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Use of 1,2,3-trisubstituted cyclopropanes as conformationally constrained peptide mimics in SH2 antagonists†

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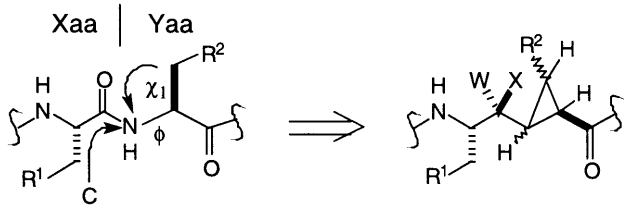
Abstract

Novel conformationally constrained phosphotyrosine pseudopeptide derivatives of the tetrapeptide pY-E-E-I were prepared and evaluated as SH2 binding antagonists.

Keywords: peptide mimics; cyclopropane; asymmetric synthesis.

Development of rigid replacements of peptide secondary structural elements is a common strategy for designing non-peptidic, small molecules that will bind to enzyme active sites and various biological receptors.[1] Because such replacements may also be exploited to gain insights regarding the biologically active conformation of flexible peptides, we invented the cyclopropane-derived isosteres **II** as a new class of mimics of the dipeptide array **I**. These novel replacements, which may be abbreviated as –XaaΨ[COcpCO]Yaa–, were uniquely designed to orient both the peptide backbone and the amino acid side chain by varying the stereochemistry on the cyclopropane ring.[2]



I **II**: W, X = H, OH; O

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† This article is dedicated to my friend Professor Harry H. Wasserman on the occasion of his 80th birthday and for his many important contributions to organic chemistry and to the *Tetrahedron* journals.

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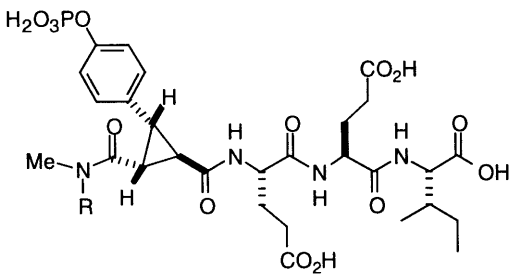
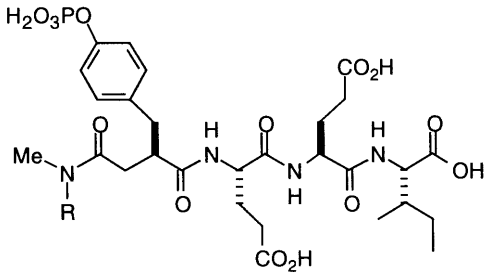
To evaluate their efficacy in biological systems, we introduced peptide replacements related to **II** into novel inhibitors of renin, HIV-1 protease, matrix metalloproteinases, Ras farnesyl-transferase as well as enkephalin analogues,[3] and others have used such cyclopropane-derived mimics in non-peptide fibrinogen receptor antagonists.[4] These studies generally established the viability of introducing trisubstituted cyclopropanes into biologically active analogues of peptides, but in contrast to expectations, the potencies of the conformationally constrained ligands were typically equal to, not superior to their flexible counterparts. Hence, the goal of preparing tighter binding pseudopeptides through conformational constraint was not achieved, and the fundamental question that arose at this juncture was: Why? Did the introduction of the cyclopropane ring into the inhibitors not result in the expected entropic advantage, or did unfavorable enthalpic factors override the entropic gains, thereby resulting in similar ΔGs of binding? To address these issues, we decided to examine the binding affinities of **1**–**4**, which are conformationally constrained and flexible analogues of the phosphotyrosine-containing tetrapeptide Ac-pTyr-Glu-Glu-Ile-OH (pY-E-E-I) (**5**), a well-known antagonist of SH2 domains.[5] There had been numerous structural and thermodynamic studies of SH2 domains complexed with various phosphotyrosine peptides,[6] so it would be possible to correlate ligand structure with the energetics of binding for this series of ligands. Using the available structural information, preliminary modeling suggested that both **1** and **2** nicely mimicked all of the critical features of a phosphotyrosine peptide ligand bound to the Src SH2 domain. We now report the details of some of these studies.



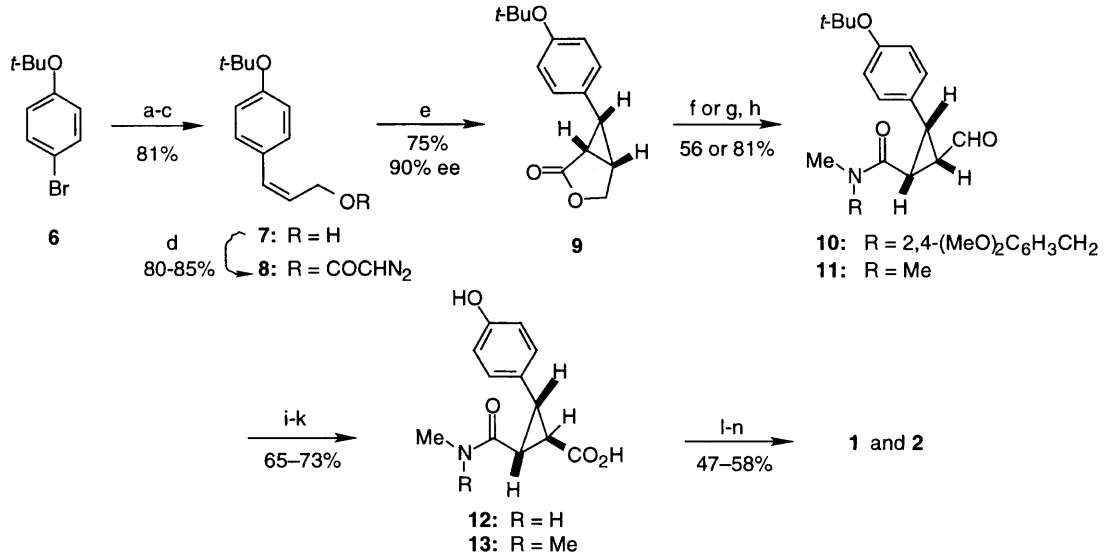
1: R = H
2: R = Me



3: R = H
4: R = Me

The synthesis of the cyclopropane containing pseudopeptides **1** and **2** commenced with conversion of 4-*tert*-butoxybromobenzene[7] (**6**) into the corresponding iodide, which underwent a palladium-catalyzed coupling with 2-propyne-1-ol to give an alkyne that was reduced to the *Z*-alkene **7** in 81% overall yield (Scheme 1). The palladium-catalyzed coupling of the aryl bromide **6** with 2-propyne-1-ol in the presence of $(Ph_3P)_4Pd$ was very slow and proceeded in only 11% yield.[8] The alcohol **7** was converted into the allylic diazoacetate **8** using a modified Corey–Myers protocol in which acetonitrile was employed as the solvent.[9] When **8** was heated in the presence of Rh_2[(5*S*)-MEPY]$_4$,[10] the cyclopropyl lactone **9** was obtained in 75% yield and 90% *ee*.

Ring opening of the lactone moiety of **9** via an aluminum mediated amidation with either 2,4-dimethoxybenzylmethylamine[11] or dimethylamine according to the Weinreb procedure,[12] followed by oxidation of the resulting amide alcohol with TPAP gave the all *cis* cyclopropyl aldehydes **10** and **11** in 56 and 81% overall yields, respectively. Sequential epimerization alpha to the aldehyde followed by Jones oxidation and global deprotection furnished the conforma-



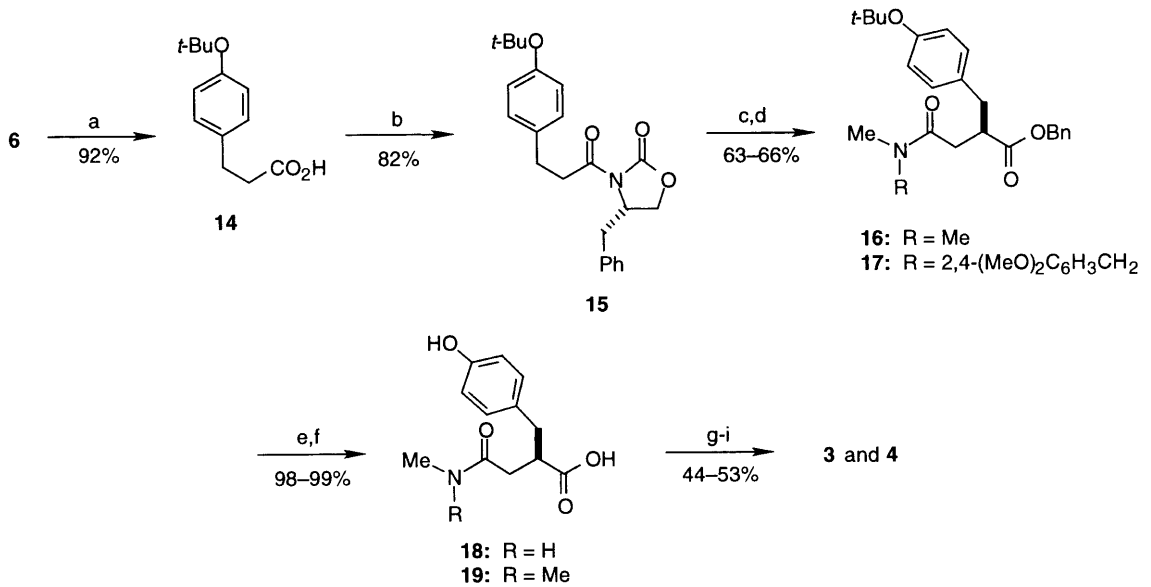
Scheme 1. (a) Mg, THF, Δ; then I_2, 0°C. (b) $Pd(PPh_3)_4$, CuI, pyrrolidine, 2-propyne-1-ol, 0°C→rt. (c) H_2, Lindlar catalyst, quinoline. (d) TsNNHCOCl, DMA, CH_3CN, –20°C; then Et_3N. (e) $Rh_2[(S)\text{-MEPY}]_4$, CH_2Cl_2, Δ. (f) $AlMe_3$, 2,4-$(MeO)_2C_6H_3CH_2NH_2$MeCl. (g) $AlMe_3$, Me_2NH_2Cl. (h) TPAP, NMO. (i) Et_3N, MeOH. (j) Jones oxidation. (k) CF_3CO_2H. (l) TBS–Cl, NMM; 1*H*-tetrazole, *i*-$Pr_2NP(OBn)_2$ then *t*-BuOOH. (m) DCC, HOBT, CH_2Cl_2, H–E(OBn)–E(OBn)–I(OBn). (n) H_2, Pd

tionally constrained tyrosine acids **12** (65% yield) and **13** (73% yield). The phenolic groups of **12** and **13** were phosphorylated,[13] and subsequent coupling of the cyclopropanecarboxylic acids thus obtained with the benzyl protected tripeptide H_2N-Glu(OBn)-Glu(OBn)-Ile-CO_2Bn in the presence of DCC and HOBT followed by exhaustive debenzylation by hydrogenolysis furnished the targeted pseudopeptides **1** and **2** in 47 and 58% overall yields.

The syntheses of the flexible analogues of **5** began with the conversion of **6** into **14** via a one-pot Heck/hydrogenation procedure (Scheme 2). The acid **14** was converted to the chiral imide **15** in 82% yield following a procedure by Mathre.[14] Reaction of the sodium enolate of **15** with either α-bromo-*N*,*N*-dimethylacetamide[15] or α-bromo-*N*-(2,4-dimethoxybenzyl)-*N*-methylacetamide[16] gave the alkylated products with high diastereoselectivity (>95%); removal of the chiral auxiliary with LiOBn provided **16** and **17** in 63 and 66% overall yields, respectively. Global deprotection by sequential reaction with CF_3CO_2H and catalytic hydrogenolysis gave the flexible tyrosine acid replacements **18** and **19** in nearly quantitative yield (98–99%). Transformation of **18** and **19** into the flexible pseudopeptides **3** and **4** was achieved by sequential phosphorylation, peptide coupling and deprotection in 44–53% overall yields according the procedure developed for the syntheses of **1** and **2**.

Once compounds **1**–**5** were in hand, their binding affinities for the $p56^{lck}$ SH2 domain were determined in a competitive binding assay using the Pharmacia Biacore instrument according to a standard experimental protocol.[17] The measured affinities are summarized in Table 1.

The conformationally constrained analogues **1** and **2** are both slightly more potent than their flexible counterparts **3** and **4**, and the binding affinities of **1** and the parent tetrapeptide **5** are comparable. These results suggest that **1**–**5** bind in similar geometries to the SH2 domain, so it seems likely that the cyclopropane ring in **1** and **2** correctly mimic the bound orientation of the phosphotyrosine moiety. The biggest difference in binding potency is a factor of about 2.7



Scheme 2. (a) $PdCl_2$, $CH_2{=}CHCO_2H$, H_2O, Na_2CO_3, Δ, 24 h; then H_2, Pd/C, 8 h. (b) Me_3CCOCl, Et_3N, 0°C then (*S*)-(−)-4-benzyl-2-oxazolidinone, LiCl, rt. (c) NaHMDS, −78°C then α-bromo acetamide. (d) LiOBn, −78°C→rt. (e) CF_3CO_2H, rt. (f) H_2, Pd/C, EtOH. (g) TBS–Cl, NMM; 1*H*-tetrazole, *i*-$Pr_2NP(OBn)_2$ then *t*-BuOOH. (h) DCC, HOBT, CH_2Cl_2, H–E(OBn)–E(OBn)–I(OBn). (i) H_2, Pd

Table 1
Competitive binding assays with $p56^{lck}$ SH2 domain

Entry	Dissociation constant (K_d nM)	Relative error (nM)
1	119	18
2	255	25
3	318	30
4	389	31
5	119	24

favoring the cyclopropane derivative **1** over its flexible counterpart **3**. This corresponds to an approximate energy difference of 0.6 kcal/mol, somewhat less than would be expected purely on the basis of the number of restricted rotors in **1**.[18] Owing to the limitations of a competitive binding assay, it is impossible to determine if any of this energy is a result of an entropic effect due to the pre-organization induced by the cyclopropane. On the other hand, the technique of isothermal titration calorimetry provides an efficient means of obtaining the entire thermodynamic profile of the binding event. However, because there is a significant conformational change that occurs in the $p56^{lck}$ SH2 domain upon complexation with phosphotyrosine ligands,[16] interpreting the results of any calorimetric studies with this protein would be problematic. Hence, the thermodynamics associated with the formation of complexes of **1** and **3** with the Src SH2 domain, which does not to undergo any significant conformational changes upon ligand binding,[6b] will be pursued instead. The results of these investigations will be reported in due course.

Acknowledgements

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