Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Molecular Structure 655 (2003) 59–68
Journal of MOLECULAR STRUCTURE
www.elsevier.com/locate/molstruc

Cyclotetrapeptides with alternating D-Ala residues: synthesis and spectroscopic studies

Maria Ngu-Schwemlein[a,*], Zhe Zhou[b], Toni Bowie[a], Rebecca Eden[a]

[a]*Department of Chemistry, University of South Alabama, Mobile, AL 36688, USA*
[b]*Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, USA*

Received 2 January 2003; revised 10 March 2003; accepted 18 March 2003

Abstract

Three cyclotetrapeptides, c[Leu-D-Ala-Xaa-D-Ala], where Xaa is Leu (**P1**), Lys (**P2**) and Glu (**P3**) were synthesized and studied by ^{1}H and ^{13}C NMR and CD spectroscopy. These cyclotetrapeptides exhibit similar coupling constants, $^{3}J_{HNH\alpha}$, in the range of 8.56–9.93 Hz, commonly observed for β-turn structures. All amide proton chemical shifts for **P1**, **P2** and **P3** exhibited linear dependence on temperature with moderate temperature coefficients ranging from −3.1 to −9.8 ppb/K. Amide proton signal broadening was observed for all residues in **P1**, **P2** and **P3**, indicating that they are solvent accessible. The number of resonance observed for **P1** was half of the total counts, indicating a C2 symmetric conformation. **P2** and **P3** exhibit similar CD in solvents of varying dielectric constants and dilutions, with characteristic positive CD bands at ca. 210 and 222 nm, which correspond to a β-turn type structure. Small CD/temperature effect was also observed with isodichroic points, consistent with conformational stability and a well-populated cyclotetrapeptide energy state. These heterochiral cyclotetrapeptides consisting of alternating D-Ala residues adopt stabilized open β-turn conformations and may be useful as a ligand template for further functionalization.

Keywords: Cyclotetrapeptides; Circular dichroism; Nuclear magnetic resonance spectroscopy; Beta structure

1. Introduction

Small cyclopeptides have been reported to exhibit interesting biological activities that are frequently more specific and of higher efficacy than their linear analogues. This is generally attributed to the inherent conformational constraints experienced by the small cyclic peptide, which enhances selective molecular interactions and recognition as opposed to that experienced by their relatively more flexible linear congeners. Some naturally occurring cyclopeptides that exhibit biological activities includes the cytotoxic tetrapeptides, Tentoxin [1] and the AM and HC toxins [2–4], the antibiotic dodecapeptide, Gramicidin S [5] and the ionophoric depsipeptide, Valinomycin [6]. In general, macrocyles are attractive template for attaching donor atoms for selective ligand–substrate interactions. Moreover, the size of the macrocycle can be tailored to encapsulate substrates of varying sizes as demonstrated by the studies conducted on the crown ethers and aza crowns [7,8].

* Corresponding author. Tel.: +1-251-460-7424; fax: +1-251-460-7359.
E-mail address: mngu@usouthal.edu (M. Ngu-Schwemlein).

0022-2860/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0022-2860(03)00207-2

The cyclotetrapeptide scaffold, a conformationally rigid 12-membered macrocyclic structure with four donor oxygen atoms and four nitrogen atoms, offers structural and chemical features that are favorable for metal ion chelation. In addition, the ease in chemical modification of the peptide backbone offers additional versatility in changing the donor atoms, allowing the design of macrocyclic derivatives with selectivity for different types of metal ions based on the ligand preferences and rates of complex formation of individual metal ions. Moreover, a cyclotetrapeptide can be synthesized, incorporating the natural amino acid, Trp. The indole heterocycle of Trp can function as an intrinic fluorophore to signal metal ion binding. Additionally, chromophoric groups can be covalently and strategically attached to the macrocyclic template to act as a signal for ligand–substract interactions. Therefore, fluorophore tagged cyclopeptides and their derivatives are attractive candidates as chemosensors.

We are interested in studying the secondary structural preference and stability of small cyclopeptides containing D-Ala residues to explore the potential of using the heterochiral cyclopeptide as a ligand template for attaching fluorophores. The incorporation of alternating D-residues should enhance the stability of the peptide backbone towards hydrolysis by proteases. Additionally, heterochiral peptide sequences, compared to their homochiral analogues, have been shown to have a greater tendency to form β-turns, which are stable under a variety of environments [9,10]. In this paper, we describe the synthesis and spectroscopic studies of three cyclotetrapeptides containing D-Ala residues and functionalized side chain groups for fluorophore attachment.

2. Experimental

2.1. Materials and methods

All chemicals were obtained from commercial suppliers and used without further purification. All spectra were obtained on peptide samples prepared in $(CD_3)_2SO$ or $CDCl_3/CF_3CD_2OH$ (8:1), 5 mg/ml, on a Bruker 300 spectrometer, with TMS as an internal standard. Sequential assignments of all ^1H and ^{13}C resonances were accomplished by through bond connectivities from HSQC and HMBC spectra. Dihedral angles (ϕ) were calculated from the Karplus equation [11], $^3J_{HNH\alpha}$ (Hz) $= A\cos^2\theta + B\cos\theta + C$, where $A = 6.4$, $B = -1.4$ and $C = 1.9$, and $\theta = |\phi - 60^\circ|$ [12]. Temperature dependence of the amide hydrogen chemical shifts was studied for every 10 K increment from 303 to 353 K.

MALDI-TOF mass spectra were recorded on a Perseptive Biosystem MALDI-TOF mass spectrometer at Louisiana State University. The matrix compound was 2,5-dihydroxybenzoic acid (DHB). Crude cyclotetrapeptides were purified on a Vydac C-4 reversed-phase column (300 Å). The mobile phase was H_2O/0.1% trifluoroacetic acid, TFA (A), and CH_3CN (50%)/isopropanol (50%)/0.1% TFA (B) delivered by the Rainin Dynamax HPLC system with UV monitoring at 220 nm. CD measurements were carried out on an Aviv 62DS Spectrophotometer. Spectra were recorded using 1 mg/ml solutions of **P2** and 0.5 mg/ml solutions of **P3** by sampling every 1 nm with an averaging time of 1 s, employing a 0.1 cm path length quartz cell. DPC in methanol was prepared as 1 mM solutions. The spectra were smoothed by the Savitzky–Golay algorithm.

2.2. General method employed for the preparation of *P1-3*

Cyclotetrapeptides **P1-3** were prepared by convergent synthesis as shown in Fig. 1 for **P1**. The linear tetrapeptides were synthesized using the N^{α}-*t*-Boc/C^{α}-*O*-phenacyl ester protecting group combination. Amino acid and peptide couplings were mediated by 1-hydroxy-7-azabenzotriazole(HOAT)/1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI)/*N*-methylmorpholine (NMM) in dichloromethane or by *N*-{(dimethylamino)-*H*-1,2,3-triazolo[4,5-b]pyridin-1-ylmethylene}-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU)/collidine in dimethylformamide following the procedure reported earlier [13]. Head-to-tail cyclization of the tetrapeptides was accomplished by cyclization of the pentafluorophenyl (Pfp) ester of the linear precursor, Leu-D-Ala-Xaa-D-Ala-*O*-Pfp, using the Schmidt method [14]. The crude peptides were purified by semi-preparative reversed-phase HPLC and characterized by MALDI-MS. The details are given below and Tables 1–3 shows the ^1H and ^{13}C NMR assignments for each cyclotetrapeptide.

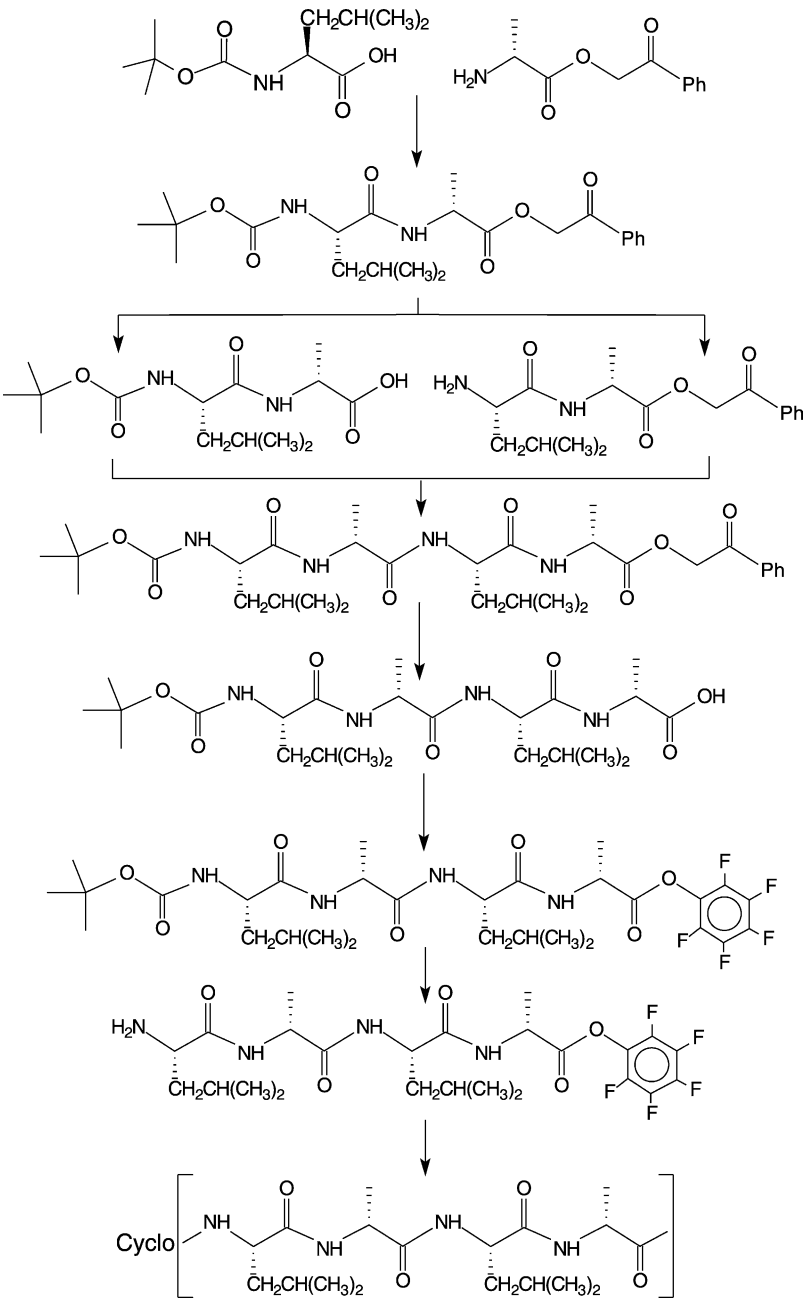

Fig. 1. Synthesis of **P1** by convergent approach.

2.3. *Cyclo[Leu-D-Ala]$_2$* ***P1***

Convergent approach to tetrapeptide preparation: coupling of the protected D-Ala and Leu, with HOAT/EDCI/NMM in CH_2Cl_2 gave the dipeptide, *t*-Boc-Leu-D-Ala-*O*-phenacyl ester (81%). Reaction of half of the dipeptide with Zn in AcOH gave *t*-Boc-Leu-D-Ala-OH, and deprotection of the second half with TFA/CH_2Cl_2 (7:3) gave TFA.Leu-D-Ala-*O*-phenacyl ester. Coupling of these dipeptides with

Table 1
^{1}H and ^{13}C Chemical Shifts (ppm) of P1 in $CDCl_3/CF_3CD_2OH$

Residue	H^N	H^α	H^β	H^γ	H^δ
Leu-1,3	6.65	4.36	1.56	1.56	0.89/0.92
Ala-2,4	6.75	4.43	1.29		
Residue	C=O	C^α	C^β	C^γ	C^δ
Leu-1,3	173.7	50.9	37.0	24.7	22.5/22.1
Ala-2,4	174.1	48.4	13.7		

HOAT/EDCI/NMM in CH_2Cl_2 gave the tetrapeptide in 81% yield. C-terminal deprotection with Zn in AcOH, followed by transesterification with pentafluorophenyl trifluoroacetate, and treatment with TFA/CH_2Cl_2 (7:3) gave TFA.Leu-D-Ala-Leu-D-Ala-*O*-Pfp (85%, over 3 steps).

Cyclization of the tetrapeptide: a solution of TFA.Leu-D-Ala-Leu-D-Ala-*O*-Pfp (0.13 g, 0.2 mmol), DMAP (0.16 mmol) in anhydrous dioxane (200 ml) and trifluoroethanol (4.5 ml) was treated at 85 °C with a solution of pyridine (3 ml) in dioxane (100 ml), over 17 h. After a further 4 h of stirring, the solution was concentrated under reduced pressure. The residue was dissolved in chloroform and extracted with 10% HCl, sat. NaCl, sat. $NaHCO_3$, H_2O, sat. NaCl, and dried over Na_2SO_4 and evaporated. Recrystallization from chloroform and methanol (1:1) gave fine white needles of cyclo[Leu-D-Ala-Leu-D-Ala] (24 mg, 33%). The characterization of this hydrophobic cyclotetrapeptide by optimized MALDI-MS techniques has been described [15]. MALDI-MS $[M + Na]^+$/392 (calculated 392.6).

Table 2
^{1}H and ^{13}C chemical shifts (ppm) of P2 in $(CD_3)_2SO$

Residue	H^N	H^α	H^β	H^γ	H^δ	H^ϵ
Leu-1	7.14	4.36	1.43	1.45	0.88/0.84	
Ala-2	8.30	4.39	1.12			
Lys-3	7.21	4.24	1.61/1.49	1.26/1.17	1.52	2.76
Ala-4	8.25	4.40	1.12			
Residue	C=O	C^α	C^β	C^γ	C^δ	C^ϵ
Leu-1	173.0	50.9	38.2	24.1	22.5/22.1	
Ala-2	172.8	46.9/46.8	14.1/14.0			
Lys-3	172.7	52.3	28.9	22.1	26.7	38.5
Ala-4	172.6	46.9/46.8	14.1/14.0			

Table 3
^{1}H and ^{13}C chemical shifts (ppm) of P3 in $(CD_3)_2SO$

Residue	H^N	H^α	H^β	H^γ	H^δ
Leu-1	7.23	4.2	1.44	1.44	0.87/0.84
Ala-2	8.16	4.3	1.12		
Glu-3	7.27	4.2	1.75	2.16	
Ala-4	8.05	4.3	1.12		
Residue	C=O	C^α	C^β	C^γ	C^δ
Leu-1	172.94	50.98	38.2	24.2	22.6/22.1
Ala-2	173.02	47.0/47.1	14.3/14.2		
Glu-3	172.45	51.72	24.6	29.7	173.76
Ala-4	172.69	47.0/47.1	14.3/14.2		

2.4. *Cyclo[Leu-D-Ala-Lys-D-Ala]* ***P2***

Cyclo[Leu-D-Ala-Lys-D-Ala] was purified on a Vydac C-4 column (10 × 250 mm^2, 300 Å, 5 μm), to give a white fluffy peptide. Conditions were 5% B to 15% B over 35 min with a flow rate of 5 ml/min. RP-HPLC $t_r = 27.1$ min. MALDI-MS $[M + H]^+$/384 (calculated 384.6). $[M + Na]^+$/405 (calculated 405.6).

2.5. *Cyclo[Leu-D-Ala-Glu-D-Ala]* ***P3***

Cyclo[Leu-D-Ala-Glu-D-Ala] was purified on a Vydac C-4 column (10 × 250 mm^2, 300 Å, 5 μm), to give a white fluffy peptide. Conditions were 10% B to 25% B over 30 min with a flow rate of 5 ml/min. RP-HPLC $t_r = 18.7$ min. MALDI-MS $[M + H]^+$/385 (calculated 385.5). $[M + Na]^+$/406 (calculated 406.5).

3. Results and discussion

3.1. Synthesis

The preparation of the pentafluorophenyl ester derivatives of the tetrapeptides were readily conducted using the general N^α-*t*-Boc/C^α-*O*-phenacyl ester protecting groups [13]. This convergent approach allows for the mixing and matching of dipeptides for [2 + 2] fragment couplings to prepare the tetrapeptide analogs. Head-to-tail cyclization of the tetrapeptide to form the constrained 12-membered ring was conducted under dilute conditions to avoid

cyclodimerizarion [16]. Although cyclization of these tetrapeptides is facilitated by the presence of D-Ala, which stabilize turn structures, this reaction turned out to be the yield-limiting step for the preparation of these three cyclotetrapeptides. The hydrophobic side chain protecting group (N^{ϵ}-2-Cl-CBZ or O^{γ}-Bzl) of the cyclopeptide precursors was removed by hydrogenation over Pd/C in trifluoroethanol. Although **P1** was readily purified by crystallization, the pure peptide was insoluble in most common solvents. **P2** and **P3** did not readily crystallize and were purified by reversed-phase HPLC.

3.2. NMR studies

Sequential assignments of all ^{1}H and ^{13}C resonances for **P1** (Table 1), **P2** (Table 2) and **P3** (Table 3) were accomplished by through bond connectivities from HSQC and HMBC spectra. The ^{1}H and ^{13}C resonances for **P1** were half of the total counts, indicating that the peptide macrocylic ring adopts a C2 symmetry. The chemical shifts of the amide proton and the carbonyl carbon of Ala are more deshielded than that of Leu. The low field resonance observed for the amide proton of Ala in P2 and P3, (8.1–8.3 ppm) parallels that reported for the NH of the i + 3 residue (~8 ppm) in type II β-turns [17]. In order to investigate the dipolar couplings between protons, which are within 3 Å of each other in the low energy conformation of the peptide, NOESY as well as ROESY spectra were acquired. Relatively strong cross peaks between all four amide protons, NH(i), and the alpha proton of the adjacent residue, $H_{\alpha}(i+1)$, are observed in the NOESY and ROESY spectra of **P1**, **P2** and **P3** (Fig. 2), which is indicative of a cyclotetrapeptide backbone consisting of trans peptide bonds. Unfortunately, no meaningful quantitative distance geometrical values can be derived from these spectra at this time since the distance between any two protons in these peptides cannot be unambiguously assigned. An analogue of these peptides containing an indole ring is in preparation and the results of the distance geometry analysis will be reported then.

The $^{3}J_{HNH\alpha}$ coupling constants for **P1**-**3** are as shown in Table 4. All four residues in each cyclotetrapeptide exhibit a relatively large coupling constant value ranging from 8.56 to 9.93 Hz. This supports a well-populated low energy cyclotetrapeptide backbone conformation since inter-converting multiple conformations would have yielded an average coupling constant of lower value (<7 Hz) [17]. Phi angle (ϕ) constraints from $^{3}J_{HNH\alpha}$ values, as calculated from the Karplus equation, yielded similar values for all three cyclopeptides. These large phi angle values (Table 4) are typically observed in peptides adopting β-turn structures. However, the interpretation of the above computed phi angles for structure analysis must be taken with caution as low level conformational averaging is not precluded from the observed $^{3}J_{HNH\alpha}$ coupling constants values.

To further investigate the secondary structure of this cyclotetrapeptide scaffolding, the temperature dependence of the amide protons were studied by conducting variable temperature ^{1}H NMR experiments at 10 K intervals from 303 to 353 K. All amide proton chemical shifts for **P1**, **P2** and **P3** exhibited linear dependence on temperature, suggesting no significant conformational changes occurred as the temperature is raised. The coefficient for the change in chemical shift per degree K for each amide proton is shown in Table 4. The chemical shifts of the non-exchangeable protons did not exhibit any significant temperature dependence. In general, coefficients greater than 3 ppb/K indicate solvent exposed amide protons [18]. The chemical shift of the amide protons of Ala residues (range from −3.1 to −3.7 ppb/K) have a moderate, and slightly lower temperature dependence than that of the other residues (−5.1 to −9.8 ppb/K) in the cyclotetrapeptides. The intermediate temperature coefficients observed for Ala residue do not exclude the possibility of their participation in weak or transient intramolecular or intermolecular hydrogen bonding. However, amide proton signal broadening was observed for all amide protons (Fig. 3) indicating that they are solvent accessible.

3.3. CD studies

P2 and **P3** showed similar CD spectra with characteristic positive CD bands at ca. 190, 210 and 222 nm and negative bands at ca. 200 and 238 nm (Fig. 4a and b). The CD spectra of these heterochiral peptides show a marked negative, red shifted, $n\pi^{*}$

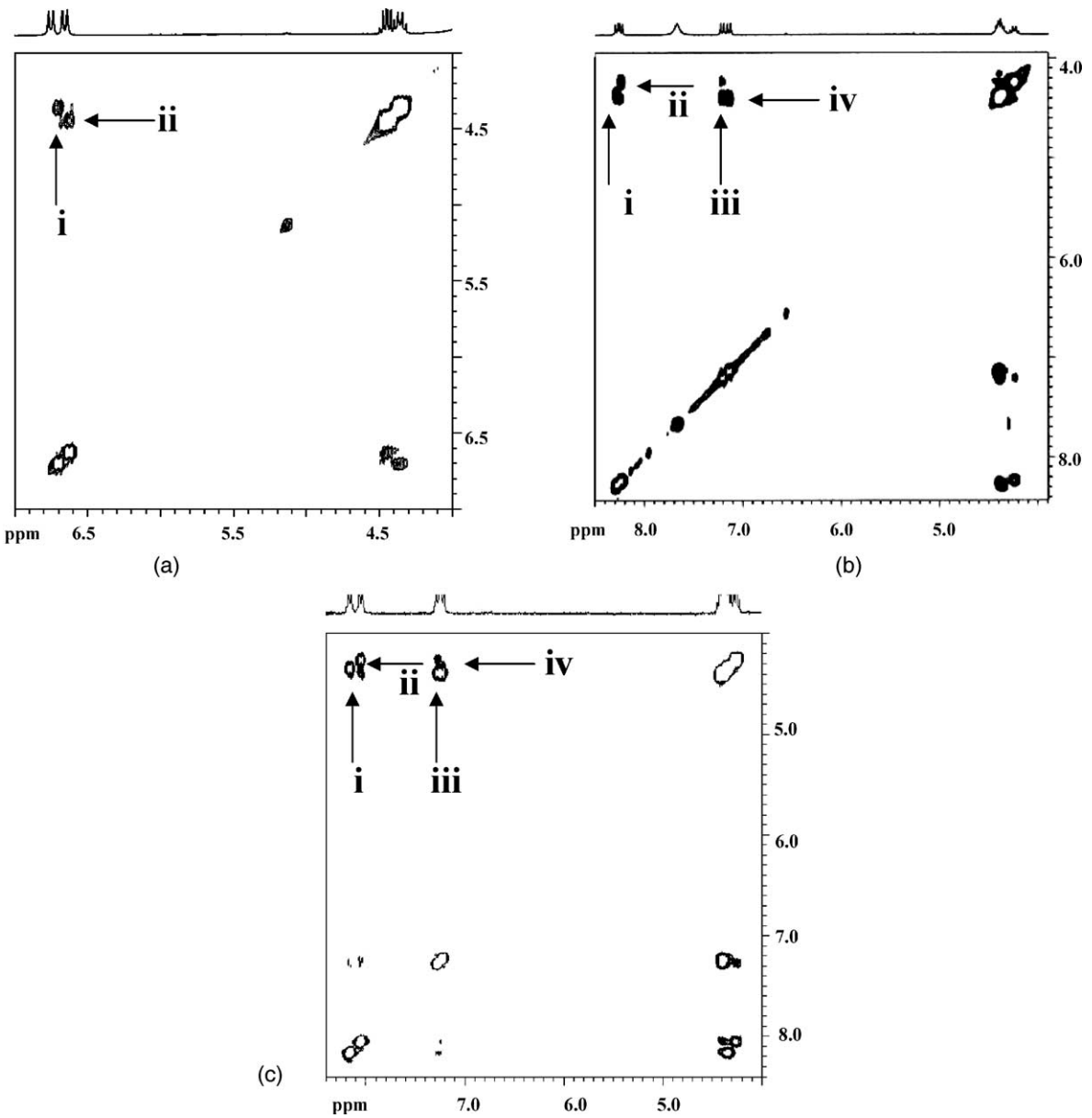

Fig. 2. (a) ROESY spectrum of **P1**, (i) $NH_{Ala} \leftrightarrow H_{\alpha(Leu)}$: (ii) $NH_{Leu} \leftrightarrow H_{\alpha(Ala)}$, (b) NOESY spectrum of **P2**, (i) $NH_{Ala2} \leftrightarrow H_{\alpha(Leu1)}$: (ii) $NH_{Ala4} \leftrightarrow H_{\alpha(Lys3)}$: (iii) $NH_{Lys3} \leftrightarrow H_{\alpha(Ala2)}$: (iv) $NH_{Leu1} \leftrightarrow H_{\alpha(Ala4)}$, and (c) ROESY spectrum of **P3**, (i) $NH_{Ala2} \leftrightarrow H_{\alpha(Leu1)}$: (ii) $NH_{Ala4} \leftrightarrow H_{\alpha(Glu3)}$: (iii) $NH_{Glu3} \leftrightarrow H_{\alpha(Ala2)}$: (iv) $NH_{Leu1} \leftrightarrow H_{\alpha(Ala4)}$.

band at 238 nm which is attributable to the contribution by the D-Ala residues. Two positive $n\pi^*$ bands at 210 and 222 nm resembles the mirror image of the α-helix similar to that reported for peptides turns containing the sequence L-Pro-D-Xaa [19]. The $\pi\pi^*$ transitions occur as a moderately strong positive band at ca. 190 nm and a negative, red shifted, band at 200 nm. The general CD profile of both peptides corresponds to a β-turn type structure. Comparative data for **P1** was not obtained because it was insoluble in common solvents. Relatively small CD/temperature effect is observed for **P2** and **P3**, accompanied by

Table 4
$^{3}J_{HNH\alpha}$ coupling constants and temperature coefficients of amide chemical shifts

Cyclopeptide	Residue				
		Leu-1	Ala-2	Xaa-3	Ala-4
P1	$^{3}J_{HNH\alpha}$	9.93	9.52	9.93	9.52
	ϕ (±20°)	−120°	−110/ −130°	−120°	−110/ −130°
	$-\Delta\delta/\Delta T$ (ppb/K)	3.1	5.1	3.1	5.1
P2	$^{3}J_{HNH\alpha}$	9.44	8.74	9.50	8.84
	ϕ (±20°)	−110/ −130°	−100/ −140°	−110/ −130°	−100/ −140°
	$-\Delta\delta/\Delta T$ (ppb/K)	3.2	9.4	3.1	9.8
P3	$^{3}J_{HNH\alpha}$	9.45	8.56	9.47	8.82
	ϕ (±20°)	−110/ −130°	−100/ −140°	−110/ −130°	−100/ −140°
	$-\Delta\delta/\Delta T$ (ppb/K)	3.7	8.9	3.5	9.0

moderate decrease in molar ellipticity at higher temperatures, with common isodichroic points at 205, 226 and 246 nm, which is consistent with conformational stability and a single well-populated cyclotetrapeptide energy state. These peptides exhibit similar circular dichroism spectra in solvents of different polarity (Fig. 5a). Decrease in solvent polarity is accompanied by a decrease in molar ellipticity, with a relatively larger decrease of the positive band at 210 nm. Conversely, a moderate increase in molar ellipticity at 210 and 222 nm is observed following peptide dilution in water (Fig. 5b).

The heterochiral cyclotetrapeptide CD is independent of the change of one side chain of the L-amino acid from Lys (**P2**) to Glu (**P3**). It is also almost independent of solvent type and concentration, unlike the solvent dependent cyclotetrapeptides reported by Tamaki et al. [20]. This result is consistent with previous reports that β-turn structures are stable in various conditions, including strong solvents. Gierasch et al. has reported that intramolecular hydrogen bond does not play a major role in stabilizing β-turn structures, although it is necessary to maintain γ-turn structures [17]. The above CD data show that there is

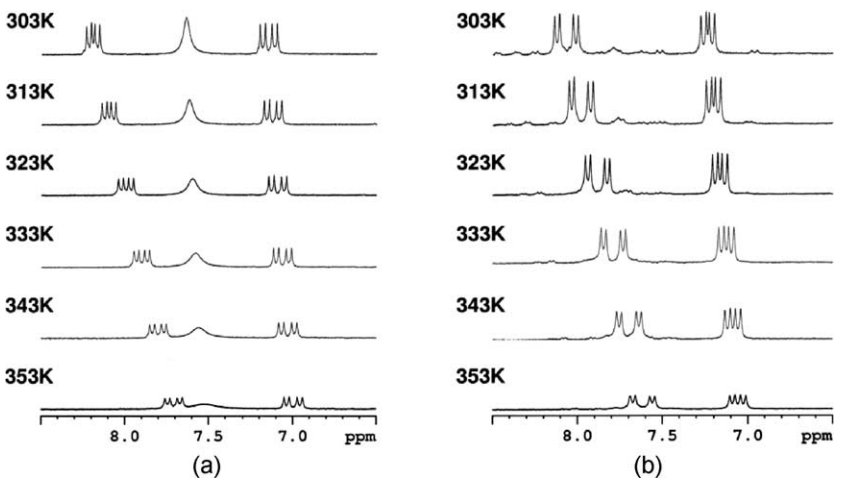

Fig. 3. Amide proton region of the ^{1}H NMR spectra of **P2** and **P3**-temperature shifts and line broadening.

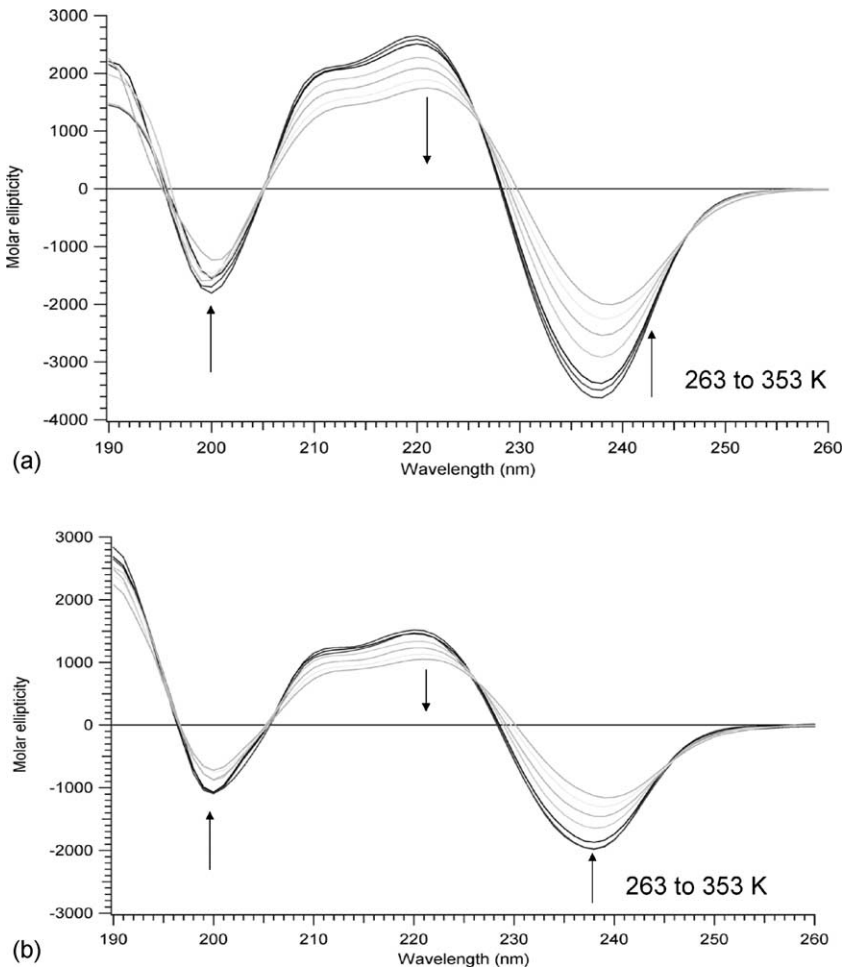

Fig. 4. Variable Temperature CD spectra of (a) **P2**, and (b) **P3** in trifluoroethanol/water (1:1), from 263 to 353 K at 10 K increments as indicated by arrow.

no remarkable difference in the conformations of these cyclotetrapeptides when the solvent, concentration or one of the L-amino acid is varied.

4. Conclusion

We have reported the synthesis of three cyclotetrapeptides consisting of alternating D-Ala residues. Nuclear magnetic resonance and circular dichroism spectroscopy showed that these cyclotetrapeptides with alternating D-Ala residues are structurally similar, with a well-populated cyclotetrapeptide backbone energy-state, which resembles an open (not stabilized by intramolecular hydrogen bondings) β-turn structure. The large coupling constant values, $^{3}J_{HNH\alpha}$, supports a preferred peptide backbone structure with considerable rigidity. Consistent with the general parallel observed for preferred conformations of cyclized turn models, peptides containing defined inserts of alternating D-Ala residues maybe useful in initiating this variant β-turn structure. Additionally, cyclotetrapeptides with such a motif maybe useful as a molecular template for attaching chemical groups or chromophores, with applications in molecular recognition or chemosensing studies. We propose that this stable heterochiral cyclotetrapeptide backbone structure could be particularly useful for further

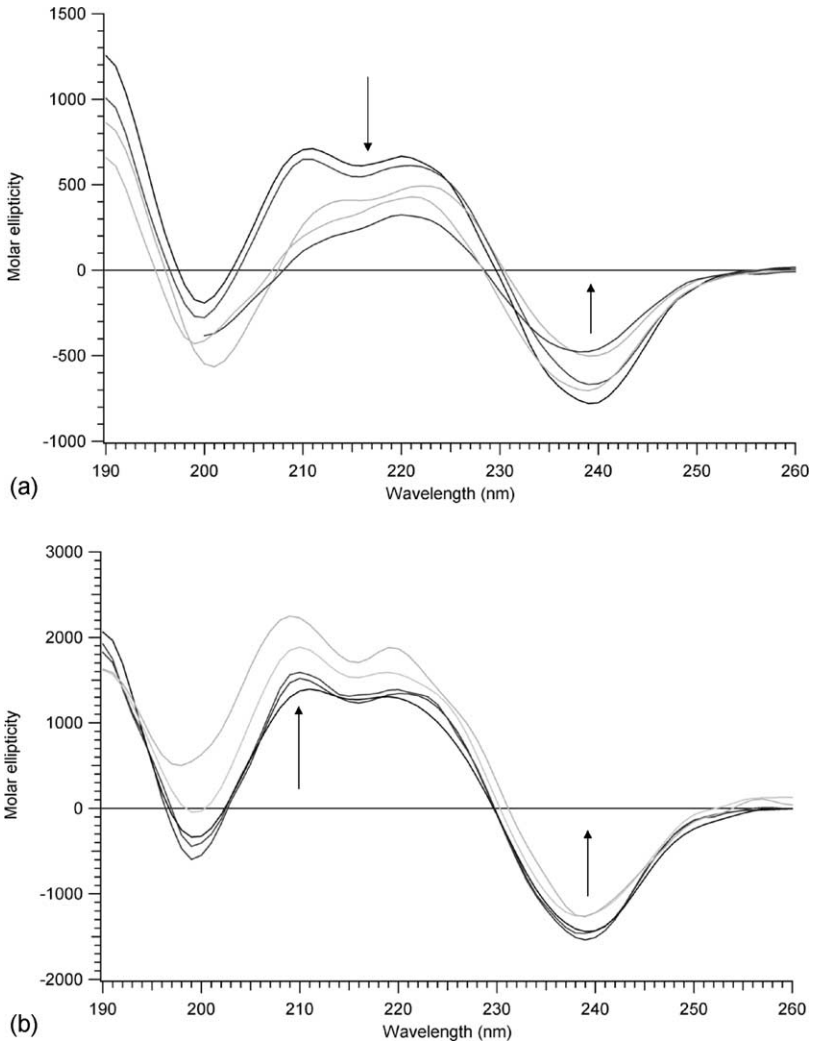

Fig. 5. CD spectra of (a) **P2** in water, 1 mM DPC, methanol, TFE, TFE/THF (1:1), and; (b) **P3** at 3.5, 0.7, 0.35, 0.17, 0.08 mM in water, sequentially as indicated by arrow.

functionalization and might be useful for the development of peptide-based ligands.

Acknowledgements

This work was supported by the National Science Foundation, grant # CHE 0071632. We also wish to express our appreciation to Kari GreenChurch and Steven Macha of Louisiana State University for the MALDI TOF mass spectra. We acknowledge helpful discussions with Dr. Karl Markos of Tulane University.

References

[1] M. Santolini, C. Minoletti, J.M. Gomis, C. Sigalat, F. Andre, F. Haraux, Biochemistry 41 (2002) 6008–6018.
[2] H. Otani, K. Kohmoto, M. Kodama, Can. J. Bot. 73 (1995) S453–S458. Suppl. 1 A–D.
[3] M. Miyashita, T. Nakamori, T. Murai, T. Yonemoto, H. Miyagawa, M. Akamatsu, T. Ueno, C. Naturforsch, Z. Naturforsch. C 56 (2001) 1029–1037.

[4] Z. Jiang, M.O. Barret, K.G. Boyd, D.R. Adams, A.S.F. Boyd, J.G. Burges, Phytochemistry 60 (2002) 33–38.
[5] E.J. Prenner, R.N.A.H. Lewis, R.N. McElhaney, Biochim. Biophys. Acta 1462 (1999) 201–221.
[6] W.L. Duax, J.F. Griffin, D.A. Langs, G.D. Smith, P. Grochulski, V. Pletnev, V. Ivanov, Biopolymers 40 (1996) 141–155.
[7] C.J. Pedersen, J. Inclus. Phenom. Mol. Recognit. Chem. 12 (1992) 7–10.
[8] J.S. Bradshaw, R.M. Izatt, Acc. Chem. Res. 30 (1997) 338–345.
[9] C.M. Wilmot, J.M. Thorton, J. Mol. Biol. 203 (1988) 221–232.
[10] C.M. Venkatachalam, Biopolymers 6 (1968) 1425–1436.
[11] M. Karplus, J. Chem. Phys. 30 (1959) 11–15.
[12] A. Pardi, M. Billiter, K. Wüthrich, J. Mol. Biol. 180 (1984) 741–751.
[13] X. Wang, W. Daly, P. Russo, M. Ngu-Schwemlein, Biomacromolecules 2 (4) (2001) 1214–1219.
[14] U. Schmidt, A. Lieberknecht, H. Griesser, J. Tabiersky, J. Org. Chem. 47 (1982) 3261–3264.
[15] K.B. Green-Church, P.A. Limbach, Anal. Chem. 70 (1998) 5322–5325.
[16] M. El Haddadi, F. Cavelier, E. Vives, A. Azmani, J. Verducci, J. Martinez, J. Peptide Sci. 6 (2000) 560–570.
[17] G.D. Rose, L.M. Gierasch, J.A. Smith, Adv. Protein Chem. 37 (1985) 1–109.
[18] H. Kessler, Angew. Chem. Int. Ed. 21 (1982) 512–523.
[19] A. Perczel, M. Hollosi, in: G.D. Fasman (Ed.), Circular Dichroism and Conformational Analysis of Biomolecules, Plenum Press, New York, 1996, pp. 285–380.
[20] T. Makoto, S. Komiya, M. Yabu, E. Watanabe, S. Akabori, I. Muramatsu, J. Chem. Soc., Perkins Trans. I 23 (1997) 3497–3500.