Effective α-Helix Stabilization *via* Hexenyl Propionate Cross-Link

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In this study we examined two ester-containing cross-links, hex-2-enyl acetate and hex-2-enyl propionate, as new cross-linking systems for helix stabilization of short peptides. We demonstrated that these hexenyl ester cross-links can be readily installed *via* a ruthenium-mediated ring-closing metathesis reaction of L-aspartic acid 4-allyl ester or L-glutamic acid 5-allyl ester at position *i* and (*S*)-2-(4'-pentenyl)alanine at position *i*+4 using second generation Hoveyda-Grubbs catalyst at 60 °C. Between these two cross-links, we found that the hex-2-enyl propionate significantly stabilizes the α-helical conformations of short model peptides. The helix-stabilizing effects of the hex-2-enyl propionate tether appear to be as powerful as Verdine's *i,i*+4 all-hydrocarbon stapling system, which is one of the most widely used and the most potent helix-stabilizing cross-linking systems. Furthermore, the hex-2-enyl propionate bridge is reasonably robust against non-enzymatic hydrolytic cleavage at a physiological pH. While extended studies for probing its chemical scopes and biological applications are needed, we believe that this new helix-stabilizing system could serve as a useful chemical tool for understanding protein folding and designing conformationally-constrained peptide drugs.

Key Words : α-Helix, Stapled peptides, Ring-closing metathesis, Hexenyl propionate, Peptide drugs

Introduction

The α-helix is the most abundant structural element of proteins and is therefore often implicated in many biomolecular recognition events.[1] For this reason, an α-helical segment that directly mediates a protein-macromolecule interaction could potentially modulate the functions of its target macromolecule.[2] However, it is instinctively difficult for a short stretch of peptide to form an α-helical conformation. Therefore, a variety of strategies to enforce short peptides to adopt an α-helical conformation *via* chemical modifications have been actively investigated.[3-12] These methods have been valuable chemical tools for better understanding protein folding, probing signal transduction pathways, and providing important information useful for the development of therapeutics.

Currently the most common strategy for α-helix stabilization is connecting two side-chains on the same face of the helix *via* a cross-link that contains an amide,[4] disulfide,[6] diphenylazo,[9] ether,[10] tetrazole,[12] or hydrocarbon[13] moiety. However, an ester-containing cross-link has never been studied for helix-stabilization. In this study, we investigated a new *i,i*+4 cross-linking strategy involving hexenyl acetate or hexenyl propionate. We found that these hexenyl ester cross-links can be readily incorporated *via* a ruthenium-mediated ring-closing metathesis reaction of L-aspartic acid 5-allyl ester or L-glutamic acid 5-allyl ester at position *i* and (*S*)-2-(4'-pentenyl)alanine at position *i*+4 using second generation Hoveyda-Grubbs catalyst at a elevated temperature. Our experimental data indicate that the hexenyl propionate cross-link effectively stabilizes an α-helical conformation of short peptides.

Experimental

General. Commercially available chemicals were used as received. Piperidine, *N*-methyl-2-pyrrolidinone (NMP), dimethylformamide (DMF), *N,N*-diisopropylethylamine (DIEA), bis(tricyclohexylphosphine)benzylidine ruthenium (IV) dichloride (first generation Grubbs catalyst), (1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(*o*-isopropoxyphenylmethylene)ruthenium (second generation Hoveyda-Grubbs catalyst), dichloromethane (DCM), 1,2-dichloroethane (DCE), triisopropylsilane (TIS), and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich. Fmoc-protected (*S*)-2-(4'-pentenyl)alanine, was purchased from Okeanos Tech Co. Ltd. All other Fmoc-protected α-amino acid building blocks, 2-(6-chloro-1-*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU), (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU), and Rink Amide MBHA resin were purchased from NovaBiochem.

Peptide Synthesis. All the peptides were synthesized on Rink Amide MBHA resin with a loading capacity of 0.6 mmol/g using typical Fmoc chemistry. Briefly, after the dry resin (50 mg, 30 μmol) was swelled in NMP for 10 min, the Fmoc protecting group was removed by treatment with 25% piperidine in NMP (2 × 10 min). Then an amino acid building block was coupled for 30 min using HCTU as an activating agent (4.75 equiv.), 5 equiv. of Fmoc-protected amino acid, and 10 equiv. of DIEA in NMP. In the case of the α,α-bisalkylated amino acid ((*S*)-2-(4'-pentenyl)alanine), the coupling was conducted for 2 hours using Fmoc-protected amino acid (3 equiv.), COMU (2.85 equiv.), and DIEA (6 equiv.). After each step of coupling or deprotecting reactions,

the resin was thoroughly washed with DCM (1 × 2 min), NMP (1 × 2 min), DCM (1 × 2 min), and NMP (1 × 2 min).

Metathesis and Purification. Ring-closing metathesis of resin-bound *N*-Fmoc, side-chain protected peptides was performed using 20 mol % of first generation Grubbs catalyst or second generation Hoveyda-Grubbs catalyst in degassed DCE for 2 h at room temperature or 60 °C. For the reactions at 60 °C, the reaction vessels were shaken in a temperature-controlled environment. The reactions were monitored by liquid chromatography-mass spectrometry (LC/MS) (Shimadzu LCMS-2020) after cleavage of the peptides from a resin aliquot. Upon the reaction completed, the reaction solution was drained, and the resin was washed with DCE (3 × 2 min) and then with DCM (3 × 2 min). After the final Fmoc-deprotection reaction, the *N*-terminal amino group was capped with acetyl group by treating the resin with 30 equiv. of acetic anhydride and 60 equiv. of DIEA in NMP for 45 min. Resin was washed with DCM (2 × 2 min), NMP (2 × 2 min), and DCM (3 × 2 min) and dried *in vacuo* overnight. The peptide products were deprotected and cleaved from the resin by treating with a mixture of TFA/TIS/water (95/2.5/2.5) for 2 hours and precipitated by adding a 1:1 mixture of *n*-pentane and diethyl ether. The precipitate was collected by centrifugation, air-dried for 2 h, dissolved in a 1:1 mixture of acetonitrile and water, and filtered to remove resin. The products in the solution were purified by reserve phase high-performance liquid chromatography (HPLC) using a Zorbax C18 column (Agilent, 5 μm, 9.4 × 250 mm), and then LC/MS (Shimadzu LCMS-2020).

RA-WT. ESIMS *m/z* for $C_{75}H_{110}N_{20}O_{21}$ $[M+2H]^{2+}$/2 calcd 814.42, found 814.85.

RA-55U. ESIMS *m/z* for $C_{83}H_{124}N_{20}O_{19}$ $[M+2H]^{2+}$/2 calcd 853.48, found 853.85.

RA-55X. ESIMS *m/z* for $C_{81}H_{120}N_{20}O_{19}$ $[M+2H]^{2+}$/2 calcd 839.46, found 839.85.

RA-E5U. ESIMS *m/z* for $C_{83}H_{122}N_{20}O_{21}$ $[M+2H]^{2+}$/2 calcd 868.46, found 868.80.

RA-E5X. ESIMS *m/z* for $C_{81}H_{118}N_{20}O_{21}$ $[M+2H]^{2+}$/2 calcd 854.45, found 854.65.

RA-D5U. ESIMS *m/z* for $C_{82}H_{120}N_{20}O_{21}$ $[M+2H]^{2+}$/2 calcd 861.45, found 861.75.

RA-D5X. ESIMS *m/z* for $C_{80}H_{116}N_{20}O_{21}$ $[M+2H]^{2+}$/2 calcd 847.44, found 847.65.

MX-WT. ESIMS *m/z* for $C_{62}H_{102}N_{18}O_{21}$ $[M+2H]^{2+}$/2 calcd 718.38, found 718.65.

MX-55U. ESIMS *m/z* for $C_{67}H_{108}N_{16}O_{20}$ $[M+2H]^{2+}$/2 calcd 729.40, found 729.75.

MX-55X. ESIMS *m/z* for $C_{65}H_{104}N_{16}O_{20}$ $[M+2H]^{2+}$/2 calcd 715.39, found 715.65.

MX-E5U. ESIMS *m/z* for $C_{67}H_{106}N_{16}O_{22}$ $[M+2H]^{2+}$/2 calcd 744.39, found 744.85.

MX-E5X. ESIMS *m/z* for $C_{65}H_{102}N_{16}O_{22}$ $[M+2H]^{2+}$/2 calcd 730.38, found 730.60.

Circular Dichroism. Peptides were dissolved in 25 mM potassium phosphate buffer, and the concentrations were determined by absorbance spectroscopy at 280 nm (extinction coefficient for Tryptophan, $\lambda_{280} = 5690$ cm^{-1} at pH 6.5).

Circular dichroism spectra were collected on a Chirascan HP dual polarization circular dichroism spectrometer equipped with a temperature controller using the following standard measurement parameters: 1 nm step resolution, 3 accumulations, 0.5 sec response, 1 nm bandwidth, and 0.1 cm path length. All spectra were converted to a uniform scale of molar ellipticity after subtracted background. Curves shown were smoothed with standard parameters.

Hydrolytic Stability. Peptides were dissolved in 25 mM potassium phosphate buffer (pH 7.2) and kept at an ambient temperature. Hydrolytic degradation of the peptides was monitored by HPLC after every 24 hours for seven days.

Results and Discussion

Among the various strategies to stabilize α-helical conformations of peptides, the all-hydrocarbon stapling system developed by Verdine and his colleagues[13,14] has been the most widely applied method in biological systems. This special cross-linking system is a powerful chemical tool that effectively enforces a short peptide into an α-helical conformation by an all-hydrocarbon tether formed *via* ruthenium-mediated ring-closing olefin metathesis (RCM) (Figure 1).[15,16]

One of many advantages of Verdine's all-hydrocarbon stapling system is that it exploits a templating effect when installing a hydrocarbon cross-link onto peptides; the hydrophobic environment provided by the non-polar solvent (typically 1,2-dichloroethane) promotes an α-helix formation of peptide substrates during the RCM reaction.[17,18] Therefore, among many possible ways of introducing a covalent tether
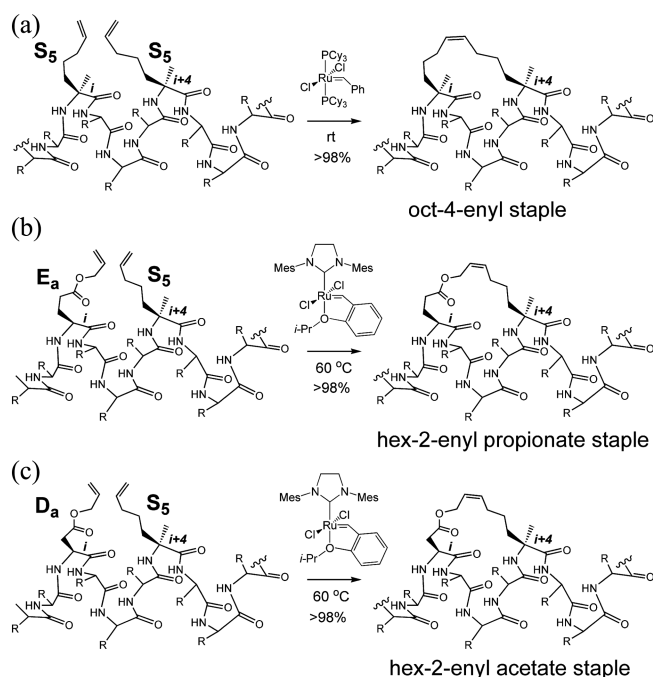

Figure 1. Schematic presentation of ring-closing olefin metathesis reactions for the incorporation of (a) Verdine's oct-4-enyl, (b) hex-2-enyl propionate, and (c) hex-2-enyl acetate staples. S_5, D_a, and E_a represent (*S*)-2-(4'-pentenyl)alanine, L-aspartic acid 4-allyl ester, and L-glutamic acid 5-allyl ester, respectively.

Table 1. Sequences of peptide substrates, conversion rates in ring-closing metathesis reactions, and percent helicity

Entry	Pre-stapled peptide[a]		% Conversion[b] after 2 h-RCM[c]			Stapled Product
			G-I, rt	G-I, 60 °C	HG-II, 60 °C	
1	RA-WT	EWAETAAAKFLAAHA	N/A[d]	N/A	N/A	N/A
2	RA-55U	EWA**S₅**TAA**S₅**KFLAAHA	> 90%	N/D[e]	N/D	RA-55X
3	RA-D5U	EWA**Dₐ**TAA**S₅**KFLAAHA	No reaction[f]	49%	> 98%	RA-D5X
4	RA-E5U	EWA**Eₐ**TAA**Dₐ**KFLAAHA	No reaction[f]	70%	> 98%	RA-E5X
5	RA-5DU	EWA**S₅**TAA**S₅**KFLAAHA	No reaction[f]	Decomposed[g]	Decomposed[g]	N/A
6	RA-5EU	EWA**S₅**TAA**Eₐ**KFLAAHA	No reaction[f]	Decomposed[g]	Decomposed[g]	N/A
7	MX-WT	SRAQILDKATEY	N/A	N/A	N/A	N/A
8	MX-55U	SRA**S₅**ILD**S₅**ATEY	> 90%	N/D	N/D	MX-55X
9	MX-E5U	SRA**Eₐ**ILD**S₅**ATEY	N/D	N/D	> 98%	MX-E5X

[a]**S₅ Dₐ**, and **Eₐ** represent (*S*)-2-(4'-pentenyl)alanine, L-aspartic acid 4-allyl ester, and L-glutamic acid 5-allyl ester, respectively. All the peptides are *N*-terminally acetylated and *C*-terminally amidated. [b]RCM product/(RCM product + starting material) as determined by reverse-phase high-performance liquid chromatography following cleavage from resin. [c]Metathesis was conducted on resin with the fully protected substrates in the presence of 20 mol % first generation Grubbs catalyst (G-I) or second generation Hoveyda-Grubbs catalyst (HG-II). [d]Not applicable. [e]Not determined. [f]RCM products were not detected. [g]Starting material was consumed without producing the corresponding RCM products.

bearing an ester moiety, we sought a strategy that allows us to use similar hydrophobic reaction conditions. We chose hex-2-enyl acetate and hex-2-enyl propionate since we expected that they would be easy to build by a ruthenium-mediated metathesis reaction between commercially available (*S*)-2-(4'-pentenyl)alanine (**S₅**) and L-aspartic acid 4-allyl ester (**Dₐ**) or L-glutamic acid 5-allyl ester (**Eₐ**) (Figure 1).

To investigate the chemical formation of these new cross-links and their helix-stabilizing effects, we prepared two RNase A-based model peptide substrates[13,14] **RA-D5U** and **RA-E5U**, incorporating **Dₐ** or **Eₐ** at position *i* and **S₅** at position *i*+4 (Table 1, entry 3 and 4). In addition, we prepared substrates **RA-5DU** and **RA-5EU**, which contain the same set of the amino acids but with their positions switched, to examine the significance of cross-link directions (Table 1, entry 5 and 6). For comparison, we also prepared the corresponding unmodified peptide (**RA-WT**) as well as substrate **RA-55U**, which contains **S₅** at both positions and forms the Verdine's oct-4-enyl cross-link. All of the peptide substrates were synthesized using the typical Fmoc/*t*-Bu-based strategy on solid support.[19]

With all the substrate peptides prepared on solid support, we first examined the efficiency of the cross-linking reaction of each substrate under the typical reaction conditions utilized for preparing Verdine's stapled peptides; when the fully-protected, resin-bound substrate peptides were subjected to RCM (using 20 mol % of first generation Grubbs catalyst in 1,2-dichloroethane at room temperature for two hours),[19] Verdine's substrate **RA-55U** underwent a smooth RCM to form its stapled product **RA-55X**. However, none of the **Dₐ**- or **Eₐ**-containing substrates produced corresponding cross-linked products under the same reaction conditions.

It has generally been observed that the RCM of substrates bearing an allyl ester moiety does not easily proceed at room temperature.[20,21] Therefore, we next attempted to promote RCM reactions for the allyl ester-containing substrates by elevating temperature.[22,23] When the RCM reactions were carried out at 60 °C in the presence of the first generation

Grubbs catalyst, both substrates **RA-D5U** and **-E5U** underwent RCM with a conversion rate of 49 and 70%, respectively. Finally, at this elevated temperature, when we switched the catalyst to the second generation Hoveyda-Grubbs catalyst, the RCM reaction of each **RA-D5U** and **-E5U** was completely proceeded yielding a single corresponding stapled product whose olefin geometry is yet to be determined. This result directly parallels previous studies in which the second generation Hoveyda-Grubbs catalyst was highly effective in mediating RCM reactions of peptide substrates at elevated temperatures.[25,26] Interestingly, substrates **RA-5DU** and **-5EU**, which only differ from **RA-D5U** and **-E5U** in the relative positions of the ally ester and pentenyl groups, respectively, did not yield the cross-linked products under any of these conditions but instead appeared to be degraded. While further studies are required to precisely elucidate the reasons, the result clearly indicates that the relative positions of this specific set of olefinic side-chains are another critical factor for effective RCM in this series of substrates.

We then examined the conformational consequences of introducing a hex-2-enyl acetate or hex-2-enyl propionate cross-link by measuring the far ultraviolet circular dichroism (CD) spectra in an aqueous environment. To our pleasant surprise, the peptide installed with the hex-2-enyl propionate tether, **RA-E5X**, clearly displaying a dramatically increase in helical content (68.4% helicity) compared to the unmodified control **RA-WT** (32.9% helicity) (Figure 2(a)).[27] As its pre-tethered counterpart **RA-E5U** only exhibited a slight increase in helical content (40.3%) compared to that of **RA-WT**, the significantly increased helicity from the cross-linked analog **RA-E5X** demonstrates the highly effective helix-stabilization by the hex-2-enyl propionate cross-link. More importantly, the helix-inducing capability of the hex-2-enyl propionate staple appears to be as potent as Verdine's all-hydrocarbon staple, as the helicity of **RA-E5X** is similar to that of **RA-55X** (70.1%) (Figure 2(c)).

Unlike the hex-2-enyl propionate, the hex-2-enyl acetate bridge did not exhibit any notable helix-stabilizing effects;
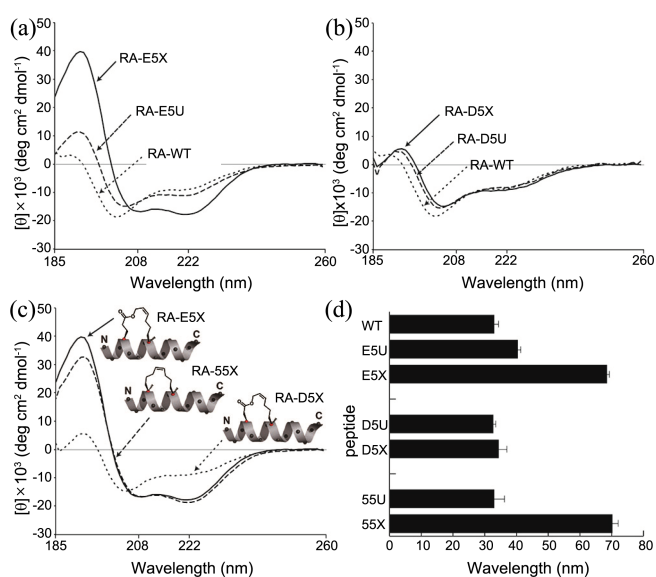
Figure 2. Circular dichroism spectra measured at 20 °C (a-c) and percent helicity (d) of the RNase A-based model peptides cross-linked by a hex-2-enyl propionate (**RA-E5X**), a hex-2-enyl acetate (**RA-D5X**), or an oct-4-enyl (**RA-55X**) staple and their pre-stapled or unmodified analogs. Error bars represent the mean ± standard deviation of two independent experiments. % Helicities were calculated from mean-residue ellipticities at 222 nm ($[\theta]_{222}$) using -31,500(1-2.5/n) and 0 deg cm^2 dmol^{-1} as the values for 100 and 0% helicity, respectively; n is the number of amino acid residues in the peptide.

the cross-linked product **RA-D5X** showed only 34.3% of helicity, similar to that of its unmodified control **RA-WT** (32.9%) or its pre-tethered control **RA-D5U** (32.6%) (Figure 2(b)). These results indicate that, in the presence of an ester moiety in the *i*,*i*+4 cross-link, an 9-atom tether is more optimal than an 8-atom one for helix-stabilization, whereas Verdine's stapling system requires an 8-atom hydrocarbon for the highest enhancement in helical contents *via* *i*,*i*+4 cross-linking.[4] We assume that, due to the incorporation of the relatively less flexible ester moiety, an ester containing tether may need a longer cross-link for effective helix-stabilization than all-hydrocarbon staple.

A potential concern about the hex-2-enyl propionate staple is its susceptibility to hydrolysis. Therefore, we next tested the hydrolytic stability of the hex-2-enyl propionate tether in a physiological pH. **RA-E5X** was incubated in a 20 mM phosphate buffer (pH 7.2) at ambient temperature and the potential hydrolytic reaction was monitored by HPLC analysis temporally. Surprisingly, no hydrolysis was observed even after seven days, indicating that the hex-2-enyl propionate cross-link is reasonably stable against non-enzymatic hydrolysis at physiological pH.

Finally, we tested whether this highly potent helix stabilization by hex-2-enyl propionate staple can be observed for sequences other than the model RNase A peptide. For this purpose, we prepared another panel of peptides based on a sequence derived from a helical segment of myc associated factor X (MAX) (Table 1, entry 7-9).[28] Like RNase A model peptides, the hex-2-enyl propionate-forming RCM of **MX-**
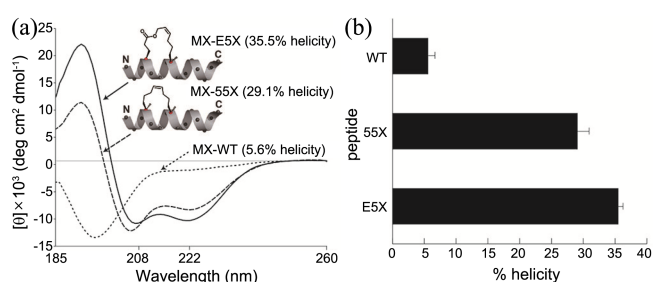

Figure 3. Circular dichroism spectra measured at 20 °C (a) and percent helicity (b) of the MAX-based model peptides cross-linked by a hex-2-enyl propionate (**MX-E5X**), or an oct-4-enyl (**MX-55X**) staple and their unmodified analog (**MX-WT**). Error bars represent the mean ± standard deviation of two independent experiments.

E5U completely proceeded in two hours at 60 °C in the presence of the second generation Hoveyda-Grubbs catalyst, affording the corresponding cross-linked peptide, **MX-E5X**. The unmodified MAX-derived 12-residue control **MX-WT** displayed a CD spectrum typically observed from random coil; its measured percent helicity was 5.6% (Figure 3). The all-hydrocarbon stapled analog **MX-55X** showed an 5.2-fold increase in helical content (29.1%) over that of the unmodified **MX-WT**. In this specific series of peptides, the hex-2-enyl propionate stapled analog **MX-E5X** exhibited the highest increase in helical content (35.5%), a 6.3-fold higher than that of the unmodified **MX-WT** (5.6%). These CD data clearly confirmed that the hex-2-enyl propionate staple, is comparable to, if not more potent than, that of Verdine's all-hydrocarbon stapling system for helix-stabilization of short peptides.

Conclusion

We examined two ester-containing cross-links, hex-2-enyl acetate and hex-2-enyl propionate, for their formation and helix-stabilizing potential. We have demonstrated that the two hexenyl ester cross-links can be readily installed onto peptides *via* a ruthenium-mediated RCM reaction of L-aspartic acid 5-allyl ester or L-glutamic acid 5-allyl ester at position *i* and (*S*)-2-(4'-pentenyl)alanine at position *i*+4 using second generation Hoveyda-Grubbs catalyst at 60 °C. We further showed that the incorporation of hex-2-enyl propionate can stabilize the α-helical conformation of short peptides as effectively as Verdine's *i*,*i*+4 all-hydrocarbon stapling system, which has been one of the most widely used and the most potent helix-stabilizing cross-linking systems. We also found that hex-2-enyl propionate is robust against non-enzymatic hydrolytic cleavage at a physiological pH. While extended studies for probing its chemical scopes and biological applications may be necessary, we believe that this new cross-linking system will find many useful applications as a reliable alternative to Verdine's stapling system especially when the hydrophobic hydrocarbon staple is not preferred for the optimal aqueous solubility of the cross-linked peptides.

References

1. Creighton, T. E. *Proteins: Structures and Molecular Properties*, Freeman and Co.: New York, 1984.
2. Fairlie, D. P.; West, M. L.; Wong, A. K. *Curr. Med. Chem.* **1998**, *5*, 29-62.
3. Marqusee, S.; Baldwin, R. L. *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 8898-8902.
4. Osapay, G.; Taylor, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 6046-6051.
5. Ghadiri, M. R.; Choi, C. *J. Am. Chem. Soc.*, **1990**, *112*, 1630-1632.
6. Jackson, D. Y.; King, D. S.; Chmielewski, J.; Singh, S.; Schultz, P. G. *J. Am. Chem. Soc.* **1991**, *113*, 9391-9392.
7. Kemp, D. S.; Allen, T. J.; Oslick, S. L. *J. Am. Chem. Soc.* **1995**, *117*, 6641-6657.
8. Cabezas, E.; Satterthwait, A. C. *J. Am. Chem. Soc.* **1999**, *121*, 3862-3875.
9. Kumita, J. R.; Smart, O. S.; Woolley, G. A. *Proc. Natl. Acad. Sci.* **2000**, *97*, 3803-3808.
10. Blackwell, H. E.; Sadowsky, D. J.; Howard, R. J.; Sampson, J. N.; Chao, J. A.; Steinmetz, W. E.; O'Leary, D. J.; Grubbs, R. H. *J. Org. Chem.* **2001**, *66*, 5291-5302.
11. Chapman, R. N.; Dimartino, G.; Arora, P. S. *J. Am. Chem. Soc.* **2004**, *126*, 12252-12253.
12. Cantel, S. *et al*. *J. Org. Chem.* **2008**, *73*, 5663-5674.
13. Schafmeister, C. E.; Po, J.; Verdine, G. L. *J. Am. Chem. Soc.* **2000**, *122*, 5891-5892.
14. Kim, Y.-W.; Kutchukian, P. S.; Verdine, G. L. *Org. Lett.* **2010**, *12*, 3046-3049.
15. Verdine, G. L.; Walenski, L. D. *Clin. Cancer Res.* **2007**, *13*, 7264-7270.
16. Verdine, G. L.; Hilinski, G. J. *Methods Enzymol.* **2012**, *503*, 3-33.
17. Toniolo, C.; Crisma, M.; Formaggio, F.; Peggion, C. *Biopolymers* **2001**, *60*, 396-419.
18. Urnes, P.; Doty, P. *Adv. Protein Chem.* **1961**, *16*, 401-544.
19. Kim, Y.-W.; Grossmann, T. N.; Verdine, G. L. *Nat. Proc.* **2011**, *6*, 761-771.
20. Andreana, P. R.; McLenllan, J. S.; Chen, Y.; Wang, P. G. *Org. Lett.* **2002**, *4*, 3875-3878.
21. Christoffers, J.; Oertling, H.; Fischer, P.; Frey, W. *Synlett* **2002**, 957-961.
22. Lin, Y. A.; Chalker, J. M.; Davis, B. G. *ChemBioChem* **2009**, *10*, 959-969.
23. Creighton, C. J.; Reitz, A. B. *Org. Lett.* **2001**, *3*, 893-895.
24. Stymiest, J. L.; Mitchell, B. F.; Wong, S.; Vederas, J. C. *Org. Lett.* **2003**, *5*, 47-49.
25. Dimartino, G.; Wang, D.; Chapman, R. N.; Arora, P. S. *Org Lett.* **2005**, *7*, 2389-2392.
26. Shim, S. Y.; Kim, Y.-W.; Verdine, G. L. *Chem. Biol. Drug Des.* **2013**, *82*, 635-642.
27. Chen, Y.-H.; Yang, J. T.; Martinez, H. M. *Biochemistry* **1972**, *11*, 4120-4131.
28. Ferré-d'Amaré, A. R.; Prendergast, G. C.; Ziff, E. B.; Burley, S. K. *Nature* **1993**, *363*, 38-45.