*J. Chem. Sci.* Vol. 127, No. 9, September 2015, pp. 1563–1569. 
DOI 10.1007/s12039-015-0922-y

# Synthetic strategy for bicyclic tetrapeptides HDAC inhibitors using ring closing metathesis

MD NURUL ISLAM[a,b,*], MD SHAHIDUL ISLAM[a,b], MD ASHRAFUL HOQUE[a,c], TAMAKI KATO[a] and NORIKAZU NISHINO[a]

[a]Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, Kitakyushu 808-0196, Japan
[b]Department of Chemistry, Faculty of Science, University of Rajshahi, Rajshahi 6205, Bangladesh
[c]Department of Biochemistry and Molecular Biology, Faculty of Science, University of Rajshahi, Rajshahi 6205, Bangladesh
e-mail: mnurulchem@gmail.com

MS received 9 February 2015; revised 27 May 2015; accepted 27 May 2015

**Abstract.** Cyclic peptides show diverse biological activities and are considered as good therapeutic agents due to structural rigidity, receptor selectivity and biochemical stability. We have developed bicyclic tetrapeptide HDAC inhibitors based on different cyclic tetrapeptide scaffolds. For the synthesis of these bicyclic tetrapeptides, two cyclization steps, namely, peptide cyclization and fusion of aliphatic side chains by ring closing metathesis (RCM) were involved. In the course of these syntheses, we have established two facts: a lower limit of aliphatic loop length and better synthetic route for bicyclic tetrapeptide synthesis. It was found that nine methylene loop length is the lower limit for aliphatic loop and the synthetic route selection depended on the configuration of amino acids in the cyclic tetrapeptide scaffold. RCM followed by peptide cyclization was the proper route for LDLD configuration and the reverse route was suitable for LLLD configuration.

**Keywords.** Bicyclic tetrapeptides; ring-closing metathesis; aliphatic loop length; HDAC inhibitors.

## 1. Introduction

Cyclic peptides show a wide spectrum of biological activity, such as antibacterial activity, immunosuppressive activity, anti-tumor activity, and so on.[1–5] Structural rigidity, receptor selectivity and biochemical stability are general features of cyclic peptides. These features allow cyclic peptides to be good therapeutic agents, and efforts have been made to develop synthetic cyclic and bicyclic peptides with biological activity.[5,6]

Ring closing metathesis (RCM) have recently appeared as a versatile, chemo-selective means to create carbon-carbon bonds in a range of molecules including natural products, polymers, and biomolecules.[7–14] We also designed and synthesized cyclic tetrapeptides Cyl-1, CHAP31, trapoxin B and HC-toxin I (figure 1) based bicyclic tetrapeptide HDAC inhibitors using RCM.[15–18]

For the synthesis of these bicyclic tetrapeptides, two cyclization steps i.e., peptide cyclization and fusion of aliphatic side chains by RCM were involved. Selection of the synthetic route depended on the configuration of the amino acids whose side chains take part in RCM. We herein describe the establishment of a lower limit of aliphatic loop length for RCM and the order of the two cyclization steps involved in bicyclic tetrapeptide synthesis.

## 2. Experimental

### 2.1 *General*

Unless otherwise noted, all solvents and reagents were reagent grade and used without purification. Flash chromatography was performed using silica gel 60 (230–400 mesh) eluting with solvents as indicated. All compounds were routinely checked by thin layer chromatography (TLC) and/or high performance liquid chromatography (HPLC). TLC was performed on aluminum-backed silica gel plates (Merck DC-Alufolien Kieselgel 60 $F_{254}$) with spots visualized by UV light or charring. Analytical HPLC was performed on a Hitachi instrument equipped with a chromolith performance RP-18e column (4.6 × 100 mm, Merck). The mobile phases used were A: $H_2O$ with 10% $CH_3CN$ and 0.1% TFA, B: $CH_3CN$ with 0.1% TFA using a solvent gradient of A-B over 15 min with a flow rate of 2 mL/min, with detection at 220 nm. HR FAB-mass spectra

*For correspondence

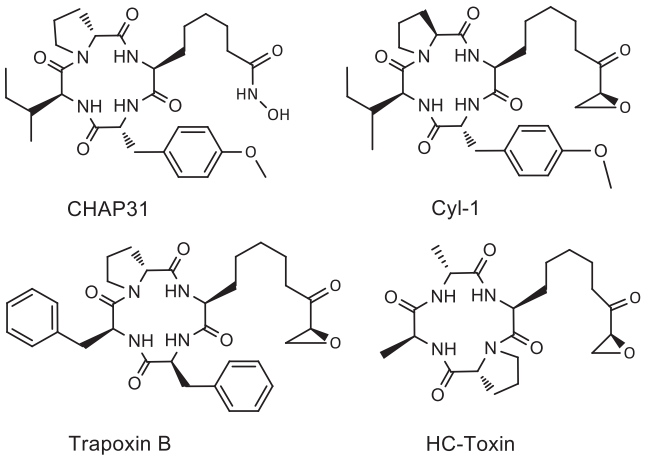



**Figure 1.** Some natural and synthetic cyclic tetrapeptide HDAC inhibitors.

were measured on a JEOL JMS-SX 102A instrument. NMR spectra were recorded on a JEOL JNM A500 MHz spectrometer in DMSO-$d_6$ solutions with reference to TMS. All $^1$H shifts are given in parts per million (s = singlet; d = doublet; t = triplet; m = multiplet). Assignments of proton resonances were confirmed, when possible, by correlated spectroscopy.

Ring closing metathesis was performed by the aid of benzylidene-bis(tricyclohexylphosphine)dichlororuthenium (Grubbs' first generation ruthenium catalyst). Coupling reactions were performed by standard solution-phase chemistry using dicyclohexyl-carbodiimide (DCC) and 1-hydroxybenzotriazol (HOBt). Peptide cyclization was mediated by *N*−[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-b]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate*N*-oxide (HATU). The artificial amino acids Boc-L-2-amino-6-heptenoic acid (Boc-L-Ae7-OH), Boc-L-2-amino-7-octenoic acid (Boc-L-Ae8-OH), Boc-L-2-amino-8-nonenoic acid (Boc-L-Ae9-OH), Boc-D-2-amino-7-octenoic acid (Boc-D-Ae8-OH) and Boc-D-2-amino-8-nonenoic acid (Boc-D-Ae9-OH) used for the synthesis of bicyclic tetrapeptides were synthesized by the reported procedure.[12]

2.1a *Synthesis of bicyclic tetrapeptide hydroxamic acid with ten $CH_2$ loop length (**4**)*: To a cooled solution of H-D-Pro-O$^t$Bu (0.855 g, 5 mmol), Boc-L-Ae8-OH (1.29 g, 5 mmol) and HOBt.$H_2O$ (0.766 g, 5 mmol) dimethylformamide (DMF) (10 mL), DCC (1.24 g, 6 mmol) were added. The mixture was stirred for 12 h at room temperature. After completion of the reaction, DMF was evaporated and the residue was dissolved in ethyl acetate (EtOAc) and successively washed with 10% citric acid, 4% sodium bicarbonate and brine. The EtOAc solution was dried over anhydrous $MgSO_4$ and concentrated to remain an oily substance, which was purified by silica gel chromatography using a mixture of chloroform and methanol (99:1) to yield Boc-L-Ae8-D-Pro-O$^t$Bu (1.60 g, 78%) as an oil. The protected dipeptide (1.55 g, 3.8 mmol) was dissolved in 4 M HCl/dioxane (16 mL) and the mixture was kept at room temperature for 30 min. The reaction was monitored by TLC. After completion of the reaction HCl/dioxane was evaporated. The residue was dissolved in EtOAc and washed with saturated $Na_2CO_3$, dried over anhydrous $Na_2CO_3$ and EtOAc was evaporated to get H-L-Ae8-D-Pro-O$^t$Bu as a heavy oil (0.930 g, 79%). To a cooled solution of H-L-Ae8-D-Pro-O$^t$Bu (0.930 g, 3 mmol), Boc-D-Ae8-OH (0.940 g, 3.3 mmol) and HOBt.$H_2O$ (0.460 g, 3 mmol) in DMF (6 mL), DCC (0.742 g, 3.6 mmol) was added and stirred for 12 h at room temperature. The product Boc-D-Ae8-L-Ae8-D-Pro-O$^t$Bu was obtained in the same manner as described earlier as a heavy oil (1.40 g, 84%). HPLC, retention time ($t_R$) 8.02 min. To a solution of linear tripeptide Boc-D-Ae8-L-Ae8-D-Pro-O$^t$Bu (1.10 g, 2 mmol) in anhydrous and degassed dichloromethane (DCM) (250 mL), a solution of Grubbs first generation ruthenium catalyst (0.330 g, 0.4 mmol) in anhydrous and degassed DCM (50 mL) was added. The reaction mixture was stirred at room temperature for 48 h. After the completion of reaction, DCM was evaporated and the residue was purified by silica gel chromatography using a mixture of chloroform and methanol (99:1) to yield linear tripeptide with unsaturated fused ring as a foam which on catalytic hydrogenation in presence of Pd-C (0.100 g) in acetic acid (10 mL) yield both side protected tripeptide with saturated fused ring (0.920 g, 87%). HPLC, $t_R$ 7.96 min. The protected tripeptide (0.530 g, 1 mmol) was dissolved in 4 M HCl/dioxane (5 mL) and the mixture was kept at room temperature for 30 min. The reaction was monitored by TLC. After completion of the reaction HCl/dioxane was evaporated. The residue was dissolved in EtOAc and washed with saturated $Na_2CO_3$, dried over anhydrous $Na_2CO_3$ and EtOAc was evaporated to get tripeptide free amine (0.310 g, 73%). To a cooled solution of the free amine (0.310 g, 0.73 mmol), Boc-L-Asu(OBzl)-OH (0.295 g, 0.78 mmol) and HOBt.$H_2O$ (0.112 g, 0.73 mmol) in DMF (2 mL), DCC (0.161 g, 0.78 mmol) was added and stirred for 12 h at room temperature. The product linear tetrapeptide with fused ring (0.450 g, 79%) was obtained in the same manner as described earlier as a foam. HPLC, $t_R$ 10.42 min. The protected tetrapeptide (0.445 g, 0.57 mmol) was dissolved in TFA (3 mL) at 0 °C and kept for 3 h. After evaporation of TFA, the residue was solidified using ether and petroleum ether to yield TFA salt of the linear tetrapeptide (0.425 g, 100%). To DMF

solvent (570 mL), the TFA salt (0.425 g, 0.57 mmol), HATU (0.326 g, 0.86 mmol), and DIEA (0.24 mL, 1.42 mmol) were added in separate five portions in every 30 min with stirring for the cyclization reaction. After completion of the reaction, DMF was evaporated under vacuum; the residue was dissolved in ethyl acetate and washed with citric acid (10%) solution, sodium bicarbonate (4%) solution, and brine, successively. It was then dried over anhydrous $MgSO_4$ and filtered. After evaporation of ethyl acetate, the residue was purified by silica gel chromatography using a mixture of chloroform and methanol (99:1) to yield the bicyclic tetrapeptide (0.143 g , 41%). HPLC, $t_R$ 7.85 min. The bicyclic tetrapeptide (0.125 g, 0.2 mmol) was dissolved in acetic acid (5 mL) and Pd-C (0.050 g) was added. The mixture was stirred under $H_2$ for 10 h. After filtration of Pd-C, acetic acid was evaporated to yield bicyclic tetrapeptide carboxylic acid (0.104 g, 100%). The bicyclic tetrapeptide carboxylic acid (0.090 g, 0.17 mmol) was dissolved in DMF (0.5 mL) at 0C, and O-benzylhydroxylamine hydrochloride (0.041 g, 0.26 mmol), $HOBt.H_2O$ (0.026 g, 0.17 mmol), triethylamine (0.037 mL, 0.26 mmol) and DCC (0.054 g, 0.26 mmol) were added. The mixture was stirred for 24 h. The product (0.062 g, 60%) obtained was dissolved in acetic acid (1 mL), and Pd-$BaSO_4$ (0.020 g) was added. The mixture was stirred under $H_2$ for 24 h. After filtration of Pd-$BaSO_4$, acetic acid was evaporated and crystallized with ether to yield compound **4** (0.036 g, yield: 70%, HPLC, $t_R$ 6.03 min). $^1$H NMR (500 MHz, DMSO-$d_6$, 40 C): $\delta_H$ 1.14–1.28 (m, 16H), 1.31–1.38 (m, 4H), 1.42–1.54 (m, 6H), 1.64–1.79 (m, 4H), 1.85–2.00 (m, 4H), 3.38–3.45 (m, 2H), 3.57 (dd, $J$ = 18, 8.2 Hz, 1H), 3.92 (ddd, $J$ = 9.5, 9.5, 3.5 Hz, 1H), 4.21 (m, 1H), 4.44 (m, 1H), 4.58 (d, $J$ = 7.9 Hz, 1H), 4.70 (m, 1H), 7.13 (d, $J$ = 9.0 Hz, 1H), 7.26 (d, $J$ = 10.1 Hz, 1H), 8.29 (d,$J$ = 9.1 Hz, 1H), 8.64 (s, 1H), 10.33 (s, 1H); HR FAB-MS $[M+Na]^+$ 558.3263 for $C_{27}H_{45}N_5O_6Na$ (calcd 558.3268).

2.1b *Synthesis of bicyclic tetrapeptide hydroxamic acid with nine $CH_2$ loop length **(3)***: Compound **3** (0.010 g, yield: 73%, HPLC, $t_R$ 4.74 min) was synthesized following the same procedure as described in case of compound **4** using Boc-L-Ae7-OH instead of Boc-L-Ae8-OH. $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta_H$ 1.15–1.40 ( m, 18H), 1.47-1.78 (m, 10H), 1.83–1.98 (m, 4H), 3.60 (dd, $J$ = 17.5, 8.7 Hz, 1H), 3.85 (ddd, $J$ = 9.7, 9.5, 3.5 Hz, 1H), 4.06 (m, 1H), 4.13 (m, 1H), 4.20 (dd, $J$ = 17.3, 8 Hz, 1H), 4.61 (d, $J$ = 7.9 Hz, 1H), 7.18 (d, $J$ = 9.5, 1H), 7.91 (s, 1H), 8.09 (s, 1H), 8.66 (s, 1H), 10.36 (s, 1H); HRFAB-MS $[M+Na]^+$ 544.3141 for $C_{26}H_{43}N_5O_6Na$ (calcd 544.3111).

2.1c *Synthesis of bicyclic tetrapeptide hydroxamic acid with eleven $CH_2$ loop length **(5)***: Compound **5** (0.195 g, yield: 75%, HPLC, $t_R$ 7.39 min) was synthesized following the same procedure as described in case of compound **4** using Boc-L-Ae9-OH instead of Boc-L-Ae8-OH. $^1$H NMR (500 MHz, DMSO-$d_6$ 30 C): $\delta_H$ 1.10–1.99 (m, 32H), 2.50 (m, 4H), 3.57 (dd, $J$ = 18.2, 8.4 Hz, 1H), 3.86 (ddd, $J$ = 9.5, 9.5, 3.8 Hz, 1H), 4.19 (dd, $J$ = 16.7, 7.7 Hz, 1H), 4.41 (m, 1H), 4.53 (d, $J$ = 7.5 Hz, 1H), 4.70 (d, $J$ = 7.6 Hz, 1H), 7.13 (d, $J$ = 9.1 Hz, 1H), 7.23 (d, $J$ = 9.8 Hz, 1H), 8.42 (d, $J$ = 9.2 Hz, 1H), 8.61 (s, 1H), 10.31 (s, 1H); HR FAB-MS $[M+H]^+$ 550.3603 for $C_{28}H_{48}N_5O_6$ (calcd 550.3605).

2.1d *Synthesis of bicyclic tetrapeptide hydroxamic acid with twelve $CH_2$ loop length (**6**)*: Compound **6** (0.195 g, yield: 71%, HPLC, $t_R$ 7.52 min) was synthesized following the same procedure as described in case of compound **4** using Boc-D-Ae9-OH instead of Boc-D-Ae8-OH. $^1$H NMR (500 MHz, DMSO-$d_6$ 30 C): $\delta_H$ 1.17–2.10 (m, 34H), 2.50 (m, 4H), 3.41 (m, 1H), 3.58 (dd, $J$ = 17.7, 8.7 Hz, 1H), 3.74 (m, 1H), 3.81 (ddd, $J$ = 9.7, 9.5, 3.5 Hz, 1H), 4.06 (m, 1H), 4.19 (dd, $J$ = 16.5, 7.5 Hz, 1H), 4.34 (t, $J$ = 10.7 Hz, 1H), 4.55 (d, $J$ = 8.2 Hz, 1H), 4.70 (d, $J$ = 7.3 Hz, 1H), 7.16 (d, $J$ = 9.5 Hz, 1H), 7.23 (d,$J$ = 10.0 Hz, 1H), 8.44 (d, $J$ = 9.2 Hz, 1H), 8.62 (s, 1H), 10.34 (s, 1H); HR FAB-MS $[M+H]^+$ 564.3691 for $C_{29}H_{50}N_5O_6$ (calcd 564.3761).

2.1e *Synthesis of HC-toxin-I and trapoxin based bicyclic tetrapeptide hydroxamic acid (**7** and **8**)*: Detailed synthetic procedures for these compounds have been published.[17]

2.1f *Synthesis of CHAP31 based cyclic tetrapeptide hydroxamic acid **(9)***: Compound **9** (0.250 g, yield: 91%, HPLC, $t_R$ 7.51 min) was synthesized by the same solution phase synthetic method described for compound **4** using Boc-L-Ae7-OH and Boc-D-Ae7-OH in place of Boc-L-Ae8-OH and Boc-D-Ae8-OH respectively, and by skipping RCM step. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta_H$ 0.81–0.88 (m, 6H),1.18-1.30 (m, 18H), 1.44 (m, 4H), 1.62–1.74 (m, 4H), 1.87–1.97 (m, 4H), 3.52 (dd, = 18.5, 8.5 Hz, 1H), 3.77 (ddd, $J$ = 10, 9.5, 4 Hz, 1H), 4.19–4.27 (m, 2H), 4.63 (dd, $J$ = 17.3, 8 Hz, 1H), 4.71 (d, $J$ = 7.3 Hz, 1H), 7.06 (d, $J$ = 10.1 Hz, 1H), 7.21 (d, $J$ = 9.5 Hz, 1H), 8.43 (d, $J$ = 9.2 Hz, 1H), 8.63 (s, 1H), 10.31 (s, 1H); HR FAB-MS $[M+H]^+$ 538.3543 for $C_{27}H_{48}N_5O_6$ (calcd 538.3605).

2.1g *Synthesis of HC-toxin-I and trapoxin based cyclic tetrapeptide hydroxamic acid **(10 and 11)***: Detailed synthetic procedures of these compounds have been published.[17]

### 2.2 *Circular dichroism (CD) measurements*

CD spectra were recorded on a JASCO J-820 spectropolarimeter using a quartz cell of 1 millimeter path length at room temperature. Peptide solutions (0.1 mM) were prepared in methanol and CD spectra were recorded in terms of molar ellipticity, $[\theta]_M$ (deg cm$^2$ dmol$^{-1}$).

## 3. Results and Discussion

### 3.1 *Design and synthesis*

We first designed Cyl-1-based (LLLD configuration) bicyclic tetrapeptide with ten methylene loop length (**1**) and tried to synthesize through RCM followed by peptide cyclization but failed in the peptide cyclization step; whereas, peptide cyclization was successful in case of cyclic tetrapeptide with the same configuration. Komatsu *et al*. also synthesized cyclic tetrapeptide CHAP30 with the same configuration.[19] We assumed that cyclization of Cyl-1 based tetrapeptide with unfavourable configuration (LLLD) is impossible due to the steric hindrance imposed by the aliphatic loop. By changing the configuration, we successfully synthesized CHAP31-based (LDLD configuration) bicyclic tetrapeptide with the same loop length of ten methylene units (**4**). Subsequently, a series of CHAP31-based bicyclic tetrapeptides with different loop lengths (**2, 3, 5** and **6**) were designed to determine the lower limit of the aliphatic loop length and favourable route for bicyclic tetrapeptide synthesis. Finally, we designed HC-toxin and trapoxin B based bicyclic tetrapeptides with ten methylene loop length (**7** and **8**, figure 2) to confirm the favourable synthetic route. We also designed and synthesized compounds **9**, **10** and **11** as open chain reference of compounds for **4**, **7** and **8**, respectively, for comparing peptide backbone conformational changes.

After successful synthesis of CHAP31 based bicyclic tetrapeptide with ten methylene loop length (**4**) through RCM followed by peptide cyclization (scheme 1), we reduced the loop length to nine methylene units and synthesized bicyclic tetrapeptide (**3**). However, synthesis of bicyclic tetrapeptide with eight methylene loop length (**2**) failed in the RCM step. Instead of obtaining expected linear tripeptide with fused ring, two dimers with two different HPLC retention times were obtained corresponding to parallel and anti-parallel arrangement of the monomer during RCM (figure 3).

The dimers were confirmed by LC-MS ([M+H]$^+$ 987.4, calcd. 987.64). We changed the route and synthesized cyclic tetrapeptide first and then RCM was applied to obtain bicyclic tetrapeptide (scheme 2). Once again, we obtained dimer instead of monomer. As the bicyclic tetrapeptides having nine or more methylene units were successively synthesized, nine methylene units is the minimum loop length for bicyclic tetrapeptides using Grubbs first generation ruthenium catalyst. We have also synthesized two more CHAP31 based bicyclic tetrapeptides with eleven (**5**) and twelve (**6**) methylene loop length to optimize the cyclization yield and to check the better route for bicyclic tetrapeptide synthesis.

For CHAP31 based bicyclic tetrapeptide series, RCM and peptide cyclization yields were affected by the aliphatic loop length (table 1). In general, both RCM and peptide cyclization yields were increased with increasing aliphatic loop length. This may be due to the increase in flexibility of loop that facilitates the RCM and peptide cyclization. As for compound **5** and **6**, RCM and peptide cyclization yields were almost the same, eleven methylene loop length was sufficient to remove stress imposed by the loop. Our previous report[16] on these compounds showed that the loop length has a significant impact on *in vivo* biological

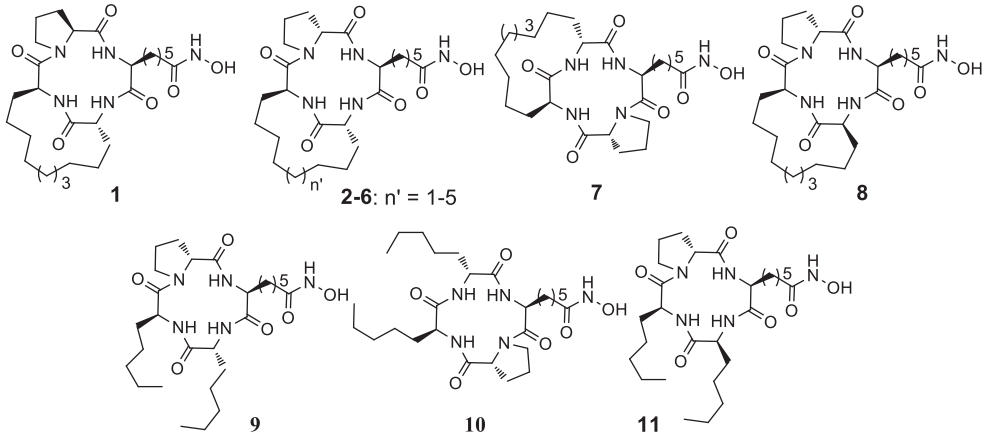
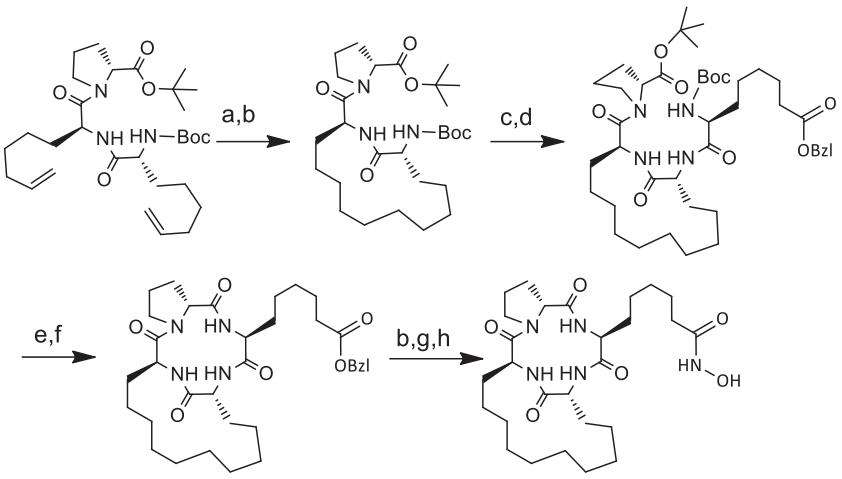



**Figure 2.** Designed bicyclic and cyclic tetrapeptides.




**Scheme 1.** Synthesis of CHAP31 based bicyclic tetrapeptide with ten methylene loop length (**4**). Reagents and conditions: (a) Grubbs first generation catalyst, DCM, 48 h; (b) AcOH, Pd-C, $H_2$, 12 h; (c) 4 M HCl/dioxane, 30 min; (d) Boc protected amino suberic acid benzyl ester (Boc-L-Asu(OBzl)-OH), DCC, HOBt, DMF, 12 h; (e) TFA, 3 h; (f) HATU, DIEA, DMF, 4 h; (g) $HCl.H_2NOBzl$, DCC, HOBt, TEA, DMF, 24 h; (h) Pd-$BaSO_4$, AcOH, $H_2$.

activity. A sharp increase in p21 promoter-inducing activity was observed with the increase in loop length from nine to eleven methylene groups. The change in biological activity may be due to the change in both the hydrophobicity and stress on cyclic tetrapeptide backbone. To check the better route for bicyclic tetrapeptide synthesis, compound **6** was synthesized through a different route i.e., through peptide cyclization followed by RCM. In this case, the RCM yield was remarkably low (40%). This may be due to the spatial arrangement of the two aliphatic side chains of the adjacent amino acids (LD configuration) taking part in RCM. In this case, the side chains are directed to the opposite sides of the cyclic tetrapeptide scaffolds which is unfavourable for RCM. Therefore, RCM followed by peptide cyclization can be considered as the proper synthetic route for bicyclic tetrapeptides with LDLD configuration. The successful synthesis with appreciable yields in peptide cyclization and RCM steps for compound **7** (LDLD configuration, reverse to CHAP31) supports our proposed route.

As mentioned above, LLLD configuration is unfavourable for peptide cyclization; RCM before peptide cyclization imposes an extra hindrance for the

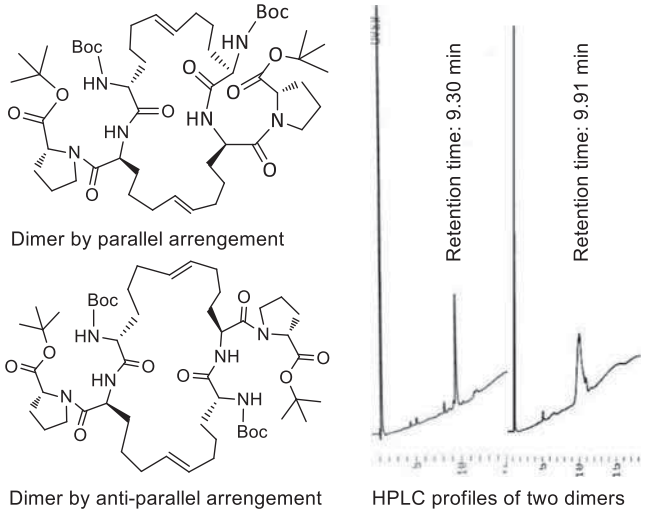
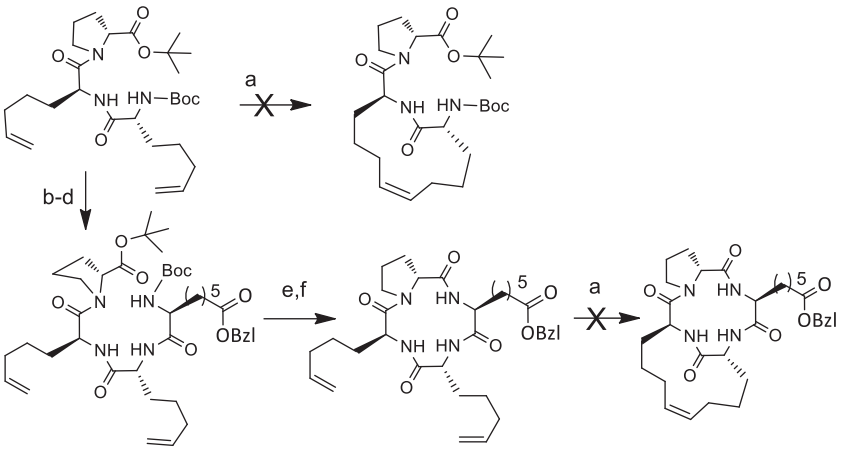



**Figure 3.** Dimers obtained from RCM and their HPLC profiles.




**Scheme 2.** Determination of lower limit for aliphatic loop length of bicyclic tetrapeptides. Reagents and conditions: (a) Grubbs first generation catalyst, DCM, 48 h; (b) 4 M HCl/dioxane, 30 min; (c) saturated $Na_2CO_3$; (d) Boc-L-Asu(OBzl)-OH, DCC, HOBt, DMF; (e) TFA, 3 h; (f) HATU, DIEA, DMF, 4 h.

**Table 1.** Effect of loop length and synthetic route on RCM and peptide cyclization.

| Compd. No. | Origin | Configuration | Loop size | Route | RCM yield (%) | PC yield (%) |
|---|---|---|---|---|---|---|
| 1 | Cyl-1 | LLLD | $-(CH_2)_{10}-$ | RCM→PC* | 90 | Failed |
| **2** | CHAP31 | LDLD | $-(CH_2)_8-$ | RCM→PC | Failed | – |
| **3** | CHAP31 | LDLD | $-(CH_2)_9-$ | RCM→PC | 50 | 15 |
| **4** | CHAP31 | LDLD | $-(CH_2)_{10}-$ | RCM→PC | 87 | 40 |
| **5** | CHAP31 | LDLD | $-(CH_2)_{11}-$ | RCM→PC | 85 | 65 |
| **6** | CHAP31 | LDLD | $-(CH_2)_{12}-$ | RCM→PC | 85 | 67 |
| **6** | CHAP31 | LDLD | $-(CH_2)_{12}-$ | PC→ RCM | 40 | 65 |
| **7** | HC-toxin I | LDLD | $-(CH_2)_{10}-$ | RCM→PC | 63 | 51 |
| **8** | Trapoxin B | LLLD | $-(CH_2)_{10}-$ | PC→ RCM | 40 | 41 |

*PC: Peptide cyclization

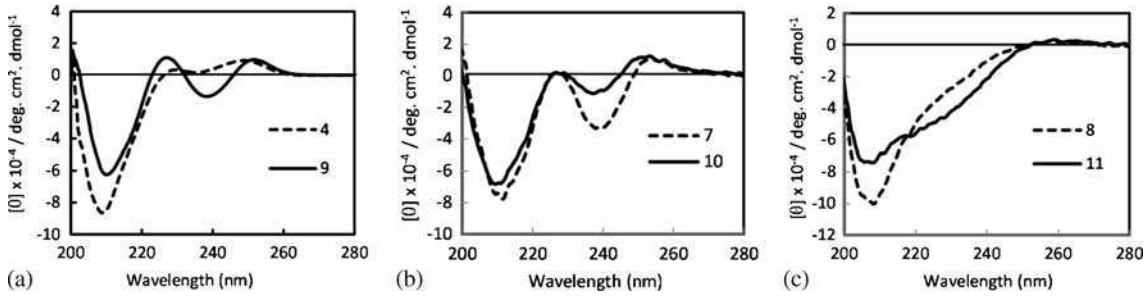



**Figure 4.** CD spectra of (**a**) CHAP31 analogs; (**b**) HC-toxin analogs and (**c**) Trapoxin B analogs.

latter step. As a result, peptide cyclization becomes almost impossible whatever be the configuration of the adjacent amino acid bearing the aliphatic side chains. The success in RCM followed by the failure in the peptide cylcization step for Cyl-1 based compound **1** is an important evidence in favour of this speculation. In such cases, bicyclic tetrapeptides are expected to be obtained if RCM is carried out after peptide

cyclization. Moreover, in this route, there is an extra advantage if the aliphatic side chain bearing amino acids have the same configuration (LL or DD). The side chains are positioned in the same side of the cyclic tetrapeptide scaffolds which make the RCM step easier. Therefore, RCM after peptide cyclization can be considered as the proper synthetic route for bicyclic tetrapeptides with LLLD configuration. The successful synthesis with appreciable yields in peptide cyclization and RCM steps for compound **8** (LLLD configuration) strengthen our proposed route.

### 3.2 *Conformational analysis by CD*

CD is a sensitive, qualitative tool for monitoring peptide conformational changes.[20] CD measurements of the synthesized compounds were carried out in methanol as solvent for analyzing peptide backbone conformation. For CHAP31 and HC-toxin analogs (LDLD configuration), the difference in CD spectra is quite remarkable between open and fused chain compounds. In case of CHAP31 analogs, the negative CD band near 240 nm disappeared and the positive band near 228 nm was shifted to longer wavelength (figure 4a), while the positive band near 250 nm shifted to longer wavelength for HC-toxin analogs upon ring closing (figure 4b). In case of trapoxin B analogs (LLLD configuration), similar CD spectra for both open and closed chain compounds were observed (figure 4c). It implies that ring closing imposes a stress on the peptide backbone of CHAP31 and HC-toxin analogs (LDLD configuration) but not on that of trapoxin B analogs (LLLD configuration) with the same loop length. Therefore, RCM faced hindrance in case of cyclic tetrapeptides with LDLD configuration.

## 4. Conclusion

In conclusion, we have established the lower limit of aliphatic loop length and a better synthetic route for bicyclic tetrapeptide synthesis. We have found that nine methylene loop length is the lower limit for aliphatic loop and the synthetic route selection depends on the configuration of amino acids in the cyclic tetrapeptide scaffold. Ring closing metathesis followed by peptide cyclization is the favourable route for bicyclic tetrapeptide with LDLD configuration and the reverse route for the LLLD configuration. Yields in peptide cyclization and ring closing metathesis steps and CD spectral analysis have been used as evidence in favour of our conclusion. This information is of immense importance for those working in the field of peptide-based drug design and synthesis.

## Supplementary Information

The electronic supplementary information (NMR spectra, figures S1-S5) is included which is available at www.ias.ac.in/chemsci.

## Acknowledgement

This study was supported by Kitakyushu Foundation for the Advancement of Industry, Science and Technology (FAIS) and Japan Student Services Organization (JASSO).

## References

1. Yokoyama F, Suzuki N, Haruki M, Nishi N, Oishi S, Fujii N, Utani A, Kleinman H K and Nomizu M 2004 *Biochemistry* **43** 13590
2. Hruby V J 2001 *Acc. Chem. Res.* **34** 389
3. Bolla M L, Azevedo E V, Smith J M, Taylor R E, Ranjit D K, Segall A M and McAlpine S R 2003 *Org. Lett.* **5** 109
4. Humphrey J M and Chamberlin A R 1997 *Chem. Rev.* **97** 2243
5. Joo S H 2012 *Biomol. Ther.* **20** 19
6. Baeriswyl V and Heinis C 2013 *Chem. Med. Chem.* **8** 377
7. Grubbs R H 2003 In *Handbook of Metathesis* (Weinheim, Germany: Wiley-VCH)
8. Grubbs R H 2006 *Angew. Chem. Int. Ed.* **45** 3760
9. Schrodi Y and Pederson R L 2007 *Aldrichimica Acta* **40** 45
10. Hoveyda A H and Zhugralin A R 2007 *Nature* **450** 243
11. Prescher J A and Bertozzi C R 2005 *Nat. Chem. Biol.* **1** 13
12. Miller S J, Blackwell H E and Grubbs R H 1996 *J. Am. Chem. Soc.* **118** 9606
13. Ghalit N, Reichwein J F, Hilbers H W, Breukink E, Rijkers D T S and Liskamp R M J 2007 *Chem. Bio. Chem.* **8** 1540
14. Berezowska I, Chung N N, Lemieux C, Wilkes B C and Schiller P W 2006 *Acta Biochim. Pol.* **53**(1) 73
15. Nishino N, Shivashimpi G M, Soni B, Bhuiyan M P I, Kato T, Maeda S, Nishino T G and Yoshida M 2008 *Bioorg. Med. Chem.* **16** 437
16. Islam N M, Kato T, Nishino N, Kim H J, Ito A and Yoshida M 2010 *Bioorg. Med. Chem. Lett.* **20** 997
17. Islam M N, Islam M S, Hoque M A, Kato T, Nishino N, Ito A and Yoshida M 2014 *Bioorg. Med. Chem.* **22** 3862
18. Hoque M A, Islam M S, Islam M N, Kato T, Nishino N, Ito A and Yoshida M 2014 *Amino Acids* **46** 2435
19. Komatsu Y, Tomizaki K, Tsukamoto M, Kato T, Nishino N, Sato S, Yamori T, Tsuruo T, Furumai R, Yoshida M, Horinouchi S and Hayashi H 2001 *Cancer Res.* **61** 4459
20. Bierzyñski A 2001 *Acta Biochim. Pol.* **48** 1091