

Published on Web 07/03/2007

Local and Tunable $n \rightarrow \pi^*$ Interactions Regulate Amide Isomerism in the Peptoid Backbone

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Peptoids, or N-substituted glycine oligomers, are a prominent peptidomimetic class with wide-ranging applications that reflect the diversity of monomers from which they are constructed.¹ Analogous to polyprolines, the peptoid backbone is composed entirely of tertiary amides (Figure 1A), and peptoids with N- α chiral side chains can form polyproline I-like (PPI) helices.² Conformational heterogeneity arising from backbone amide isomerism, however, has complicated the de novo design of additional peptoid structural motifs.² We hypothesized that carbonyl-carbonyl and carbonyl-aromatic $n \rightarrow \pi^*$ interactions could be harnessed to regulate this isomerization. Previous studies have shown that $n \rightarrow \pi^*$ interactions between tertiary prolyl amides $(n \rightarrow \pi^*_{Am})$ can stabilize the *trans*-amides of PPII helices.^{3,4} However, reports of $n \rightarrow \pi^*$ interactions between amides and electron-deficient aromatic rings $(n \rightarrow \pi^*_{A_T})$ and their impacts on oligomer folding are scarce.⁵ Here, we demonstrate not only that competing $n \rightarrow \pi^*_{Am}$ and $n \rightarrow \pi^*_{Ar}$ interactions operate in peptoids but also that α -chiral amide side chains can be used to tune the strengths of these interactions in both the backbone and the side chains. Such interactions have significant implications for peptoid folding and could be exploited for the design of new peptoid architectures.

The $n \rightarrow \pi^*_{Am}$ interaction is characterized by donation of electron density from a carbonyl oxygen lone pair into the π^* orbital of an adjacent amide carbonyl.3 Thus far, studies of these effects have mainly focused on proline derivatives, in which the stereoconstraints imposed by the proline ring play a pivotal role.^{3,6} In peptoids, only trans-amide donors can be stabilized by this interaction, as they satisfy the geometrical requirements for orbital overlap $(O_{i-1} \cdots C_i = O_i)$ distance ≤ 3.2 Å, angle = $109 \pm 10^{\circ}$; Figure 1B). Conversely, an $n \rightarrow \pi^*_{Ar}$ interaction between a backbone carbonyl and proximal side chain aromatic group would exclusively stabilize the cis-amide, again due to the geometrical considerations (carbonyl oxygen to ring centroid distance 2.8-3.8 Å, dihedral angle between aryl and carbonyl planes ≤90°; Figure 1C).^{5d} We hypothesized that competing $n \rightarrow \pi^*_{Am}$ and $n \rightarrow \pi^*_{Ar}$ interactions could be tuned to regulate $K_{\text{cis/trans}}$ in peptoids. We therefore designed a peptoid model system that allowed us to probe for these $n \rightarrow \pi^*$ interactions while mimicking the local environment in a typical N- α -chiral polypeptoid. Both new and previously reported peptoid side chains and C-termini were incorporated (Tables 1 and 2), and their impacts on K_{cis/trans} were assessed by ¹H NMR.^{3,6}

Examination of a series of piperidine-capped, benzyl peptoids (1-4, Table 2) revealed that, relative to pe(3), electron-withdrawing groups on the aromatic ring (*fe* and *np*; **1**, **2**) stabilize the *cis*-amide, suggesting a role for $n \rightarrow \pi^*_{\text{Ar}}$ interactions in these systems. Indeed, pyridinium 4mpy (**8**) afforded an exceptional increase in $K_{\text{cis/trans}}$ (270%) relative to *pe*. In contrast, the bulkier cyclohexyl (*ch*, **4**/**7**) and more electron-rich phenolate (*mph*, **9**) side chains demoted the *cis*-amide rotamer by 40% relative to *pe*. The *para* positioning of the aryl substituents in **2** and **9** excluded a purely steric rationale



Figure 1. (A) Generic peptoid structure. (B) Three-dimensional representations of $n \rightarrow \pi^*_{Am}$ and (C) $n \rightarrow \pi^*_{Ar}$ interactions (indicated by yellow arrows) in the peptoid model systems examined in this study.

Table 1. Structure of the Peptoid Model System and the Structures of the C-Termini and Amide Side Chains Examined in This Study^a



^{*a*} Single stereocenters do not influence $K_{\text{cis/trans}}$ in these systems and are not shown. The *cis*-rotamer was defined as that which orients the side chain and the oxygen atom on the same side of the amide bond.

for these effects; $n \rightarrow \pi^*_{Am}$ interactions between carbonyls in the piperidinyl series were also excluded because removal of the C-terminal carbonyl (methyl amide series 5–9) did not affect the rotameric ratios.

Having investigated the impact of $n \rightarrow \pi^*_{Ar}$ interactions on $K_{cis/trans}$ in these peptoid model systems, we next examined the C-terminal carbonyl in order to probe for $n \rightarrow \pi^*_{Am}$ interactions. We anticipated that dimethyl amides would be less effective than piperidinyl amides in stabilizing positive charge at the nitrogen. Therefore, donation of electron density by the dimethyl amide nitrogen to the carbonyl should be reduced, increasing its acceptance of $n \rightarrow \pi^*_{Am}$ interactions

Table 2. Peptoid Model System Structures, Amide cis/trans Ratios in CD₃CN, and Corresponding Free Energy Differences

peptoid	R ₁	R ₂	$K_{ m cis/trans}{}^a$	$\Delta G_{ m cis/trans}$ (kcal/mol) ^b
1	Pip	fe	3.84 ± 0.19	-0.79
2	Pip	np	3.43 ± 0.19	-0.73
3	Pip	pe	2.04 ± 0.27	-0.42
4	Pip	ch	1.22 ± 0.01	-0.12
5	Мe	np	3.27 ± 0.18	-0.70
6	Me	pe	2.12 ± 0.07	-0.44
7	Me	ch	1.30 ± 0.05	-0.15
8	Me	4mpy	7.82 ± 0.52	-1.22
9	Me	mph	1.25 ± 0.02	-0.13
10	dma	np	3.06 ± 0.01	-0.66
11	dma	pe	1.69 ± 0.14	-0.31
12	dma	ch	0.58 ± 0.06	+0.33
13	MeO	np	1.05 ± 0.01	-0.03
14	MeO	pe	0.67 ± 0.04	+0.24
15	MeO	ch	0.29 ± 0.04	+0.73
16	Pip	fpnan	>10	<-1.4

^a Determined by integrating ¹H NMR spectra of 15 mM solutions at 24 °C. ^b $\Delta G = -RT \ln(K_{\text{cis/trans}})$.



Figure 2. (A) X-ray crystal structure of 16. The $n \rightarrow \pi^*$ interaction is shown as a yellow line with associated angles and distances. The hydrogen bond is shown as a green line. (B) Newman projections depicting the most populated conformations for cis-6 and -8, as indicated by the NOEs shown: red = stronger for 8 vs 6, black = identical for 8 and 6.

and decreasing $K_{\text{cis/trans}}$.^{3a} Examination of a series of such peptoids (10-12, Tables 1 and 2) indeed revealed 10, 20, and 50% reductions in K_{cis/trans} for np, pe, and ch, respectively, compared to the analogous piperidinyl series (2–4). The large decrease in $K_{\text{cis/trans}}$ observed for *ch* suggests that the $n \rightarrow \pi^*_{Am}$ interaction is significantly enhanced by ch relative to pe and np. We speculate that ch sterically enforces a conformational bias that facilitates $n{\rightarrow}\pi^*{}_{Am}$ interactions and stabilizes the trans-amide rotamer. In order to further demonstrate the potential for $n \rightarrow \pi^*_{Am}$ interactions in these systems, we also synthesized a series of C-terminal methyl esters (13-15), which we expected to function as excellent $n \rightarrow \pi^*_{Am}$ interaction acceptors.³ As predicted, the $K_{\text{cis/trans}}$ values for 13–15 were further reduced (50-65%) relative to the dimethyl amide series (10-12), indicating an even stronger $n \rightarrow \pi^*_{Am}$ interaction.

In addition to amides in the peptoid backbone, we discovered that amides in peptoid side chains can also participate in $n \rightarrow \pi^*_{Am}$ interactions (e.g., in peptoid anilide 16, Tables 1 and 2). The transrotamer of 16 appears to be completely suppressed in solution, and its solid-state structure reveals a strong $n{\rightarrow}\pi^*{}_{Am}$ interaction from the N-terminal acetamide to the anilide carbonyl (O····C=O distance and angle of 2.6 Å and 106°, respectively; Figure 2A).7 In order to confirm the conformational plausibility of this side chain to backbone $n \rightarrow \pi^*_{Am}$ interaction in solution, we obtained NOESY NMR spectra for 16. The NOE contact patterns are qualitatively consistent with the solid-state conformation exhibiting the $n \rightarrow \pi^*_{Am}$ interaction. We also obtained NOESY NMR data for cis-6 and cis-8 that provide additional evidence of $n \rightarrow \pi^*_{Ar}$ interactions in these model systems. These data indicate that the conformation in which the aromatic ring eclipses the carbonyl oxygen (Figure 2B) is significantly populated by 8 but not by 6, suggesting that $n \rightarrow \pi^*_{Ar}$ interactions stabilize the cis-amide. Calculations of partial charges for the atoms participating in the $n \rightarrow \pi^*_{Ar}$ interactions suggest that electrostatics do not contribute appreciably to this stabilization.5a Additional calculations of LUMO energies for cis-(5-8) and interaction distances and angles for trans-(13-15) also corroborate the existence and tunability of $n \rightarrow \pi^*$ interactions in these peptoid systems (see Supporting Information).

The data presented herein strongly suggest that $n \rightarrow \pi^*$ interactions play a significant role in controlling peptoid amide isomerism, both in solution and in the solid state. To our knowledge, this work represents the first report of $n \rightarrow \pi^*_{Am}$ interactions outside of prolyl systems and expands their relevance beyond the scope of peptides. Furthermore, we have characterized an $n \rightarrow \pi^*_{Ar}$ interaction that may promote PPI helices in peptoids by stabilizing cis-amides and disrupting hydrogen bonds between the peptoid termini and backbone carbonyls.⁸ The strengths of the $n \rightarrow \pi^*$ interactions can be effectively tuned by careful selection of α -chiral side chains, which can be installed using standard peptoid synthesis methods.^{1,8a} The energetic significance of the interactions (up to 1.4 kcal or greater) is remarkable, considering the flexibility of the peptoid glycine unit relative to proline. Ongoing work in our laboratory is directed toward elucidating the role and significance of $n \rightarrow \pi^*$ interactions in related polypeptoids in order to facilitate the design of new peptoid structures.

Acknowledgment. We thank the NSF (CHE-0449959), Research Corporation, and the Shaw Scientist Program of the Greater Milwaukee Foundation for financial support of this work, Dr. Ilia Guzei for X-ray crystallographic analyses, and Prof. Ronald Raines for numerous helpful discussions. H.E.B. is an Alfred P. Sloan Foundation Fellow.

Supporting Information Available: Peptoid synthesis and characterization, X-ray and NMR data, and calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA071310L