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Modification of Constrained Peptides by Ring-Closing Metathesis Reaction†

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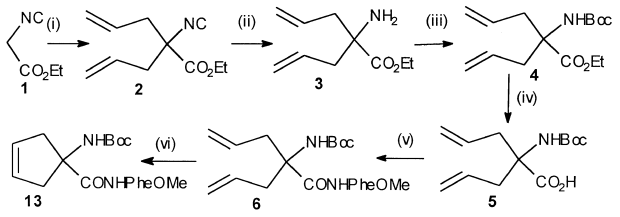
Abstract—Ring-closing metathesis (RCM) with α,α-diallylglycyl peptides is shown to furnish α,α-cyclopentenylglycyl peptides as conformationally restrained analogues in the form of post-translational type peptide modification suitable for both peptidomimetic and combinatorial chemistry applications.

Peptides, because of their potent physiological activities, are valuable lead compounds in drug discovery research, requiring molecular re-engineering to improve properties like oral absorptivity, metabolic stability, function specificity, etc., without compromising the pharmacological activity of the parent pharmacophore.[1] Combinatorial chemistry has emerged as a powerful adjunct to peptidomimetic chemistry that aims to harness the peptidic pharmacophores in alternative conformationally restrained molecular frameworks. The value of combinatorial chemistry in peptidomimetic applications is likely to be enhanced by methodologies wherein precursor peptides, made by conventional peptide chemistry methodology, may be modified in a post-translational type manner,[2] resulting in structurally critical side-chain elements to be incorporated into rigid molecular frame works. Although ring-closing metathesis (RCM) reaction is applied to the readily available *O*-alkylated or *N*-alkylated peptides, the RCM of αα-dialkylated peptides is not known in the literature. Moreover, synthesis of αα-dialkylated peptides is not a trivial exercise. Motivated by such a need for post-translational type peptide modification strategies suitable for combinatorial chemistry research,[3] we have explored RCM as a possible reaction.[4] Herein, we demonstrate the application of RCM strategy to produce α,α-cyclic peptides with restrained conformations for the first time.

Allylation of the ethyl isocyanoacetate **1**, under phase transfer catalysis (PTC) conditions (tetrabutylammonium hydrogensulphate, K_2CO_3, CH_3CN) gave the diallylated product **2** in 80% yield (Scheme 1)[5] which, on treatment with a few drops of concentrated HCl in absolute ethanol, gave the amino ester **3** in 95% yield.

The reaction of **3** with Boc anhydride in refluxing chloroform gave the Boc derivative **4** in 96% yield. (Scheme 1). Hydrolysis of the Boc protected ester **4** with 1 N NaOH in dioxane gave the acid derivative **5** (99%). The IR spectrum of **5** showed a characteristic absorption band at 1789 and 3505 cm^{-1} due to the carboxylic group. The ^1H NMR spectrum showed a broad signal at δ 8.44 due to the carboxylic proton, and a characteristic singlet at δ 1.43 due to Boc group, and multiplets at δ 5.11–5.16 and 5.16–5.76 due to the olefin functionality confirm the formation of the product **5**.



Scheme 1. Reagents: (i) allyl bromide, K_2CO_3, PTC; (ii) HCl, EtOH; (iii) $(Boc)_2O/CHCl_3$; (iv) 1 N NaOH/dioxane; (v) $HClH_2NPheOMe$, DCC, HOBt, NMM, THF; (vi) Grubbs catalyst/CH_2Cl_2.

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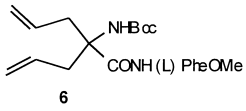
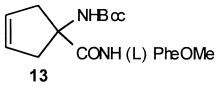
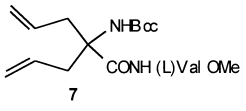
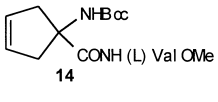
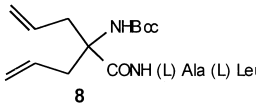
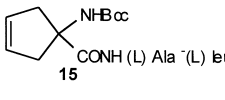
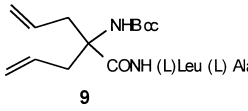
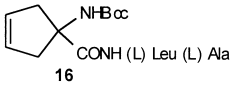
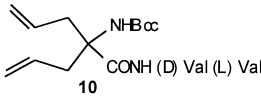
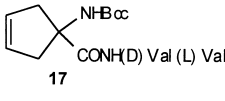
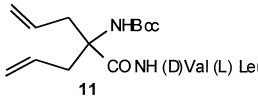
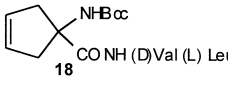
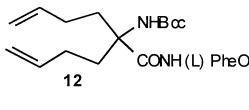
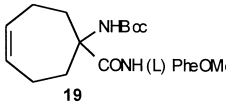
Table 1.

S. No.	Peptide	RCM product	Yield[a] (%)
1	 **6**	 **13**	75
2	 **7**	 **14**	90
3	 **8**	 **15**	50
4	 **9**	 **16**	49
5	 **10**	 **17**	53
6	 **11**	 **18**	75
7	 **12**	 **19**	69

[a]Yield refers to the yield of the RCN reaction.

Later on, we turned our attention to couple compound **5** with various proteinogenic α-amino acid (AAA) derivatives. In this regard, the reaction of **5** with the phenylalanine methyl ester hydrochloride in the presence of DCC/HOBt gave the dipeptide **6** in 63% yield (Scheme 1).[6] Along similar lines we have also prepared the compound **12** (Table 1).

The structure of **6** was fully in consonance with its spectral data. Various peptide couplings of the key building block **5** with other proteinogenic AAAs have been performed using the standard DCC-mediated coupling conditions.

Having prepared the unsaturated building blocks **6**–**12** (Table 1), attention was then focused to carry out the RCM reaction. Thus, treatment of the dipeptide **6** with the Grubbs ruthenium catalyst [$Cl_2(PCy_3)_2Ru{=}CHPh$] in refluxing toluene gave the RCM product **13** in 75% yield (Scheme 1). Similarly, the exposure of the other peptide building blocks (**7**–**12**) to Grubbs catalyst conditions gave the corresponding cyclized products (**14**–**19**) in good yields and the results are summarized in Table 1.

In conclusion, for the first time we have developed a simple method for the preparation of cyclic peptide derivatives by the RCM reaction in post-translational modification form. Since αα-dialkylated constrained peptides play an important role in peptidomimetics, our approach for modification of αα-dialkylated peptides is unique and possible incorporation of the present method into combinatorial chemistry format should be of interest to bioorganic and medicinal chemists wanting to modify peptides in a peptidomimetic application.

General Experimental Procedure for the RCM Reaction

To the dipeptide **6** (30 mg, 0.074 mmol) in toluene (3.5 mL) was added Grubbs catalyst [$Cl_2(PCy_3)_2Ru{=}CHPh$] (10% mol) and then the reaction mixture was refluxed for 12 h. Then, the reaction mixture was concentrated and the crude product was purified by a silica gel column (1:4 ethyl acetate–hexane) to give **13** as a white crystalline solid (21 mg, 75%). Mp 162–163 °C; $[\alpha]_D^{26}$ +2.40 (*c* 1, EtOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (s, 9H), 2.72–2.91 (m, 4H), 3.07 (d 1/2ABq, J = 6.6, 14.0 Hz, 1H), 3.15 (d 1/2ABq, J = 5.7, 6.0, 14.0 Hz, 1H), 3.71 (s, 3H), 4.83 (1/2ABq, J = 5.7, 6.3 Hz, 1H), 5.47 (s, 1H), 5.57 (s, 2H), 6.66 (s, 1H), 7.08–7.11 (m, 2H), 7.21–7.31 (m, 3H); HRMS *m*/*z* for $C_{21}H_{28}N_2O_5$ ($M-C_4H_9$), calcd 332.1372. Found 332.1357.

Spectral Data for Selected Compounds

14. Mp 123–124 °C; $[\alpha]_D^{26}$ +2.85 (*c* 0.7, EtOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (d, *J* = 7.2 Hz 3H), 0.95 (d, *J* = 6.9 Hz, 3H), 1.43 (s, 9H), 2.15–2.19 (m, 1H), 2.80–3.08 (m, 2H), 3.09–3.14 (m, 2H), 3.72 (s, 3H), 4.54 (dd, *J* = 4.8, 8.7 Hz, 1H), 5.37 (s, 1H), 5.70–5.75 (m, 2H), 6.86 (s, 1H).

15. $[\alpha]_D^{26}$ +14.44 (*c* 1.8, EtOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.91 (d, *J* = 4.2 Hz, 6H), 1.30–1.40 (m, 3H), 1.43 (s, 9H), 1.50–1.75 (m, 3H), 2.56–2.66 (m, 2H), 3.03–3.08 (m, 2H), 3.73 (s, 3H), 4.43–4.58 (m, 2H), 5.25 (s, 1H), 5.69 (s, 2H), 6.79 (d, *J* = 6.9 Hz, 1H), 6.87 (s, 1H); HRMS: for *m*/*z* $C_{21}H_{35}N_3O_6$, calcd 425.2525. Found 425.2505.

16. $[\alpha]_D^{26}$ −34.16 (*c* 1.2, EtOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.91–0.94 (m, 6H), 1.39–1.41 (m, 3H), 1.43 (s, 9H), 1.51–1.73 (m, 3H), 2.55–2.66 (m, 2H), 3.03–3.12 (m, 2H), 3.73 (s, 3H), 4.42–4.45 (m, 2H), 5.23 (s, 1H), 5.69 (s, 2H), 6.61 (d, *J* = 8.1 Hz, 1H), 6.97 (s, 1H); HRMS: *m*/*z* for $C_{21}H_{35}N_3O_6$ ($M-C_4H_9$), calcd 369.1899. Found 369.1909.

17. Mp 138–139 °C; $[\alpha]_D^{26}$ −18.49 (*c* 2, EtOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90–0.99 (m, 12H), 1.44 (s, 9H), 2.15–2.23 (m, 1H), 2.36–2.38 (m, 1H), 2.53–2.60 (m, 2H), 3.03–3.19 (m, 2H), 3.70 (s, 3H), 4.30 (dd, *J* = 4.5, 7.9 Hz, 1H), 4.45 (dd, *J* = 5.7, 8.4 Hz, 1H), 5.22 (s, 1H), 5.65–5.72 (m, 2H), 6.76 (d, *J* = 6.0 Hz 1H), 6.95 (d, *J* = 7.2 Hz, 1H). HRMS: *m*/*z* for $C_{22}H_{37}N_3O_6$ ($M-C_4H_9$), calcd 383.2056. Found 383.2065.

18. $[\alpha]_D^{26}$ +7.33 (*c* 1.5, EtOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85–0.99 (m, 12H), 1.44 (s, 9H), 1.59–1.77 (m, 3H), 2.54 (1/2 ABq, *J* = 16.5 Hz, 2H), 2.57 (1/2 ABq, *J* = 18.0 Hz, 1H), 2.94 (1/2 ABq, *J* = 17.1 Hz, 1H), 3.34 (1/2 ABq, *J* = 17.4 Hz, 1H), 3.67 (s, 3H), 4.30 (dd, *J* = 3.9, 8.1 Hz, 1H), 4.47–4.54 (m, 1H), 5.22 (s, 1H), 5.70 (d, *J* = 34.0, 2H), 6.67 (d, *J* = 6.0 Hz, 1H), 7.30 (d, *J* = 6.9 Hz, 1H); HRMS: *m*/*z* for $C_{23}H_{39}N_3O_6$ ($M-C_2H_4$), calcd 425.2505. Found 425.2525.

19. IR (neat): ν_{max} 3340 (NH), 1736 (CO_2Et) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (s, 9H), 2.00–2.22 (8H), 3.12 (d, *J* = 5.7 Hz, 2H), 3.68 (s, 3H), 4.73 (s, 1H), 4.86 (dd, *J* = 6.3, 7.5 Hz, 1H), 5.67 (s, 2H), 7.11 (s, 1H), 7.11–7.24 (5H). HRMS: *m*/*z* for $C_{23}H_{32}N_2O_5$ ($M-OC_2H_5$), calcd 360.1658. Found 360.1667.

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