Synthesis and Properties of Cyclopropane-Derived Peptidomimetics

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ABSTRACT
Small peptides exhibit a wide range of biological activities, but although there are some notable exceptions, they are not generally useful as drugs. This has spurred widespread interest in designing peptidomimetics and introducing them as replacements of portions of native peptides to enhance their biological properties. Special attention has been focused upon rigid replacements because of their potential to preorganize the resulting pseudopeptide in a conformation corresponding to its bound structure. Toward this goal, we invented trisubstituted cyclopropanes as novel peptidomimetics, anticipating that the cyclopropane ring would locally orient the backbone and the corresponding amino acid side chain in the biologically active conformation. Selected aspects of the syntheses and applications of these cyclopropane-derived peptidomimetics are presented in this Account.

Introduction

Designing small molecules that bind to therapeutically important biological targets with high affinity and selectivity is a major goal in contemporary bioorganic and medicinal chemistry,[1] and peptides often serve as leads in these endeavors. A widely cited guiding principle is that preorganizing a flexible peptide in the conformation it adopts upon binding to a biomacromolecule, often called the biologically active conformation, will give a constrained derivative having higher affinity because the preorganized molecule is expected to pay a lower entropic cost upon complexation.[2−4] It is implicit that the flexible and constrained molecules interact in the same way with solvent and the macromolecular target. Conformationally constrained molecules are also known to exhibit improved selectivity profiles, and there is evidence that preorganizing small molecules will lead to compounds exhibiting improved bioavailability.[5]

One general strategy for introducing conformational constraints into flexible peptides involves synthesizing cyclic derivatives, and three cyclization modes have been commonly explored (Figure 1). These entail bond formation between two side chains (path a), a side chain and the backbone (path b), or the N- and C-termini of the backbone (path c). Such cyclizations are primarily intended to constrain the backbone into a predefined secondary structure, corresponding to an α-, β-, or γ-turn or a β-strand, structural motifs that are frequently found in the biologically active conformations of peptide-derived ligands.[6,7]

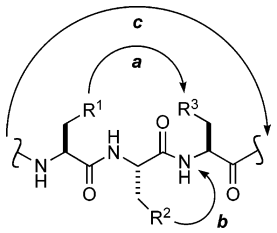

FIGURE 1. Cyclization modes to constrain peptides.

Introducing cyclic constraints into peptides has proven to be an effective strategy for preorganizing the backbone, but such cyclizations are generally not designed to preferentially orient the amino acid side chains. Because these side chains contribute significantly to specificity and affinity, controlling their spatial orientation is an important, but often overlooked, aspect in the design of peptidomimetics.[8] We thus initiated a program to invent a novel class of peptidomimetics that would constrain the peptide backbone while simultaneously projecting the side chains in defined orientations. We now present a synopsis of these efforts.

Design Rationale for Cyclopropane-Derived Peptide Replacements

We conducted molecular modeling studies in 1989 that suggested 1,2,3-trisubstituted cyclopropanes of the general structures **2**−**5** might serve as novel, rigid replacements of peptide **1** (Scheme 1).[9] Peptidomimetic **2** was derived from **1** by a side chain to backbone cyclization in which the nitrogen atom was replaced with a carbon atom and a new bond formed between this atom and the C(β) atom (mode a). Mimic **3** was similarly derived from **1** by replacing the carbonyl carbon atom with a sp^3-carbon atom and connecting it to C(β) on the side chain (mode b). The *trans* relationship of the backbone substituents of **2** and **3** was envisioned to locally stabilize a β-strand

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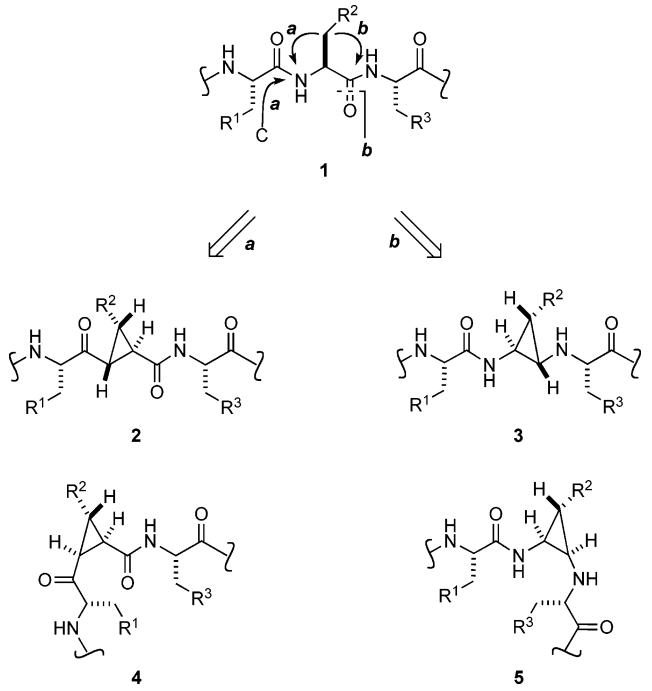
Scheme 1



conformation that would be additionally stabilized by interactions of the carbonyl π-orbitals with the carbon−carbon σ-bonds of the cyclopropane in **2**. The alternative *cis* arrangement of the backbone substituents in **4** and **5** was envisioned to induce turned structures. We recognized that deletion of the amide linkage in **2**−**5** might result in the loss of hydrogen bonds with the protein target, but we reasoned that restricting at least two rotors by forming a cyclopropane ring might afford an entropic advantage that would offset this enthalpic cost.[10]

The cyclopropane rings in **2**−**5** not only constrain the backbones but orient the side chains in defined directions. Modeling suggests that the R^2 group in **2** is oriented so it occupies a region of space *relative to the backbone* that approximates a χ_1-angle of *gauche*(−) (−60°) for the corresponding amino acid residue in **1** (Figure 2a). Similarly, R^2 in **3** is projected in an orientation similar to that of a χ_1-angle of *anti* (180°) (Figure 2b).

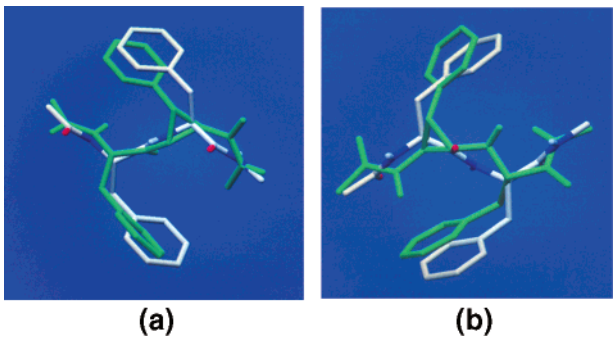
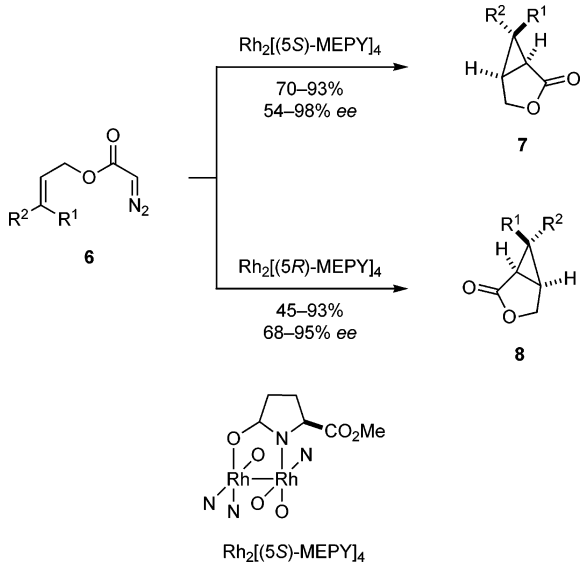


FIGURE 2. Superimposition of Phe−Phe (white) wherein the backbone is in a β-strand conformation with a cyclopropane-derived analogue: (a) phenyl group of one phenylalanine in *gauche*(−) orientation is overlaid with peptidomimetic **2** (green); (b) phenyl group of one phenylalanine in *anti* conformation is overlaid with peptidomimetic **3** (green).

Enantioselective Synthesis of 1,2,3-Trisubstituted Cyclopropanes

Having designed 1,2,3-trisubstituted cyclopropanes as potential peptidomimetics, a method for their stereoselective synthesis was required. We first established tactics to control the relative stereochemistry of substituents on racemic cyclopropanes,[9] and then we directed our efforts toward developing a route to enantiomerically pure cyclopropanes that could be transformed into replacements related to **2**−**5**. We discovered that chiral rhodium(II) carboxamide catalysts originally developed by Doyle for bimolecular cyclopropanations could be used to induce efficient cyclizations of diazoacetates **6** to give lactones **7** and **8** with ≥94% enantioselectivities in all cases except where R^1 = H and R^2 = alkyl or aryl (Scheme 2).[11,12]

Scheme 2



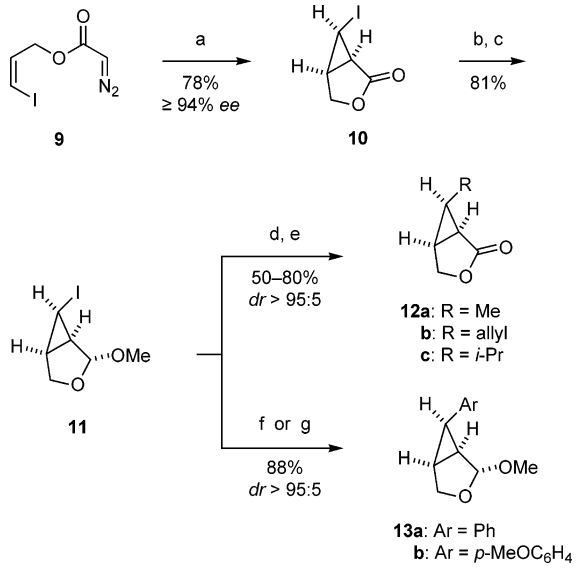
To facilitate introducing different substituents on the cyclopropane rings of compounds related to **7** and **8**, we prepared cyclopropyl iodide **11** by a process that featured the highly enantioselective cyclization of **9** (Scheme 3).[13] The acetal **11** was then transformed via alkylations to deliver **12a**−**c** and by Negishi cross-couplings with aryl iodides or Suzuki reaction with phenylboronic acid to give **13a**,**b**.

Biologically Active Cyclopropane-Derived Peptidomimetics

Having developed the requisite methods for the enantioselective synthesis of trisubstituted cyclopropanes, analogues of biologically active pseudopeptides containing replacements related to **2**−**5** were prepared to evaluate the viability of these peptidomimetics. Because protease inhibitors typically bind to the active sites of their respective enzymes in extended conformations,[7] several proteases were chosen as initial testing grounds. Other types of enzyme inhibitors and protein-binding ligands were then surveyed.

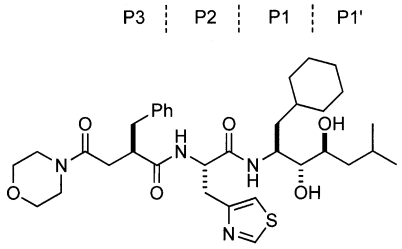
Cyclopropane-Derived Renin Inhibitors. The aspartate protease renin was selected as the first enzyme to test the

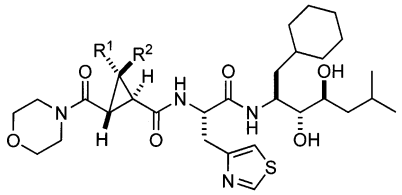
Scheme 3[a]



[a] (a) Rh_2[5*S*-MEPY]$_4$, Δ; (b) DIBAL-H; (c) TsOH, MeOH; (d) *t*-BuLi, 2-Th-Cu(CN)Li, R−X; (e) Jones oxidation; (f) *t*-BuLi, $ZnCl_2$, Ar−I, $(Ph_3P)Pd$; (g) $PhB(OH)_2$, $Pd(OAc)_2$, Ph_3P, K_2CO_3, Bu_4NCl, Δ.

efficacy of cyclopropane-containing peptidomimetics related to **2**.[14] Compound **14** was a known and potent inhibitor of renin. We reasoned that if the IC_{50} values of **15** or **16** were comparable to **14**, we might conclude that the cyclopropane ring preorganized the substituents at the P3 subsite of **14** in a manner that corresponded to their orientations in the bound conformation of **14**. This was an important question because no structural data for renin−inhibitor complexes were available at the time. The substituents corresponding to the peptide backbone in **15** and **16** are *trans*, thereby locally rigidifying the backbone in an extended conformation. The phenyl group in **15** is oriented to mimic the conformer of **14** in which the phenyl group is in the *gauche*(−) conformation; in **16** the phenyl group is positioned to mimic the *gauche*(+) conformation. It is noteworthy for determining the energetic consequences of preorganization that **14**−**16** have

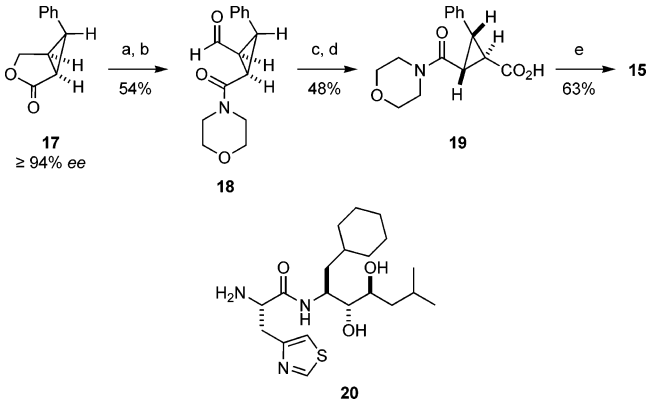




the same number and types of heavy atoms and the same number of hydrogen bond donors and acceptors.

The readily available lactone **17** was transformed in two straightforward steps into aldehyde **18**, base-promoted epimerization of which established the requisite *trans* relationship of the backbone substituents. Further oxidation delivered acid **19**, which was coupled with the P2−P1′ tripeptide replacement **20** to furnish **15** (Scheme 4).[14] A similar series of reactions was used to prepare **16**.

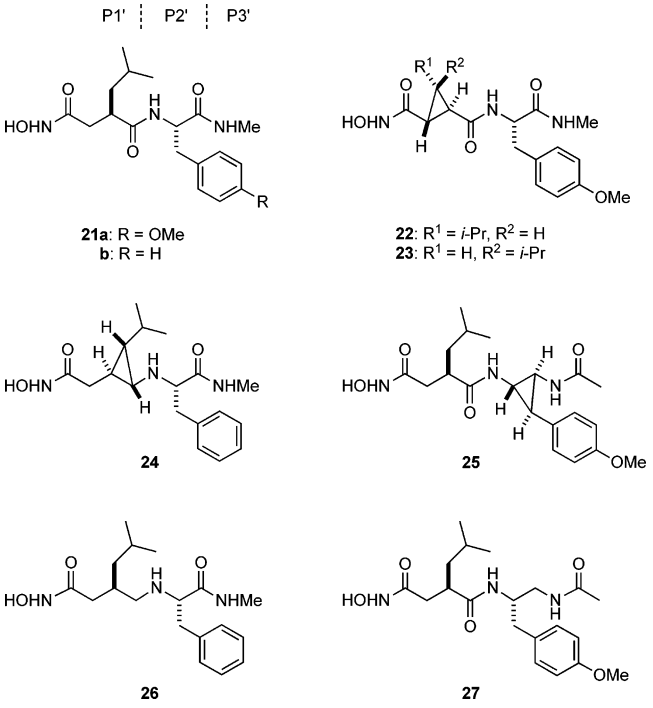
Scheme 4[a]



[a] (a) Morpholine, Me_3Al; (b) PCC; (c) K_2CO_3, MeOH; (d) Jones oxidation; (e) EDC, HOBt, **20**.

In biological assays, **15** was found to be approximately equipotent to the flexible analogue **14** as an inhibitor of renin, whereas **16** was about 300-fold less potent. Based upon these results, we concluded that **15** and **14** bound similarly to renin, suggesting that the cyclopropane in **15** positions its substituents in orientations that correspond closely to the biologically active conformation of **14**. Even though the increase in potency that was expected by restricting rotors was not observed,[10] this initial study clearly supported our hypothesis that cyclopropane-derived peptide replacements were valid as peptidomimetics and that their use could lead to insights regarding the biologically active conformations of flexible enzyme inhibitors. Further experiments were clearly indicated.

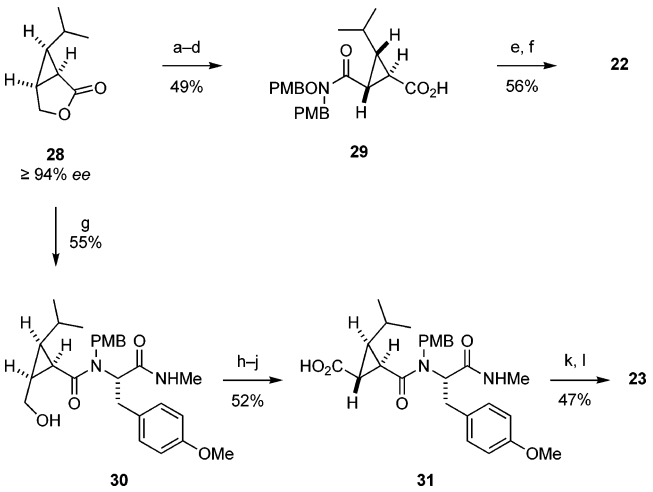
Cyclopropane-Derived Inhibitors of Matrix Metalloproteases. We turned our attention to replacing segments of matrix metalloprotease (MMP) inhibitors with substituted cyclopropanes to further probe the utility of cyclopropane-derived peptidomimetics. For example, the orientation of substituents on the cyclopropane ring in **22** and **23** would probe the topographical preferences at the P1′ subsite of the bound conformation of the known MMP inhibitor **21a**.[15,16] The backbone of **21a** is locally restricted in extended conformations in both **22** and **23**, and the isopropyl groups in **22** and **23** are oriented to mimic *gauche*(−) and *gauche*(+) conformations, respectively, of the P1′ side chain of **21a**. Importantly, **21a**−**23** possess the same number and types of heavy atoms and the same number of hydrogen bond donors and acceptors. We also examined cyclopropane-containing peptidomimetics derived from **3** (Scheme 1, path b) because these were predicted to project the side chains in *anti* conformations.[17] Introducing cyclopropanes related to **3** into **21a**,**b**



led to **24** and **25**, both of which incorporate significant structural changes relative to **21b** and **21a**. Namely, the P1′−P2′ amide bond in **21b** is replaced with an amine in **24**, whereas in **25**, the C-terminal methyl amide of **21a** is replaced with a retro acetamide moiety to retain the amide character of the nitrogen atom. It was therefore necessary to prepare **26** and **27** as the corresponding flexible controls of **24** and **25** to evaluate explicitly the consequences of the conformational constraints.

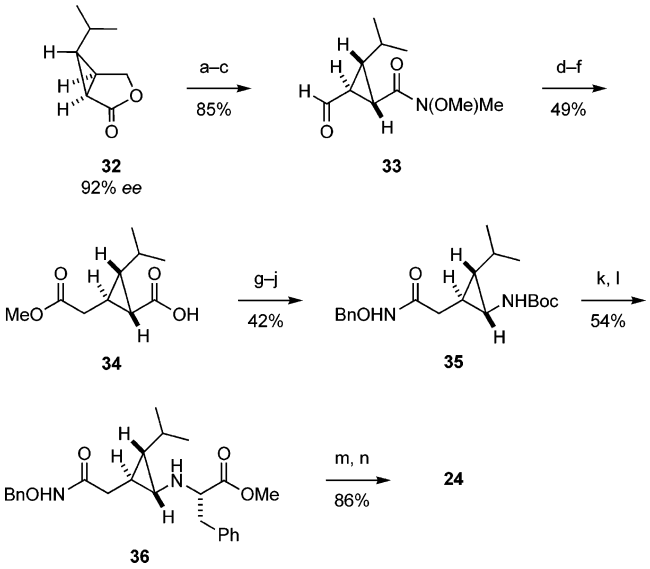
The synthesis of **22** from **28** followed the basic strategy outlined in Scheme 4, whereas preparation of **23** featured conversion of cyclopropyl lactone **28** into hydroxy amide **30** using a protocol that was developed specifically to effect direct coupling of amino acid derivatives with esters and lactones (Scheme 5).[18]

Scheme 5[a]



[a] (a) PMBONHPMB, Me_3Al, Δ; (b) PCC; (c) K_2CO_3, MeOH; (d) Jones oxidation; (e) EDC, HOBt, H-Tyr(Me)-NHMe; (f) $MeSO_3H$, TFA; (g) PMB-Tyr(Me)-NHMe, Me_3Al, Δ; (h) PCC; (i) K_2CO_3, MeOH; (j) Jones oxidation; (k) *i*-BuO_2CCl, Et_3N, H_2NOH; (l) TFA.

Scheme 6[a]



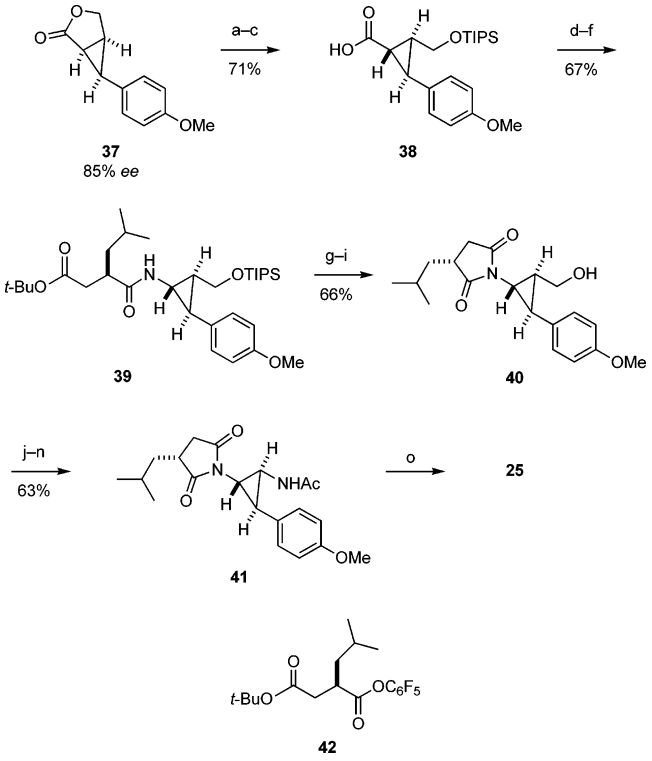
[a] (a) MeONHMe, Me_3Al; (b) PCC; (c) Et_3N, MeOH, Δ; (d) 2-lithio-2-trimethylsilyl-1,3-dithiane; (e) aq KOH, Δ; (f) $HgCl_2$, aq MeOH; (g) *i*-BuO_2CCl, Et_3N, NaN_3; (h) *t*-BuOH, Δ; (i) aq NaOH; (j) *i*-BuO_2CCl, Et_3N, $BnONH_2$; (k) HCl; (l) methyl D-phenyl lactate, Tf_2O, 2,6-lutidine, *i*-Pr_2NEt; (m) $MeNH_2$, NaCN, Δ; (n) H_2, Pd/$BaSO_4$.

Key steps in the synthesis of **24** were the one-carbon homologation of aldehyde **33**, which was derived from lactone **32**, and transformation of the carboxylic acid in **34** into a protected amine via a stepwise Curtius procedure to give **35** (Scheme 6). *N*-Deprotection of **35** followed by *N*-alkylation with the triflate of methyl D-phenyl lactate furnished **36**, which was readily transformed into **24**.

The synthesis of **25** was more challenging as tactics for preparing 1,2-diaminocyclopropanes were not well precedented. Acid **38** was converted into **39** via a novel sequence that featured a Curtius reaction wherein the intermediate isocyanate was trapped with allyl alcohol to give an allyl carbamate that was subsequently deprotected in the presence of **42** (Scheme 7). Initial attempts to refunctionalize **39** to a diaminocyclopropane were unsuccessful, so the cyclopropylamide group was protected as an imide. The acetamido group was introduced into **40** following the protocol for converting **38** into **39**, and succinimide **41** was opened with hydroxylamine to give a mixture of regioisomers of which **25** was the major product.

In assays against several MMPs, **22** and **23** were found to be significantly less potent than **21a**, leading us to conclude that neither **22** nor **23** mimicked the bound conformation of **21a**. At the time, there were no structures of a MMP−inhibitor complex, and we speculated that the side chain of the P1′ amino acid in **21a** might adopt an *anti* orientation upon binding to MMPs. This hypothesis was subsequently confirmed by a crystallographic study of a complex of **21b** bound to MMP-1 that showed that the isopropyl and aromatic groups at the P1′ and P2′ sites were both in *anti* conformations.[19] Even though the cyclopropane rings in **24** and **25** mimic this *anti* conformation, **24**−**27** were 2−4 orders of magnitude less potent than **21a**,**b**, indicating that reducing the P1′−P2′ amide linkage, which forms highly conserved hydrogen bonds

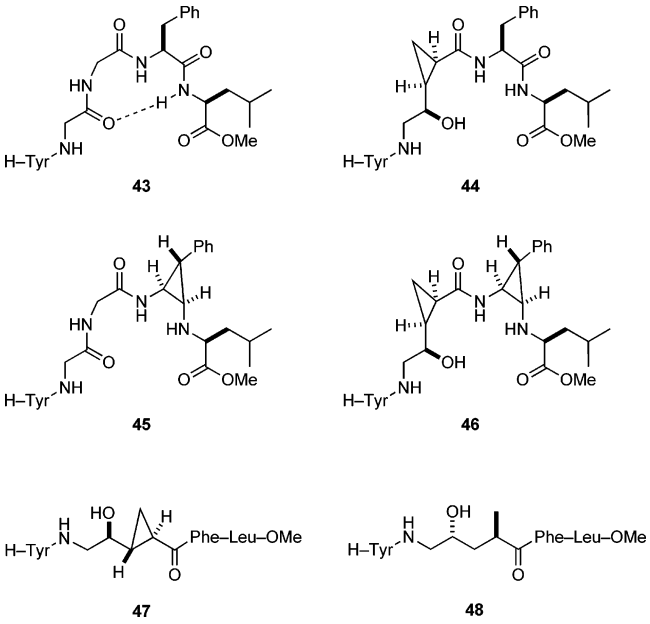
Scheme 7[a]



[a] (a) MeONHMe, Me_3Al; (b) TIPSOTf, 2,6-lutidine; (c) *t*-BuOK, H_2O; (d) EtO_2CCl, Et_3N, NaN_3; (e) allyl alcohol, Δ; (f) **42**, $(Ph_3P)_4Pd$, Bu_3SnH; (g) HCO_2H; (h) AcCl, Δ; (i) MeOH, Δ; (j) Dess–Martin periodinane; (k) $NaClO_2$, H_2O_2; (l) EtO_2CCl, Et_3N, NaN_3; (m) allyl alcohol, Δ; (n) Ac_2O, $(Ph_3P)_4Pd$, Bu_3SnH; (o) $HONH_2$.

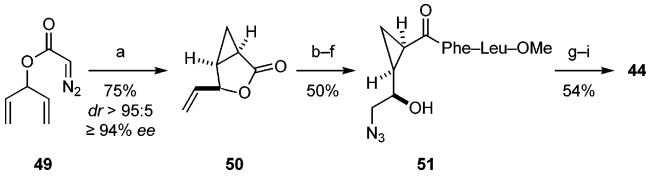
with Leu-181 and Pro-238 of MMPs,[19] and inverting the P2′–P3′ amide moiety of **21b** were detrimental to biological activity. Compounds **24** and **26** were approximately equipotent, but **25** was 5–35 times more potent than its flexible counterpart **27**. This is a significant result because it clearly shows that introducing a cyclopropane ring as a conformational constraint into a flexible ligand can provide a preorganized pseudopeptide having significantly higher activity.

Cyclopropane-Derived Enkephalin Analogues. Structural and computational studies suggest that a β-turn is a structural element in the biologically active conformation of enkephalins.[20] We thus decided to prepare derivatives of Leu-enkephalin methyl ester (**43**) in which the Gly^3 residue, the Phe^4 residue, or both were substituted with cyclopropanes related to **4** and **5** to probe whether such replacements could stabilize β-turns (cf. Scheme 1).[21] The pseudopeptides **44**–**46** were selected as targets for this study, whereas compound **47** would serve as an extended control for **44**. Compounds **44**–**47** differ significantly from **43** owing to the modifications at the Gly^2–Gly^3 or the Phe^4–Leu^5 amide linkages, so compound **48**, which has the same number and types of heavy atoms and functional groups as **44** and **47**, was prepared as a flexible control. Even though these studies were considered risky, we were intrigued by the possibility that loss of the hydrogen bond between Glu^2 and Leu^5 that stabilizes the putative β-turn in **43** might be offset by introducing rigid cyclopropanes related to **4** and **5**.



Two key steps in the synthesis of **44** included desymmetrization of the divinyl diazoacetate **49** using $Rh_2[(5S)\text{-MEPY}]_4$ to give **50**[22] and an application of our published procedure to open lactones with dipeptides leading to **51** (Scheme 8).[18]

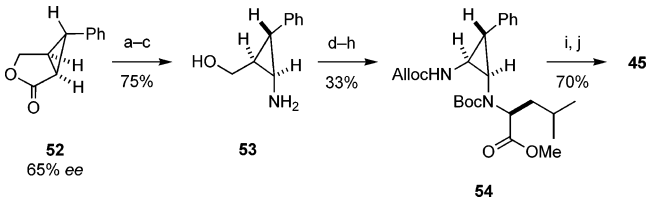
Scheme 8[a]



[a] (a) $Rh_2[(5S)\text{-MEPY}]_4$, Δ; (b) O_3, $NaBH_4$; (c) MsCl, Et_3N; (d) NaN_3, Δ; (e) H-Phe-Leu-OH, Me_3Al; (f) CH_2N_2; (g) H_2, Pd/C; (h) Boc-Tyr-OH, EDC, HOBt; (i) TFA.

Synthesis of **45** from **52** featured *N*-alkylation of the amino group in **53**, which was produced by hydrolysis of an intermediate cyclic carbamate. The second amino substituent was introduced onto the cyclopropane ring by a stepwise Curtius procedure (cf. **38** → **39**) leading to **54**, which was easily transformed into **45** (Scheme 9).

Scheme 9[a]



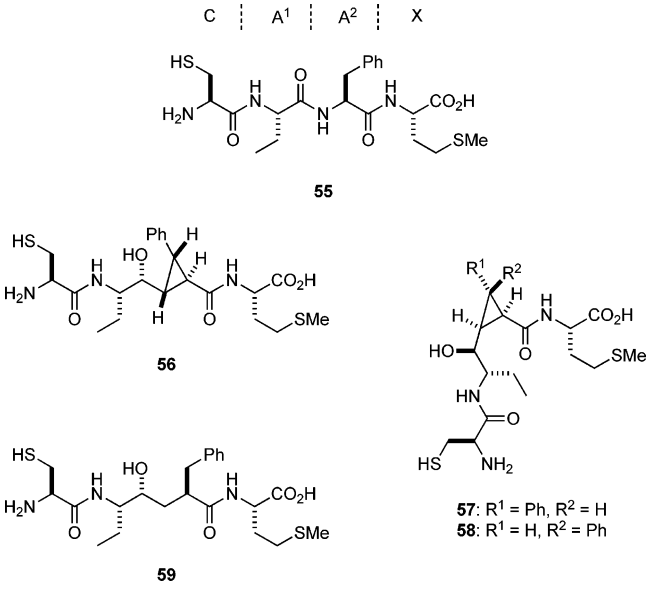
[a] (a) H_2NNH_2; (b) HONO, Δ; (c) $Ba(OH)_2$, H_2O, Δ; (d) methyl (2*S*)-2-hydroxy-6-methylpentanoate, Tf_2O, 2,6-lutidine, *i*-Pr_2NEt; (e) Boc_2O; (f) $RuCl_3$, $NaIO_4$; (g) EtO_2CCl, Et_3N, NaN_3; (h) allyl alcohol, Δ; (i) Boc-Tyr-(*t*-Bu)-Gly-Gly-OH, HOBt, $(Ph_3P)_4Pd$, Bu_3SnH; (j) TFA.

Compound **46** was prepared by coupling a cyclopropyl acid derived from **50** with **54**, and **47** was synthesized from the enantiomer of **50**.

The binding affinities of the enkephalin analogues **44**–**48** for the μ- and δ-opiate receptors were determined.

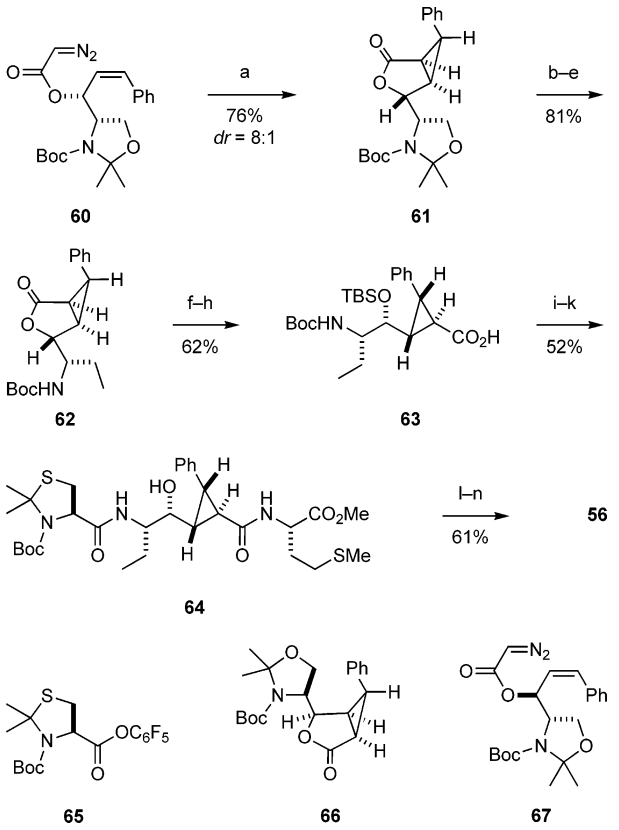
Compounds **45** and **46** did not bind to either receptor. Pseudopeptides **44**, **47**, and **48** had no detectable affinity for the δ-receptor, but they exhibited comparable affinities for the μ-receptor that were 100−500-fold less than Leu-enkephalin **43**. Introducing either a *cis*- or a *trans*-disubstituted cyclopropane ring into **48** had little effect upon receptor binding. Only **44** exhibited activity in functional assays, being a weak agonist for both μ- and δ-receptors with a 7-fold selectivity for the former. These studies demonstrated that replacing the amide linkages at Gly^2-Gly^3 and Phe^4-Leu^5 with flexible *and* constrained peptidomimetics was not tolerated by opioid receptors. Our initial hypothesis that substituted cyclopropanes related to **4** and **5** might stabilize β-turn conformations could not be corroborated by these experiments.

Cyclopropane-Derived Ras Farnesyltransferase Inhibitors. Early NMR studies of peptides and peptidomimetics related to **55**, which comprises a CA^1A^2X motif, suggested that substrates and inhibitors of ras farnesyltransferase (FTase) might adopt β-turn conformations upon binding;[23] recent evidence, however, supports extended structures.[24] We decided to synthesize **56**−**58** as conformationally restricted analogues of **55** that might stabilize extended and turn-like conformations.[25] The phenyl groups are projected in approximately *gauche*(−) orientations in **56** and **57** and in a *gauche*(+) conformation in **58**. Compound **59** would serve as the flexible control for **56**−**58**.



We developed several useful procedures to build substituted N-terminal side chains of cyclopropanes related to **2** and **4**,[26] and preparation of **56**−**58**, which is illustrated by the preparation of **56**, featured one of these (Scheme 10). The synthesis commenced with cyclization of **60** in the presence of $Rh_2[(5R)\text{-MEPY}]_4$ to deliver a separable mixture (8:1) of **61** and **66**.[27] Use of a chiral catalyst was essential since achiral catalysts such as $Cu(TBS)_2$ gave decreased ratios of **61** and **66**. Interestingly, cyclization of **67**, which was a precursor of **58**, in the presence of

Scheme 10[a]

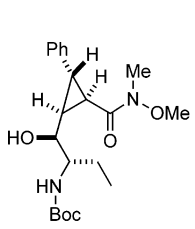


[a] (a) $Rh_2[(5R)\text{-MEPY}]_4$, Δ; (b) HCl; (c) Boc_2O, Et_3N; (d) DEAD, Ph_3P; (e) Me_2CuLi; (f) MeONHMe, Me_3Al; (g) TBSOTf, 2-6-lutidine; (h) *t*-BuOK, H_2O; (i) EDC, HOBt, H-Met-OMe; (j) HCl; (k) **65**, Et_3N; (l) LiOH, H_2O; (m) TFA; (n) MeOH, H_2O, Δ.

$Cu(TBS)_2$ delivered a mixture (8:1) of diastereomers; this ratio did *not* improve upon use of a chiral catalyst. The *N*,*O*-acetal in **61** was then transformed into an aziridine that underwent reaction with Gilman's reagent to give **62**, which was elaborated into the constrained A^1-A^2 replacement **63** by lactone opening and epimerization of the C-terminal carboxyl group. The remaining steps in the synthesis of **56** were straightforward, and **57** and **58** were prepared by similar sequences.

Pseudopeptides **56**−**59** were all less potent than the tetrapeptide **55** as inhibitors of FTase. The flexible control **59** was about 8-fold less potent than **55**, indicating that the reduction of the A^1-A^2 amide linkage to a hydroxyethylene moiety was detrimental to binding. Compounds **56** and **57** exhibited similar activities, but they were about 2-fold weaker than **59**; **58** was about 22-fold less active than **59**. Hence, introducing a cyclopropane ring as a conformational constraint did not compensate the activity that was lost upon introducing a hydroxymethylene isostere at the A^1-A^2 subsite.

The observation that **56** and **57** were approximately equipotent was unexpected because we predicted that the *cis* relationship of the backbone chains in **57** might favor a turned conformation. To explore the conformational preferences of derivatives of **57**, we conducted several structural studies. An X-ray crystal structure of **68** revealed that the O−H was *not* hydrogen-bonded to the *cis*-amide carbonyl group and that the N-terminal side chain was



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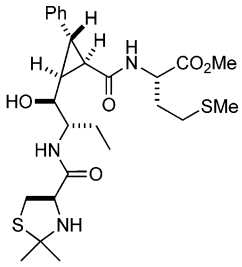
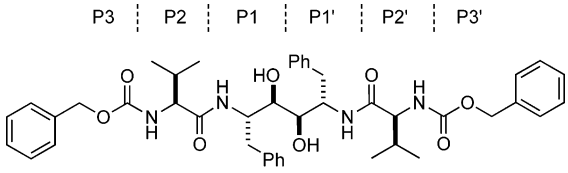


69

extended *away* from the C-terminus. Moreover, in NMR studies on **69**, there were no intrastrand NOEs involving the C-terminal methionine and the N-terminal subunit. These studies do not provide any compelling evidence that cyclopropane rings in **68** and **69** locally induce a turned structure. Subsequent modeling studies suggested that both **56** and **57** are capable of adopting similar extended conformations for binding to the active site cleft of FTase, thereby accounting for their comparable activities.

Cyclopropane-Derived HIV-1 Protease Inhibitors. Inhibitors of HIV-1 protease are known to bind in β-strand conformations,[7] and we envisioned that introducing cyclopropane rings at both the P2 *and* the P2′ sites of the C-2 symmetric subnanomolar inhibitor **70**[28] might enforce



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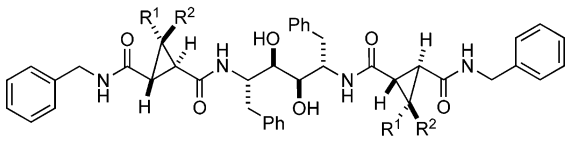
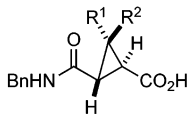


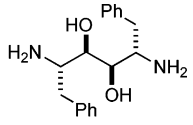
71: $R^1 = R^2 = Me$
72: $R^1 = Me$, $R^2 = H$
73: $R^1 = H$, $R^2 = Me$
74: $R^1 = R^2 = H$

an extended conformation upon the P2−P2′ core. Compounds **71**−**74** were identified to test this hypothesis and to evaluate the effects of varying the orientation and number of methyl groups.[29]

The syntheses of **71**−**74** were readily achieved by coupling the diamine **76** with the acids **75**, which were



75: R^1, R^2 = Me, H combinations



76

readily available using methods we had previously established. Biological assays of these compounds using recombinant wild-type HIV-1 protease revealed that they were all approximately equipotent with **70**, thereby indicating that introducing two cyclopropane rings into **70** was well tolerated by the protease.

It was beginning to appear that cyclopropane-derived pseudopeptides were at best only slightly more potent than their flexible counterparts. We were not observing the expected benefits of preorganization and naturally wondered why. In some cases, as for **71**−**74**, the number and types of heavy atoms and hydrogen bond donors and acceptors in the flexible and constrained ligands varied, so the effects of ligand preorganization could not be explicitly evaluated. However, some important questions had not been addressed: Do compounds containing cyclopropanes and their flexible analogues bind to their biological targets in the same way and make the same contacts? Are the structures of cyclopropane-derived ligands complexed with proteins similar to their preferred solution conformations?

Toward addressing these critical structural issues, X-ray crystallographic data were collected for the complex of **72** and HIV-1 protease.[29] These data revealed that the bound conformation of the central P2−P2′ core of **72** and its interactions with the protease were similar to other bound inhibitors. The most notable differences were at the P3 and P3′ subsites where the hydrogen bonding interactions between **72** and the protease are at slightly different distances and angles relative to those made by inhibitors such as **70** as a result of the nitrogen atoms in **72** being shifted. The preferred structure of **71** in solution was then determined by NMR, and there was a close correspondence between this structure and the bound conformation of **72** (Figure 3). These structural studies

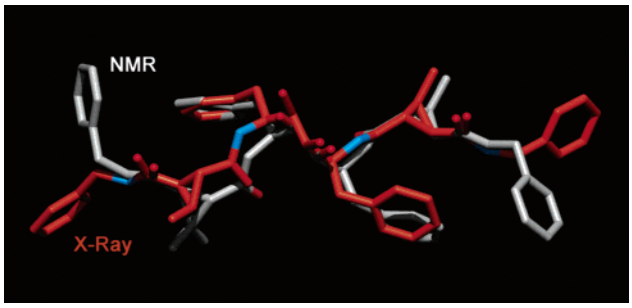
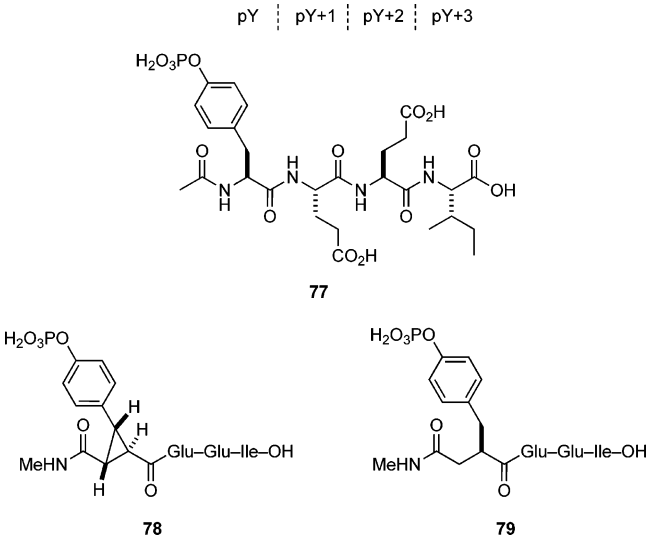


FIGURE 3. Comparison of bound structure of **72** (gray) complexed with HIV-1 protease (X-ray data) with an averaged, minimized structure of **71** (burnt orange) as determined by NMR. Oxygen and nitrogen atoms are colored red and blue, respectively. In the solution structure of **71**, the benzyl groups at P3 and P3′ are disordered.

thus provided some answers to our queries: Pseudopeptides containing cyclopropane rings may bind to their protein targets in conformations closely resembling those adopted by their more flexible counterparts, and the bound and preferred solution structures of cyclopropane-derived ligands can be closely comparable. Despite the similarities, there were differences that were difficult to quantify, and the question of why cyclopropane-derived ligands did not bind with increased affinities relative to their flexible controls as expected based upon the principle of preorganization remained unanswered.

Cyclopropane-Derived Antagonists of SH2 Domains. Our studies had advanced to the stage where it was essential to identify a well-defined biological system so that we could correlate structure and energetics in protein−ligand complexes involving flexible and constrained ligands. After considering numerous options, we initiated investigations of the complexes formed between phosphotyrosine-derived compounds and the Src homology-2 (SH2) domain of Src kinase. Structural studies revealed that peptides related to **77** bound in extended conformations



that were anchored by interactions between the phosphotyrosine (pY) and pY + 3 residues and the corresponding pockets of the domain.[30] Modeling studies suggested **78** might serve as a partially constrained analogue of the tetrapeptide **77** because the cyclopropane ring in **78** positioned its substituents in a manner that corresponded closely to the orientation of a phosphotyrosine residue bound to the domain. The amide N−H of the phosphotyrosine residue is not involved in hydrogen bonding, so editing this group from **77** in forming **78** should not decrease interactions with the Src SH2 domain. Nevertheless, **79** is the appropriate flexible control for **78** because these two compounds contain the same number and type of heavy atoms.

The thermodynamic parameters for binding of **78** and **79**, which were prepared via coupling of the appropriate phosphotyrosine replacement with a protected tripeptide,[31] and **77** to the Src SH2 domain were determined using isothermal titration calorimetry (ITC).[32] The constrained and flexible compounds **78** and **79**, respectively, exhibited comparable binding affinities with both being slightly more potent than the tetrapeptide **77**. The favorable entropy of binding that was expected from restricting rotors in **79** was indeed observed as **78** bound with an approximately 9 eu/mol advantage relative to **79**. However, this entropic gain was offset by a balancing enthalpic penalty, so there was little *net* energetic advantage attending the preorganization of **79**. Entropy−enthalpy compensation is a common but poorly understood phenomenon in protein−ligand interactions.[33] Preliminary, unpublished work with pairs of compounds related to **78** and **79** containing different amino acids at the pY + 1 to pY + 3 positions showed similar trends: the flexible and constrained ligands always exhibited approximately equal affinities as a consequence of entropy−enthalpy compensation, and even though the constrained compound in each pair always bound with a more favorable entropy of binding, the entropic advantage varied unpredictably over a range of 3−13 eu/mol with changes in the amino acid substitution.

We determined the structure of the complex of **78** bound to the Src SH2 domain by X-ray crystallography with the goal of elucidating the origin of the enthalpic differences in binding of **78** and **79**.[32] Although we have not yet been able to obtain crystals of **79** complexed with the Src SH2 domain, we were able to compare the structures of the complexes of this domain with **78** and with an 11-mer peptide having the same four N-terminal amino acids.[30] An overlay of the ligands in these structures revealed that they bound in similar conformations, the most notable differences being in the orientations of the solvent-exposed side chains of the pY + 1 and pY + 2 glutamic acid residues (Figure 4). There were other minor differences in the two structures, but the interactions of these ligands with the SH2 domain, especially the key phosphotyrosine (pY) and isoleucine (pY + 3) subsites, were essentially the same,[30] suggesting that the substituted cyclopropane in **78** was a good mimic for the bound structures of the phosphotyrosine residue in **77** and its replacement in **79**.

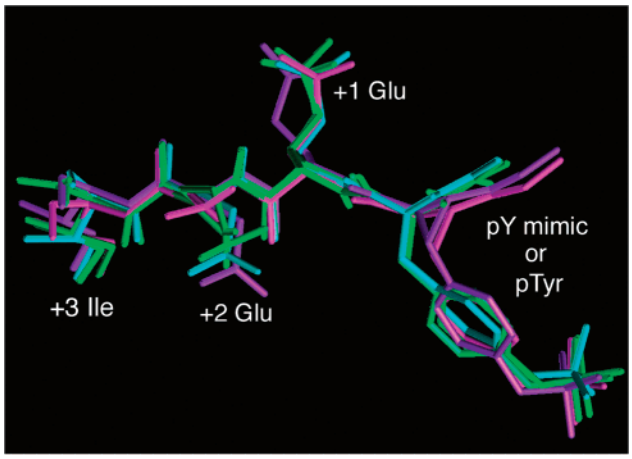
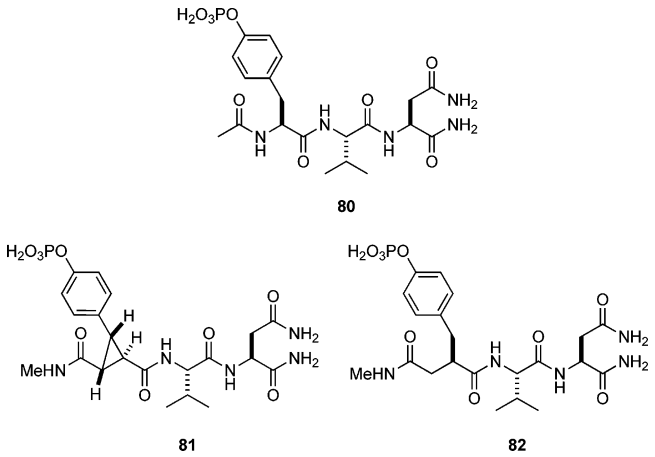


FIGURE 4. Overlay of Src-SH2 domain-bound structures of **78** (two complexes in asymmetric unit in purple and magenta) and the 11-mer peptide, which is truncated for clarity to show only the pYEEI core (three complexes in asymmetric unit in cyan and green).

We have begun to examine the structural and energetic effects of introducing cyclopropane-derived phosphotyrosine replacements into peptides that bind to the SH2 domain of the mammalian growth receptor bound protein (Grb2).[34] Although peptides related to **80** bind to the Grb2 SH2 domain in a turned conformation, the interactions between the phosphotyrosine residue and this domain are similar to those observed between phosphotyrosine peptides and the Src SH2 domain.[35] It therefore occurred to



us that **81** might be a constrained analogue of **80**, although **82** would serve as the appropriate flexible control for thermodynamic and structural studies. In ITC experiments, we recently found that the cyclopropane-derived ligand **81** bound to the Grb2 SH2 domain 5-fold better than **82**.[36] Surprisingly, however, the entropy of binding of **81** was about 3 eu/mol *less* favorable than **82**. Hence, the increased affinity of **81** relative to **82** arose from a more favorable binding enthalpy that overrode an unfavorable binding entropy! Structural and dynamic studies are underway to elucidate the origin of this unexpected discovery that stands in stark contrast to conventional wisdom regarding the putative entropic benefits of ligand preorganization in biological systems.

Summary

1,2,3-Trisubstituted cyclopropanes were conceived as novel rigid peptidomimetics that were designed anticipating that the cyclopropane ring might locally enforce extended or turned conformations while projecting the amino acid side chains in orientations approximating selected χ_1-angles. After development of methodologies for the enantioselective synthesis of trisubstituted cyclopropanes, cyclopropane-containing analogues of a number of pseudopeptides were prepared and their affinities for their respective biological targets were determined. In a number of cases, the cyclopropane-containing pseudopeptides were highly active, sometimes more potent than their flexible counterparts. However, the changes in the peptide backbone that were required to introduce the cyclopropane ring in other cases were detrimental to binding affinity. Although placing the substituents corresponding to the peptide backbone *trans* on the cyclopropane ring appears compatible with locally extended structures, there is no evidence that the corresponding *cis* orientation enforces a turned structure.

NMR and X-ray structural studies revealed that introducing a cyclopropane subunit into a peptide or pseudopeptide may provide a constrained derivative in which the cyclopropane ring locally positions substituents in spatial orientations that approximately mimic the bound conformation of the more flexible parent. ITC studies of complex formation between pairs of flexible and constrained ligands and the Src SH2 domain showed that cyclopropane-derived ligands bound to this protein with more favorable entropies of binding than their flexible analogues, an observation consistent with the expected entropic benefit commonly associated with restricting rotors. Because this entropic advantage was universally accompanied by a corresponding enthalpic penalty, there was, however, little or no gain in binding affinity. In a parallel study with Grb2 SH2 binding ligands, we discovered that constraining a flexible molecule can provide a more potent ligand even though the entropy of binding of the preorganized molecule is unfavorable relative to its flexible counterpart. This finding is inconsistent with the conventional thinking that presumes, perhaps too simplistically, that preorganizing a ligand in its biologically active conformation will be entropically favorable. We are conducting detailed thermodynamic and structural studies of other complexes of proteins with constrained and flexible ligand pairs to probe the generality of our findings and to determine explicitly the consequences of ligand preorganization upon energetics, kinetics, structure, and dynamics in protein−ligand interactions.

I am especially grateful to have had the exceptionally good fortune to work with an outstanding group of undergraduate and graduate students and postdoctoral associates over the years. They have contributed enormously to the intellectual and experimental development of cyclopropane-derived peptidomimetics, and their names are found in the numerous papers cited herein. I also wish to thank the National Institutes of Health, the Robert A. Welch Foundation, Pfizer, Inc., and Merck Research Laboratories for their generous support of our research.

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