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Mimicry of a β-Hairpin Turn by a Nonpeptidic Laterally Flexible Foldamer

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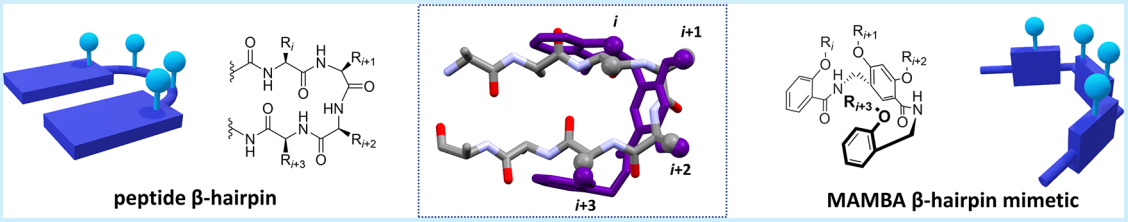
Supporting Information



ABSTRACT: The design and characterization of a proteomimetic foldamer that displays lateral flexibility endowed by intramolecular bifurcated hydrogen bonds is reported. The MAMBA scaffold, derived from *meta*-aminomethylbenzoic acid, adopts a serpentine conformation that mimics the side chain projection of all four residues in a β-hairpin turn.

Synthetic structures that emulate the arrangement and chemical recognition features of peptide and protein surfaces are known as peptidomimetics and proteomimetics, respectively.[1] The development of peptidomimetics to modulate protein−protein interactions (PPIs) is of particular interest for the study and treatment of disease, especially where traditional medicinal chemistry approaches have been unsuccessful.[2] PPIs are mediated over large surface areas by many weak interactions, with some "hot-spot" residues contributing more than others.[3] Optimal peptidomimetics and proteomimetics should possess large, densely functionalized surfaces that can mimic and compete with PPI interface motifs.[4] Small-molecule pharmaceuticals typically have a comparatively reduced surface area and may not contribute the binding interactions sufficient for high affinity or specificity for a protein surface.[5]

A variety of protein structure motifs participate in binding interactions at the interface of PPIs.[6] Therefore, peptidomimetic scaffolds have been designed to mimic α-helices, β-strands, or various turn elements.[7] Peptide-based systems often attempt to mitigate excessive flexibility by using macrocyclization[8] and rigid template methods.[9] Conformational control in nonpeptidic systems is achieved by restricting flexibility through conjugation, steric effects, dipolar repulsion, and hydrogen bonds. We have previously utilized these approaches in indolinone[10] and pyridyl-linked imidazolidin-2-one[11] β-strand mimetics and in terphenyl[12] and pyridylamide[13] α-helix mimetic foldamer scaffolds (Figure 1). Peptidomimetics and proteomimetics have successfully been applied to the study of intrinsically disordered proteins,[14] in cancer cell signaling,[15] and as antibiotics.[16]

The arrangement of rigid and flexible components in peptidomimetics and proteomimetics endows different modes of folding and therefore defines which protein motifs can be mimicked. A unifying feature of many nonpeptidic scaffolds is that they are generally linear, conjugated systems that tend toward planarity. These scaffolds possess an axis along which

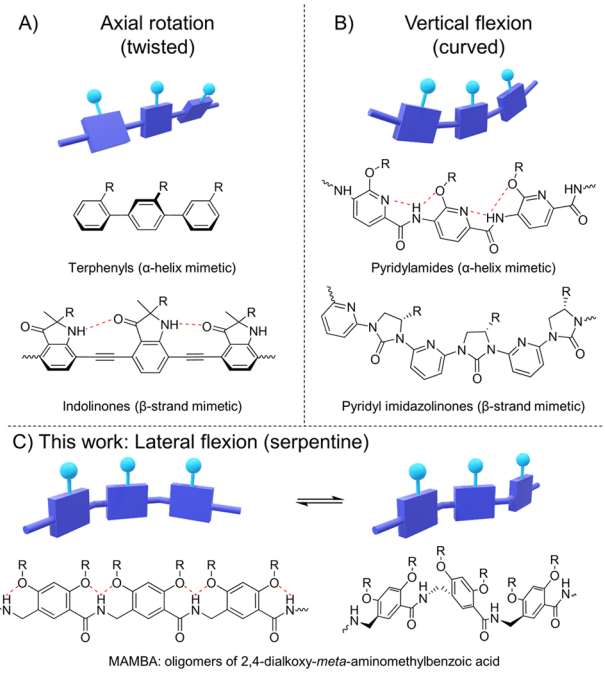


Figure 1. Peptidomimetic conformational flexibility. (A) Terphenyls and indolinones twist along the oligomer axis. (B) Pyridylamides and pyridylimidazolinones curve vertically to form a concave binding surface. (C) MAMBA oligomers are laterally flexible and form *cis* (left schematic) and *trans* (right schematic) conformers.

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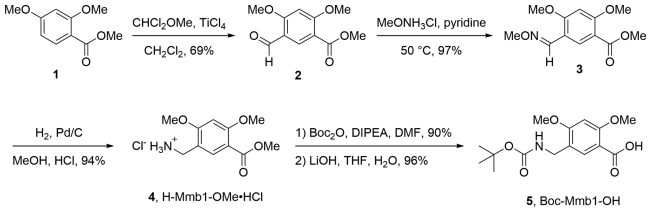
the oligomer chain extends and a vertical plane from which the side chains project. Using these directional standards, one can characterize flexibility in these systems by axial rotation (Figure 1A) and vertical flexion (Figure 1B), which produces twisted and curved structures, respectively. Acute vertical flexion gives rise to helical structures.[17] The extended conjugation of many scaffolds derived from aromatic monomers generates rod-like structures, which have proven effective in reproducing the side chain orientation of a single face of an α-helix or β-strand.[18] However, mimicking broader protein surfaces and nonlinear motifs with nonpeptidic scaffolds still remains a challenge.

In this work, we report a nonpeptidic scaffold based on oligoamides of 2,4-dialkoxy-*meta*-aminomethylbenzoic acid (MAMBA) that demonstrates lateral flexibility (Figure 1C), allowing the mimicry of more extended protein surfaces. A MAMBA trimer mimics the position and orientation of all four side chains in a β-hairpin turn motif. By combining conformation-rigidifying hydrogen bonds with flexible methylene groups, this scaffold can adopt folded conformations that emulate the three-dimensional array of amino acid side chains on protein surfaces.

The MAMBA scaffold is designed for facile functionalization and elongation. It is composed of oligomeric *meta*-aminomethylbenzoic acid units with alkoxy groups appended in positions 2 and 4 on the benzene ring. The benzylic methylene is intended to disrupt conjugation in order to increase scaffold flexibility. Oligomers of the closely related 2,4-dialkoxy-*meta*-aminobenzoic acid are fully conjugated and therefore lack lateral flexibility. These stuctures form crescent oligoamides and helices due to vertical flexion.[19] Coupling of MAMBA monomers forms a secondary amide whose proton participates in bifurcated hydrogen bonds with the phenolic ether oxygen atoms of adjacent monomers.[20] This creates a hydrogen bond assisted hinge that directs alkoxy groups to one face of the foldamers, while maintaining flexibility lateral to the extending chain—similar to the serpentine form of a snake in motion.

In order to test the scaffold design, we prepared a dimethoxy-functionalized MAMBA-1 monomer, which we abbreviated as Mmb1. As MAMBA monomers are δ-amino acids, we chose to use conventional amino acid nomenclature for describing carboxy and amino protecting groups. Thus, the *tert*-butylcarbamate-protected Mmb1 compound **5** is abbreviated Boc-Mmb1-OH. Monomer **5** was prepared from commercially available methyl 2,4-dimethoxybenzoate in five steps (Scheme 1). Methyl ester **1** was formylated regioselectively at the 5-

Scheme 1. Synthesis of MAMBA-1 Monomers



position under Rieche conditions[21] to give aldehyde **2**. Methoxime **3** was prepared using methoxyamine and was subsequently converted to benzylamine **4** (H-Mmb1-OMe·HCl) by catalytic hydrogenation under acidic conditions. Amino group protection with di-*tert*-butyl dicarbonate, followed by saponification of the methyl ester, gave Boc-Mmb1-OH monomer **5** in good yield.

A critical aspect of peptidomimetic design is the scaffold conformation, which specifies the spacing and orientation of amino acid side chain isosteres. To investigate these properties of MAMBA, oligomers were prepared using a solution-phase Boc/COOMe protection strategy (Figure 2A). The dimer Boc-

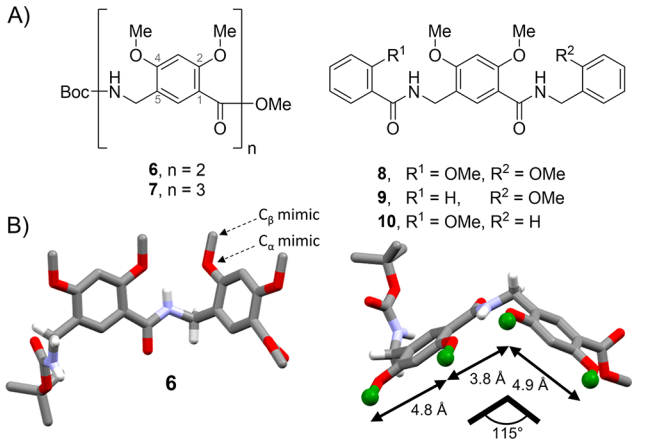


Figure 2. (A) Chemical structures of MAMBA dimer, trimer, and truncated trimers. (B) X-ray diffraction structure of dimer **6** as viewed from the side (left) and the top (right) with methoxy carbon atoms highlighted in green to illustrate the side chain spatial orientation.

Mmb1$_2$-OMe (**6**) and trimer Boc-Mmb1$_3$-OMe (**7**) were prepared by amino acid coupling under the standard conditions (see Supporting Information (SI)). These compounds were examined by single-crystal X-ray diffraction. The expected intramolecular bifurcated hydrogen bond was observed in the crystal structure of dimer **6**, causing all methoxy groups to be displayed on a single face of the structure (Figure 2B). The benzylic methylene group permits the scaffold to deviate from planarity, creating a 115° hinge between monomers. A nonplanar scaffold is advantageous, as it permits mimicry of nonlinear protein motifs.

The phenolic oxygen atoms of the MAMBA monomer serve as locations for diverse functionalization and side chain mimicry. The oxygen atom simulates the α-carbon of an amino acid side chain with the attached methyl group simulating the β-carbon (Figure 2B). Interatom distances are between 3.8 and 4.9 Å for adjacent groups. In the crystal structure of **6**, the terminal *tert*-butylcarbamate is rotated out of plane to form an intermolecular hydrogen bond with the carbonyl oxygen of an adjacent molecule. This was observed for both the dimer **6** and the trimer **7** (see SI) crystal structures. The intermolecular contact is mediated by the carbamate proton, causing the intramolecular hydrogen bond to the 4-methoxy group to be broken. This suggests that the benzylamide hydrogen bond to the 4-methoxy (4-OMe) group is weaker than the benzamide hydrogen bond to the 2-methoxy (2-OMe) oxygen atom. To minimize the potential for intermolecular contacts between terminal groups in the solid state, a truncated trimer **8** was prepared lacking terminal carboxy, aminomethyl, and methoxy groups. In addition, truncated trimer analogues **9** and **10** were prepared lacking an internal methoxy group to investigate the relative hydrogen bond strengths of the 2-OMe and 4-OMe groups (Figure 2A).

A truncated MAMBA trimer such as **8**, with each set of bifurcated hydrogen bonds intact, can display a surface with four different functional groups. A nontruncated trimer

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contains six customizable positions. The benzylic methylene spacers endow the hydrogen-bonded structure with lateral flexibility; thus, a trimer can adopt *cis* or *trans* conformations, which are likely in equilibrium. The *trans* conformation arranges functional groups in a pleated pattern (Figure 1C, right), while the *cis* trimer will adopt a U-shaped turn (Figure 3A). Crystals of truncated trimer **8** suitable for structure

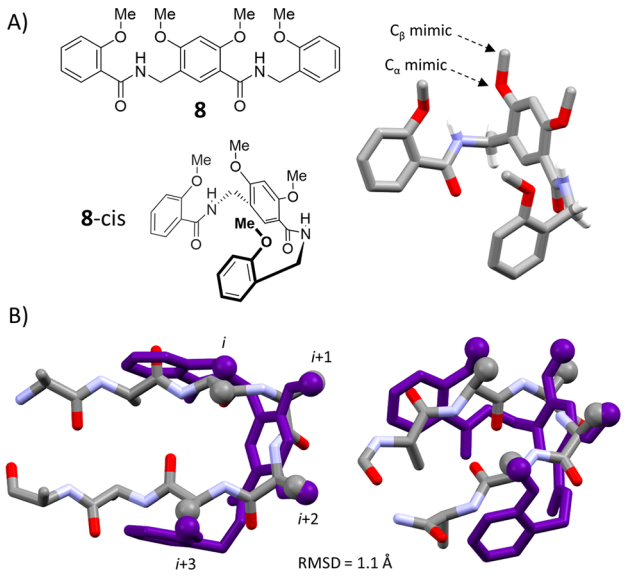

Figure 3. (A) Chemical structures (left) and crystal structure (right) of truncated trimer **8** in a fully hydrogen-bonded *cis* conformation. (B) Overlay of **8** (violet) aligned with a canonical type II β-hairpin (extracted from PDB 1fth). Hydrogen atoms and side chains are truncated for clarity. An eight-point RMSD of 1.1 Å was calculated from C_α–C_α and C_β–C_β distances.

determination by X-ray diffraction were obtained and showed the compound in the *cis* conformation with all four methyl groups pointing to the same face of the foldamer, similar to a β-hairpin turn.

The β-hairpin turn motif consists of four amino acids with each side chain directed to a single face of the β-sheet. The β-hairpin has been shown to be an important motif in loop-mediated protein–protein interactions.[6c] A key structural aspect of the hairpin is a hydrogen bond between the carbonyl oxygen of residue *i* and the N-terminal amide proton of residue *i* + 3. β-Hairpin turn mimetics that induce the interaction between these two residues have been developed using a variety of scaffolds, including dibenzofuran,[22] azobenzene,[23] and many others.[24] These structures are often synthetically challenging or functionally limited. By omitting the side chains of interceding residues *i* + 1 and *i* + 2, many hairpin-templating scaffolds potentially forsake important binding interactions. To determine the potential for mimicry of all four turn residues by the MAMBA scaffold, the *cis* isomer of **8** was aligned to a canonical type II β-hairpin turn (Figure 3B). The methoxy group of the N-terminal benzamide cap mimics the *i* residue. The 4-OMe and 2-OMe groups of the central MAMBA unit overlay with the *i* + 1 and *i* + 2 residues, and the C-terminal benzylamide cap completes the turn to mimic the *i* + 3 side chain. An eight-point RMSD of 1.1 Å was determined by using the methoxy group oxygen and carbon atoms as amino acid C_α and C_β isosteres, respectively. A similar overlap was observed for type I, type I′, and type II′ β-hairpins (see SI, section 6).

In order to be an effective proteomimetic, the structure must be capable of adopting the appropriate binding conformation in solution. Under most biological conditions, this implies a necessarily polar environment, which may disrupt the intramolecular hydrogen bonding of the MAMBA foldamer. To probe the hydrogen bond in solution, truncated trimers **8**–**10** were characterized by ^{1}H NMR experiments in various solvent mixtures. Figure 4 depicts the change in chemical shift for the

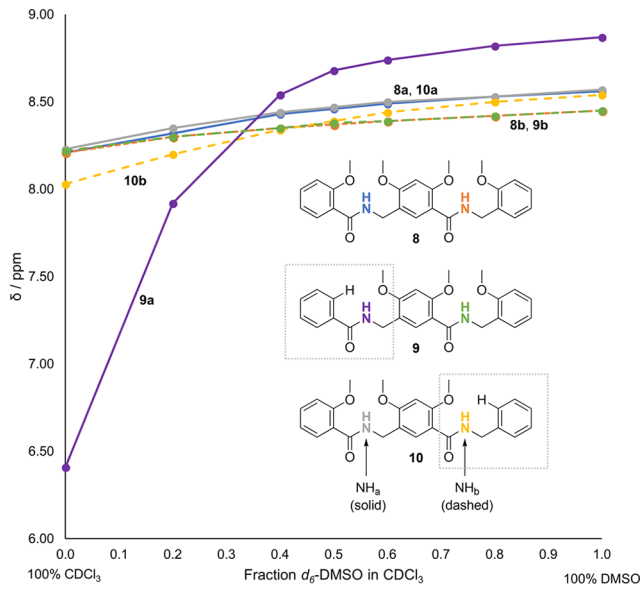

Figure 4. Amide ^{1}H NMR chemical shift dependence on solvent polarity for hydrogen-bonded and nonbonded truncated trimers.

two amide protons of **8**–**10** as a function of increasing the fraction of dimethyl sulfoxide in chloroform. For the tetrafunctionalized **8** the chemical shift values for NH_a and NH_b are nearly identical in chloroform and shift only slightly downfield as the solvent polarity increases. Truncated trimer **9** lacks the N-terminal 2-OMe group as a hydrogen bond acceptor for NH_a, which results in a significant upfield shift in chloroform. As the DMSO fraction increases, this proton is free to interact with polar DMSO molecules, and the chemical shift is observed to move downfield to a greater extent than both amide protons in **8**. A similar, albeit less dramatic, trend is observed for NH_b in **10**. These data confirm that the benzamide indeed forms the stronger hydrogen bond and account for the observation that the hydrogen bond to the 4-OMe in some crystal structures is preferentially disrupted. We interpret the lower chemical shifts in **8** compared to **9**-NH_a and **10**-NH_b in 100% DMSO to indicate that even in this polar solvent the hydrogen-bonded structure is a significant contributor to the overall conformational equilibrium.

Additional conformational analysis was conducted by 2D NOESY NMR experiments. Truncated trimers **8**–**10** were examined in chloroform and dimethyl sulfoxide for interactions between the amide, benzylic, and aromatic ring hydrogens (see SI, section 4.2). In chloroform, the signal of an amide with two adjacent hydrogen bond acceptors (**8**, **9**-NH_b, and **10**-NH_a) overlaps with the *ortho*-aryl hydrogen, thus preventing substantive interpretation. However, in DMSO, these peaks are well resolved and show strong NOE correlations between benzylic protons and the *ortho*-aryl hydrogen and weak or nonexistant crosspeaks for NH–aryl pairs, indicating the

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bifurcated hydrogen bonds maintain the predicted conformation. The amides with a single hydrogen bond acceptor (**9**-NH_a and **10**-NH_b) show stronger NH−aryl crosspeaks, especially in the absence of the 2-OMe group in **9**-NH_a. This evidence confirms that the planar 2-OMe hydrogen bond is stronger than the 4-OMe bond and that the MAMBA scaffold is flexible, yet retains its hydrogen-bonded character in polar solvents.

In conclusion, a proteomimetic scaffold MAMBA was designed with lateral flexibility provided by a tripartite bifurcated hydrogen bond hinge. The foldamer is extended through amide bonds to form the conformation-directing hydrogen bond motif, which is maintained in polar solvents. MAMBA foldamers display a broad and densely functionalized proteomimetic surface and are synthetically accessible, and the *cis* conformation was shown to replicate the position and orientation of all four residues in a β-hairpin turn. Synthetic methods are currently being developed in our laboratory to incorporate chemically diverse side chains and to accommodate solid-phase synthetic methods. The development of MAMBA oligomers as customizable macromolecular recognition tools and as modulators of biomedically relevant PPIs is underway.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b01463.

Experimental details, synthesis and characterization of all new compounds, 1D and 2D NMR spectra, and crystallographic information (PDF)

Accession Codes

CCDC 1828445−1828448 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Jayatunga, M. K. P.; Thompson, S.; Hamilton, A. D. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 717−724. (b) Fletcher, S.; Hamilton, A. D. *Curr. Opin. Chem. Biol.* **2005**, *9*, 632−638.

(2) (a) Ivanov, A. A.; Khuri, F. R.; Fu, H. *Trends Pharmacol. Sci.* **2013**, *34*, 393−400. (b) Vagner, J.; Qu, H.; Hruby, V. J. *Curr. Opin. Chem. Biol.* **2008**, *12*, 292−296.

(3) Thangudu, R. R.; Bryant, S. H.; Panchenko, A. R.; Madej, T. *J. Mol. Biol.* **2012**, *415*, 443−453.

(4) Corbi-Verge, C.; Kim, P. M. *Cell Commun. Signaling* **2016**, *14*, 8.

(5) Scott, D. E.; Bayly, A. R.; Abell, C.; Skidmore, J. *Nat. Rev. Drug Discovery* **2016**, *15*, 533−550.

(6) (a) Watkins, A. M.; Wuo, M. G.; Arora, P. S. *J. Am. Chem. Soc.* **2015**, *137*, 11622−11630. (b) Watkins, A. M.; Arora, P. S. *ACS Chem. Biol.* **2014**, *9*, 1747−1754. (c) Gavenonis, J.; Sheneman, B. A.; Siegert, T. R.; Eshelman, M. R.; Kritzer, J. A. *Nat. Chem. Biol.* **2014**, *10*, 716−722.

(7) Pelay-Gimeno, M.; Glas, A.; Koch, O.; Grossmann, T. N. *Angew. Chem., Int. Ed.* **2015**, *54*, 8896−8927.

(8) (a) Patgiri, A.; Jochim, A. L.; Arora, P. S. *Acc. Chem. Res.* **2008**, *41*, 1289−1300. (b) Walensky, L. D.; Bird, G. H. *J. Med. Chem.* **2014**, *57*, 6275−6288. (c) Favre, M.; Moehle, K.; Jiang, L.; Pfeiffer, B.; Robinson, J. A. *J. Am. Chem. Soc.* **1999**, *121*, 2679−2685.

(9) Nowick, J. S.; Lam, K. S.; Khasanova, T. V.; Kemnitzer, W. E.; Maitra, S.; Mee, H. T.; Liu, R. *J. Am. Chem. Soc.* **2002**, *124*, 4972−4973.

(10) Wyrembak, P. N.; Hamilton, A. D. *J. Am. Chem. Soc.* **2009**, *131*, 4566−4567.

(11) German, E. A.; Ross, J. E.; Knipe, P. C.; Don, M. F.; Thompson, S.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2015**, *54*, 2649−2652.

(12) Orner, B. P.; Ernst, J. T.; Hamilton, A. D. *J. Am. Chem. Soc.* **2001**, *123*, 5382−5383.

(13) Ernst, J. T.; Becerril, J.; Park, H. S.; Yin, H.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2003**, *42*, 535−539.

(14) (a) Kumar, S.; Hamilton, A. D. *J. Am. Chem. Soc.* **2017**, *139*, 5744−5755. (b) Oh, M.; Lee, J. H.; Wang, W.; Lee, H. S.; Lee, W. S.; Burlak, C.; Im, W.; Hoang, Q. Q.; Lim, H.-S. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 11007−11012.

(15) Chang, Y. S.; Graves, B.; Guerlavais, V.; Tovar, C.; Packman, K.; To, K.-H.; Olson, K. A.; Kesavan, K.; Gangurde, P.; Mukherjee, A.; Baker, T.; Darlak, K.; Elkin, C.; Filipovic, Z.; Qureshi, F. Z.; Cai, H.; Berry, P.; Feyfant, E.; Shi, X. E.; Horstick, J.; Annis, D. A.; Manning, A. M.; Fotouhi, N.; Nash, H.; Vassilev, L. T.; Sawyer, T. K. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, E3445−E3454.

(16) Zerbe, K.; Moehle, K.; Robinson, J. A. *Acc. Chem. Res.* **2017**, *50*, 1323−1331.

(17) Jiang, H.; Léger, J.-M.; Huc, I. *J. Am. Chem. Soc.* **2003**, *125*, 3448−3449.

(18) (a) Azzarito, V.; Prabhakaran, P.; Bartlett, A. I.; Murphy, N. S.; Hardie, M. J.; Kilner, C. A.; Edwards, T. A.; Warriner, S. L.; Wilson, A. J. *Org. Biomol. Chem.* **2012**, *10*, 6469−6472. (b) Volonterio, A.; Moisan, L.; Rebek, J. *Org. Lett.* **2007**, *9*, 3733−3736. (c) Li, X.; Miltschitzky, S.; König, B. *Bioorg. Med. Chem.* **2006**, *14*, 6075−6084.

(19) Gong, B. *Acc. Chem. Res.* **2008**, *41*, 1376−1386.

(20) Du, P.; Jiang, X.-K.; Li, Z.-T. *Tetrahedron Lett.* **2009**, *50*, 316−319.

(21) Rieche, A.; Gross, H.; Höft, E. *Chem. Ber.* **1960**, *93*, 88−94.

(22) Diaz, H.; Espina, J. R.; Kelly, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 8316−8318.

(23) Aemissegger, A.; Kräutler, V.; van Gunsteren, W. F.; Hilvert, D. *J. Am. Chem. Soc.* **2005**, *127*, 2929−2936.

(24) (a) Eckhardt, B.; Grosse, W.; Essen, L.; Geyer, A. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 18336−18341. (b) Burgess, K. *Acc. Chem. Res.* **2001**, *34*, 826−835. (c) Whitby, L. R.; Boger, D. L. *Acc. Chem. Res.* **2012**, *45*, 1698−1709.

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