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# *N*-Aminoglycine and Its Derivatives Stabilize PPII Secondary Structure

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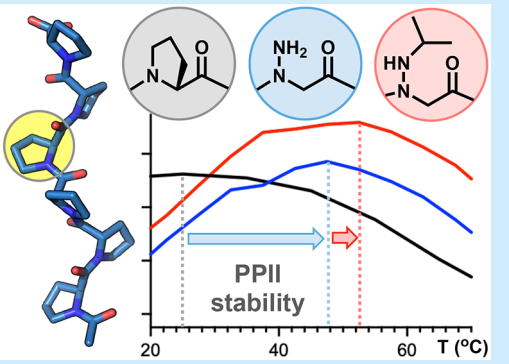
**ABSTRACT:** The identification of unnatural residues that stabilize polyproline type 2 (PPII) folds can aid in the design of peptidomimetics targeting PPII-binding domains. Here, we examine the impact of peptide backbone N-amination on PPII helix stability and find *N*-aminoglycine (aGly) to be an effective PPII promoter. Further derivatization of an aGly-containing peptide affords *N*′-alkylated analogues with increased helical propensity. Backbone N-amination of glycine represents a convenient approach to stabilize PPII conformation and allows for the diversity-oriented synthesis of optimally constrained folds.






The left-handed polyproline type 2 (PPII) helix is a secondary structure motif important for modulating a broad array of biomolecular interactions. Nearly 10% of all residues in known protein structures are found in the PPII conformation.[1] The PPII helix is now accepted as a major ordered component of protein "unfolded" states[2] and is thought to precede the conformational transition of several amyloidogenic proteins into $\beta$-rich fibrils.[3] It is also the major secondary structural feature of the collagen triple helix, the most abundant protein in vertebrates.[4]

PPII helices are defined by $\psi$ and $\phi$ dihedral angles near +150° and −75°, resulting in a rigid structure with precisely 3 residues per turn and a helical pitch of 9.4 Å. Despite the increased propensity for prolyl amide bonds to adopt the less favored *cis* rotamer geometry, the PPII helix is comprised entirely of *trans* amides ($\omega$ = 180°). Unlike $\alpha$-helices and $\beta$-sheets, PPII folds are not stabilized by inter-residue H-bonds involving the backbone NH, even when comprised of nonproline residues. Recent studies have identified through-space electron delocalization events and their solvation-mediated enhancement as key factors in enforcing PPII structure.[5]

The stabilization of PPII folds through covalent constraint or substitution with nonproline residues remains a challenge. The identification of PPII-promoting residues that both increase unnatural amino acid content and provide opportunities for further peptide diversification will enable the design of new PPII mimics. Toward this end, $\gamma$-substituted prolines, often derived from naturally occurring 4-hydroxyproline, have been employed as diversifiable surrogates in PPII model systems.[5a,6] Replacement of proline with select peptoid (*N*-alkylated glycine) and azapeptide residues has also recently been shown to stabilize PPII secondary structure.[7] Our interest in the effect of amide N-heteroatom substitution on peptide folding prompted us to investigate the PPII propensity of $\alpha$-hydrazino acids. Beyond its impact on the local conformation, backbone amide-to-hydrazide replacement introduces a nucleophilic handle amenable to chemoselective diversification in aqueous environments. Here, we demonstrate that *N*-aminoglycine promotes PPII helicity in a secondary structure model and that late-stage derivatization can be used to identify analogous residues with even higher PPII propensity.

To assess the impact of $\alpha$-hydrazino acids on PPII structure, we employed an 8-residue peptide reported by Brown and Zondlo[8] which is, in turn, based on the longer polyproline model system originally developed by Creamer and co-workers.[9] This host−guest peptide features a pentaproline core, two flanking glycine residues, and a C-terminal tyrosine to facilitate quantification by UV. Substitution of the central proline (Pro4) for other residues breaks the ordered core into

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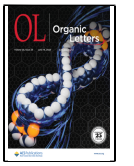




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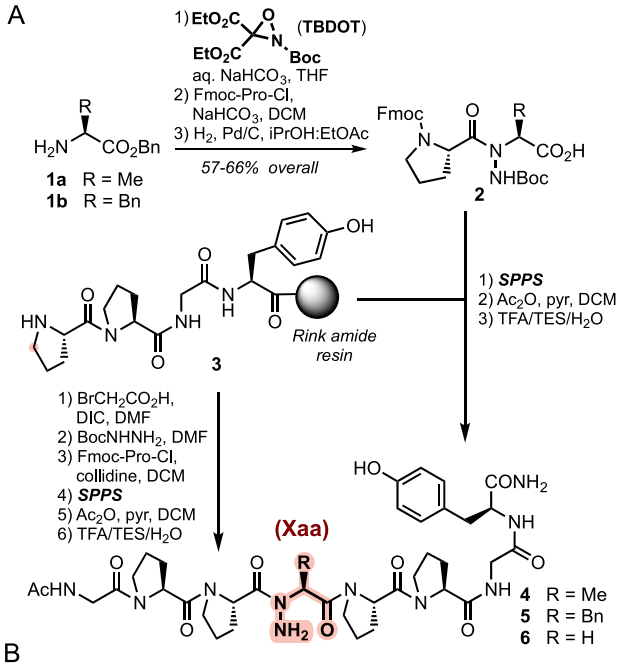
two diproline segments. This 8-residue model is particularly sensitive to the guest since a stable PPII fold generally requires a minimum of 3 contiguous prolines.[10] We synthesized peptides in which Pro4 was substituted for *N*-aminoalanine (aAla), *N*-aminophenylalanine (aPhe), or *N*-aminoglycine (aGly). Synthesis of the aAla- and aPhe-containing peptides commenced with the 3-step conversion of amino esters **1** into dipeptide building blocks **2** via electrophilic amination, condensation with Fmoc-protected proline acid chloride, and benzyl ester hydrogenolysis (Figure 1A). These dipeptide




B

| peptide | Xaa | $[\theta]_{228}$ | $\lambda_{max}$ | ρ |
|---|---|---|---|---|
| **4** | aAla | 2200 | 228 | 0.08 |
| **5** | aPhe | 4000 | 225 | 0.25 |
| **6** | aGly | 4000 | 227 | 0.15 |
| **7** | Pro | 3100 | 228 | 0.10 |
| **8** | Ala | 1900 | 227 | 0.08 |
| **9** | Phe | 3200 | 224 | 0.33 |
| **10** | Gly | 1000 | 228 | 0.05 |

**Figure 1.** (A) Synthesis of host−guest PPII octapeptides **4**−**6**. (B) CD data obtained for **4**−**10** at 150 $\mu$M in aqueous buffer (5 mM sodium phosphate, 25 mM KF). $[\theta]_{228}$ values are reported to 2 significant digits.

building blocks were incorporated into the growing peptide by standard Fmoc SPPS. The N-terminus was acetylated prior to cleavage, and the crude peptides were purified by RP-HPLC to afford **4** and **5**. The aGly analogue (**6**) was synthesized via displacement of a resin-bound bromacetamide with *tert*-butyl carbazate. We also prepared the parent Pro4 peptide (**7**) as well as the corresponding Ala4 (**8**), Phe4 (**9**), and Gly4 (**10**) controls for comparison with backbone N-aminated derivatives.

All peptides were analyzed by far-UV circular dichroism (CD) spectroscopy. Pro-based peptides that adopt a PPII conformation exhibit a characteristic negative band at ∼204 nm and a smaller but distinct positive band at ∼228 nm corresponding to $\pi \rightarrow \pi^*$ and n $\rightarrow \pi^*$ electronic transitions, respectively.[11] The intensity of the positive band at 228 nm has been used to assess the stability of the PPII fold in this and related peptide model systems.[8,9,12] While the intensity of the negative band near 204 nm is not strongly correlated to PPII stability, a high maximum/minimum band strength ratio ($\rho$) may indicate a distortion of canonical PPII conformation or a change in backbone solvation.[13] Figure 1B depicts molar ellipticities at 228 nm ($[\theta]_{228}$, in deg·cm$^2$·dmol$^{-1}$), wavelengths of maximum ellipticity ($\lambda_{max}$ in nm), and band strength ratios ($\rho$) for all synthesized peptides.

As shown in Figure 2A, the CD spectrum of aAla peptide **4** exhibited a diminished positive molar ellipticity relative to parent peptide **7**. Ala substitution (**8**) resulted in even more pronounced destabilization of the PPII helix. Aromatic residues are known to be disruptive to PPII folds due in part to an enhanced population of the *cis* amide rotamer.[8] In agreement with previous studies, Phe analogue **9** exhibited a noticeable blue shift in $\lambda_{max}$ and a significant increase in band strength ratio ($\rho$ = 0.33). Here, aPhe substitution (**5**) appears to partially restore native-like structure (Figure 2B). Substitution of the central Pro residue in **7** for Gly (**10**) severely destabilized the PPII fold as evidenced by the near total loss of a positive Cotton effect (Figure 2C). However, N-amination of this largely unstructured peptide resulted in remarkable enhancement of molar ellipticity at 228 nm ($[\theta]_{228}$ = 4000 for **6** vs 1000 for **10**). The apparent helicity of **6** also exceeded that of parent peptide **7**, suggesting aGly to be a stabilizing Pro surrogate in the PPII fold. This result is particularly interesting considering the *de*stabilizing effect of sarcosine (Sar) recently demonstrated in the same PPII model system.[7]

We previously invoked increased 1,3-allylic strain as a factor contributing to the propensity for non-Gly $\alpha$-hydrazino acids to adopt $\beta$-sheet-like $\psi$ and $\phi$ dihedral angles.[14] This effect is also observed in peptide tertiary amides and is dependent on substitution at C$\alpha$ as well as the amide N.[15] Accordingly, *N*-methylalanine is known to be particularly disruptive to the PPII fold,[7] presumably due to a strong preference for extended

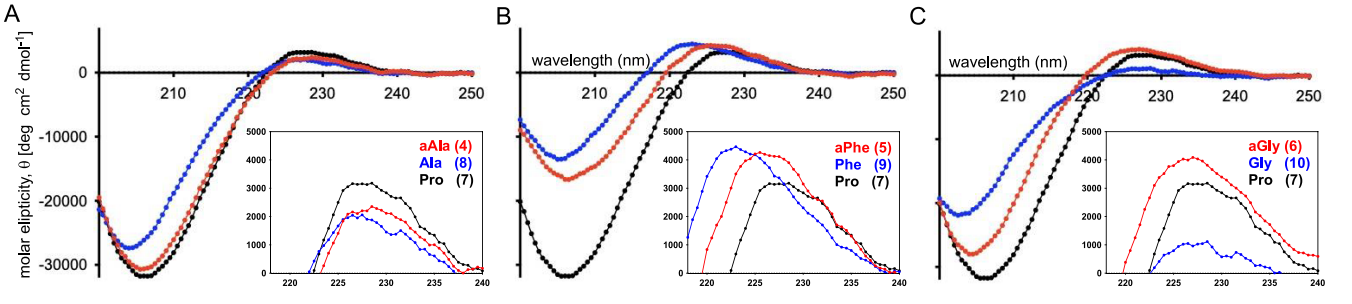



**Figure 2.** CD spectra of peptides **4**−**10** at 150 $\mu$M in aqueous buffer (5 mM sodium phosphate, 25 mM KF).



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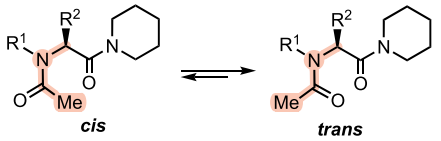
backbone conformations. The trend with *N*-amino derivatives thus mirrors that of *N*-methylation: while the C$\alpha$-substituted Ala guest is less disruptive to PPII than Gly, the combination of C$\alpha$ *and* N substitution generally disfavors PPII conformation.

We considered the possibility that aGly may promote PPII through simple enhancement of the *all-trans* amide population relative to peptide tertiary amides such as Pro. This impact on the $\omega$ *trans*/*cis* equilibrium ratio in *N*-amino peptides stems from lone-pair repulsion between the hydrazide $NH_2$ group and the preceding carbonyl O.[16] To investigate this, we obtained rotamer ratios for a series of *N*-acetyl piperidinyl amide monomers in $D_2O$ (Table 1). Although we previously

**Table 1. Equilibrium Trans/Cis Amide Rotamer Ratios in $D_2O$ (Derived from $^1H$ NMR Peak integration)**




| Compound | $R^1$ | $R^2$ | $K_{trans/cis}$ |
|---|---|---|---|
| **11** Ac-aAla-NPip | $NH_2$ | Me | 12.1 |
| **12** Ac-(N-Me)Ala-NPip | Me | Me | 7.8 |
| **13** Ac-aGly-NPip | $NH_2$ | H | 2.3 |
| **14** Ac-Sar-NPip | Me | H | 1.9 |
| **15** Ac-Pro-NPip | $-CH_2CH_2CH_2-$ | | 3.0 |
| **16** Ac-Ala-NPip | H | Me | >99 |
| **17** Ac-Gly-NPip | H | H | >99 |

observed that N-amination of Ala (**11**) results in markedly higher *trans* amide bias relative to its *N*-methyl analogue (**12**),[14] we found that the same is not true for aGly. Surprisingly, **13** exhibited a *trans* propensity similar to that of Sar (**14**) and less than that of Pro (**15**). The enhanced PPII helicity of **6** relative to **7** can therefore not be attributed to effects on preferred $\omega$ geometry.

The electron-withdrawing effect of the backbone $NH_2$ group in *N*-amino peptides results in a lower barrier of rotation for the $\omega$ bond and greater electrophilicity of the preceding carbonyl carbon.[16] Given the importance of $n_O \rightarrow \pi^*_{C=O}$ interactions in stabilizing PPII folds,[17] we expected that N-amination of the *i*+1 residue would enhance the $\pi^*_{C=O}$ acceptor capacity of the *i* residue. However, this would be accompanied by compromised donor capacity of the *i* residue $n_O$ electrons (Figure 3A). To explore this further, we synthesized peptides **18** and **19**, which feature N-amination of the Gly residues flanking the ordered polyproline core (Figure 3B). In the case of **18**, the *N*-amino substituent should perturb the N-terminal n $\rightarrow$ $\pi^*$ interaction by lowering acetyl $n_O$ donor capacity without providing any $\pi^*$ acceptor benefit (no preceding residue donor). In peptide **19**, the same substituent should lower the $\pi^*_{C=O}$ energy level of the C-terminal Pro without impacting a carbonyl donor in the ordered core. As shown in Figure 3C, N-terminally modified peptide **18** exhibited reduced PPII helicity relative to the parent structure. However, N-amination of Gly7 (peptide **19**) enhanced PPII folding, beyond even that of aGly4 analogue **6**. These data suggest that the PPII-promoting effect of aGly is largely attributable to enhancement of $\pi^*_{C=O}$ acceptor capacity and that placement of aGly at the C-terminus of a PPII motif may result in an optimal stabilizing effect.

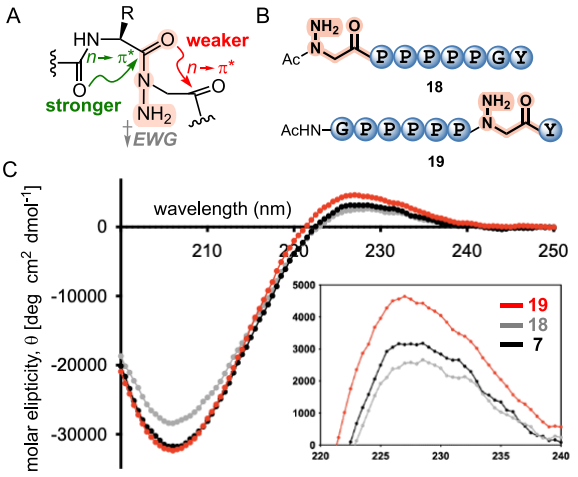
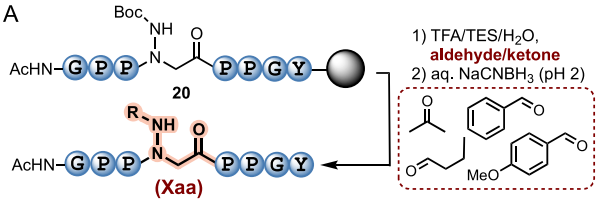



**Figure 3.** (A) Impact of amide N-amination on electron delocalization. (B) N- and C-terminally modified peptides **18** and **19**. (C) CD spectra of **18** and **19**.

We next leveraged the reactivity of hydrazides to prepare a series of functionalized derivatives of the PPII model sequence. Intermediate resin-bound peptide **20** was thus subjected to cleavage and deprotection in the presence of aldehydes/ketone to generate the crude hydrazones (Figure 4A). Addition of sodium cyanoborohydride in aqueous buffer provided alkylated peptides **21**−**24** following RP-HPLC purification. We discovered that *N*′-acylation of the Boc-protected hydrazide in **20** could be achieved by treatment with acetic anhydride




B

| peptide | Xaa | $[\theta]_{228}$ | $\lambda_{max}$ | ρ |
|---|---|---|---|---|
| **21** | iPr-aGly | 4700 | 227 | 0.18 |
| **22** | Bu-aGly | 4200 | 227 | 0.17 |
| **23** | Bn-aGly | 2800 | 228 | 0.21 |
| **24** | PMB-aGly | 6800 | 227 | 0.41 |
| **25** | Ac-Gly | 3000 | 225 | 0.15 |
| **26** | $CF_3CO$-aGly | 6600 | 225 | 0.37 |

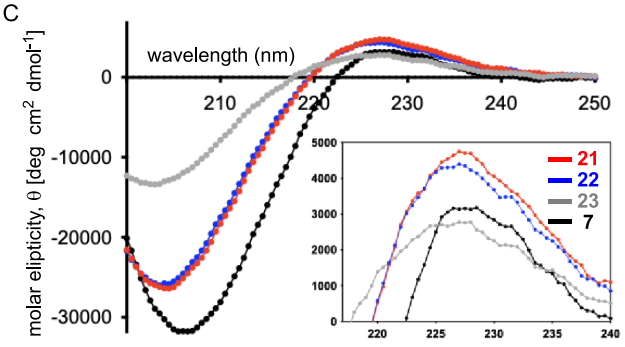



**Figure 4.** (A) Late-stage derivatization of hydrazide **20**. (B) CD data obtained for **21**−**26** at 150 $\mu$M in aqueous buffer (5 mM sodium phosphate, 25 mM KF). $[\theta]_{228}$ values are reported to 2 significant digits. (C) CD spectra of **21**−**23**.



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and pyridine in DMF (rather than DCM) to generate **25** following cleavage and purification (see the Supporting Information). We also found that TFA-mediated cleavage of **20** in the absence of aldehyde/ketone additives produced a significant amount of trifluoroacetylated byproduct **26** in addition to **6**. We isolated **26** for comparison to N-acetylated and N-alkylated derivatives by CD spectroscopy.

As shown in Figure 4B,C, peptides featuring *N*′-isopropyl-*N*-aminoglycine (iPr-aGly; **21**) and *N*′-butyl-N-aminoglycine (Bu-aGly; **22**) residues at position 4 showed considerable enhancement of PPII helicity relative to **6** and **7**. In contrast, incorporation of a central *N*′-benzyl-*N*-aminoglycine residue (Bn-aGly; **23**) resulted in significant disruption of the PPII fold. Although the analogues harboring *N*′-(*p*-methoxy)benzyl-*N*-aminoglycine (PMB-aGly; **24**) and *N*′-trifluoroacetyl-*N*-aminoglycine ($COCF_3$-aGly; **25**) residues exhibited intense positive molar ellipticities at 228 nm, their unusually high $\rho$ values suggest deviation from canonical PPII conformation (Figure 3B). Acylated derivatives **25** and **26** also featured noticeable blue shifts in $\lambda_{max}$, similar to those observed for aPhe and Phe analogues **5** and **9** (Figure S2).

To further support the helicity trends obtained from maximum molar ellipticity measurements, we carried out variable-temperature CD on *N*-amino peptides **6** and **21** as well as the Pro4 parent sequence (**7**). Each of the peptides demonstrated two-state denaturation behavior, with isodichroic points at or near 214 nm (Figure S3). Obtaining accurate melting temperatures ($T_m$) from PPII models is notoriously difficult due to noncooperative transition that involves desolvation followed by gradual unfolding of the helix.[5a,6d,13] Previous studies with short polyproline sequences have thus resorted to estimation of $T_m$ from analysis of first derivatives ($\delta[\theta]/\delta T$) across the temperature range.[5a,6d] Using the method described by Horng and Raines,[5a] in conjunction with average minimum CD band intensities (202−204 nm), the Pro4 control peptide was found to have a $T_m$ of ∼25 °C (Figures 5A and S4). This value is consistent with the previously reported melting temperatures of Ac-$(Pro)_9$-$NH_2$ ($T_m$ ∼ 23 °C)[6d] and H-$(Pro)_{10}$GlyTyr-OH ($T_m$ ∼ 27 °C).[5a] The analogues harboring aGly4 (**6**) and iPr-aGly4 (**21**) residues exhibited markedly enhanced thermal stability, with estimated $T_m$ values of 47.5 and 52.5 °C, respectively. As expected, nonlinear curve-fitting was not possible for peptides **6** and **7** due to the lack of plateau regions corresponding to their fully folded states. However, iPr-aGly4 analogue **21** exhibited a discernible melting transition when molar ellipticity changes were monitored near the maximum band wavelength (226−228 nm). Sigmoidal regression analysis yielded a $T_m$ value of 53.8 °C, which is in close agreement with the value estimated from the first derivatives. These data demonstrate that substitution of Pro4 for aGly or iPr-aGly leads to a considerable increase in PPII thermal stability. Notably, incorporation of a single iPr-aGly affords a helix with stability exceeding that reported for a nonapeptide composed entirely of PPII-promoting 4-hydroxyproline residues (H-$(Hyp)_9$-$NH_2$).[6d]

In summary, we have carried out the first conformational investigation of $\alpha$-hydrazino acids in a model PPII fold. In contrast to its C$\alpha$-substituted analogues, aGly was found to stabilize the PPII conformation in an 8-residue guest−host peptide. Our studies suggest that enhanced helicity is the result of stronger electronic delocalization upon substitution of Pro for aGly. An important feature of aGly is the ability to carry out late-stage chemoselective reactions on the pendant hydrazide $NH_2$. We demonstrate that some *N*′-alkylated aGly derivatives, readily obtained via reductive alkylation in aqueous solution, further enhance PPII helicity relative to the unsubstituted parent residue. This work sets the stage for diversity-oriented conformational screening of *N*-amino peptides in the context of more complex PPII domains, including triple helical collagen. More broadly, we expect this approach will enable the rapid identification of $\alpha$-hydrazino acid residues that stabilize other biologically relevant folds.

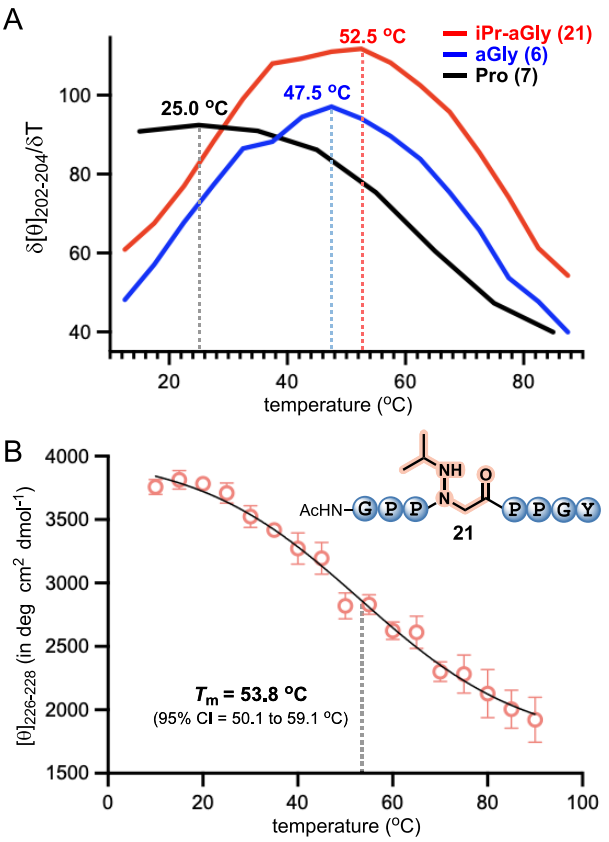


**Figure 5.** (A) Plot of rate-of-change in minimum molar ellipticity for **6**, **7**, and **21** as a function of temperature. Melting temperatures are estimated from maximum first derivative ($\delta[\theta]/\delta T$) values. (B) Plot of maximum molar ellipticity for **21** as a function of temperature. Melting temperature and 95% confidence interval are derived from sigmoidal 4-parameter regression analysis (black curve).

## ■ ASSOCIATED CONTENT

### Data Availability Statement

The data underlying this study are available in the published article and its Supporting Information.

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.3c01502.

Supplementary figures, detailed experimental procedures, characterization data for novel compounds, copies of RP-HPLC, and NMR spectra (PDF)



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### Notes

The authors declare no competing financial interest.

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