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Tailoring Reaction Selectivity by Modulating a Catalytic Diad on a Foldamer Scaffold

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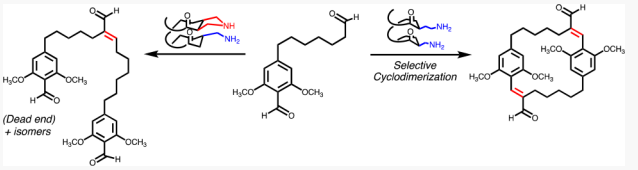
ABSTRACT: Use of a tunable molecular scaffold to align a reactive diad for bifunctional catalysis can reveal relationships between functional group identity and reactivity that might otherwise be impossible to identify. Here we use an α/β-peptide helix to show that an aligned pair of primary amine groups is uniquely competent to catalyze crossed aldol condensations with an aryl aldehyde as the electrophile. Geometrically similar diads in which one amine group is secondary, or both are secondary, are good catalysts for other types of aldol condensations but not those involving an aryl aldehyde. Catalytic efficacy requires β-amino acid residues that are preorganized for helix formation via cyclic constraint. Conventional peptides (exclusively α-amino acid residues) that display the primary amine diad are poor catalysts, which highlights the critical role of the foldamer scaffold.



INTRODUCTION

Coordinated action by multiple catalytic groups to facilitate conversion of substrate(s) to product(s) is a hallmark of enzymatic catalysis. Efforts to develop non-macromolecular catalysts often derive inspiration from enzymatic prototypes, although small molecular frameworks are limited in the number of catalytic groups that can be deployed and the orientations among those groups that can be achieved. Conventional peptides (α-amino acid residues) and other oligomers are useful scaffolds for catalyst development because their modular nature enables serial variation of the reactive groups and the covalent connectivity between groups.[1−6] Synthetic oligomers with strong and well-characterized conformational behavior ("foldamers") offer the benefit that reactive group location along the sequence translates into predictable three-dimensional positioning in the folded state.[7−10] Here we use a foldamer-based approach to show how changes in a pair of amine groups can alter the aldol condensation selectivity profile displayed by bifunctional catalysts. Effective catalysis required the foldamer scaffold; a conventional peptide backbone was inadequate.

Our efforts focused on a specific foldamer helix formed by a backbone with a repeating motif of one α-amino acid residue and two β-amino acid residues ($\alpha\beta\beta$).[11,12] This α/β-peptide helix has approximately three residues per turn and is stabilized by β residues with a five-membered-ring constraint, such as (*S*,*S*)-*trans*-2-amino-cyclopentanecarboxylic acid (ACPC). Placing amine-bearing residues in an *i*,*i*+3 sequence relationship, as in α/β-peptides **A**−**C** (Figure 1), causes the amine diad to be aligned along one side of the helix, which promotes bifunctional catalysis.[13] The foldamer scaffold allows us to ask how changes in the identity of the catalytic amine groups influence reactivity.

Previously, we found that a diad comprising two secondary amines (as in **A**) supports bifunctional catalysis of crossed aldol reactions with formaldehyde as electrophile.[13] Replacement of one secondary amine with a primary amine (α/β-peptide **B**) generated a catalyst for macrocyclization via aldol condensation, but catalysis of crossed aldol reactions with formaldehyde was lost.[14] α/β-Peptide **A** was not competent in the macrocyclization reactions. Here we report the unexpected discovery that a diad with two primary amine groups (as in α/β-peptide **C**) is required for bifunctional catalysis of crossed aldol reactions in which an aryl aldehyde serves as the electrophile. The effect of amine identity on reaction selectivity could not have been elucidated without the use of a modular and well-folded scaffold.

The present work emerged from a regiochemical challenge. When dialdehyde **1a** (Figure 2) was used as a substrate for intramolecular aldol catalysis by α/β-peptide **B**, two isomeric enals were obtained (Figure 1),[14] each containing the 18-membered-ring core of the natural product nostocyclyne A.[15] Alternative substrate **1b** has only one site for enamine formation (the alkyl aldehyde) and should undergo selective cyclization; however, α/β-peptide **B** was a poor catalyst for this reaction, as shown below. Our effort to discover an effective catalyst for the cyclization of **1b** was not motivated by an intent to synthesize the natural product, but rather by a desire

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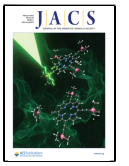

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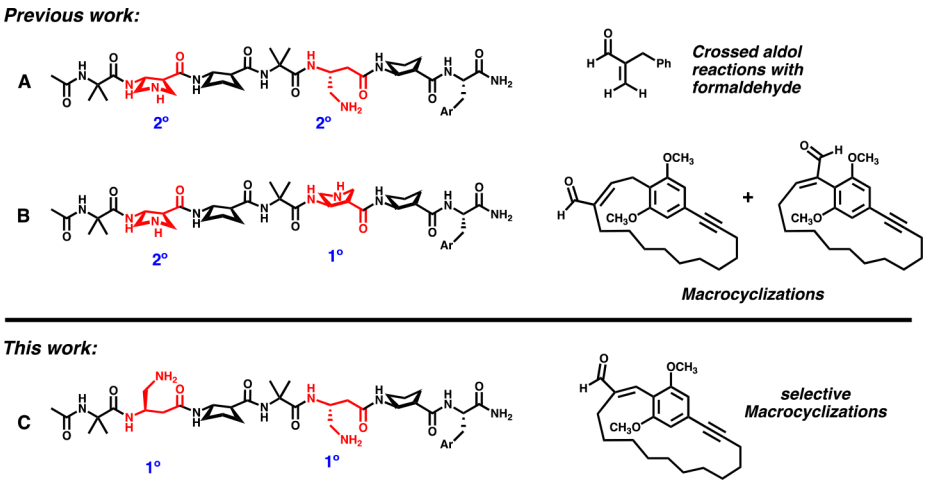

Figure 1. Overview of distinct catalytic activities displayed by foldamers that present different amine diads on a 1:2 α/β-peptide scaffold.

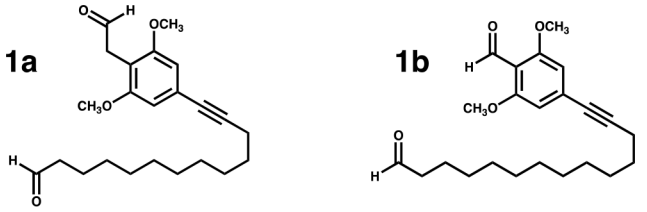

Figure 2. Isomeric dialdehydes that can form macrocyclic products corresponding to the core of nostocyclyne A.

to understand how altering amine identity and the scaffold affects reaction outcome.

RESULTS AND DISCUSSION

Intermolecular Crossed Aldol Reactions. Aryl aldehydes are often less electrophilic than alkyl aldehydes.[16] We used a mixture of hydrocinnamaldehyde and benzaldehyde (1:2) to seek catalysts that favor the crossed aldol product (**2**) relative to the homoaldol product from hydrocinnamaldehyde (**3**) under established conditions (Figure 3).[17] A diad composed of two secondary amines (α/β-peptide **A**) was ineffective for either reaction pathway, and a diad with one secondary and one primary amine (α/β-peptide **B**) strongly favored the homoaldol pathway. A diad with two primary amines (α/β-peptide **C**) supported catalysis of both the crossed aldol and homoaldol reactions. Parallel studies with β-peptides (pure β-amino acid residue backbone; **D1** and **D2**) showed that a primary amine diad provided the crossed aldol and homoaldol products in similar amounts in these cases as well; however, the β-peptide catalysts were inferior to α/β-peptide counterpart **C** in terms of yield.

The discovery that the best catalytic diad contained two primary amines led us to consider new α/β-peptides in which reactive side chains project from α rather than β residues. We examined a set of α/β-peptides in which all possible $i,i+3$ combinations of α residues derived from (*S*)-2,3-diaminopropanoic acid (Dap), (*S*)-2,4-diamino-butanoic acid (Dab), ornithine (Orn), and lysine (Lys) were represented (Figure 3). All of the β residues were preorganized (ACPC) in each of these α/β-peptides, with the exception of the C-terminal β^3-homotyrosine residue, which was included to provide a UV chromophore. This series systematically varied spacing of each amine group from the backbone (one to four intervening CH_2 units). Significant crossed aldol product was formed with each α/β-peptide, but the highest yields were observed with Dab at position 3 and either Dap or Orn at position 6 (α/β-peptides **E2** and **F5**). In each case, swapping the positions of the amine-bearing residues generated an α/β-peptide (**E3** or **F2**) that was less effective at promoting the crossed aldol reaction and less selective for crossed aldol vs homoaldol of hydrocinnamaldehyde. Thus, catalytic activity in this series is sensitive to the three-dimensional arrangement of the two catalytic amine groups, as has been observed among α-peptide catalysts.[18]

The relationship between side chain lengths and catalytic activity seems complex, although some general conclusions are evident. All six Lys-containing α/β-peptides (**G1**–**G6**) are relatively poor catalysts. This trend suggests the general conclusion that a side chain can be too long (four CH_2 units between backbone and the amine) to support effective catalysis. On the other hand, the series **E2**, **E4**, **F5**, in which the N-terminal amine is presented by Dab, while the C-terminal amine side chain is incrementally lengthened (from one to three intervening CH_2 units), manifests a non-monotonic trend, with the highest yields of cross aldol product for **E2** and **F5** (one or three intervening CH_2 units, respectively). This behavior may reflect the operation of competing structure–reactivity relationships. For example, lengthening a side chain by adding CH_2 units increases overall flexibility, which should increase the entropic cost of coordinated action by the two amine groups. On the other hand, longer side chains might allow the diad to achieve a more favorable geometry relative to shorter side chains.

α/β-Peptide **E2** was the focus of subsequent studies because this catalyst provided the most favorable combination of crossed aldol yield and selectivity among the set shown in Figure 3. The major products obtained from the reaction involving hydrocinnamaldehyde and benzaldehyde were assigned an *E* configuration, as shown in Figure 3, based on NOE measurements with purified products (Figures S36 and S37). Careful inspection of the initial product mixture revealed that a small proportion of the *Z* isomer was formed in each case (4–6% relative to the *E* isomer; Figure S28). When purified **2** and **3** were subjected to the reaction conditions in the presence of **E2**, these enals were recovered unchanged after 24 h. This observation indicates that the aldol products are stable under the reaction conditions (Figure S1).

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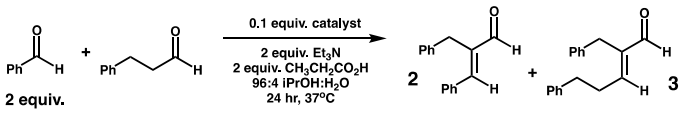

αββ scaffold, catalytic side chains on β residues

	2	3
A	3%	4%
B	4%	54%
C	33%	33%

β Scaffold

	2	3
D1	15%	14%
D2	12%	9%

αββ scaffold, catalytic side chains on α residues

	2	3
E1	29%	25%
E2	46%	33%
E3	25%	25%
E4	31%	25%
F1	24%	21%
F2	24%	23%
F3	25%	25%
F4	32%	26%
F5	49%	40%
G1	17%	12%
G2	13%	13%
G3	17%	19%
G4	18%	12%
G5	12%	9%
G6	21%	15%

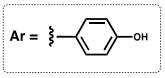

Figure 3. Reaction outcomes used to identify bifunctional catalysts that favor crossed aldol condensation (to form enal **2**) relative to homoaldol condensation (to form enal **3**). Yields were determined via LC-MS based on external calibration. Catalysts included 1:2 α/β-peptides bearing amines on β residues (**A**−**C**), β-peptides with primary amine residues (**D1** and **D2**), and 1:2 α/β-peptides that present a primary amine from α amino acid residues including 2,3-diaminopropanoic acid (Dap; green), 2,4-diaminobutanic acid (Dab; blue), ornithine (orange), and lysine (purple) (**E1**−**G6**).

α/β-Peptides **H1** and **H2** (Figure 4) are analogues of **E2** in which one of the primary amine groups has been removed (Dab or Dap replaced by alanine). To test the hypothesis that **E2** is a bifunctional catalyst, we conducted a reaction in which 0.1 equiv of **E2** was replaced by a 1:1 mixture of **H1** and **H2** (0.1 equiv each). Only trace quantities of aldol products **2** and **3** were obtained in the presence of this pair of monofunctional α/β-peptides, which is consistent with the bifunctional catalysis hypothesis for **E2**. As a further test of this hypothesis, we monitored the initial rate of crossed aldol product formation as the concentration of **E2** was varied (0.02, 0.05, and 0.1 equiv relative to the limiting reagent, hydrocinnamaldehyde (Figure 5)). The linear relationship between initial rate and α/β-peptide concentration indicates first-order catalysis by **E2**, which is consistent with a bifunctional catalytic mechanism.

Additional control experiments were conducted with amine-containing small molecules, pyrrolidine (2°) and *n*-butylamine (1°). Amines of this type have been widely explored as catalysts of aldol reactions.[19] Very little product was detected from these reactions (Figure 4), which highlights the importance of a scaffold that controls the positioning of the two amine groups in a catalyst such as **E2**.

To ask whether the critical feature of the Dap and Dab residues in α/β-peptide **E2** is the presence of a primary amine group rather than the lack of a cyclic constraint (as found in **A** or **B**), we examined α/β-peptide **I**, an analogue of **E2** in which one of the primary amine groups was replaced by a secondary

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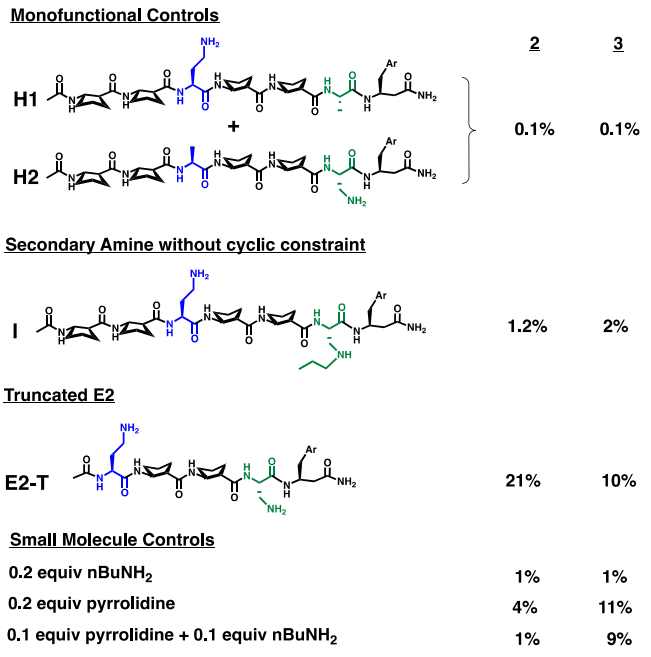

Figure 4. Control studies used to probe the mechanism of crossed aldol catalysis. Monofunctional α/β-peptides **H1** and **H2** are analogues of catalyst **E2** in which one residue bearing a primary amine has been replaced by alanine; these monofunctional peptides were used in combination, 0.1 equiv each, relative to the limiting starting material (hydrocinnamaldehyde). Peptide **I** is an analogue of **E2** that has a propyl group appended to the side chain amine of the Dap residue. Peptide **E2-T** is a shortened version of **E2** in which the ACPC residues preceding Dab have been removed. Amines of low molecular weight, pyrrolidine (0.2 equiv), *n*-butyl amine (0.2 equiv), and a combination of these two (0.1 equiv each), were compared with peptide catalysts.

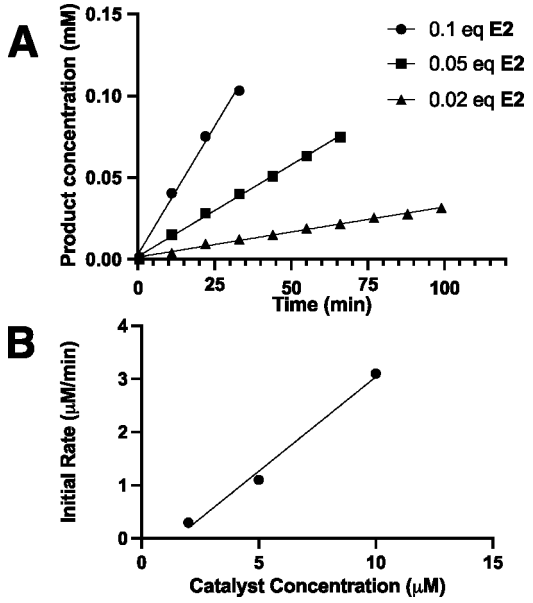

Figure 5. (A) Reaction progress for the crossed aldol condensation (to form **2**) as a function of the loading of α/β-peptide **E2** (0.02, 0.05, or 0.1 equiv relative to hydrocinnamaldehyde). (B) Initial rates of formation of product **2** as a function of α/β-peptide **E2** concentration.

amine. Specifically, the Dap residue of **E2** was replaced with an analogous residue bearing an *n*-propyl substituent on the side chain nitrogen. α/β-Peptide **I** was ineffective as a catalyst for the crossed aldol or homoaldol reaction, which indicates the importance of a diad composed exclusively of primary amines.

The observations summarized in Figures 3−5 support our hypothesis that catalysis of the crossed aldol reaction requires the coordinated action of two primary amine groups. We propose that the catalytic mechanism proceeds via condensation of one primary amine with benzaldehyde to form an iminium and condensation of the other primary amine with hydrocinnamaldehyde to form an enamine. Such a hypothetical intermediate, illustrated in Figure 6, positions the two

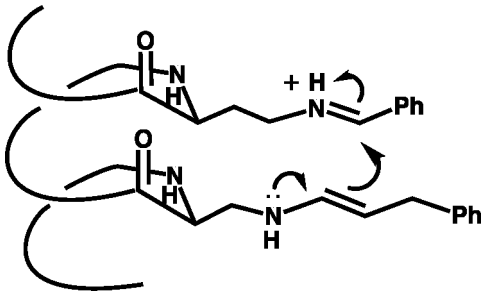

Figure 6. Cartoon showing a hypothetical intermediate in the crossed aldol reaction catalyzed by α/β-peptide **E2**. In this scenario, bifunctional catalysis would result from simultaneous activation of benzaldehyde as an iminium (electrophile) and hydrocinnamaldehyde as an enamine (nucleophile).

reactive moieties for formation of the new carbon−carbon bond. Subsequent hydrolytic steps would liberate the crossed aldol product from the catalyst. Evidence to support this hypothesis was obtained from LC-MS analysis of the reaction mixture after 1 h of reaction time (Figures S15−S17). We detected low levels of two species with m/z values that are consistent with singly charged M + H ions corresponding to predicted enamine/iminium intermediates in a bifunctional catalytic cycle. When $NaBH_4$ was added to this mixture, to reduce any enamine or iminium moieties, we detected low levels of new species with m/z values consistent with singly charged M + H ions of expected reduction products (Figures S18−S20).

Structural Characterization of E2. Our hypothesis regarding the catalytically active conformation of α/β-peptides such as **E2** is based on crystal structures of α/β-peptides featuring the 1:2 α:β backbone, which uniformly display a helical secondary structure containing C═O(i)−H−N(i+3) H-bonds.[11,12] The crystallized oligomers, however, contained α-amino isobutyric acid (Aib) at α positions. We therefore used 2D NMR to characterize the folding of α/β-peptide **E2** in isopropanol (1 mM). Several nuclear Överhauser effects (NOEs) were observed between protons from residues with i,i+3 or i,i+2 spacing (Figure 7). These nonsequential NOEs are consistent with formation of the expected helix secondary structure under the reaction conditions. Further support for the expected helix conformation is provided by a set of i,i+1 NH−NH NOEs. However, the lack of NOEs in the N-terminal segment of **E2** raises the possibility that this portion of the molecule is disordered under reaction conditions.

To explore the possibility, suggested by the NOE analysis, that the two N-terminal β residues in **E2** do not contribute to catalyst efficacy, we examined truncated analogue **E2-T** (Figure 4). This shortened peptide was less effective than **E2** itself in terms of the overall yield of crossed aldol product **2** (46% for **E2** vs. 21% for **E2-T**). However, truncated peptide

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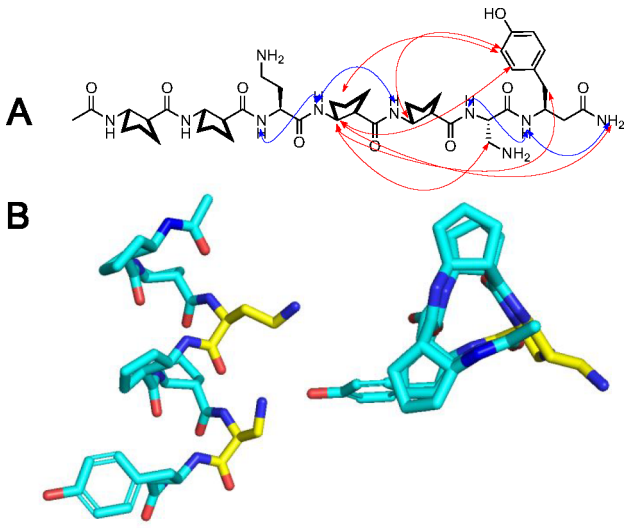

Figure 7. (A) Graphic summary of NOEs observed for α/β-peptide **E2** (1 mM in d_7-iPrOH) between protons from residues that are not adjacent in sequence (red arrows) or between NH groups on adjacent residues (blue arrows). All NOEs are consistent with the expected helical conformation. (B) Model of the expected helical conformation of α/β-peptide **E2** based on the crystal structure of a similar 1:2 α/β-peptide (CSD:685819). The image on the left is a view perpendicular to the helix axis, and the image on the right is the same structure viewed along the helix axis. β residues are cyan, and α residues are yellow. The α/β-peptide crystallized contained 2-aminoisobutyric acid (Aib) residues in the α positions. These residues were replaced by a Dap and a Dab residue to generate the model shown.

was slightly more selective for crossed aldol vs homo-aldol reaction than the full-length peptide. This comparison indicates that the two N-terminal ACPC residues of α/β-peptide **E2** influence catalytic activity despite the lack of NOE evidence that these two residues are involved in helix formation.

Macrocyclization. Results of intermolecular crossed aldol condensations led us to examine α/β-peptide **E2** as a catalyst for macrocyclization of dialdehyde **1b**. With 10 mM dialdehyde and 1 mM **E2**, we obtained a 76% yield of **4**, according to LC-MS analysis (Figure 8). A second, minor product had m/z corresponding to a cyclodimer. With 2.5 mM dialdehyde and 0.25 mM **E2**, the yield of **4** was 92% after 24 h by LC-MS. A reaction conducted under these conditions with 0.13 mmol of **1b** provided **4** in 84% isolated yield after 48 h.

Optimal catalysis of this macrocyclization required a primary amine diad, as established with reactions involving 10 mM **1b** and 1 mM peptide. Use of α/β-peptide **A**, with a secondary amine diad, resulted in relatively little consumption of dialdehyde **1b**, and minimal formation of macrocycle **4** was detected via LC-MS. The α/β-peptide with a primary amine/secondary amine diad (**B**) was much more effective at inducing reaction of dialdehyde **1b**, but a complex product mixture was formed that contained only a modest amount of **4**. Other products appeared to include cyclodimer(s) and linear dimer(s), based on m/z values. α/β-Peptide **C**, which presents a primary amine diad with amine groups on β residue side chains, gave **4** in 66% yield. To our knowledge, it would not have been possible to predict the substantial reactivity differences among the three types of amine diads displayed by these α/β-peptides from the extensive literature on amine-catalyzed aldol condensations.[19−22]

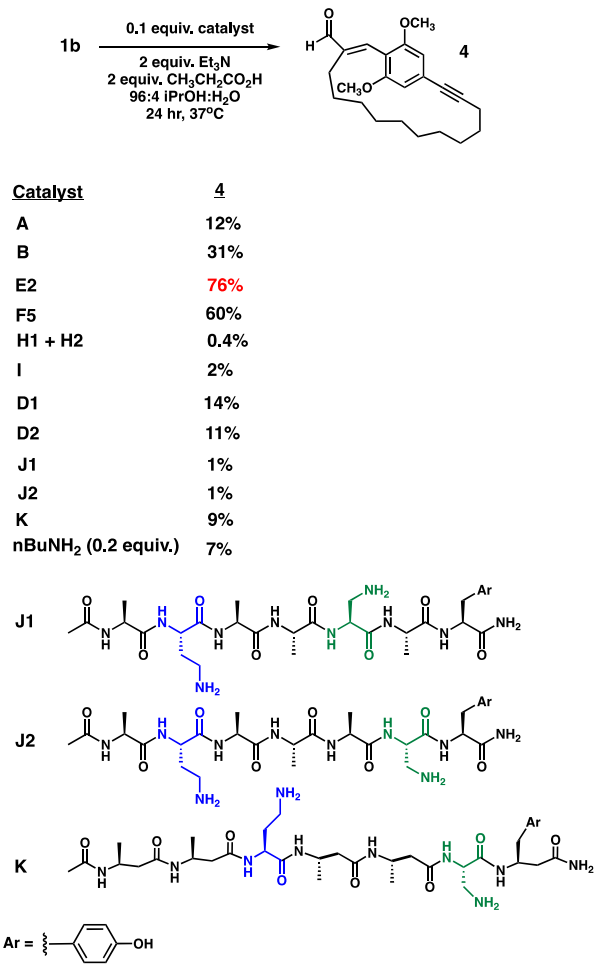

Figure 8. Cyclization of dialdehyde **1b** to form **4** as catalyzed by various peptides. Yields were determined via LC-MS based on external calibration. The structures of most peptides are shown in previous figures. Structures are shown here for peptides with conformationally flexible backbones, composed of either exclusively α-amino acid residues (**J1** and **J2**) or a combination of α and acyclic β residues (**K**).

α/β-Peptide **F5** contains a primary amine diad and matched **E2** in terms of intermolecular crossed aldol yield (Figure 3); however, **F5** was less effective than **E2** in terms of the macrocyclization reaction, providing a 60% yield. The contrast between the intermolecular and intramolecular reactions suggests that deleterious effects of increased side chain flexibility (as in **F5** relative to **E2**) are more pronounced for macrocycle formation than for intermolecular crossed aldol reaction.

To ask whether **E2** serves as a bifunctional catalyst for the macrocyclization of **1b**, we evaluated a 1:1 mixture of monofunctional α/β-peptides **H1** and **H2** (0.1 equiv each). In this case, only a trace amount of **4** was obtained. α/β-Peptide **I**, in which one of the primary amines of **E2** is replaced with an acyclic secondary amine, was a poor catalyst as well.

We used the cyclization of **1b** to evaluate the effect of altering the peptide backbone on bifunctional catalysis by a primary amine diad (Figure 8). Peptides composed entirely of α-amino acid residues have been extensively studied for catalytic activity,[1−6,23−26] and we therefore examined α-peptides **J1** and **J2**. The i,i+3 Dab/Dap spacing in **J1** would cause the two amine groups to be slightly less than one turn

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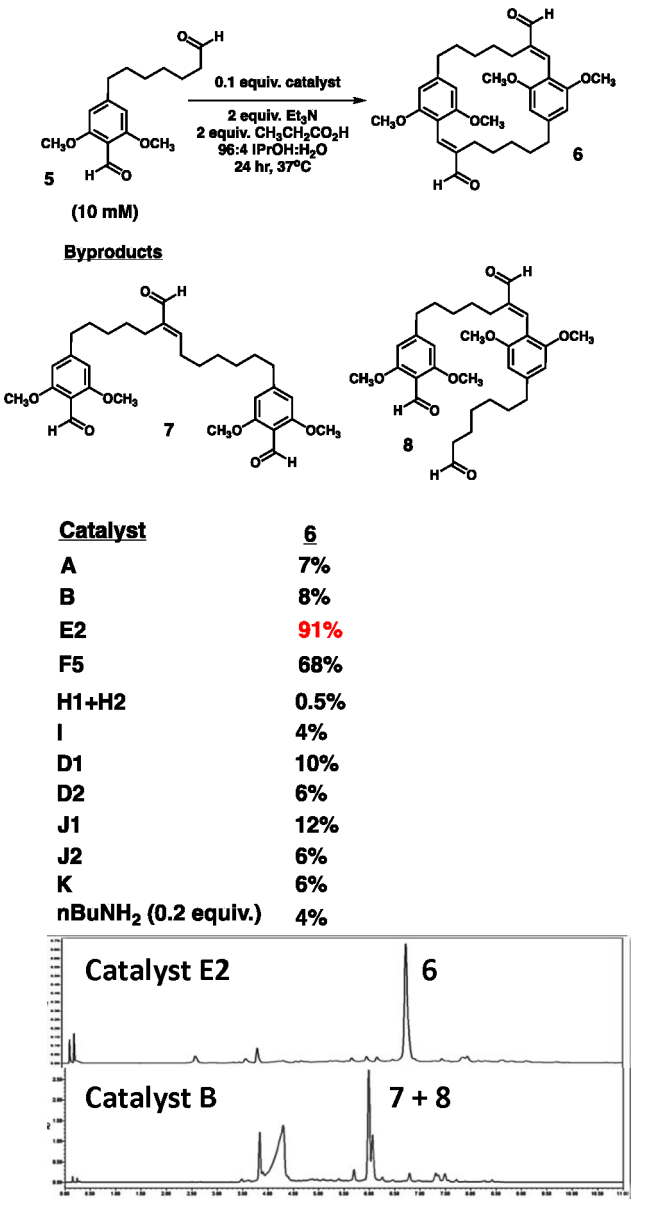
apart in an α-helix (~3.6 residues per turn) and almost exactly one turn apart in a 3_{10}-helix (~3 residues per turn). The *i*,*i*+4 spacing in **J2** would lead to a diamine separation of slightly more than one α-helical turn but would cause misalignment in a 3_{10}-helix. 2D NMR analysis of **J2** in isopropanol revealed several *i*,*i*+3 and *i*,*i*+4 NOEs that are consistent with an α-helical conformation (Figure S30). Both of these α-peptides proved to be extremely poor catalysts, each providing only 1% yield of macrocycle **4**. It is noteworthy that replacing 0.1 equiv of either α-peptide-diamine with 0.2 equiv of *n*-butylamine led to a slightly higher yield of **4** (~7%). It appears that the amine groups in peptide side chains are intrinsically less reactive than a simple amine, which is consistent with prior observations and may reflect a more congested steric environment in peptides.[13]

The failure of the conventional peptides as catalysts for cyclization of **1b** might arise because of imperfect alignment of the primary amine groups or because the backbone is too flexible to support high population of a helical conformation, or from a combination of these two factors. The evidence of helicity provided by 2D NMR of **E2** and **J2** is qualitative and does not allow determination of helix population in either case. To explore the role of helix stability in the observed catalysis, we evaluated α/β-peptide **K**, the analogue of **E2** in which all four preorganized ACPC residues have been replaced by flexible β^3-homoalanine residues. Helix formation should be substantially diminished for **K** relative to **E2**.[27] Consistent with this expectation, no NOEs involving sequentially nonadjacent residues were detected for **K** in isopropanol.

Flexible α/β-peptide **K** proved to be a poor catalyst for macrocyclization of **1b** (Figure 8), although **K** was superior to α-peptides **J1** and **J2** in this regard. We attribute the poor performance of **K** to the greater conformational flexibility relative to **E2**, which is confirmed by differences in 2D NMR data between these two α/β-peptides. Differences in macrocyclization yield among the flexible peptides **J1**, **J2**, and **K** suggest that the diad arrangements provided by either α- or 3_{10}-helices are inferior to the diad arrangement provided by the $\alpha\beta\beta$ foldamer helix.

The substantially lower macrocyclization yield obtained with α/β-peptide **K** relative to α/β-peptide **E2** demonstrates the benefit of local, residue-based conformational stabilization in the development of peptide catalysts. Rings and other sources of local rigidity can be readily incorporated into β-amino acids and higher homologues, but α-amino acids offer very limited prospects for residue-based conformational stabilization.

Cyclodimerization. We turned to a different macrocycle-forming process, cyclodimerization of dialdehyde **5** (Figure 9), to ask whether the reactivity trends manifested in the macrocyclization of **1b** are general. In particular, we wondered whether **E2** would maintain distinctive reactivity relative to α/β-peptides containing different amine diads (**A** and **B**) and whether the preorganized scaffold would retain its superiority relative to more flexible α/β- or α-peptide scaffolds (**K**, **J1**, or **J2**). Conversion of **5** to **6** is more challenging than the cyclization of **1b** because the first intermolecular step must be chemoselective. If an alkyl aldehyde serves as the electrophile at this point (to form **7**), then the cyclodimer will be inaccessible; intermolecular reaction with the aryl aldehyde as electrophile (to form **8**) is a prerequisite for cyclodimerization. Cyclodimer **6** contains the core of the cylindrocyclophane natural product family,[28] but the goal of this study was not to provide a new synthesis of cylindrocyclophanes, for which several creative routes are available.[29−31] Instead, we viewed



Catalyst	6
A	7%
B	8%
E2	91%
F5	68%
H1+H2	0.5%
I	4%
D1	10%
D2	6%
J1	12%
J2	6%
K	6%
$nBuNH_2$ (0.2 equiv.)	4%

Figure 9. Cyclodimerization of dialdehyde **5** to form **6**, as catalyzed by various peptides. In some reactions, significant amounts of **7**, **8**, and/or other products were observed, as described in the text. Yields were determined via LC-MS based on external calibration. The structures of the peptides are shown in previous figures. LC-MS data are shown for representative product mixtures formed with α/β-peptide **E2** or **B**.

the cyclodimerization of **5** as an opportunity to deepen our understanding of the catalytic capabilities of a primary amine diad.

α/β-Peptide **E2** was a very effective catalyst for cyclodimerization of dialdehyde **5** to **6** (Figure 9; 10 mM **5**), while **A** and **B** were poor catalysts, a pattern similar to that found for macrocyclization of dialdehyde **1b** (Figure 8). When this reaction was conducted with catalyst **E2** and 0.14 mmol of **5**, macrocycle **6** was isolated in 86% yield.

The secondary amine diad (**A**) induced little reaction of **5**, while the secondary amine/primary amine diad (**B**) caused extensive reaction of **5** but led to a complex product mixture. LC-MS analysis suggested that byproducts formed in the presence of **B** included **7** and **8**. α/β-Peptide **F5** provided a

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68% yield of cyclodimerization product **6**; the diminution in yield relative to **E2** parallels the trend between these two catalysts in the cyclization of **1b**. This comparison indicates that cyclodimerization is more sensitive to side chain length than is the intermolecular crossed aldol reaction (Figure 3).

The pair of monoamine α/β-peptides, **H1** and **H2**, provided very little of the cyclodimerization product, which is consistent with the hypothesis that **E2** functions as a bifunctional catalyst. Replacing one of the primary amines in **E2** with an acyclic secondary amine (**I**) profoundly hindered catalytic activity, which parallels observations with other aldol reactions. Collectively, these observations support the hypothesis that a diad composed of two primary amines is critical for crossed aldol reactions involving an aryl aldehyde as electrophile.

The α-peptides **J1** and **J2** and flexible α/β-peptide **K** were all poor catalysts for cyclodimerization of **5**, as they had been for macrocyclization of **1b**. These observations strengthen the general conclusion that the $\alpha\beta\beta$ backbone is superior to a conventional all-α backbone for catalysis of macrocyclization via aldol reaction. The benefit of the $\alpha\beta\beta$ backbone appears to arise at least in part from the ability to implement local preorganization via incorporation of cyclic β residues.

CONCLUSIONS

The striking differences in aldol reactivity among different amine diads, as manifested by α/β-peptides **A** vs **B** vs **C**/**E2**, could not have been predicted despite extensive prior study of aldol condensations catalyzed by monofunctional amines.[19−21,32−34] Discovery of these differences depended on the use of a specific foldamer backbone that features an $\alpha\beta\beta$ backbone repeat and preorganized β residues. This molecular scaffold ensures a robust alignment of the catalytic diad. Optimizing crossed aldol reactivity involving an aryl aldehyde as the electrophile required the development of previously unknown α/β-peptides in which the critical primary amine groups are provided by side chains of α-amino acid residues. The α/β-peptide series from which **E2** emerged (Figure 3) revealed a complex relationship among the length of the amine-bearing side chains, overall aldol reactivity, and crossed aldol selectivity. Optimal intermolecular crossed aldol reactivity was observed for two side chain combinations (**E2** and **F5**), but only the combination in **E2** was optimal for macrocyclization of **1b** (Figure 8) or cyclodimerization of **5** (Figure 9).

The catalytic efficacy of α/β-peptide **E2** for macrocyclization of **1b** to generate **4** and for cyclodimerization of **5** to generate **6** depends upon the use of β residues that are preorganized via a five-membered ring. Replacement of these preorganized residues by flexible β residues (as in **K**) caused a dramatic decline in yield for both reactions. Comparable residue-based preorganization strategies are not available for α-amino acid residues, and this distinction suggests that β-amino acid residues and other extended residues offer advantages relative to α residues in efforts to develop peptidic catalysts.

The substantial difference in catalytic efficacy between **E2** (preorganized β residues) and **K** (flexible β residues) correlates with the substantial difference in 2D NMR data: only **E2** displays NOEs characteristic of the expected helix conformation in isopropanol. Since this helix should bring the two amine groups into proximity, because of their $i,i+3$ sequence spacing (Figures 6 and 7), these observations collectively support our hypothesis that helical folding is important for catalytic activity. However, it is very likely that α/β-peptide **E2** explores other conformations in solution, because this molecule contains many backbone bonds that have low-energy barriers to rotation. Nonhelical conformations may play significant roles in the catalytic cycle for the crossed aldol reactions we have explored.

Our results show that proper placement of the components of a catalytic diad on a foldamer scaffold can engender catalytic activity that is inaccessible with simpler potential catalysts, such as *n*-butyl amine, or with α-peptides, such as **J1** or **J2**. The diversity of known foldamer backbones[7−10,13,35,36] offers many geometries for reactive group orientation and should therefore provide a fertile basis for continued exploration of multifunctional catalysis.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.1c11542.

Experimental procedures, calibration curves, LC-MS spectra, NMR data, and MALDI-TOF spectra (PDF)

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Notes

The authors declare no competing financial interest.

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