

DESIGN OF SYNTHETIC α -HELIX MIMICS FOR DISRUPTION OF PROTEIN-PROTEIN INTERACTIONS

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INTRODUCTION

Protein-Protein Interactions

Protein-protein interactions play crucial roles in a variety of biological activities, including cell growth, differentiation, and death, and are very important in disease proliferation. While a large body of work has been dedicated to examining the factors that control them, disruption of protein-protein interactions remains a difficult challenge.² Unlike enzyme-substrate interactions, which are often composed of binding “pockets” that are amenable to small molecule screening, many protein-protein interactions contain broad, shallow interfacial surfaces ($>500 \text{ \AA}^2$) with unique topological designs that cannot be easily probed using small molecule screens. Despite these challenges, knowledge of the crucial residues located within protein-protein interfaces has led to the development of a number of molecules that can interrupt a variety of interactions. One such class of molecules utilizes synthetic secondary structural motifs to mimic the surface of a protein involved in a particular protein-protein interaction to disrupt binding to its target. In particular, molecules based on α -helix secondary structure have been widely used.^{4,5}

Properties of α -Helices

The α -helix is a right-handed, coiled polypeptide that is stabilized by intramolecular hydrogen-bonding (Figure 1). α -Helices are important secondary structural motifs in peptides and proteins, making up approximately 40% of protein structure. Helices often contain non-polar residues in the i , $i+3/4$, $i+7$, and $i+11$ positions, creating a hydrophobic face along one side of the helix that facilitates interactions with “hydrophobic clefts” of other proteins. For example, the tumor suppressor protein p53 is a transcription factor that controls cellular response to stress and is down-regulated by human double minute 2 (HDM2) protein.⁶ HDM2 binds the N-terminal α -helical transactivation domain of p53 via hydrophobic interactions. Overproduction of HDM2 inhibits the p53 pathway, leading to the uncontrolled cell proliferation observed in certain tumors. Design and synthesis of molecules that can mimic the α -helical structure of molecules such as the p53 transactivation domain requires an understanding of several factors, including the ability of the native peptide to form helices in solution

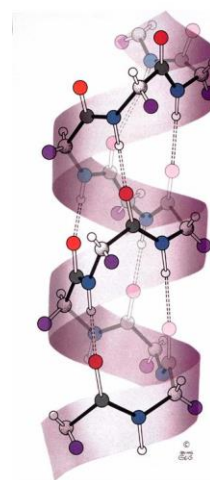


Figure 1: α -Helix structure.³

and the binding strength between the native peptide and its target.^{2,3,6} Molecules with better protease stability and ability to bind to protein substrates than native peptides can be useful in a variety of settings, particularly in the interruption of the protein-protein interactions that lead to disease states. Many of these synthetic α -helix mimics have been developed, and three classes are presented.

LOCKED SYNTHETIC α -HELICES

When designing synthetic α -helices as modulators of protein-protein interactions, inclusion of α -amino acids has several major drawbacks, including low cell permeability, high proteolytic sensitivity, and poor pharmacokinetic properties. A further disadvantage is the frequent loss of helical structure in the absence of a binding partner due to sequence bias toward random coiled structures. If the sequence of the peptide prevents helix formation, the molecule will have no utility in disrupting protein-protein interactions. These issues can be addressed, however, via the addition of several olefin-containing building blocks which can be connected to “lock” the synthetic helices into pre-determined conformations through ring closing metathesis (RCM) techniques, which were first developed by Grubbs and co-workers.⁷ RCM utilizes olefin-containing building blocks substituted for amino acids that are not involved in binding, ensuring side chain availability. In addition, locked peptides have increased protease resistance and better cell membrane permeability than α -peptides.⁸ The functional group tolerance of this technique allows for incorporation of a variety of side-chains.⁹

Utilizing these “locking” methods, several research groups have developed synthetic α -helices that retain secondary structure regardless of building block composition. For example, Verdine and coworkers developed a strategy termed “hydrocarbon stapling”, in which RCM utilizing Grubbs-I catalyst was used to lock a peptide containing several α,α -disubstituted non-natural amino acids into a helical conformation (Figure 2).⁸ The peptide mimicked the α -helical BH3 domain of BID, one of several pro-apoptotic proteins (Bak, Bad, Bim) in the B-cell lymphoma 2 (Bcl-2) family that interacts with a hydrophobic cleft on the anti-apoptotic Bcl-x_L protein. Overproduction of Bcl-x_L prevents pro-apoptotic proteins from initiating programmed cell death in tumor cells. Utilizing circular dichroism (CD), the authors showed the locked peptides were more helical than their unlocked counterparts (87% vs. 16%) in aqueous solution, as well as being more stable.⁸ Competitive fluorescence polarization (FP) assays, which measure the decrease in fluorescence of a fluorophore when it is displaced from a target, indicated that the best locked derivative bound Bcl-x_L with high affinity ($K_d = 38.8$ nM) compared to the unmodified BID^{BH3} peptide ($K_d = 269$ nM). More importantly, this derivative was permeable to Jurkat

leukemia cells by endocytosis and suppressed leukemia cell proliferation in mouse models compared to controls as measured by total body luminescence of leukemia cells expressing luciferase.⁸

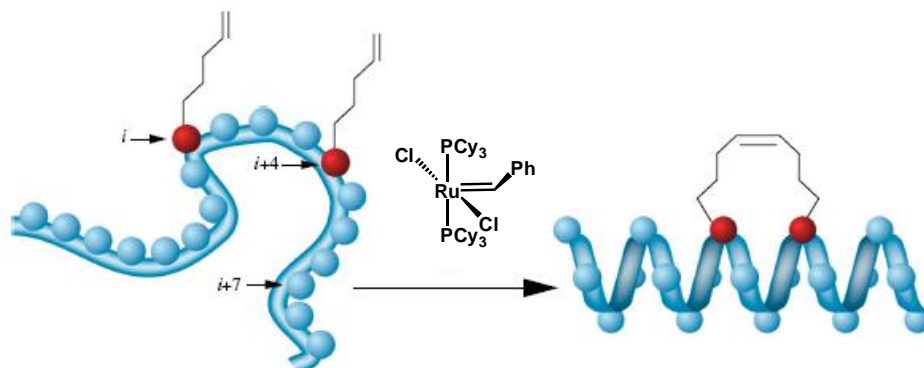


Figure 2: Method of “hydrocarbon stapling” to stabilize α -helical structure.⁸

Arora and coworkers have utilized RCM to develop hydrogen bond surrogates (HBS), in which the hydrogen bond between i and $i+4$ residues is replaced with an olefin (Figure 3). Using RCM, the authors were able to synthesize several short α -helices.¹⁰⁻¹³ The HBS motif was chosen because of its ability to maintain helical structure without sacrificing helix surface functionality, leaving the surface available for binding. In addition, HBS molecules have increased protease resistance and helical character.¹¹ HBS syntheses combined solid phase peptide synthesis (SPPS) with RCM utilizing the Hoveyda-Grubbs ruthenium catalyst, under both standard¹³ and microwave⁹ heating conditions. Microwave synthesis not only increased yields and decreased reaction times, but also increased functional group tolerance so bulky *t*-butyl protecting groups could be included.⁹ The locked α -helix mimic of Bak^{BH3} displayed increased affinity for the anti-apoptotic Bcl-x_L protein compared to the native Bak^{BH3} peptide ($K_d = 69$ nM vs. 264 nM) in a competitive FP binding assay.¹² Overall, it is evident from these studies that the addition of a lock to the α -helix enhances binding affinity, in addition to increasing protease stability and cell permeability.

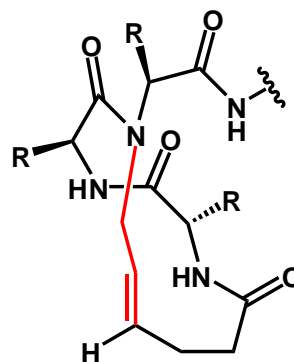


Figure 3: Hydrogen Bond Surrogate.¹

FOLDAMERS - SYNTHETIC α -HELICES WITH UNNATURAL AMINO ACIDS

As a second route to α -helix mimics, many researchers have turned to foldamers – unnatural oligomers with discreet folding properties containing building blocks such as peptoids and β -peptides¹⁴ – to mimic the spatial arrangement of residues on the α -helix structure. These building blocks offer

several advantages, including increased protease and metabolic stability, making them promising building blocks for α -helix mimicry.^{15,16} This class of molecules has been utilized to disrupt protein-protein interactions, and several examples of molecules containing β -peptides are highlighted below.

($\alpha/\beta+\alpha$) Peptide Foldamer Motif

Recognizing the increased stability of β -peptides, Gellman and co-workers set out to find foldamers that mimic the Bak^{BH3} motif. After analyzing the binding affinities of over one hundred different β -amino acid polypeptides and finding no strong binders, they designed a molecule containing alternating α - and β -amino acids, which showed increased affinity in fluorescence polarization ($IC_{50} = 40 \mu M$) and binding studies ($K_i = 1.5 \mu M$).¹⁷ The addition of a 6-residue exclusively α -amino acid region from the N-terminus of the native Bak^{BH3} peptide (Figure 4) increased binding substantially ($IC_{50} = 0.059 \mu M$, $K_i = 0.0019 \mu M$), as indicated by competitive FP assays.¹⁷ ¹⁵N-¹H HSQC NMR spectroscopy indicated that the derivative matched the binding location of the native Bak^{BH3} peptide ($IC_{50} = 0.67 \mu M$, $K_i = 0.025 \mu M$). A recent study examined a large library of derivatives and found this foldamer to be the best binder.¹⁸ The exclusively α -amino acid region was highly susceptible to protease degradation, however, indicating further study and optimization of derivatives is still necessary.

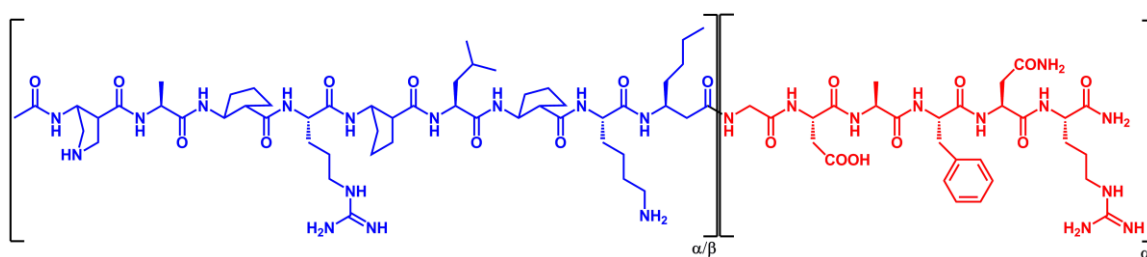


Figure 4: ($\alpha/\beta+\alpha$) Peptide foldamer motif.¹⁷

β -Peptide Foldamer Motif

Despite the lack of success using β -peptide foldamers by Gellman and co-workers, Schepartz and co-workers have found successes in producing all β -peptide foldamers that mimic α -helices.¹⁵ Using an exclusively β -amino acid motif to mimic the α -helical transactivation domain of the p53 protein, the authors were able to interrupt binding of the native p53 to HDM2 with relatively high affinity ($IC_{50} = 94.5 \mu M$, $K_d = 368 \text{ nM}$) compared to the native α -peptide sequence ($IC_{50} = 2.47 \mu M$, $K_d = 233 \text{ nM}$) as measured by competitive FP assays.¹⁹ In a second study, Schepartz and co-workers designed β -peptide foldamers for binding of gp41, a protein involved in fusion of virus-protein cell walls which leads to proliferation of HIV.²⁰ Mimicking the spatial orientation of key residues in the interaction (as verified by CD and NOESY 2D-NMR spectroscopy), the researchers obtained K_d values for four derivatives (0.75-1.5 μM) that were similar to those for a similarly sized α -peptide ($K_d = 1.2 \mu M$) and better than those for

non-specific hydrophobic residue binders carbonic anhydrase II and calmodulin.²⁰ Gp41-mediated cell-cell fusion was also inhibited in HeLa cell assays with micromolar EC₅₀ values.²⁰

SYNTHETIC NON-PEPTIDE α -HELIX MIMICS

As the study of protein-protein interactions and search for disruptors of these interactions has grown, the factors determining the molecular recognition have become better understood. While binding surfaces are often broad and shallow, indicating many possible contact points, often only a few key residues play important roles in binding. This indicates that the continuous surface of α -helix may not be required for strong binding. By analyzing the target protein surface, the binding properties of α -helix residues can be identified and mimicked by synthetic molecules containing little or no helical structure. Since Willems and coworkers discovered that small molecules such as indanes can mimic the *i* and *i*+1 residues on a peptide surface,²¹ many research groups have extensively studied similar α -helix mimics. Hamilton and co-workers have been particularly active in this field⁵ (Figure 5), and several examples developed by this group are highlighted below.

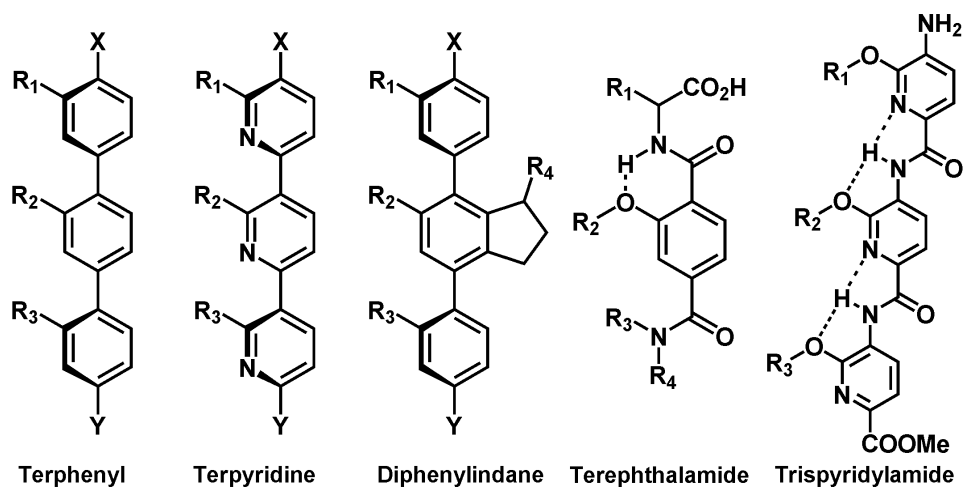


Figure 5: α -helix mimics developed by Hamilton and co-workers.⁵

Terphenyl Derivatives

Building upon research done by Willems and coworkers²¹, Hamilton and co-workers devised a 3,2',2''-substituted terphenyl structure that closely mimicked the *i*, *i*+3, and *i*+7 positions of a native α -helix.²²⁻²⁷ The structure was synthesized using iterative Suzuki couplings to couple *ortho*-substituted phenyl rings, with side-chain R-groups based on the desired target structure. These low molecular weight molecules retained key points for contact with protein substrates, as indicated by docking and

^{15}N - ^1H HSQC NMR spectroscopy studies. Terphenyl derivatives were synthesized and tested in a variety of systems, including smooth muscle myosin light chain kinase (smMLCK)-calmodulin binding,²⁵ gp41 binding,²³ p53-HDM2 binding^{22,26} and Bak^{BH3}/Bcl-x_L binding.^{24,27} In all cases, these molecules exhibited micromolar- to nanomolar-level affinity, displacing the native ligand as measured primarily by competitive FP assays. The specificity of these molecules could be attenuated based on inclusion of certain side chains. For example, a 1-naphthalenemethylene-containing derivative with high affinity for Bcl-x_L ($K_d = 114$ nM) could be altered to bind to HDM2 with high affinity ($K_d = 182$ nM) by simply changing the connection of the naphthalene side-chain to 2-naphthalenemethylene.²⁶

Terephthalamide Derivatives

In examining the properties of the terphenyl scaffold (Figure 5), Hamilton and coworkers noted several issues, including low levels of solubility and relatively cumbersome synthetic procedures. The researchers sought to design easily synthesized molecules that were better able to dissolve into aqueous solution while still retaining an α -helix side-chain arrangement. One new strategy involved replacing several phenyl rings with carboxamide groups containing alkyl substituents to develop a class of molecules termed terephthalamides.^{28,29} Synthesis of these molecules involved *O*-alkylation of the core phenyl ring, followed by amide bond formations using molecules containing the desired alkyl substituents.^{28,29} The spatial orientation of side chains on one face of the molecule was maintained by an intramolecular hydrogen bond, which withstood both fluctuating concentration and temperature conditions as monitored by ^1H NMR (amide H $\delta=8.54$ ppm). The solubility level of these molecules increased substantially compared to the terphenyls, as measured by log P values (4.4 for terephthalamide compared to 9.3 for terphenyl – a value within the range of -2 to 6 constitutes a relatively soluble molecule).²⁹ Utilizing fluorescence polarization assays, the researchers studied a set of derivatives mimicking the Bak^{BH3} domain. The best derivative had a K_i value of 0.78 μM , comparable to the Bak^{BH3} peptide ($K_i = 0.122$ μM). Further study using ^{15}N - ^1H HSQC-NMR spectroscopy and computational methods confirmed that the binding location of these molecules mimicked that of the native peptide, indicating that terephthalamides represented potential inhibitors of the Bak^{BH3}/Bcl-x_L interaction.

Trispyridylamide Derivatives

In addition to the terephthalamide derivatives (Figure 5), Hamilton and co-workers synthesized trispyridylamides, α -helix mimics comprised of a series of pyridine rings linked by amide bonds.³⁰ Monomers were synthesized through *O*-alkylation of functionized pyridine rings, followed by iterative amide bond formation between aryl amines and aryl acid chlorides using standard coupling conditions³⁰.

The connection of side-chain functionality via aryl ether bonds aided in the formation of an extensive hydrogen-bonding network that helped to maintain structural rigidity. A combination of 2D NOESY ^1H NMR spectroscopy and X-ray crystallography confirmed the presence of the extended H-bonded network. Trispyridylamide derivatives were designed to mimic the Bak^{BH3} domain, and the binding preference for the hydrophobic cleft of Bcl-x_L was examined. Competitive FP assay analysis led to the discovery of three derivatives that bound to the cleft with micromolar affinity ($K_i = 1.6 \mu\text{M}$ for best inhibitor). 2D-NMR spectroscopy verified the binding site. Analysis of derivatives containing two or four pyridine rings did not give molecules with better binding constants, indicating that a three-residue mimic is ideal.³⁰ These studies indicate that trispyridylamide derivatives represent yet another approach to designing inhibitors of the Bak^{BH3}/Bcl-x_L interaction.

CONCLUSIONS

Synthetic α -helices and α -helix mimics provide a useful means of interrupting certain protein-protein interactions. Since these protein-protein interactions do not contain the “pockets” of enzymes, small molecule screening must be replaced by rational design of molecules based on the native peptides, leading to a variety of molecules that are able to interact with protein targets. As research moves forward, the design of these molecules will continue to evolve in order to ease synthesis and increase binding affinity and specificity. Starting with synthetic α -helices from native protein structures, the state of the art now involves molecular mimics that contain only the necessary functional groups and correct spatial arrangement. Further study into this field will likely yield better binding molecules, possibly leading to molecules that can be used as therapeutic treatments for those suffering from disease.

REFERENCES

- (1) Wang, D.; Chen, K.; Dimartino, G.; Arora, P. S. *Org. Biomol. Chem.* **2006**, *4*, 4074-81.
- (2) Yin, H.; Hamilton, A. D. *Angew. Chem. Int. Ed. Engl.* **2005**, *44*, 4130-63.
- (3) Che, Y.; Brooks, B. R.; Marshall, G. R. *J. Comput. Aided. Mol. Des.* **2006**, *20*, 109-30.
- (4) Che, Y.; Brooks, B. R.; Marshall, G. R. *Biopolymers* **2007**, *86*, 288-97.
- (5) Davis, J. M.; Tsou, L. K.; Hamilton, A. D. *Chem. Soc. Rev.* **2007**, *36*, 326-34.
- (6) Murray, J. K.; Gellman, S. H. *Biopolymers* **2007**, *88*, 657-86.
- (7) Trnka, T. M., Grubbs, R.H. *Acc. Chem. Res.* **2001**, *34*, 18-29.
- (8) Walensky, L. D.; Kung, A. L.; Escher, I.; Malia, T. J.; Barbuto, S.; Wright, R. D.; Wagner, G.; Verdine, G. L.; Korsmeyer, S. J. *Science* **2004**, *305*, 1466-70.
- (9) Chapman, R. N.; Arora, P. S. *Org. Lett.* **2006**, *8*, 5825-8.
- (10) Chapman, R. N.; Dimartino, G.; Arora, P. S. *J. Am. Chem. Soc.* **2004**, *126*, 12252-3.
- (11) Wang, D.; Chen, K.; Kulp Iii, J. L.; Arora, P. S. *J. Am. Chem. Soc.* **2006**, *128*, 9248-56.

- (12) Wang, D.; Liao, W.; Arora, P. S. *Angew. Chem. Int. Ed. Engl.* **2005**, *44*, 6525-9.
- (13) Dimartino, G.; Wang, D.; Chapman, R. N.; Arora, P. S. *Org. Lett.* **2005**, *7*, 2389-92.
- (14) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893-4012.
- (15) Kritzer, J. A.; Stephens, O. M.; Guarracino, D. A.; Reznik, S. K.; Schepartz, A. *Bioorg. Med. Chem.* **2005**, *13*, 11-6.
- (16) Sadowsky, J. D.; Murray, J. K.; Tomita, Y.; Gellman, S. H. *Chembiochem* **2007**, *8*, 903-16.
- (17) Sadowsky, J. D.; Schmitt, M. A.; Lee, H. S.; Umezawa, N.; Wang, S.; Tomita, Y.; Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 11966-8.
- (18) Sadowsky, J. D.; Fairlie, W. D.; Hadley, E. B.; Lee, H. S.; Umezawa, N.; Nikolovska-Coleska, Z.; Wang, S.; Huang, D. C.; Tomita, Y.; Gellman, S. H. *J. Am. Chem. Soc.* **2007**, *129*, 139-54.
- (19) Kritzer, J. A.; Lear, J. D.; Hodsdon, M. E.; Schepartz, A. *J. Am. Chem. Soc.* **2004**, *126*, 9468-9.
- (20) Stephens, O. M.; Kim, S.; Welch, B. D.; Hodsdon, M. E.; Kay, M. S.; Schepartz, A. *J. Am. Chem. Soc.* **2005**, *127*, 13126-7.
- (21) Horwell, D. C.; Howson, W.; Ratcliffe, G. S.; Willems, H. M. *Bioorg. Med. Chem.* **1996**, *4*, 33-42.
- (22) Chen, L.; Yin, H.; Farooqi, B.; Sebti, S.; Hamilton, A. D.; Chen, J. *Mol. Cancer. Ther.* **2005**, *4*, 1019-25.
- (23) Ernst, J. T.; Kutzki, O.; Debnath, A. K.; Jiang, S.; Lu, H.; Hamilton, A. D. *Angew. Chem. Int. Ed. Engl.* **2002**, *41*, 278-81.
- (24) Kutzki, O.; Park, H. S.; Ernst, J. T.; Orner, B. P.; Yin, H.; Hamilton, A. D. *J. Am. Chem. Soc.* **2002**, *124*, 11838-9.
- (25) Orner, B. P.; Ernst, J. T.; Hamilton, A. D. *J. Am. Chem. Soc.* **2001**, *123*, 5382-3.
- (26) Yin, H.; Lee, G. I.; Park, H. S.; Payne, G. A.; Rodriguez, J. M.; Sebti, S. M.; Hamilton, A. D. *Angew. Chem. Int. Ed. Engl.* **2005**, *44*, 2704-7.
- (27) Yin, H.; Lee, G. I.; Sedey, K. A.; Kutzki, O.; Park, H. S.; Orner, B. P.; Ernst, J. T.; Wang, H. G.; Sebti, S. M.; Hamilton, A. D. *J. Am. Chem. Soc.* **2005**, *127*, 10191-6.
- (28) Yin, H.; Hamilton, A. D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1375-9.
- (29) Yin, H.; Lee, G. I.; Sedey, K. A.; Rodriguez, J. M.; Wang, H. G.; Sebti, S. M.; Hamilton, A. D. *J. Am. Chem. Soc.* **2005**, *127*, 5463-8.
- (30) Ernst, J. T.; Becerril, J.; Park, H. S.; Yin, H.; Hamilton, A. D. *Angew. Chem. Int. Ed. Engl.* **2003**, *42*, 535-9.