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2,3-Methanoamino acid analogs of Arg stabilize secondary structures of a 13 amino acid peptide in aqueous solution

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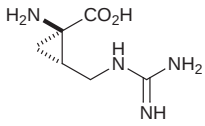
Peptidomimetics 1 and 2 of RN24 (an RNase A *C*-peptide analog) in which the Arg$^+$-10 residue is replaced by 2*R*,3*S*-cyclo-Arg′ and by 2*S*,3*S*-cyclo-Arg′, respectively, show less temperature dependence in CD studies than the parent peptide.

The *N*-terminal fragment of RNase A has been adopted as a paradigm for conformational studies of short helical peptides. For instance, CD and NMR studies of a succinimidyl-capped analog of the *C*-peptide, RN24, indicate this 13-mer is *ca*. 50% helical in aqueous buffer at around 3 °C.[1,2] One of the key intramolecular interactions that is thought to stabilize this helical ensemble of conformations is a salt bridge between Glu$^-$-2 and Arg$^+$-10.[3,4] Syntheses of two 2,3-methanoarginine stereoisomers, 2*R*,3*S*-cyclo-Arg′ and 2*S*,3*S*-cyclo-Arg′,[5] gave us a unique opportunity to manipulate the Glu$^-$-2/Arg$^+$-10 interaction by constraining the guanidine functionality to point towards the *C*- and *N*-termini, respectively. Here we report the syntheses of these RN24 peptidomimetics, and CD studies to elucidate their conformational stabilities.

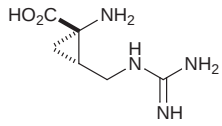
succ-A^{1}E^{2}T^{3}A^{4}A^{5}A^{6}K^{7}F^{8}L^{9}R^{10}A^{11}H^{12}A^{13}-amide RN24

succ-A^{1}E^{2}T^{3}A^{4}A^{5}A^{6}K^{7}F^{8}L^9(*2R,3S*-cyclo-R)^{10}A^{11}H^{12}A^{13}-amide **1**

succ-A^{1}E^{2}T^{3}A^{4}A^{5}A^{6}K^{7}F^{8}L^9(*2S,3S*-cyclo-R)^{10}A^{11}H^{12}A^{13}-amide **2**



2*R*, 3*S*-cyclo-Arg'
or 2*R*, 3*S*-cyclo-R'



2*S*, 3*S*-cyclo-Arg'
or 2*S*, 3*S*-cyclo-R'

Peptidomimetics **1** and **2** were prepared *via* stepwise couplings of Fmoc-amino acid derivatives[6] on Rink's amide resin[7] using a manual shaker system.[8] Typical conditions and side-chain protecting groups were used. Couplings of natural amino acids were performed by premixing the amino acid with *N*-methylmorpholine, HOBt and PyBOP[9] in DMF. This coupling protocol was modified to incorporate the hindered cyclo-Arg′ residues and the amino acid immediately following (Leu-9). For these couplings, acid fluorides were produced *in situ via* the reagent TFFH (*i.e.* tetramethylfluoroformadinium hexafluorophosphate)[10] with HOAt (1-hydroxy-7-azabenzotriazole)[11] as an activating agent. The coupling to incorporate the cyclo-Arg moieties required only 1 h, whereas the subsequent coupling was more difficult and was run for 12 h. Deprotection of the side chains and cleavage from the resin was performed using TFA and a mixture of scavengers (phenol, ethane-1,2-dithiol and thioanisole). The crude peptide was further purified by preparative RP-HPLC.† Overall yields of isolated materials were in the 10% range giving enough sample for CD studies but not for NMR analysis.

Fig. 1(*a*) compares the CD spectra obtained for RN24, and the peptidomimetics at 3 °C (pH 5.1 buffer, 1 mM in each of sodium citrate, sodium phosphate and sodium borate, was used throughout this study). Peptide/peptidomimetic concentrations were accessed by calibration of the UV absorbance at 212 nm. These data show that the two peptidomimetics adopt helical conformations, but these are less populated than for RN24. With regards to the shape of the spectra, the 2*R*,3*S*-cyclo-Arg′ derivative **1** had an accentuated negative ellipticity at 222 nm relative to a classical α-helix. The other peptidomimetic, **2**, had a CD spectrum with a shape like that of RN24.

Variable temperature CD spectra of the peptidomimetics were particularly informative. The stability of the helical ensemble can be related directly to the change in the helical CD signal with temperature. Relative changes in $[\theta]_{222}$ requires the use of only one peptide solution, thus eliminating the error in the absolute peptide concentration and the variability between peptides. Fig. 1(*b*) is an overlay of five CD spectra for RN24 recorded at 5 °C intervals.‡ The molar ellipticity at 222 nm steadily decreased as the temperature was raised, ultimately corresponding to a *ca*. 40% reduction of the helical character. However, for peptidomimetics **1** and **2** the loss was significantly less over the same temperature range. Estimates for the loss of helical character for these two compounds were 30 and 23%, respectively.

Molecular dynamics simulations of RN24 and the two peptidomimetics was performed. Briefly, CHARMm parameters and coordinates for an ideal α-helix were modified using data sets already developed for the 2,3-methanoarginine analogs.[12] A medium of relative permittivity (ε = 80), and a simulated temperature of 276 K was used throughout. Trajecto-

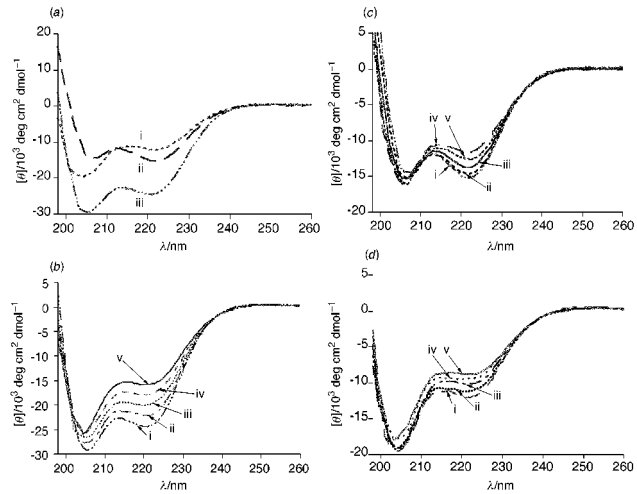
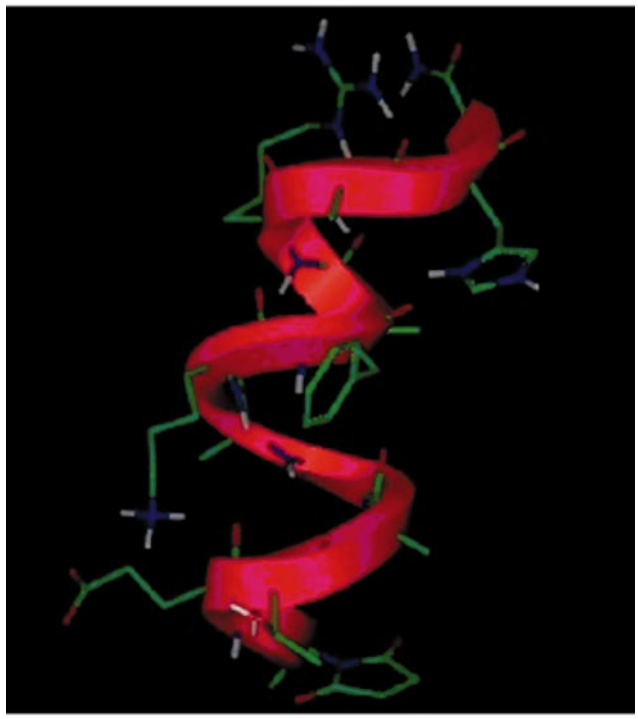


Fig. 1 (*a*) CD of (i) 2*S*,3*S*-cyclo-Arg′ **2**, (ii) 2*R*,3*S*-cyclo-Arg′ **1** and (iii) RN-24. Variable temperature CD of (*b*) RN-24, (*c*) **1** and (*d*) **2** at (i) 3, (ii) 8, (iii) 13, (iv) 18 and (v) 23 °C.

(*a*)



(*b*)

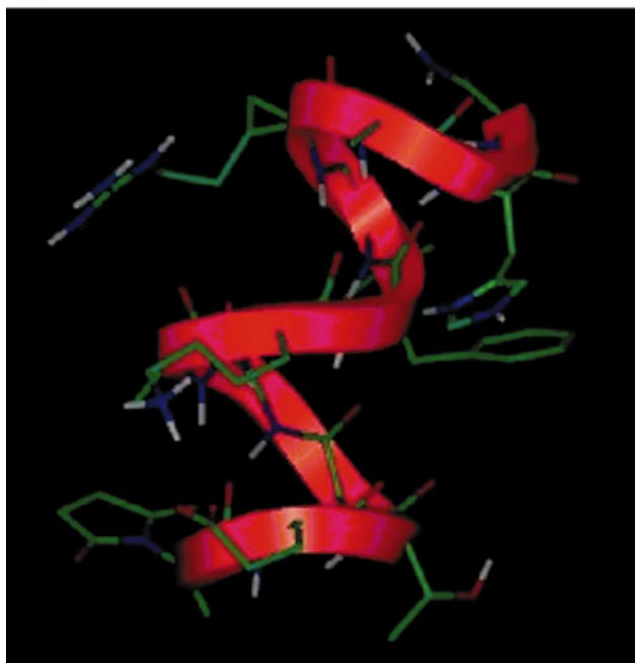


Fig. 2 Illustrative conformers after 200 ps of molecular dynamics for peptidomimetics (*a*) **1** and (*b*) **2**.

ries for the three starting structures sampled over a 200 ps interval showed that helical conformations were maintained for all three compounds throughout the dynamics run; Fig. 2 shows representative snap-shots of peptidomimetics **1** and **2**, respectively. These demonstrate a general trend observed in the molecular simulations, *i.e.* that peptidomimetic **1** tended to favor more tightly wound helical conformers than the 2*S*,3*S*-cyclo-Arg′-containing peptidomimetic **2**.§

In conclusion, we propose that the rigidity of the 2,3-methano analogs of arginine in peptidomimetics **1** and **2** can be used to impart conformational constraints. In this study, CD spectra of peptidomimetics containing these protein amino acids surrogates showed less temperature variations than that of RN24, indicative of a more stable helical structure. We also suggest that these same constraints distort the helical conformations such that atypical CD spectra were observed. The 2*R*,3*S*-cyclo-Arg′-containing peptidomimetic **1** has the Arg-side chain oriented towards the *C*-terminus where it cannot interact with the Glu-2 side-chain [Fig. 2(*a*)]. However, the guanidinium group locked in this orientation reinforces the helix dipole.[13] Conversely, 2*S*,3*S*-cyclo-Arg′ in peptidomimetic **2** presents the same side-chain in such a way that its charge opposes the helix dipole. The guanidinium moiety in this compound is oriented towards the Glu-2 residue, but the salt bridge is disrupted relative to RN24 because the cyclo-Arg′ has one less side chain methylene than natural arginine. The latter two effects result in less perfect helical conformations for peptidomimetic **2** than for **1** [Fig. 2(*b*)]. Other substitutions of 2,3-methanoamino acids into the RNase A *C*-peptide sequence are being investigated in these laboratories.[14]

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Notes and references

† HPLC conditions: Vydac C18 (22 mm × 25 cm, 10 μm) column with a linear solvent gradient; A = 0.1% TFA in H_2O, B = 0.1% TFA in MeCN; flow rate 6 ml min^{-1}; gradient 5–20% B in A over 60 min. MALDI-MS data: RN24 [M+H_2O], calc. 1455.73, found 1455.91; **1** [M+H_2O], calc. 1454.47, found 1454.80; **2**, calc. 1454.47, found 1454.73.

‡ Concentrations (mM) used in the CD studies as determined by UV analysis at 210 nm: RN24: 1.12×10^{-2}; **1**: 1.14×10^{-2}; **2**: 1.18×10^{-2}.

§ Over the 200 ps period, 200 structures in the dynamics run were sampled; in the latter 100 ps interval, when the structures were adequately equilibrated, nearly all the conformers sampled showed the same fundamental helical characteristics as shown in Fig. 2. This MD experiment was run three times in slightly different ways, and essentially the same results were obtained.

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