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Probing the Bioactive Conformation of an Archetypal Natural Product HDAC Inhibitor Using Conformationally Homogeneous Triazole-Modified Cyclic Tetrapeptides**

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Keywords

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A fundamental strategy in rationally designing synthetic compounds to bind a protein of interest is to use a known ligand as a structural model to specify the precise conformational and pharmacophoric requirements for binding. Despite the remarkable success of this approach, a significant difficulty is that free ligands (in the absence of their cognate receptors) often adopt multiple conformations in solution or in the solid state compared to the receptor-bound structure.[1] These occurrences can render design models based on the free ligand structure difficult to obtain or even misleading.[2] Here we present evidence that the more potent conformation of apicidin, an archetypal member of a family of naturally occurring cyclic tetrapeptide inhibitors of histone deacetylases (HDACs), is not the all-*trans* (*t*-*t*-*t*-*t*) structure that predominates in solution,[3,4] but rather a *cis*-*trans*-*trans*-*trans* (*c*-*t*-*t*-*t*) conformation. Our studies rely on the design, synthesis, structural characterization, and functional analysis of a series of cyclic pseudo-tetrapeptides bearing 1,4- or 1,5-disubstituted 1,2,3-triazole amino acids that serve as *trans*- or *cis*-amide bond surrogates, respectively. We show that by replacing an amide bond with a triazole, the bond in question can be fixed in either a *trans*-like or *cis*-like configuration, allowing us to individually probe the binding affinity of distinct conformations. The heterocyclic compounds adopt conformations that overlay closely with the targeted conformations of apicidin and demonstrate potent HDAC inhibitory activities, in some cases equivalent or superior to those of the natural product. This study highlights the utility of triazole-modified cyclic peptides in constructing useful

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bioactive probe molecules, supports the *c*-*t*-*t*-*t* conformation as the bioactive conformation of cyclic tetrapeptide HDAC inhibitors, and provides a useful three-dimensional pharmacophoric model for use in advancing design principles for more selective HDAC inhibitors.

HDACs play critical roles in the regulation of gene transcription[5] by cleaving the acetyl groups from specific ε-aminoacetylated lysine residues in nucleosomal histone tails and non-histone proteins.[6] Although the precise roles of HDAC isoforms in cellular function and tumorigenesis are not yet completely understood, inhibition of HDAC activity has emerged as a promising approach in anticancer chemotherapy.[6–8] An interesting family of non-ribosomal cyclic tetrapeptide natural products, including the apicidins, trapoxins, microcins, and chlamydocin, exert potent cytotoxic activities against cancer cells by inhibiting HDACs.[6–9] These natural cyclic tetrapeptides are distinguished by the presence of a decanoic acid side chain derivatized with a ketone, terminal α,β-epoxyketone, or hydroxamic acid, which mimics an acetylated lysine residue and interacts with the active site zinc ion.

Despite the considerable number of synthetic analogs that have been reported for the cyclic tetrapeptide HDAC inhibitors,[8,10] the bioactive conformation has remained rather ambiguous. Indeed, several molecular conformations have been reported for apicidin alone (Figure 1). Apicidin was originally reported to adopt a *t*-*t*-*t*-*t* conformation based on NMR experiments carried out using pyridine-d_5 or CD_2Cl_2.[4] More recently, our laboratory[11] and a group at Merck[3] independently found that apicidin adopts multiple conformations (in a ratio of ~80%:15%:5%) in the more polar solvent DMSO, with the predominant species adopting a *t*-*t*-*t*-*t* conformation and the second most populated conformation having a *cis*-configuration at the tertiary amide bond of the Pip residue. Finally, a crystallographic structure obtained from $CHCl_3$/methanol/pentane contained a *cis*-amide at the Pip residue.[3] The observation of both *cis*- and *trans*-tertiary amides in these structures of apicidin (as well as other members of the tetrapeptide natural product family[8,12]), combined with the lack of any co-crystal structure of a cyclic tetrapeptide inhibitor with an HDAC enzyme, led us to question which amide configuration is present in the dominant bioactive conformation. Although the *c*-*t*-*t*-*t* conformation had previously been tentatively identified as the bioactive structure in related cyclic tetrapeptides based on a correlation of activity with predicted or known solution conformations,[12] we aimed to carry out a more controlled study in which apicidin analogs having either a fixed *cis*- or *trans*-amide isostere could be directly compared in an HDAC inhibition assay.

We and others have shown that the 1,4-disubstituted 1,2,3-triazole regioisomer effectively mimics a *trans*-amide bond,[13] whereas the 1,5-disubstituted 1,2,3-triazole regioisomer closely models a *cis*-amide bond.[14,15] Furthermore, the requisite 1,4- and 1,5-regioisomers can both be conveniently synthesized from azide and alkyne substrates,[16,17] making the triazole an attractive choice for an amide bond surrogate. We first synthesized heterocyclic apicidin analogs **2**, **3**, and **4** (Scheme 1). Bromoketal **5** was converted to the corresponding Grignard reagent and reacted with Boc-Ser β-lactone to yield the protected L-2-amino-8-oxodecanoic acid (L-Aoda) derivative **6**.[18] Boc-Ile (**7**) was converted to propargyl amine derivative **8**,[19] which was used along with amino acid **6** in solution phase peptide synthesis to afford linear azido-alkyne tetrapeptides **9** and **10**. Copper-(I)-catalyzed intramolecular azide–alkyne cycloaddition[17] of **9** and **10** yielded heterocyclic peptides **2** and **3**, respectively, each bearing the *trans*-amide surrogate. Peptide **4**, bearing the 1,5-substituted 1,2,3-triazole, was prepared by a thermal Huisgen [3+2] dipolar cycloaddition reaction of **9** (DMF, microwave, 220 °C), which afforded a mixture of triazole regioisomers **2** and **4** in a 2:1 ratio from which **4** was isolated by preparative HPLC. The preparation of additional 1,5-regioisomers **11**–**13**, in which the Ile residue was substituted with Leu, allowed us to investigate the hypothesis that the multiple conformations in **4** (*vide infra*)

might arise from the presence of a β-substituted amino acid (Ile) adjacent to the triazole. Since Ru-mediated cyclization of linear tetrapeptides proved unsuccesful, we developed an alternative strategy employing the Ru-catalyzed formation of 1,5-disubstituted 1,2,3-triazoles on solid phase followed by macrolactamization of the linear pseudotetrapeptides **14**–**16** in solution to give **11**–**13** (Scheme 2,Figure S1).[15,16] Compounds **11**–**13** differ only in the chirality at the Leu and Ala positions of the macrocycle, making possible an examination of how chirality might affect conformational properties of the peptide ring. The Trp(OMe) residue present in apicidin was replaced with Trp in our compounds to simplify the syntheses.

Both 1,4-disubstituted regioisomers (**2** and **3**) showed a single set of sharp ^{1}H NMR peaks in DMSO-d_6, indicating their conformational homogeneity in solution.[21] Of the 1,5-disubstituted regioisomers, peptides **11** and **12** also adopted a single conformation on the NMR timescale, whereas analogs **4** and **13** showed multiple sets of ^{1}H NMR peaks indicative of multiple, slowly interconverting backbone conformations (Figure S2). The observed conformational heterogeneity for **4** and **13** possibly results from *cis–trans* isomerization of one or more amides in the macrocycles.

The bioactivities of the peptides were preliminarily assessed using an *in vitro* fluorescence assay measuring the inhibition of HDAC activity in HeLa cell nuclear extracts. Along with apicidin, 1,4-disubstituted triazole-modified peptide **2** and 1,5-disubstituted triazole-modified peptides **4**, **11**, and **12** showed IC_{50} values ≤100 nM. Compound **3** had an intermediate IC_{50} of ~200 nM, while peptide **13** exhibited an IC_{50} value >10 μM. As a control, linear azido-alkyne peptide **9**, which contains all the important functional groups present in **3** and **4** but lacks the cyclic structure, had an IC_{50} more than two orders of magnitude higher than any of the cyclic peptides. The lower inhibitory activity of **3** compared to **2** may be explained by the methyl side chain in **2** acting as a better steric mimic of Pip than the branched isobutyl in **3**. Although compound **4** exhibited an IC_{50} of <100 nM, the multiple conformations observed by ^{1}H NMR for this compound negated its utility as a probe of apicidin's bioactive conformation. For our more detailed characterizations of HDAC inhibitory activities, we therefore employed only peptides **2**, **11**, and **12** (all of which adopted a single conformation as indicated by ^{1}H NMR) using a panel of purified recombinant human HDAC enzymes (Table 1). Against HDAC1, 1,5-disubstituted 1,2,3-triazole regioisomer **11** had an IC_{50} value similar to that of apicidin, whereas the corresponding 1,4-disubstituted 1,2,3-triazole regioisomer **2** showed an eight-fold loss in inhibitory activity. Against HDAC3, the three compounds all had similar activities, although **11** was again somewhat more potent than **2**. Interestingly, compound **11** was significantly more active than apicidin against HDAC8, and somewhat more active against HDAC6. Compound **12**, which differs in stereochemistry from apicidin at the Leu residue, exhibited reduced activity compared to the other apicidin analogs for all the HDACs tested.

The observed functional data were somewhat surprising, as the greater activity of 1,5-disubstituted 1,2,3-triazole regioisomer **11** compared to 1,4-disubstituted 1,2,3-triazole **2** implicated the *c-t-t-t* even though the *t-t-t-t* conformation of apicidin predominates in solution in a variety of solvents. However, a critical requirement for the above analysis to be valid is that peptides **11** and **2** must closely mimic the targeted *c-t-t-t* and *t-t-t-t* conformations of apicidin, respectively. To confirm that the apicidin analogs adopted the intended conformations in solution, we carried out a series of structural analyses based on multidimensional NMR spectroscopy (TOCSY, COSY, and ROESY) and distance geometry calculations for compounds **2**, **11**, and **12** (which are all conformationally homogeneous in DMSO-d_6). The three–dimensional NMR structure of **2** indeed revealed that all backbone amide bonds were in the *trans* configuration (Figures 2a, S3), consistent with the observed large HN–H_α coupling constants (Trp 9.1 Hz, Ile 8.4 Hz, Aoda 8.6 Hz). An overlay of the

NMR structure of **2** with the published *t-t-t-t* NMR structure of apicidin determined in pyridine-d_5[4] indicated close similarity of the two structures with high backbone overlap and C_α–C_β vector alignments (0.55 Å backbone r.m.s.d. for C_α and C_β atoms) (Figure S4). Likewise, overlaying the NMR structure of **2** with the lowest energy calculated conformation of apicidin, which reportedly closely matches the conformation of the predominant species in DMSO-d_6,[3] indicated a significant backbone overlap and good alignment of the C_α–C_β vectors (0.52 Å r.m.s.d. for C_α and C_β atoms) (Figures 2a, S3). The reported NMR solution structures for apicidin determined in pyridine-d_5 and DMSO-d_6 differ only in the rotation of the Aoda–Trp amide bond relative to the plane of the backbone; the corresponding amide bond in compound **2** adopts the conformational rotamer present in the pyridine-d_5 structure of apicidin.

Peptides **11** and **12**, which differ only in stereochemistry at the Leu residue, adopt considerably different backbone conformations in DMSO-d_6. The three amide bonds of peptide **11** (which contains amino acid residues of the same chirality as apicidin) were all in the *trans* configuration, leading to the intended *c-t-t-t* conformation when considering the 1,5-triazole as a *cis*-amide bond isostere (Figure 2b, S3). Two families of structures were observed for **11** that differed only in the rotation of the Aoda–Trp amide bond relative to the backbone plane; however, this amide rotation has little effect on the position of other backbone atoms or on C_α–C_β vectorial alignments (0.28 Å r.m.s.d. for C_α and C_β atoms of representative members of the two families). The NMR structures for **11** overlayed very well onthe crystallographic *c-t-t-t* structure of apicidin[3] (0.30 Å and 0.51 Å r.m.s.d. for C_α and C_β atoms for representative members of the two families) (Figures 2b, S3), supporting that **11** indeed faithfully represents the *c-t-t-t* conformation of apicidin that is present as a minor component in DMSO-d_6 solution. On the other hand, peptide **12** contained a *cis* configuration at the Aoda–Trp amide, leading to a *cis–trans–cis–trans* (*c-t-c-t*) tetrapeptide conformation (Figures 2c, S3). The *c-t-c-t* structure has been the focus of several calculations and discussions in the literature,[12,22,23] likely because it is among the most common conformations observed in the structures of cyclic tetrapeptides and is reportedly the conformation having the least ring strain. Although our data indicate that the *c-t-c-t* conformation is poorly suited to exert potent HDAC inhibitory activity, peptide **12** could be useful as a lead compound in constructing conformationally restricted analogs of other cyclic tetrapeptide natural products that are known to adopt the *c-t-c-t* conformation, such as tentoxin[23] and dihydrotentoxin[24] or the symmetric natural products cyclo(L-Pro-L-Leu)$_2$, cyclo(L-Pro-L-Val)$_2$, or cyclo(L-Pro-L-Phe)$_2$.[25] Indeed, peptide **12** overlayed closely on the crystal structure of dihydrotentoxin[24] (0.24 Å r.m.s.d. for C_α and C_β atoms) (Figures 2c, S3).

Although there are subtle variations in the chemical structures of the natural product **1** and analogues **2**, **11**, and **12**, it is our hypothesis that the major determinant of the observed HDAC inhibition potencies is the respective *cis*/*trans* configurations and resulting backbone conformations in each cyclic peptide.[26] For instance, peptide **12** exhibited reduced HDAC inhibitory activity compared to **2** and **11** across our panel of HDAC enzymes. An overlay of C_α atoms for the three compounds clearly shows that compound **12** differs structurally from the other two peptides in having a significantly shorter distance between the Trp and Aoda C_α atoms (2.8 Å for **12** compared to 3.8 Å for both **2** and **11**) and, perhaps more importantly, the chair-like *c-t-c-t* conformation of **12** causes the C_α–C_β vectors for the Trp, Aoda, and Leu side chains to all project in the same plane as the backbone ring, whereas these side chains in **2** and **11** project above the plane of the ring (Figure 2d). Peptides **2** and **11** both exhibited similar IC_{50} values against HDAC3, whereas **11** was four-fold more potent than **2** against HDAC1. An overlay of C_α and C_β atoms for the two compounds indicated that the most obvious structural differences for the two compounds are the greater distance between C_α atoms of the Ile/Leu and Ala residues (5.1 Å for **2** compared to 3.2 Å for both structure

families of **11**) and the different directions at which the Leu or Ile residue is projected relative to the backbone ring (directly above the ring in **11** compared to outward from the ring in **2**) (Figure 2e). Together with the biological data, this finding suggests that the position and orientation of the Leu/Ile residue influences potency for HDAC1, but does not have a pronounced effect for HDAC3. It is also possible that the difference in the aliphatic residue for **2** and **11** (Ile in **2** and Leu in **11**) contributes to the observed differences in activity for these compounds.

In summary, the structural and functional data presented here support that a *cis*-configuration at the Pip residue in the cyclic tetrapeptide HDAC inhibitor apicidin affords improved HDAC-inhibitory activities compared to the *t*-*t*-*t*-*t* conformation that predominates in solution. We have presented the rational design of conformationally constrained, triazole modified cyclic peptide scaffolds with potent biological activity and have established the ability to probe the biologically relevant conformation of a natural peptide ligand by introducing different triazole regioisomers in place of amide bonds in its backbone. It is our hope that this study will help guide future efforts aimed at improving on the cyclic tetrapeptide HDAC inhibitors and lead to more selective HDAC ligands.

Supplementary Material

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References

1. a) Fesik SW, Neri P, Meadows R, Olejniczak ET, Gemmecker G. J Am Chem Soc 1992;114:3165. b) Kofron JL, Kuzmic P, Kishore V, Gemmecker G, Fesik SW, Rich DH. J Am Chem Soc 1992;114:2670. c) Kallen J, Spitzfaden C, Zurini MG, Wider G, Widmer H, Wuthrich K, Walkinshaw MD. Nature 1991;353:276. [PubMed: 1896075] d) Fesik SW, et al. Science 1990;250:1406. [PubMed: 2255910] e) Lautz J, Kessler H, Van Gunsteren WF, Weber HP, Wenger RM. Biopolymers 1990;29:1669. [PubMed: 2386812] f) Loosli HR, Kessler H, Oschkinat H, Weber HP, Petcher TJ, Widmer A. Helv Chim Acta 1985;68:682.
2. a) Hummel G, Reineke U, Reimer U. Mol BioSystems 2006;2:500. b) Perola E, Charifson PS. J Med Chem 2004;47:2499. [PubMed: 15115393] c) Taylor P, Mikol V, Kallen J, Burkhard P, Walkinshaw MD. Biopolymers 1996;40:585. [PubMed: 9101762]
3. Kranz M, Murray PJ, Taylor S, Upton RJ, Clegg W, Elsegood MRJ. J Pept Sci 2006;12:383. [PubMed: 16342331]
4. Singh SB, Zink DL, Polishook JD, Dombrowski AW, Darkin-Rattray SJ, Schmatz DM, Goetz MA. Tetrahedron Lett 1996;37:8077.
5. a) Biel M, Wascholowski V, Giannis A. Angew Chem, Int Ed 2005;44:3186. b) de Ruijter AJM, van Gennip AH, Caron HN, Kemp S, van Kuilenburg ABP. Biochem J 2003;370:737. [PubMed: 12429021]
6. Minucci S, Pelicci PG. Nat Rev Cancer 2006;6:38. [PubMed: 16397526]
7. Bieliauskas AV, Pflum MKH. Chem Soc Rev 2008;37:1402. [PubMed: 18568166]
8. Meinke PT, Liberator P. Curr Med Chem 2001;8:211. [PubMed: 11172676]
9. a) Shen J, Woodward R, Kedenburg JP, Liu X, Chen M, Fang L, Sun D, Wang PG. J Med Chem 2008;51:7417. [PubMed: 19007204] b) Marks PA, Breslow R. Nat Biotechnol 2007;25:84. [PubMed: 17211407] c) Singh SB, Zink DL, Liesch JM, Mosley RT, Dombrowski AW, Bills GF, Darkin-Rattray SJ, Schmatz DM, Goetz MA. J Org Chem 2002;67:815. [PubMed: 11856024]
10. a) Montero A, Beierle JM, Olsen CA, Ghadiri MR. J Am Chem Soc. 2009 In Press. b) Gomez-Paloma L, Bruno I, Cini E, Khochbin S, Rodriquez M, Taddei M, Terracciano S, Sadoul K. ChemMedChem 2007;2:1511. [PubMed: 17694590] c) Deshmukh PH, Schulz-Fademrecht C, Procopiou PA, Vigushin DA, Coombes RC, Barrett AGM. Adv Synth Catal 2007;349:175. d) Shivashimpi GM, Amagai S, Kato T, Nishino N, Maeda S, Nishino TG, Yoshida M. Bioorg Med Chem 2007;15:7830. [PubMed: 17881232] e) Murray PJ, et al. Bioorg Med Chem Lett

2001;11:773. [PubMed: 11277517] f) Furumai R, Komatsu Y, Nishino N, Khochbin S, Yoshida M, Horinouchi S. Proc Natl Acad Sci U S A 2001;98:87. [PubMed: 11134513]

11. Horne, WS. PhD thesis. The Scripps Research Institute; La Jolla, CA: 2005.
12. Shute RE, Kawai M, Rich DH. Tetrahedron 1988;44:685.
13. a) Beierle JM, Horne WS, van Maarseveen JH, Waser B, Reubi JC, Ghadiri MR. Angew Chem, Int Ed. 2009 In Press. b) Appendino G, Bacchiega S, Minassi A, Cascio Maria G, De Petrocellis L, Di Marzo V. Angew Chem, Int Ed 2007;46:9312. c) Bock VD, Speijer D, Hiemstra H, van Maarseveen JH. Org Biomol Chem 2007;5:971. [PubMed: 17340013] d) Turner RA, Oliver AG, Lokey RS. Org Lett 2007;9:5011. [PubMed: 17956112] e) Angell YL, Burgess K. Chem Soc Rev 2007;36:1674. [PubMed: 17721589] f) Bock VD, Perciaccante R, Jansen TP, Hiemstra H, van Maarseveen JH. Org Lett 2006;8:919. [PubMed: 16494474] g) Choi WJ, Shi ZD, Worthy KM, Bindu L, Karki RG, Nicklaus MC, Fisher RJ, Burke TR. Bioorg Med Chem Lett 2006;16:5265. [PubMed: 16908148] h) Angelo NG, Arora PS. J Am Chem Soc 2005;127:17134. [PubMed: 16332031] i) van Maarseveen JH, Horne WS, Ghadiri MR. Org Lett 2005;7:4503. [PubMed: 16178569] j) Angell Y, Burgess K. J Org Chem 2005;70:9595. [PubMed: 16268639] k) Brik A, Alexandratos J, Lin YC, Elder JH, Olson AJ, Wlodawer A, Goodsell DS, Wong CH. ChemBioChem 2005;6:1167. [PubMed: 15934050] l) Horne WS, Yadav MK, Stout CD, Ghadiri MR. J Am Chem Soc 2004;126:15366. [PubMed: 15563148] m) Horne WS, Stout CD, Ghadiri MR. J Am Chem Soc 2003;125:9372. [PubMed: 12889966]
14. a) Pokorski JK, Miller Jenkins LM, Feng H, Durell SR, Bai Y, Appella DH. Org Lett 2007;9:2381. [PubMed: 17506576] b) Hitotsuyanagi Y, Motegi S, Hasuda T, Takeya K. Org Lett 2004;6:1111. [PubMed: 15040735] c) Hitotsuyanagi Y, Motegi S, Fukaya H, Takeya K. J Org Chem 2002;67:3266. [PubMed: 12003534] d) Duncia JV, et al. Bioorg Med Chem Lett 1998;8:775. [PubMed: 9871540]
15. Tam A, Arnold U, Soellner MB, Raines RT. J Am Chem Soc 2007;129:12670. [PubMed: 17914828]
16. a) Boren BC, Narayan S, Rasmussen LK, Zhang L, Zhao H, Lin Z, Jia G, Fokin VV. J Am Chem Soc 2008;130:8923. [PubMed: 18570425] b) Rasmussen LK, Boren BC, Fokin VV. Org Lett 2007;9:5337. [PubMed: 18052070] c) Zhang L, Chen X, Xue P, Sun HHY, Williams ID, Sharpless KB, Fokin VV, Jia G. J Am Chem Soc 2005;127:15998. [PubMed: 16287266]
17. a) Tornoe CW, Christensen C, Meldal M. J Org Chem 2002;67:3057. [PubMed: 11975567] b) Rostovtsev VV, Green LG, Fokin VV, Sharpless KB. Angew Chem, Int Ed 2002;41:2596.
18. Kim S, Kim EY, Ko H, Jung YH. Synthesis 2003:2194.
19. a) Hauske JR, Dorff P, Julin S, Martinelli G, Bussolari J. Tetrahedron Lett 1992;33:3715. b) Ohira S. Synth Commun 1989;19:561.
20. Chan TR, Hilgraf R, Sharpless KB, Fokin VV. Org Lett 2004;6:2853. [PubMed: 15330631]
21. We define conformational homogeneity as >95% of a single conformation in the 1H NMR spectrum
22. a) Che Y, Marshall GR. J Med Chem 2006;49:111. [PubMed: 16392797] b) Seebach D, Bezencon O, Juan B, Pietzonka T, Matthews JL, Kuehnle FNM, Schweizer WB. Helv Chim Acta 1996;79:588. c) Kato T, Lee S, Shimohigashi Y, Tone A, Kodera Y, Izumiya N. Int J Pept Protein Res 1987;29:53. [PubMed: 3570655]
23. a) Loiseau N, Gomis JM, Santolini J, Delaforge M, Andre F. Biopolymers 2003;69:363. [PubMed: 12833263] b) Pinet E, Neumann JM, Dahse I, Girault G, Andre F. Biopolymers 1995;36:135. [PubMed: 7492742]
24. Swepston PN, Cordes AW, Kuyper LF, Meyer WL. Acta Crystallogr, Sect B: Struc Crystallogr Crystal Chem 1981;B37:1139.
25. Aracil JM, Badre A, Fadli M, Jeanty G, Banaigs B, Francisco C, Lafargue F, Heitz A, Aumelas A. Tetrahedron Lett 1991;32:2609.
26. Biron E, et al. Angew Chem, Int Ed 2008;47:2595.

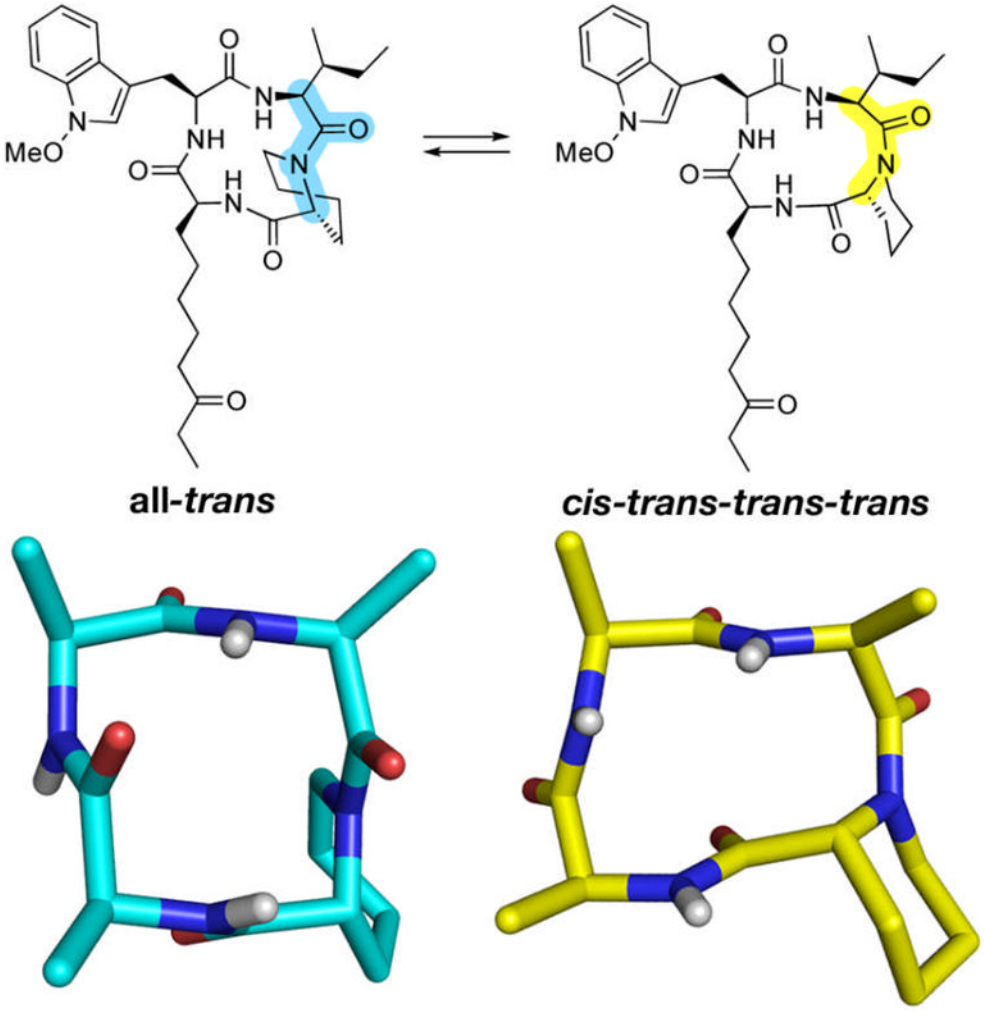


Figure 1.

Cis-trans isomerization in the cyclic tetrapeptide natural product apicidin (**1**) gives rise to an equilibrium between the predominant *t-t-t-t* conformation (80%) and a minor *c-t-t-t* conformer (15%) in DMSO.[3,11] The molecular structures shown are the *t-t-t-t* NMR structure determined in pyridine-d_5[4] and the *c-t-t-t* crystal structure.[3]

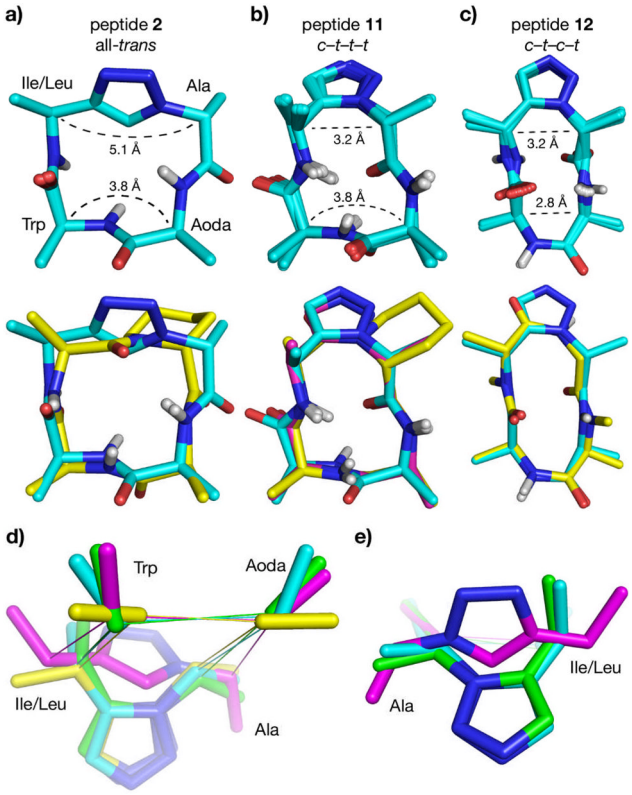
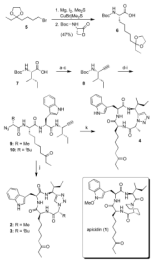


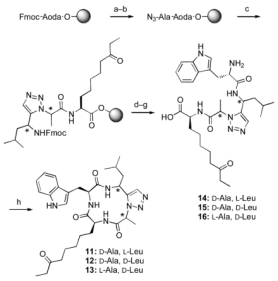
Figure 2.

NMR structures for the triazole–modified apicidin analogs. a) Peptide **2** adopted a *t-t-t-t* conformation (top) that overlays well (bottom, 0.52 Å r.m.s.d. for C_α and C_β atoms) on the lowest energy calculated conformation of apicidin (yellow) (which reportedly closely matches the predominant conformation of apicidin in DMSO).[3] b) Peptide **11** adopted a *c-t-t-t* conformation; two families of structures were observed that differ in the rotation of the Trp/Aoda amide relative to the backbone (top). The structures overlay very well on the crystal structure of apicidin[3] (bottom, 0.30 Å and 0.51 Å r.m.s.d. for C_α and C_β atoms for representative members of the two structural families). c) Peptide **12** adopted a *c-t-c-t* conformation (top), which overlays very well on the crystal structure of the natural product dihydrotentoxin[24] (yellow) (bottom, 0.24 Å r.m.s.d. for C_α and C_β atoms). d) Overlay of C_α atoms for compounds **2** (magenta), **11** (two structural families, green and cyan), and **12** (yellow). Due to the *c-t-c-t* conformation of **12**, the Aoda, Trp, and Leu side chains of this peptide project in the same plane as the backbone ring as opposed to projecting upward and out of the plane as for the other peptides. e) Overlay of C_α and C_β atoms for compounds **2** (magenta) and **11** (two structural families, green and cyan). The C_α atoms of the Ala and Ile/Leu residues are farther apart in **2** and the C_α–C_β vector of **2** directs the Ile side chain outward away from the ring as opposed to directly above the ring in **11**.



Scheme 1.

(a) *N*,*O*-dimethylhydroxylamine·HCl, EDC·HCl, $^{i}Pr_2EtN$; (b) $LiAlH_4$; (c) dimethyl (2-oxopropyl)phosphonate, *p*-TsN_3, K_2CO_3 (48% overall, 3 steps); (d) TFA; (e) Boc-Trp-OH, HBTU, $^{i}Pr_2EtN$ (94% over 2 steps); (f) TFA; (g) **6**, HBTU, $^{i}Pr_2EtN$ (74% over two steps); (h) TFA; (i) N_3-D-Ala-OH or N_3-D-Leu-OH, EDC·HCl, HOBT, $^{i}Pr_2EtN$, 0 °C (73% for Ala, 31% for Leu, overall); (j) CuI, 2,6-lutidine, $^{i}Pr_2EtN$, *tris*-(benzyltriazolylmethyl)amine[20] (50% yield by HPLC); (k) microwave, 220 °C (2:1 ratio of **4:2** by HPLC, 8% isolated yield of **4**).



Scheme 2.

a) NMP–piperidine 3:1; b) N_3-L-Ala-OH or N_3-D-Ala-OH (4 equiv), DIC (4 equiv), HOBt (4 equiv), NMP, 2 h; c) Fmoc-L-Leu-CCH or Fmoc-D-Leu-CCH (2 equiv), Cp*Ru(COD)Cl (20%), toluene, Ar, 16 h, 45 °C; d) NMP-piperidine 3:1; e) Fmoc-Trp-OH (4 equiv), HBTU (4 equiv), $^{i}Pr_2EtN$ (8 equiv), NMP, 2 h; f) NMP–piperidine 3:1; g) TFA-CH_2Cl_2 1:1; h) HATU (2 equiv), $^{i}Pr_2EtN$ (4 equiv), 1.5 h, 0.5 mM in DMF (all cyclization yields were >95% by HPLC [see Figure S1]; isolated yields based on resin loading were 9% for **11**, 9% for **12**, and 10% for **13**).

Table 1

Observed conformations in DMSO and potencies of triazole containing pseudopeptides and control compounds against recombinant human HDAC enzymes.[a]

		IC_{50}/nM			
compound	**conformation**	**HDAC1**	**HDAC3**[b]	**HDAC6**	**HDAC8**
2	*t-t-t-t*	25	16	>10,000	ND
11	*c-t-t-t*	7	9	6100	105
12	*c-t-c-t*	75	119	>10,000	ND
apicidin	*t-t-t-t* (80%) *c-t-t-t* (15%)	3	11	>10,000	750
TSA	—	ND	ND	6	32

[a] IC_{50} values were obtained by testing in triplicate. ND = Values not determined for the compound against this enzyme. TSA = trichostatin A, which was used as a positive control for HDAC6 and HDAC8 because apicidin is a poor inhibitor of these enzymes.

[b] In complex with NCoR2.