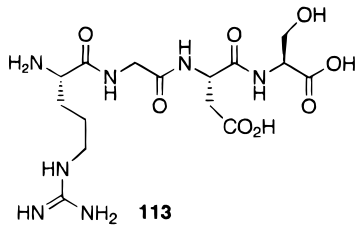
6. Tuftsin

Tuftsin, Thr-Lys-Pro-Arg, is degraded in vivo, principally between Thr and Lys, to produce tripeptides which inhibit its activity. Therefore Verdini and co-workers synthesized the PMRI tuftsin, Thrψ-(NHCO)Lys-Pro-Arg (part of their synthesis is shown in Scheme 28).[166] This PMRI tuftsin displayed prolonged in vitro stability in human plasma (less than 2% hydrolysis after 50 m *cf.* complete hydrolysis of tuftsin in less than 8 m).[166] It does, however, suffer hydrolysis to H_2N-*m*Lys-Pro-Arg (cf. section VI. A.1.a.vi).[166] Tests in vitro and in vivo (including oral administration) have demonstrated the higher immunostimulatory activity of this PMRI tuftsin compared with natural tuftsin.[166,206,207] The enhancement of activity is thought to be due to the PMRI tuftsin's resistance to peptidases and the fact that its hydrolysis product does not interfere with its activity.[166]

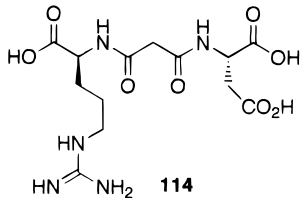
FAB tandem mass spectrometry revealed that, despite the stability described above, the PMRI tuftsin is labile under FAB conditions with the most abundant fragmentation processes involving the *gem*-diamino group.[208] Indeed, it is generally the case for PMRI peptides that ions formed by fragmentation in the vicinity of the *gem*-diamino residue are particularly abundant, which may be interpreted as indicating lower bond strengths in this region.[209]

7. RGD

Many peptides containing the Arg-Gly-Asp (RGD) cell-binding sequence inhibit tumor metastatis. However the therapeutic exploitation of RGD peptides has been complicated by their short half-lives in vivo, the Arg-Gly linkage being easily degraded. Nishikawa and co-workers tackled this problem using the partial retro-inverso modification.[162,210] They synthesized a series of partially modified retro and PMRI RGD peptides that exhibited antimetastatic effects in animal models and displayed greatly enhanced stability toward enzymatic degradation in plasma.[162,210] Their most potent antimetastatic pseudopeptide, the partially modified retro peptide **114**, showed a higher inhibitory effect on tumor metastatis and invasion than the control sequence RGDS, **113**.[162,210] The resistance to proteases displayed by *r*Arg-*m*Gly-Asp, **114**, presumably increases its biological half-life and therefore augments its antimetastatic and antiinvasive effects.[162,210] Furthermore, *r*Arg-*m*Gly-Asp (**114**) showed lower inhibitory activity on platelet aggregation than RGDS (**113**) which thus constitutes increased selectivity of antitumor action.[162,210]



113



114

8. The Renaissance of Retro-Inverso and End Group Modified Retro-Inverso Peptides

Chorev and Goodman reviewed the recent resurgence of retro-inverso and end group modified retro-inverso peptides in areas such as combinatorial chemistry and immunology.[86] In the latter application, retro-inverso, end group modified retro-inverso and (less so) PMRI peptides show great promise for the development of vaccines, immunomodulators, and immunodiagnostics.[115,211−215]

9. Taking the Concept Beyond Pseudopeptides

We have seen how the PMRI peptide concept developed from end group modified retro-inverso and linear retro-inverso peptides, which in turn developed from cyclic retro-inverso peptides. The PMRI peptide concept constituted the arrival of the retro peptide bond as a true peptide bond surrogate, which has been widely applied in various biologically relevant peptides. The retro concept has recently been applied to amide bonds other than those in simple peptides, that is in peptide nucleic acids (PNA)[216,217] and sugar−amino acid links.[218]

C. Conformational Implications of Partial Retro-Inverso Modification

In this final section of the review conformational studies of PMRI peptides are described. Many studies of specific PMRI peptides of biological interest have been undertaken,[85,86,219] but here we consider only model studies with general implications.

1. The Amide Bond Itself

The retro amide bond obviously scores highly in similarity tests against the natural peptide bond,[41,85,220] indeed, with some protocols it scores perfectly, due to symmetry.[65] Furthermore, X-ray crystal structures of PMRI peptides show that retro peptide bond lengths and angles are very similar to those of a standard peptide bond, as would be expected (see ref 85 and those cited in Table 6).

Nevertheless, despite the close geometrical mimicry of a standard peptide bond by a retro peptide bond, if a particular peptide bond is involved in a functionally significant interaction (e.g., through hydrogen bonding to other groups in the parent peptide or a receptor), then its reversal will neces-

sarily result in a change in the interaction and thus alter the PMRI peptide's secondary structure (see section VI.C.3) and/or activity.[86,221,222] This is an attribute shared by all peptide bond surrogates (as mentioned in section IV) and may, of course, be applied to investigate the functional role of peptide bonds.[86,115,222]

In terms of bond dimensions and permitting identical conformers to be adopted, it is perhaps surprising that the *trans*-alkene surrogate [ψ(*E*-CH=CH)] mimics the natural peptide bond better than does the retro amide bond.[41]

2. The Retro-Inverso Residues

PMRI peptides contain, embedded within the parent peptide sequence, a sequence of reversed and inverted residues flanked by modified residues (unless the modified residues are consecutive; see Figure 6). The reversed and inverted residues exert a conformational influence different to that of the native residues they replace, as has long been recognized in the simpler case of cyclic retro-inverso peptides.[72,221] Wermuth et al. have recently undertaken an elegant, systematic, experimental, and theoretical investigation of the conformational consequences of retro-inverso modification for *cyclo*(-Arg-Gly-Asp-D-Phe-Val-) (a selective, superactive $\alpha_V\beta_3$ integrin inhibitor) and all the other cyclic pentapeptides of sequence Arg-Gly-Asp-Phe-Val containing one D-amino acid.[222] One of these cyclic retro-inverso modified pentapeptides, *cyclo*(-D-Val-D-Phe-D-Asp-Gly-Arg-), proved to be a more potent inhibitor than the original sequence, despite existing in a somewhat different conformation.[222]

The change in conformational influence occurs because residue reversal swaps the bonds $C^\alpha-C'$ (1.53 Å) and $C^\alpha-N$ (1.45 Å) and swaps the torsion angles (ϕ,ψ) of the parent residue for (ψ,ϕ) of the D-amino acid residue (see Figure 10).[221,222]

Swapping the bonds results in an imperfect mimicry of side-chain topology (even if the same backbone torsion angles are maintained) as Freidinger and Veber demonstrated in their theoretical study of *cyclo*(-Ala-Ala-Gly-Gly-Ala-Gly-).[221] The discrepancy arises due to the interchange of bond lengths and bond angles inherent to residue reversal.[221] Nevertheless the best fit average C^α and C^β deviations Freidinger and Veber determined (0.39 and 0.20 Å respectively) still constitute quite good mimicry.[221,222]

The interchange of torsion angles, when combined with residue inversion, maps (ϕ,ψ) of a parent residue to ($-\psi,-\phi$) on the Ramachandran map[3,223] of the same residue (since a parent residue is replaced by its (reversed) D-amino acid counterpart). The net effect of this transposition on the residue's Ramachandran map is a reflection at the diagonal between (−180°,180°) and (180°,−180°).[222] Such a

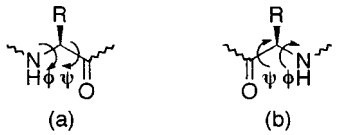


Figure 10. (a) the native L-amino acid residue, and (b) its D-amino acid residue replacement.

Table 3. Local Minima for a Native Residue

residue	ref	minima[a]	(ϕ,ψ), deg	energy, kcal/mol[b]
Ac-Ala-NHMe	226	C_7^{eq}	−86,84	0.0
		α_R	−79,−34	3.4
		C_7^{ax}	70,−66	3.5
		α'_R	−145,−66	3.8
		α_L	~60,~40	8.4

[a] The minima are described using the terms of the original authors. [b] Relative energies are included in Tables 3−5 for comparison of different minimum energy conformations within the same entry only, not between entries.

transformation in the Ramachandran map has profound consequences for a peptide's secondary structure because, other than for conformations near the diagonal (e.g., the β-sheet and γ-turns), the energies of given conformations change dramatically.[221,222] Thus if, for example, the parent peptide contains an α-helix (right-handed) then reversal and inversion of that part of the sequence would not be expected to give rise to a right-handed α-helix in the corresponding (PM)RI peptide because that part of the Ramachandran map corresponding to a right-handed α-helical conformation is now a high-energy region and conversely, that part corresponding to a left-handed α-helical conformation, a low-energy region.[222] Therefore an alternative conformation is likely to be adopted (possibly but not necessarily a left-handed α-helix, because the sequence is reversed as well as inverted).[211,222] Although the studies described considered cyclic retro-inverso peptides, the analysis also applies to the reversed and inverted portions of linear PMRI peptides. However cyclic retro-inverso peptides are poor models for linear PMRI peptides. Thus further general investigations of the extent of the topological equivalence between linear peptides and their PMRI isomers is merited.

Consideration of the impact of residue reversal and inversion on the secondary structure has prompted much discussion recently.[211,224,225] Overall, we may conclude that the implications for conformational mimicry of retro-inversion are minor if the residues affected exist in conformations in the parent peptide for which $\phi \sim -\psi$ [such as β-sheets (e.g., the cyclic β-hairpin peptide designed to mimic the α-chain of FcϵRI and its retro-inverso isomer present very similar topochemical surfaces[213]) and γ-turns].[221,222] Conversely the implications are profound for other parent peptide conformations, most importantly the α-helix.[211,212,221,222] If the residues concerned are Gly, however, then the consequences are less dire due to the absence of C^β and hence the symmetry of its Ramachandran map.

3. The New Residues

As discussed in section VI, partial retro-inverso modification generates two new residues, a *gem*-diaminoalkyl residue and a C-2-substituted malonyl residue. Their presence in a PMRI peptide exerts a profound influence upon its conformation, especially in their immediate vicinity.

Dauber-Osguthorpe et al. carried out a complete search of the conformational space available to the

Table 4. Local Minima for *gem*-Diaminoalkyl Residues[a]

entry	residue	ref	minima	(ϕ,ψ), deg	energy, kcal/mol
1	Ac-*g*Ala-Ac	226	extended	−156,140	0.0
			extended′	−140,156	0.0
			α_R	−135,−60	5.1
			α_L	60,135	5.1
2[b]	Ac-*g*Gly-Ac	227	α_L and α_R	±(80,80)	0.0
			low-energy region	±(60,−180)	1.4
			low-energy region	±(−180,60)	1.4
3[b]	Ac-*g*Ala-Ac	227	α_R	−80,−60	0.0
			α_L	60,80	0.0
			extended	−160,70	0.8
			extended	−70,160	0.8
4[c]	Ac-*g*Ala-CONHMe	79	α_L and α_R	±(45,120)	0
			β-region	−135,135	<1
5[d]	Ac-*g*Val-CONHMe	*e*		−125,130	0
6[f]	Ac-*g*Ala-Ac	235	helical	53,83	0.0
			helical	−83,−54	0.0
			semiextended	152,−79	2.2
				144,−80	3.6
				−79,142	3.6
7	Ac-*g*Gly-Ac	238	α_L and α_R	±(67,67)	0.0
			saddle point	±(−113,113)	5.3
7a	as entry 7 but including environmental effects	238	α_R	−83,−80	0.0
			α_L	80,83	0.0

[a] See notes on Table 3. [b] Stern et al. performed empirical force-field calculations of the Ramachandran maps using both a rigid geometry approximation and a flexible geometry. Only the latter results are reported in Tables 4 (entries 2 and 3) and 5 (entries 2 and 3). They also performed ab initio calculations on For-*g*Gly-Ac and H_2N-*m*Gly-NHMe,[228] the results of which were similar to those reported in Table 4 entry 2 and Table 5 entry 2, respectively. [c] The calculated Ramachandran map was generally similar to those of Stern et al. and Dauber-Osguthorpe et al., again differing only in the exact locations of the minima and their relative stabilities: *cf.* entries 1 and 3. [d] Also included in this study were Ac-*g*Gly-CONHMe, Ac-*g*Ala-CONHMe, Ac-*g*Abu-CONHMe, and Ac-*g*Tyr-CONHMe. The minimum energy conformations for Ac-*g*Gly-CONHMe and Ac-*g*Ala-CONHMe (*cf.* entry 4) were similar to those determined by Stern et al. (entries 2 and 3). Chipens and co-workers have also calculated part of the Ramachandran map for Ac-*g*Val-$CONMe_2$,[e] which differs significantly from that of Ac-*g*Val-CONHMe (entry 5). [e] Balodis, J.; Vegners, R.; Nikiforovich, G. V.; Cipens, G. *Bioorg. Khim.* **1978**, *4*, 481−488; *Chem. Abstr.* **1978**, *89*, 110306z. Mishnev, A. F.; Bleidelis, J.; Ancans, J. E.; Cipens, G. I. *J. Struct. Chem. (Engl. Transl.)* **1982**, *23*, 252−256; *Zh. Strukt. Khim.* **1982**, *23*, 101−106. [f] The results reported in entry 6 are those from Alemán and Puiggalí's molecular mechanics calculations using their newly parametrized force field.

Table 5. Local Minima for Malonyl Residues[a]

entry	residue	ref	minima	(ϕ,ψ), deg	energy, kcal/mol
1	MeHN-*m*Ala-NHMe	226	α_R	−101,−67	0.0
			α_L	67,101	0.0
			$\sim C_7^{eq}$	−89,122	3.6
			$\sim C_7^{eq'}$	−122,89	3.6
			$\sim C_7^{ax}$	63,−93	4.1
			$\sim C_7^{ax'}$	93,−63	4.1
2	MeHN-*m*Gly-NHMe	227	α_L and α_R	±(80,80)	0.0
			$\sim C_7^{eq}$	±(−120,40)	*b*
			$\sim C_7^{eq}$	±(40,−120)	*b*
3	MeHN-*m*Ala-NHMe	227	α_R	−80,−60	0.0
			α_L	60,80	0.0
			$\sim C_7^{eq}$	−160,70	*b*
			$\sim C_7^{eq}$	−70,160	*b*
			saddle point	±(85,−85)	5.9
4	MeHN-*m*Gly-NHMe	242,243	helical	±(52,111)	0.0
			helical	±(111,52)	0.0
4a	as entry 4 but hydrated	243	helical	68,80[c]	0.0
5	MeHN-D-*m*Ala-NHMe	236	helical	−55,−113	0.0
			helical	111,54	0.0
			helical	−101,−55	0.4
			helical	53,100	0.4
				24,−153	2.1
				−142,23	2.1
				39,−165	2.2

[a] See notes on Tables 3 and 4. [b] A minimum in the rigid geometry calculation only. [c] The torsion angles were calculated by hydrating MeHN-*m*Gly-NHMe in initial conformation (110°,53°) with four water molecules.

gem-diamino and malonyl residues derived by partial retro-inverso modification of Ac-Ala-NHMe (i.e., Ac-*g*Ala-Ac and MeNH-*m*Ala-NHMe) using a valence force field method.[226] The resultant Ramachandran maps (which were generally similar to the previous results of Stern et al.,[227,228] differing only in the exact locations of the minima and their relative stabilities: see entries 1 to 3 in Tables 4 and 5) were

Table 6. Backbone Torsion Angles from X-ray Crystal Structures of *gem*-Diamino and Malonyl Residues

		(ϕ,ψ), deg		
entry	residue	*g*Xaa	*m*Yaa	ref
1	$H_2NCONHCH(CO_2K)$-$NHCONH_2$	65,62		*a*
2	hydantoin fromAla-Alaψ(NHCO)Gly-OBn	54,70	84,173	*b*
3	Ac-*g*Ala-Ac	−160,80		*c*
4	Ac-*g*Val-CONHMe	−113,105		80
5	Ac-*g*Phe-CONHMe	−111,93		*d*
6	Ac-*g*Val-$CONMe_2$	−105,124 −121,90		*e*
7	Asp-*g*Ala-CO-TMCP	−100,105		202
8	Asp-D-*g*Ala-CO-TMCP	104,−97		202
9	Bu^tCO-Alaψ(NHCO)Gly-$NHPr^i$	−111,107	150,125	231
10	Bu^tCO-*g*Val-$COBu^t$	−101,99		232
11	Z-*g*Gly-$CONMe_2$	95,79		*f*
12	Ac-*g*Gly-Ac	93,77 91,80		241
13	H_2N-*m*Gly-NH_2		111,140 −115,−138	*g*
14	PhHN-*m*Gly-NHPh		109,153	*h*
15	PrHN-*m*Gly-NHPr		115,115	*h*
16	MeHN-*m*Gly-Gly-NHMe		105,112	*i*
17	PrHN-*r*Gly-*m*Gly-Gly-NHPr		113,113	154
18	MeHN-*m*Ala-Ala-NMe_2		−104,107	*i*
19	MeHN-*m*Val-NHMe		−110,110	233
20	$H_2NCOCHBrCONH_2$		−111,111	*j*

[a] Ringertz, H. *Acta Crystallogr., Sect. B: Struct. Sci.* **1968**, *24*, 1686−1692. [b] Benedetti, E.; Pedone, E. M.; Kawahata, N. H.; Goodman, M. *Biopolymers* **1995**, *36*, 659−667. [c] Kolakowski, B. *Acta Crystallogr., Sect. B: Struct. Sci.* **1969**, *25*, 1669−1671. [d] Mishnev, A. F.; Bleidelis, Ya. Ya.; Antsans, Yu. E.; Chipens, G. I. *J. Struct. Chem. (Engl. Transl.)* **1979**, *20*, 128−130; *Zh. Strukt. Khim.* **1979**, *20*, 154−157. [e] Mishnev, A. F.; Bleidelis, J.; Ancans, J. E.; Cipens, G. I. *J. Struct. Chem. (Engl. Transl.)* **1982**, *23*, 252−256; *Zh. Strukt. Khim.* **1982**, *23*, 101−106. [f] Mishnev, A. F.; Bleidelis, J.; Ancans, Y.; Cipens, G. *Latv. PSR Zinat. Akad. Vestis, Khim. Ser.* **1981**, 494−498; *Chem. Abstr.* **1981**, *95*, 195520m. [g] Chieh, P. C.; Subramanian, E.; Trotter, J. *J. Chem. Soc. A* **1970**, 179−184. [h] Tereshko, V.; Navarro, E.; Puiggalí, J.; Subirana, J. A. *Macromolecules* **1993**, *26*, 7024−7028. [i] El Masdouri, L.; Aubry, A.; Gomez, E. J.; Vitoux, B.; Marraud, M. *J. Chim. Phys. Phys.−Chim. Biol.* **1988**, *85*, 583−588. [j] Picone, R. F.; Rogers, M. T.; Neuman, M. *J. Chem. Phys.* **1974**, *61*, 4808−4813.

compared with that of the parent residue.[226] The minima found by Dauber-Osguthorpe et al. are listed in Tables 3−5, along with those found by other researchers. For both PMRI residues the C_7 regions were destabilized (C_7^{eq}, or the inverse γ-turn, being the lowest energy conformation of Ac-Ala-NHMe, see Table 3), due to the proximity of atoms with like charges.[226] The *gem*-diamino residue had its minimum energy in an extended conformation, and also local minima in equivalent helical conformations containing six-membered hydrogen-bonded rings (a C_6 arrangement) (Table 4, entry 1).[226] The malonyl residue had its minimum energy in equivalent helical conformations (C_6 arrangement) and low energy regions corresponding to extended conformations (Table 5, entry 1).[226] The reported crystal structures of appropriate *gem*-diaminoalkyl and malonyl residues (Table 6) are compatible with these results: of the *gem*-diaminoalkyl residues, two are in helical conformations (entries 1 and 2) and seven are in C_7^{eq}/extended conformations (entries 3−7, 9, and 10). Of the malonyl residues five (all −*m*Gly−) are in helical conformations (entries 13−17) and three in the C_7^{eq}/extended region (entries 18 to 20). For a brief survey of model pseudopeptide crystal structures (including most PMRI examples) see ref 220.

From these results were deduced the implications of the incorporation of the modified residues into secondary structures, on the basis of reasoning that "a residue will be likely to be part of a secondary structure element if the required conformation corresponds to a local minimum of the energy surface of the isolated residue. Conversely, if a specific conformation is very unstable for an isolated residue it is not likely to be observed in this conformation."[226] Interresidue interactions were also considered.[226]

a. β-Turns. Four residues are involved in a β-turn, with a hydrogen bond between the carbonyl of residue i and the NH of residue i + 3. The six types of ideal β-turn (types I, II, and III, and their mirror images) differ in the (ϕ,ψ) angles of residues i + 1 and i + 2.[3,223,229,230] If the first amide bond of a β-turn is reversed, then so must be the third in order to enable the i...i + 3 hydrogen bond to form.[226] The compound modeled of this type [Acψ(NHCO)Ala-Alaψ(NHCO)NHMe] formed no stable β-turns because the *gem*-diaminoalkyl residue has a very high energy in the conformations necessary for the i + 2 position.[226]

Reversal of the second amide bond (model compound Ac-Alaψ(NHCO)Ala-NHMe) resulted in a PMRI peptide that could form a stable type II β-turn, although an extended conformation was more stable.[226]

These predictions are supported by experimental studies. Solution-phase ^{1}H NMR and IR spectroscopic studies of Bu^tCO-Ala-Gly-$NHPr^i$ and its three PMRI analogues demonstrated that reversal of the middle amide bond exerts little influence on the propensity to form β-turns, in stark contrast to reversal of the other two amide bonds.[62−64,231] However, the crystal structure of Bu^tCO-Alaψ(NHCO)-Gly-$NHPr^i$ was "quasi-extended" with no intramolecular (only intermolecular) hydrogen bonding, whereas the parent peptide adopted a type II β-turn.[62,231] Intermolecular interactions in the crystal are presumably responsible for this difference. Bu^t-CO-Pro-*g*Gly-$COBu^t$ similarly showed reduced β-turn formation with respect to the parent peptide in a solution-phase ^{1}H NMR and IR spectroscopic study.[62]

For a nine-membered hydrogen-bonded ring conformation specific to PMRI peptides that resembles a native β-turn see section VI.C.3.e.ii.

b. α-Helixes. An α-helix has a regular hydrogen bond network between the carbonyl of residue i and the NH of residue i + 4, with all dipoles aligned.[3,223,230] Reversal of one amide bond disrupts this hydrogen bond network, and brings two NHs and two carbonyls close together, considerably destabilizing the helix.[226] Reversing every third amide bond (model compound Ac-[ψ(NHCO)Ala_3]$_3\psi$(NHCO)NHMe) produced a helix with a complete hydrogen bond network similar to an α-helix.[226] But it was not as stable as a native α-helix because the dipole alignment is less favorable and the *gem*-diaminoalkyl residues are not in their lowest energy conformation.[226] No experimental

studies applicable to such systems have been performed.

c. β-Sheets. β-Sheets consist of parallel or antiparallel extended peptide chains hydrogen bonded to each other.[3,223,230] The smallest unit of a β-sheet is not one strand (i.e. one extended peptide chain) but two hydrogen-bonded strands, since the hydrogen bonds go from one strand to another.[230] Dauber-Osguthorpe et al. used three strands to construct model parallel and antiparallel β-sheets and calculated the energy of sheet formation with respect to the isolated extended strands. In common with α-helixes, indiscriminate amide bond reversal results in disruption of the β-sheet's hydrogen-bond network and hence destabilization. But by reversing every second amide bond (model strands Ac-Alaψ(NHCO)-Ala-Alaψ(NHCO)NHMe and Acψ(NHCO)Ala-Alaψ-(NHCO)Ala-NHMe) fully hydrogen bonded parallel and antiparallel β-sheets were constructed.[226] Unlike natural β-sheets, these modified sheets have all their carbonyls pointing one way, and all their NHs pointing the other, which is a similarly favorable arrangement to that of a native α-helix and results in strong interstrand attraction.[226] Thus, stable parallel and antiparallel β-sheets were formed, which each had a significantly more favorable energy of formation than the native β-sheets in Dauber-Osguthorpe et al.'s study.[226] Experimental studies applicable to these PMRI peptide β-sheets are somewhat limited. El Masdouri et al. described the model PMRI peptides Bu^{t}CO-*g*Val-COBu^t and MeNH-*m*Val-NHMe, in their respective crystals, as "hydrogen bonded in such a way as to form a parallel β-sheet structure."[232,233] But the hydrogen-bonded structures in both these cases are 12-membered rings, rather than the 10-membered rings found in Dauber-Osguthorpe et al.'s model PMRI β-sheets, because the crystallized PMRI peptides do not contain both *gem*-diamino and malonyl residues.

Gardner and Gellman have investigated the stability of these 10-membered hydrogen-bonded rings formed between *gem*-diamino and malonyl residues (see section VI.C.3.e.ii). We also have attempted to synthesize PMRI peptides containing the motif [Xaaψ-(NHCO)Yaa]$_n$ ($n \geq 2$) in order to study the conformational behavior thereof.[109] Nylon 1,3 contains this structural motif but exists in a helical conformation: (see section VI.C.3.e.i). The one other literature example of a PMRI peptide containing this motif is Argψ(NHCO)(*R*,*S*)-Lys-Aspψ(NHCO)(*R*,*S*)-Val-Tyr, a biologically active PMRI analogue of the immunomodulatory peptide thymopentin.[234] However the authors made no specific mention of its synthesis or conformation, and we are therefore unable to deduce anything from it concerning the stability PMRI peptide β-sheets.

d. The Influence of Side Chains. Inspection of the modeling studies of both Stern et al. and Alemán and co-workers (see Table 4, entries 2, 3, 6, and 7; Table 5, entries 2−5; and section VI.C.3.e.i) permits the conformational influence of side chains to be deduced.

The Ramachandran maps calculated by Stern et al. for the two model compounds MeNH-*m*Xaa-NHMe (Xaa = Gly or Ala; see Table 5, entries 2 and 3), are very similar,[227] indicating that the presence of a side chain little affects the conformational preference of a malonyl residue. However, the region of the maps corresponding to the β-sheet conformation differs somewhat between the studies of Stern et al.[227] and Dauber-Osguthorpe et al.[226] In Stern et al.'s study, especially for Xaa = Gly, the minima in this region are very shallow and ill defined.[227] It is therefore difficult to deduce the effect of a side chain on the conformational preferences of malonyl residues. The available crystal structures suggest that the absence of a side chain makes an extended conformation less likely; those malonyl residues in extended conformations in the crystalline state all possess side chains (Table 6, entries 18−20).

The Ramachandran maps calculated by Stern et al. for the two model compounds Ac-*g*Xaa-Ac, Xaa = Gly or Ala, display differences; there are only minima in the extended region for Xaa = Ala (Table 4, entry 2; *cf.* entry 3).[227] Therefore the absence of a side chain in a *gem*-diamino residue disfavors an extended conformation with respect to a helical conformation. The available crystal structures of *gem*-diaminoalkyl residues support this view, for those without side chains are in helical conformations (Table 6, entries 11 and 12) and the majority of those with side chains are in more extended conformations (Table 6, entries 3−10).

e. Other Conformational Studies of PMRI Peptides. Experimental and modeling studies have been performed on various PMRI peptides of biological significance,[85,86,219] but these, not being model PMRI peptides, permit few further general conclusions to be drawn.

However two other groups of studies are generally applicable.

i. Aléman, Puiggalí, and Co-workers: The Nylon Perspective. As mentioned in section VI.A.1.a.v, nylon 1,3 is identical to [Glyψ(NHCO)Gly]$_n$ (**58**). Thus there is interest in *gem*-diamino residues as components of nylons 1,*n*; and malonyl residues as components of nylons *n*,3. Aléman, Puiggalí, and coworkers have carried out many theoretical studies on model compounds containing *gem*-diamino and malonyl residues, using AM1 SCF-MO (i.e., quantum mechanical) calculations (supported by ab initio calculations); much of this work is summarized in their recent publication, ref 235. In that study, Aléman, Puiggalí, and co-workers built upon their earlier investigations of MeHN-*m*Gly-NHMe (Table 5, entry 4), MeHN-*m*Ala-NHMe (Table 5, entry 5), MeHN-*m*Aib-NHMe,[236] and Ac-*g*Gly-Ac (Table 4, entry 7) to parametrize a force field suitable for use with *gem*-diamino and malonyl residues. Their motive for this enterprise was the discrepancy between their earlier results and the corresponding force field calculations of Dauber-Osguthorpe et al.[226] and Stern et al.[227] (discussed above).

Aléman, Puiggalí, and co-workers attributed these discrepancies to "deficiencies in the force field parameters, which give a poor description of the conformational properties of the excessive attractive interactions, that is C═O···HN, or the excessive

repulsive interactions, that is C═O|||O═C and NH|||HN".[235] But the AM1 method is not without criticism: Dado and Gellman showed, by comparison with their experimental studies (see section VI.C.3.e.ii), that AM1 overestimates the strength of intramolecular hydrogen bonds.[237]

The discrepancies reveal themselves in the computed Ramachandran maps for *gem*-diamino and malonyl residues; although there is overall similarity between the different researchers' maps, the minima are in different positions and of different relative energies (see Tables 4 and 5).

Specifically, the map of Alemán and Pérez for Ac-*g*Gly-Ac features only minima in the helical regions, with merely a saddle point in the β-sheet/extended region (Table 4, entry 7). In the minimum-energy helical conformation, Ac-*g*Gly-Ac formed two intramolecular six-membered, hydrogen-bonded rings[238] (the helical conformations found by Dauber-Osguthorpe et al. and Stern et al. form one C_6 ring; see section VI.C.3, above). This conformation changed on inclusion of environmental effects (modeled by the introduction of ammonia and formaldehyde in order to mimic intermolecular hydrogen bonding), but a helical conformation with the intramolecular hydrogen bonds was maintained (Table 4, entry 7a).[238] These results are supported by experimental studies in that −*g*Gly− in the crystalline state (Table 6, entries 11 and 12) and some nylons 1,*n*[239,240] adopt similar helical conformations; but no intramolecular hydrogen bonds were detected for Ac-*g*Gly-Ac in solution (see section VI.C.3.e.ii) or the solid state.[241]

Alemán and Pérez's maps for MeHN-*m*Gly-NHMe[242] and MeHN-D-*m*Ala-NHMe[236] resemble each other and feature four minima in the helical region (different to Stern et al.'s helical conformations but similar to those of Dauber Osguthorpe et al., and thus similarly supported by crystal structures; see Table 5 and section VI.C.3, above). The map for MeHN-*m*Gly-NHMe contains no other minima (Table 5, entry 4), but that for MeHN-D-*m*Ala-NHMe has additional local minima in more extended conformations (Table 5, entry 5). In the helical conformations the molecules form a six-membered, hydrogen-bonded ring (also found by Stern et al. and Dauber Osguthorpe et al.; see section VI.C.3, above), and detected experimentally by Gellman and co-workers (see section VI.C.3.e.ii). Alemán and Pérez found that their predicted minimum energy conformation of MeHN-*m*Gly-NHMe is changed by hydration, but remains helical (Table 5, entry 4a).[243]

Alemán and Puiggalí computed Ramachandran maps for Ac-*g*Ala-Ac and Ac-*g*Aib-Ac using the AM1 method and found reasonable agreement between them and the conformational preferences obtained using their newly parametrized force field.[235] The map for Ac-*g*Ala-Ac is broadly similar to their map for Ac-*g*Gly-Ac, previously calculated (discussed above). However, in addition to helical minimum energy conformations, Ac-*g*Ala-Ac was also found to possess semi-extended minima (different from those found by Dauber-Osguthorpe et al. and Stern et al. for Ac-*g*Ala-Ac, see Table 4, entries 1, 3, and 6 and section VI.C.3, above).[235] Alemán and Puiggalí further used their force field to model nylons 1,3 and 1,5 and found good correlation with the X-ray data,[142,244] that is a 3-fold helical structure with three hydrogen bond directions and the dihedral angles of the *gem*-diamino residues close to those found for the model compounds; as opposed to the more commonly observed nylon γ-structure, which resembles a protein β-sheet.[239,240]

Alemán and co-workers have further studied the PMRI peptides Ac-Glyψ(NHCO)Gly-NHMe[245] and Ac-Gly-Glyψ(NHCO)Gly-NHMe,[246] as nylon models, using AM1 and force field methods. Ac-Glyψ(NHCO)-Gly-NHMe was found to possess helical minima, some resulting in C_6 hydrogen-bonded rings involving the $-m\text{Gly}^2-$ residue carbonyls[245] (similar to those found by Dauber-Osguthorpe et al., see section VI.C.3, above). When applied to an infinite nylon 1,3 chain model, results consistent with previous models of the crystal structure were obtained.[245] Only the application of an unfavorable symmetry constraint produced minima in an extended/β-sheet conformation for Ac-Glyψ(NHCO)Gly-NHMe.[245] Ac-Gly-Glyψ-(NHCO)Gly-NHMe was computed to be rather flexible, but with a strong tendency to fold and adopt conformations featuring intramolecular hydrogen bonds.[246] An infinite chain model produced two low-energy conformations: an α-helix (as previously described by Dauber-Osguthorpe et al. for the corresponding alanine model sequence; (see section VI.C.3.b), and a 6-fold helix, stabilized by intra- and intermolecular hydrogen bonds, respectively.[246] A model β-sheet structure was of higher energy because of unfavorably close intraresidue contacts between C═O|||O═C and NH|||HN.[246]

Alemán has also studied the conformational impact of the combination of the retro and dehydro modifications by computing the potential energy surface of the retro-modified dehydroalanine dipeptide.[247]

The conformational predictions of Alemán, Puiggalí, and co-workers may be summarized as indicating that helical structures are always of lower energy than extended or β-sheet type structures for the *gem*-diamino and malonyl residue containing model compounds that they studied, irrespective of the nature of the side chain. This contrasts with the predictions of Dauber-Osguthorpe et al. (see section VI.C.3, above).

ii. Gellman and Co-workers: Small Molecules. Gellman and co-workers have conducted extensive studies on the conformation-directing effects of noncovalent interactions, in particular hydrogen bonds, in small molecules in order to deduce their impact on biopolymer folding. In the course of these studies they have investigated some small molecules containing a malonyl residue, which are of interest in the PMRI peptide context.

Gellman and co-workers' results from variable-temperature (VT) IR and NMR spectroscopy (in dichloromethane and acetonitrile) and X-ray crystallography imply that the nine-membered hydrogen bonded ring conformation, depicted in Figure 11, is intrinsically favorable to the *N*-malonylglycine or alanine unit.[160,248−251]

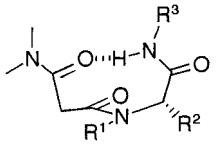


Figure 11. The favored conformation of *N*-malonylglycine (R^2 = H) or alanine (R^2 = Me) derivatives. R^1 = H, Me, or Et; R^3 = Me, Bn, 1-adamantyl.

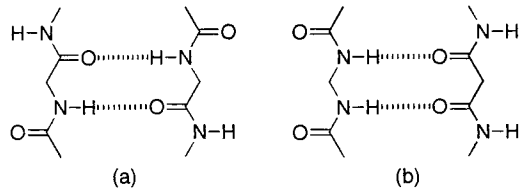


Figure 12. Ten-membered ring hydrogen bonded dimers of (a) blocked glycine and (b) Ac-*g*Gly-Ac and MeNH-*m*Gly-NHMe.

Gellman and co-workers therefore asserted that "the malonyl-*N*-methyl-amino acid subunit...represents a potential alternative to the natural dipeptide subunit at residues i + 1 and i + 2 of a β-turn".[249]

Gellman and co-workers also demonstrated, using VT IR and ^{1}H NMR spectroscopy, that *N*,*N*,*N*′-trimethylmalonamide adopts a six-membered hydrogen-bonded ring conformation in dichloromethane solution, and less so in acetonitrile.[252] A similar hydrogen-bonded ring is found in the crystal structures of some malonamide derivatives.[160] The detection of the hydrogen bond concurs with the predictions of Stern et al., Dauber-Osguthorpe et al., and Alemán and Pérez (*cf.* section VI.C.3, above).

Jorgensen and co-workers explained the variations observed among association constants for a series of triply hydrogen bonded complexes (pertinent to nucleotide base-pairing) by considering the "secondary interactions" among the hydrogen-bonded groups, that is electrostatic interactions between donor and acceptor atoms forced to approach each other due to the formation of a primary hydrogen bond.[253,254] Moreover they went on to predict, in accordance with Dauber-Osguthorpe et al., that secondary interactions would lead to greater stability for the PMRI dipeptide dimer depicted in Figure 12b, than for the corresponding glycine dimer, Figure 12a, when the hydrogen-bonding groups were constrained to be planar.[253]

Gardner and Gellman published an experimental test (using IR spectroscopy in dichloromethane solution) of the latter prediction.[255,256] The model pseudopeptide for their study was Me_2N-*m*Gly-Pro$\psi(CO_2)$-Gly-*g*Gly-Ac (**115**), which should, according to Jorgensen and co-workers' prediction, fold in a β-hairpin type fashion more readily than the corresponding parent depsipeptide, Ac-Gly-Pro$\psi(CO_2)$Gly-Gly-NMe_2. However, Gardner and Gellman found that their IR spectroscopic data, while consistent with the two state equilibrium depicted in Figure 13, yielded very similar equilibrium constants for the parent and PMRI depsipeptides ($K \approx 0.6$, in dichloromethane).[255,256]

Therefore secondary interactions do not increase the β-folding propensity of **115** over that of its parent, in this case, probably because intramolecular dipole−dipole repulsions result in nonplanarity of the amide groups,[255,256] a situation that cannot arise within the rigid heterocyclic systems found in nucleotide bases.

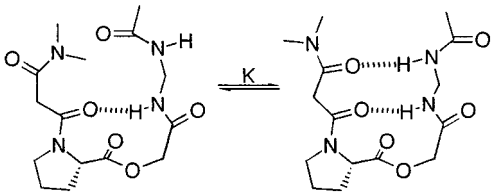


Figure 13. The two-state equilibrium found for Me_2N-*m*Gly-Pro$\psi(CO_2)$Gly-*g*Gly-Ac, **115**, (and Ac-Gly-Pro$\psi(CO_2)$-Gly-Gly-NMe_2, not shown).

In the course of their study, Gardner and Gellman also demonstrated that Ac-*g*Gly-Ac exhibits no intramolecular hydrogen bonding in dichloromethane solution.[255,256] This result accords with Dauber-Osguthorpe et al.'s modeling study, which predicted that a six-membered hydrogen bonded ring arrangement of Ac-*g*Ala-Ac is less stable than the extended conformation; but it contrasts with the predictions of Alemán and Pérez and Stern et al. that such an arrangement is the minimum energy conformation for Ac-*g*Gly-Ac (section VI.C.3, above).

The theoretical and experimental studies described in this section enable some estimation of the conformational influence of partial retro-inverso modification to be made. To clarify these influences and better inform the design of PMRI peptides, further model studies are needed.

VII. Concluding Remarks

We have charted the development of the retro-inverso concept from the originating studies on cyclic peptides to the present day, when sufficient synthetic methodology exists to apply the partial retro-inverso modification at will to virtually any peptide bond(s) within a synthetically accessible peptide. A survey of successful applications of the partial retro-inverso modification to biologically active peptides has revealed examples where it has resulted in increased biological half-life, increased activity and enhanced selectivity. Experimental and theoretical studies of model PMRI peptides have exposed the changes in conformational behavior that result from partial retro-inverso modification of a peptide. Thus the partial retro-inverso modification may continue to be gainfully employed, guided by the accumulated wisdom of the literature's examples and predictions.

VIII. Abbreviations

Abu	α-aminobutyric acid
ACE	angiotensin converting enzyme
Adoc	adamantyloxycarbonyl
Aib	α-aminoisobutyric acid
AIBN	azobisisobutyrylnitrile
AM1	Austin model 1
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Bom	benzyloxymethyl
BOP	(1-benzotriazolyloxy)tris(dimethylamino)-phosphonium hexafluorophosphate
BSA	*O*,*N*-bis(trimethylsilyl)acetamide
Bt	benzotriazole

Bz	benzoyl
cat	catalyst
CCK	cholecystokinin
CNS	central nervous system
DABCO	1,4-diazabicyclo[2.2.2]octane
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
DIBAL	diisobutylaluminum hydride
DIPEA	diisopropylethylamine
DMF	*N*,*N*-dimethylformamide
DMAP	4-(dimethylamino)pyridine
DMSO	dimethyl sulfoxide
DPPA	diphenylphosphoryl azide
FAB	fast-atom bombardment
Fmoc	9-fluorenylmethyloxycarbonyl
For	formyl
Glp	pyroglutamic acid
HIV	human immunodeficiency virus
HOBt	*N*-hydroxybenzotriazole
HOSu	*N*-hydroxysuccinimide
HPLC	high-performance liquid chromatography
IBTFA	iodobenzene bis(trifluoroacetate)
IC_{50}	median inhibitory concentration
imid	imidazole
K_i	inhibition constant
LDA	lithium diisopropylamide
LNAP	lithium naphthalenide
MMA	*N*-methylmercaptoacetamide
MNP	(2-methyl-2-*o*-nitrophenoxy)propionyl
Mor	Morpholine
MPLC	medium-pressure liquid chromatography
Mtr	4-methoxy-2,3,6-trimethylbenzenesulfonyl
NBS	*N*-bromosuccinimide
NK-1	neurokinin/substance P receptor 1
NMM	*N*-methylmorpholine
NMR	nuclear magnetic resonance
nr	not reported
O/N	overnight
OPcp	pentachlorophenyl
PAL	peptide amide linker: 5-[4-(aminomethyl)-3,5-dimethoxyphenoxy]valeric acid
PEG	poly(ethylene glycol)
PG	protecting group
Pip	piperidine
PMRI	partially modified retro-inverso
PS	polystyrene
py	pyridine
Reflux	heated under reflux
RPHPLC	reverse-phase high-performance liquid chromatography
SAR	structure activity relationship
SCF-MO	self-consistent field molecular orbital
SPS	solid-phase synthesis
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TEBA	triethylbenzylammonium chloride
TFA	trifluoroacetic acid or trifluoroacetyl
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TMCP	2,2,5,5-tetramethylcyclopentanyl
TMEDA	*N*,*N*,*N*′,*N*′-tetramethylethylenediamine
TMS	trimethylsilyl
TMSOTf	trimethylsilyl trifluoromethanesulfonate
tol	toluene
Ts	tosyl (*p*-toluenesulfonyl)
VT	variable temperature
Xaa	unspecified α-amino acid
Yaa	unspecified α-amino acid
Z	benzyloxycarbonyl
Zaa	unspecified α-amino acid

IX. Acknowledgments

The authors are indebted to the following people, whose advice, constructive comments and clarifying suggestions enabled this review to be completed: Rachael Fletcher, Anne Hackett, Richard Kinsman, Max Liu, Anne-Lise Matharu, Brian Ridge, Malcolm Sainsbury, Andrew Sutherland, and Chris Willis.

X. References

(1) Halverson, K.; Fraser, P. E.; Kirschner, D. A.; Lansbury, P. T., Jr. *Biochemistry* **1990**, *29*, 2639−2644.
(2) IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN) *Eur. J. Biochem.* **1984**, *138*, 9−37.
(3) Schulz, G. E.; Schirmer, R. H. *Principles of Protein Structure*; Springer-Verlag: New York, 1990.
(4) Plattner, J. J.; Norbeck, D. W. In *Drug Discovery Technologies*; Clark, C. R., Moos, W. H., Eds.; Ellis Horwood: Chichester, 1990; pp 92−126.
(5) Giannis, A.; Kolter, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244−1267.
(6) Davis, S. S. In *Perspectives in Medicinal Chemistry*; Testa, B., Kyburz, E., Fuhrer, W., Giger, R., Eds.; Verlag Helvetica Chimica Acta: Basel, 1993; pp 533−544.
(7) Gante, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699−1720.
(8) Grant, S. K.; Meek, T. D.; Metcalf, B. W.; Petteway, S. R., Jr. *Biomed. Appl. Biotechnol.* **1993**, *1*, 325−353.
(9) Wiley, R. A.; Rich, D. H. *Med. Res. Rev.* **1993**, *13*, 327−384.
(10) Moore, G. J. *Trends Pharmacol. Sci.* **1994**, *15*, 124−129.
(11) Morgan, B. A.; Gainor, J. A. *Annu. Rep. Med. Chem.* **1989**, *24*, 243−252.
(12) Kemp, D. S. *Trends Biotechnol.* **1990**, *8*, 249−255.
(13) Olson, G. L.; Bolin, D. R.; Bonner, M. P.; Bös, M.; Cook, C. M.; Fry, D. C.; Graves, B. J.; Hatada, M.; Hill, D. E.; Kahn, M.; Madison, V. S.; Rusiecki, V. K.; Sarabu, R.; Sepinwall, J.; Vincent, G. P.; Voss, M. E. *J. Med. Chem.* **1993**, *36*, 3039−3049.
(14) Veber, D. F. In *Peptides: Chemistry and Biology. Proceedings of the 12th American Peptide Symposium*; Smith, J. A., Rivier, J. E., Eds.; Escom: Leiden, 1992; pp 3−14.
(15) Hirschmann, R. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1278−1301.
(16) Pavia, M. R.; Sawyer, T. K.; Moos, W. H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 387−396.
(17) Jung, G.; Beck-Sickinger, A. G. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 367−383.
(18) Lowe, G. *Chem. Soc. Rev.* **1995**, *24*, 309−317.
(19) Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233−1251.
(20) Moran, E. J.; Wilson, T. E.; Cho, C. Y.; Cherry, S. R.; Schultz, P. G. *Biopolymers (Peptide Science)* **1995**, *37*, 213-219.
(21) Zuckermann, R. N. *Curr. Opin. Struct. Biol.* **1993**, *3*, 580−584.
(22) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555−600.
(23) Czarnik, A. W.; Ellman, J. A., Eds. *Acc. Chem. Res.* **1996**, *29*, 111−170 (special issue).
(24) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385−1401.
(25) Liskamp, R. M. J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 633−636.
(26) Szostak, J. W., Ed. *Chem. Rev.* **1997**, *97*, 347−348 (special issue).
(27) Bristol, J. A., Ed. *Tetrahedron* **1997**, *53*, Symposia-in-Print No. 63, 6573−6705.
(28) Dougall, W. C.; Peterson, N. C.; Greene, M. I. *Trends Biotechnol.* **1994**, *12*, 372−379.
(29) Kemp, D. S.; Bowen, B. R. In *Protein Folding. Deciphering the Second Half of the Genetic Code*; Gierasch, L. M., King, J., Eds.; American Association for the Advancement of Science: Washington, DC, 1990; pp 293−303.
(30) Freidinger, R. M. *Trends Pharmacol. Sci.* **1989**, *10*, 270−274.
(31) Horwell, D. C. *Bioorg. Med. Chem.* **1996**, *4*, 1573−1576.
(32) Kahn, M. *Synlett* **1993**, 821−826.
(33) Liskamp, R. M. J. *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 1−19.
(34) Hölzemann, G. *Kontakte (Darmstadt)* **1991**, 3−12.
(35) Hölzemann, G. *Kontakte (Darmstadt)* **1991**, 55−63.
(36) Müller, K.; Obrecht, D.; Knierzinger, A.; Stankovic, C.; Spiegler, C.; Bannwarth, W.; Trzeciak, A.; Englert, G.; Labhardt, A. M.; Schönholzer, P. In *Perspectives in Medicinal Chemistry*; Testa, B., Kyburz, E., Fuhrer, W., Giger, R., Eds.; Verlag Helvetica Chimica Acta: Basel, 1993; pp 513−531.
(37) Nowick, J. S.; Smith, E. M.; Pairish, M. *Chem. Soc. Rev.* **1996**, *25*, 401−415.
(38) Hanessian, S.; McNaughton-Smith, G.; Lombart, H.-G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789−12854.

(39) Hruby, V. J.; Kazmierski, W.; Kawasaki, A. M.; Matsunaga, T. O. In *Peptide Pharmaceuticals*; Ward, D., Ed.; Open University: Milton Keynes, 1991; pp 135−184.
(40) Rizo, J.; Gierasch, L. M. *Annu. Rev. Biochem.* **1992**, *61*, 387−418.
(41) Spatola, A. F. In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*; Weinstein, B., Ed.; Marcel Dekker: New York, 1983; Vol. 7, pp 267−357.
(42) Roberts, D. C.; Vellaccio, F. In *The Peptides: Analysis, Synthesis, Biology*; Gross, E., Meienhofer, J., Eds.; Academic: New York, 1983; Vol. 5, pp 341−449.
(43) Toniolo, C. *Int. J. Pept. Protein Res.* **1990**, *35*, 287−300.
(44) Fairlie, D. P.; Abbenante, G.; March, D. R. *Curr. Med. Chem.* **1995**, *2*, 654−686.
(45) Tourwé, D. *Janssen Chim. Acta* **1985**, *3*, 3−18.
(46) Dutta, A. S. *Adv. Drug Res.* **1991**, *21*, 145−286.
(47) Hruby, V. J. *Biopolymers* **1993**, *33*, 1073−1082.
(48) Hruby, V. J.; Al−Obeidi, F.; Kazmierski, W. *Biochem. J.* **1990**, *268*, 249−262.
(49) Hruby, V. J.; Li, G. G.; Haskell-Luevano, C.; Shenderovich, M. *Biopolymers* **1997**, *43*, 219−266.
(50) Marshall, G. R. *Tetrahedron* **1993**, *49*, 3547−3558.
(51) *Specialist Periodical Report: Amino Acids and Peptides*; Davies, J. S., Ed.; Royal Society of Chemistry: Cambridge, 1986−1997; Vols. 17−28.
(52) Patani, G. A.; LaVoie, E. J. *Chem. Rev.* **1996**, *96*, 3147−3176.
(53) Fauchère, J.-L.; Thurieau, C. *Adv. Drug Res.* **1992**, *23*, 127−159.
(54) Morley, J. S.; Hennessey, T. D.; Payne, J. W. *Biochem. Soc. Trans.* **1983**, *11*, 798−800.
(55) Smith, A. B., III; Hirschmann, R.; Pasternak, A.; Guzman, M. C.; Yokoyama, A.; Sprengeler, P. A.; Darke, P. L.; Emini, E. A.; Schleif, W. A. *J. Am. Chem. Soc.* **1995**, *117*, 11113−11123.
(56) Pandit, U. K. *Recl. Trav. Chim. Pays-Bas* **1993**, *112*, 431−443.
(57) Stewart, J. D.; Benkovic, S. J. *Chem. Soc. Rev.* **1993**, *22*, 213-219.
(58) Schultz, P. G.; Lerner, R. A. *Science (Washington, D.C.)* **1995**, *269*, 1835−1842.
(59) Jakubke, H.-D. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 175−177.
(60) Hirschmann, R.; Smith, A. B., III; Taylor, C. M.; Benkovic, P. A.; Taylor, S. D.; Yager, K. M.; Sprengeler, P. A.; Benkovic, S. J. *Science (Washington, D.C.)* **1994**, *265*, 234−237.
(61) Jacobsen, J. R.; Schultz, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5888−5892.
(62) Aubry, A.; Boussard, G.; Cung, M. T.; Marraud, M.; Vitoux, B. *J. Chim. Phys. Phys.−Chim. Biol.* **1988**, *85*, 345−359.
(63) Aubry, A.; Marraud, M. *Biopolymers* **1989**, *28*, 109−122.
(64) Marraud, M.; Dupont, V.; Grand, V.; Zekout, S.; Lecoq, A.; Bousssard, G.; Vidal, J.; Collet, A.; Aubry, A. *Bioploymers* **1993**, *33*, 1135−1148.
(65) Fincham, C. I.; Higginbottom, M.; Hill, D. R.; Horwell, D. C.; O'Toole, J. C.; Ratcliffe, G. S.; Rees, D. C.; Roberts, E. *J. Med. Chem.* **1992**, *35*, 1472−1484.
(66) Goodman, M.; Chorev, M. *Acc. Chem. Res.* **1979**, *12*, 1−7.
(67) Shemyakin, M. M.; Ovchinnikov, Yu. A.; Ivanov, V. T.; Ryabova, I. D. *Experientia* **1967**, *23*, 326.
(68) Shemyakin, M. M.; Ovchinnikov, Yu. A.; Ivanov, V. T. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 492−499.
(69) Wieland, T.; Penke, B.; Birr, C. *Liebigs Ann. Chem.* **1972**, *759*, 71−75.
(70) Snezhkova, L. G.; Shepel, E. N.; Ryabova, I. D.; Miroshnikov, A. I.; Ivanov, V. T.; Ovchinnikov, Yu. A. *Bioorg. Khim.* **1975**, *1*, 347−358; *Chem. Abstr.* **1975**, *83*, 193686y.
(71) Vogler, K.; Lanz, P.; Lergier, W.; Haefely, W. *Helv. Chim. Acta* **1966**, *49*, 390−403.
(72) Rudinger, J. In *Drug Design*; Ariëns, E. J., Ed.; Academic: New York, 1971; Vol. II, pp 319−419.
(73) Goissis, G.; Nouailhetas, V. L. A.; Paiva, A. C. M. *J. Med. Chem.* **1976**, *19*, 1287−1290.
(74) Hayward, C. F.; Morley, J. S. In *Peptides 1974, Proceedings of the 13th European Peptide Symposium*, Israel; Wolman, Y., Ed.; Wiley: New York and Israel Universities: Jerusalem, 1975; pp 287−298.
(75) Willson, C. G.; Goodman, M.; Rivier, J.; Vale, W. In *Peptides: Proceedings of the 5th American Peptide Symposium*; Goodman, M., Meienhofer, J., Eds.; Wiley: New York, 1977; pp 579−581.
(76) Chorev, M.; Willson, C. G.; Goodman, M. *J. Am. Chem. Soc.* **1977**, *99*, 8075−8076.
(77) Ancans, J.; Cipens, G. *Latv. PSR Zinat. Akad. Vestis, Khim. Ser.* **1974**, 241−243; *Chem. Abstr.* **1974**, *81*, 78221q.
(78) Ancans, J.; Afanas'eva, G. A.; Indulens, J.; Klusa, V.; Cipens, G.; Kibirev, V. K. In *Tezisy Dokl. − Vses. Simp. Khim. Pept. Belkov. 3rd*, Kiev, **1974**; p 10; *Chem. Abstr.* **1976**, *85*, 193072m.
(79) Chipens, G. I.; Afanas'eva, G. A.; Antsan, Yu. E.; Zalitis, G. M.; Vegner, R. É.; Balodis, Yu. Yu. *Biochemistry (Engl. Transl.)* **1978**, *43*, 692−698; *Biokhimiya (Moscow)* **1978**, *43*, 872−879.
(80) Kemme, A. A.; Shvets, A. E.; Bleidelis, J. J.; Ancans, Yu. E.; Cipens, G. I. *J. Struct. Chem. (Engl. Transl.)* **1976**, *17*, 965−967; *Zh. Strukt. Khim.* **1976**, *17*, 1132−1135.
(81) Ancan, J.; Cipens, G. *Bioorg. Khim.* **1975**, *1*, 1410−1417; *Chem. Abstr.* **1976**, *84*, 106104m.
(82) Ancans, J.; Makarova, N. A.; Cipens, G. *Bioorg. Khim.* **1981**, *7*, 336−341; *Chem. Abstr.* **1981**, *95*, 959f.
(83) Ancans, J.; Makarova, N. A.; Misins, I.; Cipens, G. *Bioorg. Khim.* **1979**, *5*, 1295−1301; *Chem. Abstr.* **1980**, *92*, 69995v.
(84) Zalitis, G. M.; Afanas'eva, G. A.; Chipens, G. I. *Biochemistry (Engl. Transl.)* **1982**, *47*, 220−222; *Biokhimiya (Moscow)* **1982**, *47*, 263−265.
(85) Chorev, M.; Goodman, M. *Acc. Chem. Res.* **1993**, *26*, 266−273.
(86) Chorev, M.; Goodman, M. *Trends Biotechnol.* **1995**, *13*, 438−445.
(87) Bodanszky, M. *Principles of Peptide Synthesis*, 2nd ed.; Springer-Verlag: Berlin, 1993.
(88) *The Peptides: Analysis, Synthesis, Biology*; Gross, E., Meienhofer, J., Udenfriend, S., Eds.; Academic: New York, 1979−1987; Vols. 1−9.
(89) Chorev, M.; Shavitz, R.; Goodman, M.; Minick, S.; Guillemin, R. *Science (Washington, D.C.)* **1979**, *204*, 1210−1212.
(90) Bergmann, M.; Zervas, L. *J. Biol. Chem.* **1936**, *113*, 341−357.
(91) Chorev, M.; Goodman, M. *Int. J. Pept. Protein Res.* **1983**, *21*, 258−268.
(92) Chorev, M.; MacDonald, S. A.; Goodman, M. *J. Org. Chem.* **1984**, *49*, 821−827.
(93) Meienhofer, J. In *The Peptides: Analysis, Synthesis, Biology*; Gross, E., Meienhofer, J., Eds.; Academic: New York, 1979; Vol. 1, p 197−240.
(94) Goldschmidt, S.; Wick, M. *Liebigs Ann. Chem.* **1952**, *575*, 217−231.
(95) Chaturvedi, N.; Goodman, M.; Bowers, C. *Int. J. Pept. Protein Res.* **1981**, *17*, 72−88.
(96) Moutevelis-Minakakis, P.; Photaki, I. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2277−2281.
(97) Ninomiya, K.; Shioiri, T.; Yamada, S. *Tetrahedron* **1974**, *30*, 2151−2157.
(98) Cremlyn, R. J. W. *Aust. J. Chem.* **1973**, *26*, 1591−1593.
(99) Sisto, A.; Verdini, A. S.; Virdia, A. *Synthesis* **1985**, 294−296.
(100) Shioiri, T.; Yamada, S.-I. *Chem. Pharm. Bull.* **1974**, *22*, 859−863.
(101) Shioiri, T.; Yamada, S.-I. *Chem. Pharm. Bull.* **1974**, *22*, 855−858.
(102) DeBons, F. E.; Loudon, G. M. *J. Org. Chem.* **1980**, *45*, 1703−1704.
(103) Loudon, G. M.; Almond, M. R.; Jacob, J. N. *J. Am. Chem. Soc.* **1981**, *103*, 4508−4515.
(104) Biffin, M. E. C.; Miller, J.; Paul, D. B. In *The Chemistry of the Azido Group*; Patai, S., Ed.; Interscience: London, 1971; pp 57−190.
(105) Lwowski, W. In *The Chemistry of the Azido Group*; Patai, S., Ed.; Interscience: London, 1971; pp 503−554.
(106) Rawal, V. H.; Zhong, H. M. *Tetrahedron Lett.* **1994**, *35*, 4947−4950.
(107) Scriven, E. F. V.; Turnbull, K. *Chem. Rev.* **1988**, *88*, 297−368.
(108) Campbell, M. M.; Ross, B. C.; Semple, G. *Tetrahedron Lett.* **1989**, *30*, 1997−2000.
(109) Campbell, M. M.; Fletcher, M. D. Unpublished results.
(110) Pallai, P. V.; Richman, S.; Struthers, R. S.; Goodman, M. *Int. J. Pept. Protein Res.* **1983**, *21*, 84−92.
(111) Qasmi, D.; René, L.; Badet, B. *Tetrahedron Lett.* **1993**, *34*, 3861−3862.
(112) *The Peptides: Analysis, Synthesis, Biology. Major Methods of Peptide Bond Formation*; Gross, E., Meienhofer, J., Eds.; Academic: New York, 1979; Vol. 1.
(113) Cushman, M.; Jurayj, J.; Moyer, J. D. *J. Org. Chem.* **1990**, *55*, 3186−3194.
(114) Rodriguez, M.; Galas, M.-C.; Lignon, M.-F.; Mendre, C.; Laur, J.; Aumelas, A.; Martinez, J. *J. Med. Chem.* **1989**, *32*, 2331−2339.
(115) Guichard, G.; Connan, F.; Graff, R.; Ostankovitch, M.; Muller, S.; Guillet, J.-G.; Choppin, J.; Briand, J.-P. *J. Med. Chem.* **1996**, *39*, 2030−2039.
(116) Berman, J. M.; Goodman, M. *Int. J. Pept. Protein Res.* **1984**, *23*, 610−620.
(117) IBTFA is also known as phenyl iodosyl bis(trifluoroacetate) (PIFA or PIT), [bis(trifluoroacetoxy)iodo]benzene (TIB), phenyl-bis(trifluoroacetato-*O*)iodine and iodoso benzene 1,1-bis(trifluoroacetate)! Radhakrishna, A. S.; Parham, M. E.; Riggs, R. M.; Loudon, G. M. *J. Org. Chem.* **1979**, *44*, 1746−1747.
(118) Loudon, G. M.; Radhakrishna, A. S.; Almond, M. R.; Blodgett, J. K.; Boutin, R. H. *J. Org. Chem.* **1984**, *49*, 4272−4276.
(119) Boutin, R. H.; Loudon, G. M. *J. Org. Chem.* **1984**, *49*, 4277−4284.
(120) Parham, M. E.; Loudon, G. M. *Biochem. Biophys. Res. Commun.* **1978**, *80*, 1−6.
(121) Pallai, P.; Goodman, M. *J. Chem. Soc., Chem. Commun.* **1982**, 280−281.

(122) Swaminathan, K.; Venkatasubramanian, N. *J. Chem. Soc., Perkin Trans. 2* **1975**, 1161−1166.
(123) Dürr, H.; Goodman, M.; Jung, G. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 785−787.
(124) Gazerro, L.; Pinori, M.; Verdini, A. S. In *Innovation and Perspectives in Solid-Phase Synthesis, Oxford, England*; Epton, R., Ed.; SPCC(UK): Birmingham, 1990; pp 403−412.
(125) Gazerro, L.; Pinori, M.; Verdini, A. S. In *Peptides 1990. Proceedings of the 21st European Peptide Symposium*, Platja d'Aro, Spain; Giralt, E., Andreu, D., Eds.; Escom: Leiden, 1991; pp 395−396.
(126) Katritzky, A. R.; Urogdi, L.; Mayence, A. *J. Org. Chem.* **1990**, *55*, 2206−2214.
(127) Schmidt, U.; Stäbler, F.; Lieberknecht, A. *Synthesis* **1994**, 890−892.
(128) Bock, M. G.; DiPardo, R. M.; Freidinger, R. M. *J. Org. Chem.* **1986**, *51*, 3718−3720.
(129) Zoller, U.; Ben-Ishai, D. *Tetrahedron* **1975**, *31*, 863−866.
(130) Losse, G.; Strobel, J. *J. Prakt. Chem.* **1984**, *326*, 765−778.
(131) Rivier, J. E.; Jiang, G.-C.; Koerber, S. C.; Porter, J.; Simon, L.; Craig, A. G.; Hoeger, C. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 2031−2036.
(132) Jiang, G.-C.; Simon, L.; Rivier, J. E. *Protein Peptide Lett.* **1996**, *3*, 219−224.
(133) Rivier, J.; Jiang, G.-C.; Lahrichi, S. L.; Porter, J.; Koerber, S. C.; Rizo, J.; Corrigan, A.; Gierasch, L.; Hagler, A.; Vale, W.; Rivier, C. *Hum. Reprod.* **1996**, *11*, 133−147.
(134) Kohn, H.; Sawhney, K. N.; LeGall, P.; Robertson, D. W.; Leander, J. D. *J. Med. Chem.* **1991**, *34*, 2444−2452.
(135) Kober, R.; Steglich, W. *Liebigs Ann. Chem.* **1983**, 599−609.
(136) Bergmann, M.; Grafe, K. *Hoppe-Seyler's Z. Physiol. Chem.* **1930**, *187*, 187−195; *Chem. Abstr.* **1930**, *24*, 2150.
(137) Bergmann, M.; Grafe, K. *Hoppe-Seyler's Z. Physiol. Chem.* **1930**, *187*, 196−202; *Chem. Abstr.* **1930**, *24*, 2151.
(138) Bergmann, M.; Grafe, K. *Hoppe-Seyler's Z. Physiol. Chem.* **1930**, *187*, 183−186; *Chem. Abstr.* **1930**, *24*, 2150.
(139) Lucente, G.; Lucente, G.; Romeo, A. *Ann. Chim. (Rome)* **1966**, *56*, 572−579; *Chem. Abstr.* **1967**, *66*, 85999t.
(140) Romeo, A.; Schimberni, A. M. *Atti Accad. Naz. Lincei, Rend., Sci. Fis., Mater. Nat.* **1957**, *22*, 620−625; *Chem. Abstr.* **1958**, *52*, 2837i.
(141) Magat, E. E.; Chandler, L. B.; Faris, B. F.; Reith, J. E.; Salisbury, L. F. *J. Am. Chem. Soc.* **1951**, *73*, 1031−1035.
(142) Puiggalí, J.; Muñoz-Guerra, S. *J. Polym. Sci., Polym. Phys. Ed.* **1987**, *25*, 513−523.
(143) Moad, G.; Benkovic, S. J. *J. Am. Chem. Soc.* **1978**, *100*, 5495−5499.
(144) Fife, T. H.; Hutchins, J. E. C.; Pellino, A. M. *J. Am. Chem. Soc.* **1978**, *100*, 6455−6462.
(145) Kametani, T.; Honda, T.; Sasaki, J.; Terasawa, H.; Fukumoto, K. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1884−1887.
(146) Gutman, À. L.; Boltanski, A. *Tetrahedron Lett.* **1985**, *26*, 1573−1576.
(147) The same overall reaction of a carboxylic acid and a chloroformate to yield the carboxylate ester is fairly general but requires the agency of DMAP: Kim, S.; Lee, J. I.; Kim, Y. C. *J. Org. Chem.* **1985**, *50*, 560−565.
(148) Carmona, A. K.; Juliano, L. *Biochem. Pharmacol.* **1996**, *51*, 1051−1060.
(149) Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids*; John Wiley and Sons: New York, 1961.
(150) Vul'fson, N. S. *Zh. Obshch. Khim.* **1949**, *19*, 1904−1916; *Chem. Abstr.* **1950**, *44*, 1901f.
(151) Wheelan, P.; Kirsch, W. M.; Koch, T. H. *J. Org. Chem.* **1989**, *54*, 1364−1370.
(152) Angelastro, M. R.; Baugh, L. E.; Bey, P.; Burkhart, J. P.; Chen, T.-M.; Durham, S. L.; Hare, C. M.; Huber, E. W.; Janusz, M. J.; Koehl, J. R.; Marquart, A. L.; Mehdi, S.; Peet, N. P. *J. Med. Chem.* **1994**, *37*, 4538−4553.
(153) Paiaro, G.; Pandolfo, L.; Busico, V.; Corradini, P. *Eur. Polym. J.* **1988**, *24*, 99−102.
(154) Navarro, E.; Tereshko, V.; Subirana, J. A.; Puiggalí, J. *Biopolymers* **1995**, *36*, 711−722.
(155) Perrin, C. L.; Arrhenius, T. *J. Am. Chem. Soc.* **1978**, *100*, 5249−5251.
(156) Staudinger, H.; Ott, E. *Chem. Ber.* **1908**, *41*, 2208−2217; *Chem. Abstr.* **1908**, *2*, 2808.
(157) Katagi, T.; Aoki, M.; Kashiwagi, M.; Ohata, K.; Kohno, S.; Murata, T.; Inoi, T. *Chem. Pharm. Bull.* **1985**, *33*, 4878−4888.
(158) Bezuglyi, P. A.; Treskach, V. I.; Ukrainets, I. V.; Grinenko, V. V.; Bevz, N. Yu. *J. Org. Chem. USSR (Engl. Transl.)* **1991**, *27*, 1233−1236; *Zh. Org. Khim.* **1991**, *27*, 1410−1413.
(159) Ogura, H.; Kobayashi, T.; Shimizu, K.; Kawabe, K.; Takeda, K. *Tetrahedron Lett.* **1979**, *20*, 4745−4746.
(160) Dado, G. P.; Desper, J. M.; Holmgren, S. K.; Rito, C. J.; Gellman, S. H. *J. Am. Chem. Soc.* **1992**, *114*, 4834−4843.
(161) Vedejs, E.; Campbell, J. B., Jr.; Gadwood, R. C.; Rodgers, J. D.; Spear, K. L.; Watanabe, Y. *J. Org. Chem.* **1982**, *47*, 1534−1546.
(162) Nishikawa, N.; Komazawa, H.; Orikasa, A.; Yoshikane, M.; Yamaguchi, J.; Kojima, M.; Ono, M.; Itoh, I.; Azuma, I.; Fujii, H.; Murata, J.; Saiki, I. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2725−2728.
(163) Cope, A. C.; Holmes, H. L.; House, H. O. *Org. React. (New York)* **1957**, *IX*, 107−331.
(164) Pallai, P. V.; Struthers, R. S.; Goodman, M.; Moroder, L.; Wunsch, E.; Vale, W. *Biochemistry* **1985**, *24*, 1933−1941.
(165) McNab, H. *Chem. Soc. Rev.* **1978**, *7*, 345−358.
(166) Verdini, A. S.; Silvestri, S.; Becherucci, C.; Longobardi, M. G.; Parente, L.; Peppoloni, S.; Perretti, M.; Pileri, P.; Pinori, M.; Viscomi, G. C.; Nencioni, L. *J. Med. Chem.* **1991**, *34*, 3372−3379.
(167) Wright, A. D.; Haslego, M. L.; Smith, F. X. *Tetrahedron Lett.* **1979**, *20*, 2325−2326.
(168) Hrubowchak, D. M.; Smith, F. X. *Tetrahedron Lett.* **1983**, *24*, 4951−4954.
(169) Chorev, M.; Rubini, E.; Gilon, C.; Wormser, U.; Selinger, Z. *J. Med. Chem.* **1983**, *26*, 129−135.
(170) Rigo, B.; Fasseur, D.; Cauliez, P.; Courturier, D. *Tetrahedron Lett.* **1989**, *30*, 3073−3076.
(171) Birkofer, L.; Dickopp, H. *Angew. Chem., Int. Ed. Engl.* **1964**, *3*, 514.
(172) Klebe, J. F.; Finkbeiner, H.; White, D. M. *J. Am. Chem. Soc.* **1966**, *88*, 3390−3395.
(173) Verdini, A. S.; Pinori, M.; Viscomi, G. C.; Pileri, P.; Silvestri, S.; Nencioni, L. In *Peptides 1990. Proceedings of the 21st European Peptide Symposium*, Platja d'Aro, Spain; Giralt, E., Andreu, D., Eds.; Escom: Leiden, 1991; p 397−398.
(174) Rogozhin, S. V.; Davidovich, Yu. A.; Yurtanov, A. I. *Synthesis* **1975**, 113−114.
(175) Dal Pozzo, A.; Kanai, K.; Kereszturi, G.; Calabrese, G. *Int. J. Pept. Protein Res.* **1993**, *41*, 561−566.
(176) Danishevsky, S.; Singh, R. K. *J. Am. Chem. Soc.* **1975**, *97*, 3239−3241.
(177) Campbell, M. M.; Semple, G. Unpublished results.
(178) Krapcho, A. P.; Jahngen, E. G. E., Jr.; Kashden, D. S. *Tetrahedron Lett.* **1974**, *15*, 2721−2723.
(179) Reiffers, S.; Wynberg, H.; Strating, J. *Tetrahedron Lett.* **1971**, *12*, 3001−3004.
(180) Krapcho, A. P.; Stephens, W. P. *J. Org. Chem.* **1980**, *45*, 1106−1109.
(181) Bossler, H. G.; Waldmeier, P.; Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 439−440.
(182) Padgett, H. C.; Csendes, I. C.; Rapoport, H. *J. Org. Chem.* **1979**, *44*, 3492−3496.
(183) Yamazaki, T.; Kasatkin, A.; Kawanaka, Y.; Sato, F. *J. Org. Chem.* **1996**, *61*, 2266−2267.
(184) Grossman, R. B.; Varner, M. A. *J. Org. Chem.* **1997**, *62*, 5235−5237.
(185) Chorev, M.; Yaion, M.; Wormser, U.; Levian-Teitelbaum, D.; Gilon, C.; Selinger, Z. *Eur. J. Med. Chem.−Chim. Ther.* **1986**, *21*, 96−102.
(186) Dal Pozzo, A.; Laurita, E. In *Peptides 1994. Proceedings of the 23rd European Peptide Symposium*; Maia, H. C. S., Ed.; Escom Science: Leiden 1995; pp 714−715.
(187) Buffoni, F.; Pino, R.; Dal Pozzo, A. *Arch. Int. Pharmacodyn. Ther.* **1995**, *330*, 345−360; *Chem. Abstr.* **1996**, *125*, 105019r.
(188) Abouabdellah, A.; Welch, J. T. *Tetrahedron: Asymmetry* **1994**, *5*, 1005−1013.
(189) Welch, J. T.; Araki, K.; Kawecki, R.; Wichtowski, J. A. *J. Org. Chem.* **1993**, *58*, 2454−2462.
(190) Pessi, A.; Pinori, M.; Verdini, A. S.; Viscomi, G. C. *J. Chem. Soc., Chem. Commun.* **1983**, 195−197.
(191) Bonelli, F.; Pessi, A.; Verdini, A. S. *Int. J. Pept. Protein Res.* **1984**, *24*, 553−556.
(192) Di Gregorio, G.; Pinori, M.; Verdini, A. S. In *Innovation and Perspectives in Solid-Phase Synthesis: Peptides, Polypeptides and Oligonucleotides*, Canterbury, England; Epton, R., Ed.; Intercept: Andover, 1992; pp 311−318.
(193) Albericio, F.; Kneib-Cordonier, N.; Biancalana, S.; Gera, L.; Masada, R. I.; Hudson, D.; Barany, G. *J. Org. Chem.* **1990**, *55*, 3730−3743.
(194) Roques, B. P.; Lucas-Soroca, E.; Chaillet, P.; Costentin, J.; Fournié-Zaluski, M.-C. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 3178−3182.
(195) Roderick, S. L.; Fournié-Zaluski, M. C.; Roques, B. P.; Matthews, B. W. *Biochemistry* **1989**, *28*, 1493−1497.
(196) Hernandez, J. F.; Soleilhac, J. M.; Roques, B. P.; Fournié-Zaluski, M. C. *J. Med. Chem.* **1988**, *31*, 1825−1831.
(197) Fournié-Zaluski, M.-C.; Hernandez, J.-F.; Soleilhac, J.-M.; Renwart, N.; Peyroux, J.; Xie, J.; Roques, B. P. *Int. J. Pept. Protein Res.* **1989**, *33*, 146−153.
(198) Chorev, M.; Willson, C. G.; Goodman, M. In *Peptides: Proceedings of the 5th American Peptide Symposium*; Goodman, M., Meienhofer, J., Eds.; Wiley: 1977; pp 572−574.
(199) Yamazaki, T.; Benedetti, E.; Kent, D.; Goodman, M. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1437−1451.
(200) Fuller, W. D.; Goodman, M.; Verlander, M. S. *J. Am. Chem. Soc.* **1985**, *107*, 5821−5822.

(201) Goodman, M.; Coddington, J.; Mierke, D. F.; Fuller, W. D. *J. Am. Chem. Soc.* **1987**, *109*, 4712−4714.
(202) Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C.; Fuller, W. D.; Mierke, D. F.; Goodman, M. *J. Am. Chem. Soc.* **1990**, *112*, 8909−8912.
(203) Rodriguez, M.; Dubreuil, P.; Bali, J.-P.; Martinez, J. *J. Med. Chem.* **1987**, *30*, 758−763.
(204) Charpentier, B.; Durieux, C.; Pelaprat, D.; Dor, A.; Reibaud, M.; Blanchard, J.-C.; Roques, B. P. *Peptides (New York)* **1988**, *9*, 835−841.
(205) Ruiz-Gayo, M.; Dauge, V.; Menant, I.; Begue, D.; Gacel, G.; Roques, B. P. *Peptides (New York)* **1985**, *6*, 415−420.
(206) Paulesu, L.; Di Stefano, A.; Luzzi, E.; Bocci, V.; Silvestri, S.; Nencioni, L. *Immunol. Lett.* **1992**, *34*, 7−11.
(207) Becherucci, C.; Perretti, M.; Nencioni, L.; Silvestri, S.; Parente, L. *Agents Actions* **1992**, C115−117; *Chem. Abstr.* **1992**, *116*, 248087m.
(208) De Angelis, F.; Nicoletti, R.; Kuster, T.; Heizmann, C. W.; Pinori, M.; Verdini, A. S. *Biol. Mass Spectrom.* **1994**, *23*, 262−266.
(209) De Angelis, F. In *NATO ASI Series, Series C: Mass Spectrometry in the Biological Sciences: A Tutorial*; Gross, M. L., Ed.; Kluwer Academic: Netherlands, 1992; Vol. 353, pp 357−369.
(210) Fujii, H.; Nishikawa, N.; Komazawa, H.; Orikasa, A.; Ono, M.; Itoh, I.; Murata, J.; Azuma, I.; Saiki, I. *Oncol. Res.* **1996**, *8*, 333−342.
(211) Guichard, G.; Muller, S.; van Regenmortel, M.; Briand, J. P.; Mascagni, P.; Giralt, E. *Trends Biotechnol.* **1996**, *14*, 44−45.
(212) Carver, J. A.; Esposito, G.; Viglino, P.; Fogolari, F.; Guichard, G.; Briand, J. P.; Van Regenmortel, M. H. V.; Brown, F.; Mascagni, P. *Biopolymers* **1997**, *41*, 569−590.
(213) McDonnell, J. M.; Fushman, D.; Cahill, S. M.; Sutton, B. J.; Cowburn, D. *J. Am. Chem. Soc.* **1997**, *119*, 5321−5328.
(214) Bartnes, K.; Hannestad, K.; Guichard, G.; Briand, J. P. *Eur. J. Immunol.* **1997**, *27*, 1387−1391.
(215) Hervé, M.; Maillére, B.; Mourier, G.; Texier, C.; Leroy, S.; Ménez, A. *Mol. Immunol.* **1997**, *34*, 157−163.
(216) Krotz, A. H.; Buchardt, O.; Nielsen, P. E. *Tetrahedron Lett.* **1995**, *36*, 6937−6940.
(217) Krotz, A. H.; Buchardt, O.; Nielsen, P. E. *Tetrahedron Lett.* **1995**, *36*, 6941−6944.
(218) Frey, O.; Hoffmann, M.; Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2026−2028.
(219) Esposito, G.; Settembri, L.; Viscomi, G. C.; Niccolai, N. *J. Chem. Soc., Perkin Trans. 2* **1988**, 1313−1318.
(220) Marraud, M.; Aubry, A. *Biopolymers (Peptide Science)* **1996**, *40*, 45−83.
(221) Freidinger, R. M.; Veber, D. F. *J. Am. Chem. Soc.* **1979**, *101*, 6129−6131.
(222) Wermuth, J.; Goodman, S. L.; Jonczyk, A.; Kessler, H. *J. Am. Chem. Soc.* **1997**, *119*, 1328−1335.
(223) Creighton, T. E. *Proteins. Structures and Molecular Properties*, 2nd ed.; W. H. Freeman: New York, 1993. In all the discussions of Ramachandran maps herein, the following terms are used, rather loosely, to describe the given, general regions of the maps: helical, the lower left and upper right-hand quadrants [in the general vicinity of the α-helical conformation, ca. ±(60°, 60°)];[3] extended, the upper left and lower right-hand corners; *β*-sheet region, the upper left-hand corner, near the protein *β*-sheet conformation, ca. (−130°, 120°)].[223]
(224) Chorev, M.; Goodman, M. *Trends Biotechnol.* **1996**, *14*, 43−44.
(225) Guptasarma, P. *Trends Biotechnol.* **1996**, *14*, 42−43.
(226) Dauber-Osguthorpe, P.; Campbell, M. M.; Osguthorpe, D. J. *Int. J. Pept. Protein Res.* **1991**, *38*, 357−377.
(227) Stern, P. S.; Chorev, M.; Goodman, M.; Hagler, A. T. *Biopolymers* **1983**, *22*, 1885−1900.
(228) Stern, P. S.; Chorev, M.; Goodman, M.; Hagler, A. T. *Biopolymers* **1983**, *22*, 1901−1917.
(229) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1−109.
(230) Richardson, J. S.; Richardson, D. C. In *Prediction of Protein Structure and the Principles of Protein Conformation*; Fasman, G. D., Ed.; Plenum: New York, 1989; pp 1−98.
(231) Gomez, E. J.; Vitoux, B.; Marraud, M.; Sakarellos, C.; El Masdouri, L.; Aubry, A. *Int. J. Pept. Protein Res.* **1989**, *34*, 480−486.
(232) El Masdouri, L.; Aubry, A.; Gomez, E.; Vitoux, B.; Marraud, M. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1992**, *48*, 175−176.
(233) El Masdouri, L.; Aubry, A.; Gomez, E.; Vitoux, B.; Marraud, M. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1992**, *48*, 178−179.
(234) Sisto, A.; Mariotti, S.; Groggia, A.; Marcozzi, G.; Villa, L.; Nencioni, L.; Silvestri, S.; Verdini, A. S.; Pessi, A. In *Peptides: Chemistry, Structure and Biology, Proceedinds of the 11th American Peptide Symposium, 1989*; Rivier, J. E., Marshall, G. R., Eds.; Escom: Leiden, 1990; pp 772−773.
(235) Alemán, C.; Puiggalí, J. *J. Org. Chem.* **1995**, *60*, 910−924.
(236) Alemán, C.; Pérez, J. J. *Int. J. Pept. Protein Res.* **1994**, *43*, 258−263.
(237) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1992**, *114*, 3138−3139.
(238) Alemán, C.; Pérez, J. J. *J. Mol. Struct. (THEOCHEM)* **1994**, *304*, 17−24.
(239) Bella, J.; Subirana, J. A. *Polym. Prepr. (Am. Chem. Soc. Div. Polym. Chem.)* **1992**, *33*, 276−277.
(240) Franco, L.; Aceltuno, J. E.; Subirana, J. A.; Puiggalí, J. *Polym. Prepr. (Am. Chem. Soc. Div. Polym. Chem.)* **1992**, *33*, 325−326.
(241) El Masdouri, L.; Aubry, A.; Gomez, E.; Vitoux, B.; Marraud, M. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1992**, *48*, 176−178.
(242) Alemán, C.; Pérez, J. J. *J. Mol. Struct. (THEOCHEM)* **1993**, *285*, 221−227.
(243) Alemán, C.; Pérez, J. J. *Int. J. Pept. Protein Res.* **1993**, *41*, 606−610.
(244) Franco, L.; Navarro, E.; Subirana, J. A.; Puiggalí, J. *Macromolecules* **1994**, *27*, 4284−4297.
(245) Alemán, C.; Bella, J. *Biopolymers* **1995**, *35*, 257−269.
(246) Alemán, C.; Puiggalí, J. *J. Polym. Sci., Polym. Phys. Ed.* **1996**, *34*, 1327−1338.
(247) Alemán, C. *J. Biomol. Struct. Dyn.* **1996**, *14*, 193−199.
(248) Gellman, S. H.; Adams, B. R.; Dado, G. P. *J. Am. Chem. Soc.* **1990**, *112*, 460−461.
(249) Dado, G. P.; Desper, J. M.; Gellman, S. H. *J. Am. Chem. Soc.* **1990**, *112*, 8630−8632.
(250) Liang, G.-B.; Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1991**, *113*, 3994−3995.
(251) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 4228−4245.
(252) Gellman, S. H.; Dado, G. P.; Liang, G.-B.; Adams, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 1164−1173.
(253) Jorgensen, W. L.; Pranata, J. *J. Am. Chem. Soc.* **1990**, *112*, 2008−2010.
(254) Pranata, J.; Wierschke, S. G.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1991**, *113*, 2810−2819.
(255) Gardner, R. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, *117*, 10411−10412.
(256) Gardner, R. R.; Gellman, S. H. *Tetrahedron* **1997**, *53*, 9881−9890.

CR970468T