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Synthesis of Macrocyclic β-Strand Templates by Ring Closing Metathesis

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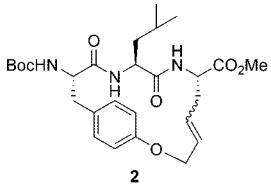

2
(*E*) : (*Z*)
9 : 1

Ring Closing Metathesis	% Conversion
Thermal	22
Microwave	37
Thermal + Lewis Acid	82
Microwave + Lewis Acid	91

We report the synthesis of macrocycles **1**−**6** via ring closing metathesis of dienes **7**−**12**. Addition of chlorodicyclohexylborane to thermal and microwave assisted RCM of dienes **8** and **9** markedly improves the yield. The geometric isomers of macrocycles **1**−**3** and **5** have been assigned using X-ray crystallography and NMR.

Proteases are known to bind their substrates and inhibitors in a β-strand geometry.[1] On the basis of this observation, we recently reported[2] the design and synthesis of macrocyclic β-strand templates (**1**−**3** and **6**) for use in the development of cysteine protease inhibitors. A key step in their synthesis required thermal ring-closing metathesis (RCM) of an appropriate peptide-based diene (**7**−**9** and **12**), however, yields were generally moderate.

Factors that influence the efficiency of RCM, such as the nature of the solvent and reaction temperature, have been well studied.[3,4] In addition, the effect of microwave irradiation has been reported to increase the yield and the rate of RCM.[5] For example, improved yields and short reaction times have been reported for microwave assisted RCM in the solid phase synthesis of α-helices, with an optimum temperature of 120 °C using Grubbs second generation catalyst.[6] Despite such reports of improvements, some structural features within the substrate still limit the efficiency of RCM. For example, co-ordination of a ruthenium intermediate with a proximate polar functional group has been reported[7] to be detrimental to RCM because of the formation of a stable intramolecular ruthenium carbene chelate. In these cases, the addition of a Lewis acid can improve yields by suppressing the formation of this chelate.[8] Chlorodicyclohexylborane has been reported to be particularly good at facilitating such metathesis reactions.[9] In this paper we report efforts to improve the efficiency of the key RCM macrocyclisation of an extended set of peptide based dienes (**7**−**12**, Scheme 1), using chlorodicyclohexylborane as an additive under thermal and microwave-assisted conditions.

The starting dienes **7**−**12** used in the study were prepared by standard peptide coupling of either *O*-allyl or *O*-homoallyl *N*-Boc-Tyr-LeuOH with an appropriate alkene containing amino acid, Scheme 2. All dienes were then cyclized by RCM (Scheme 1) using the optimum thermal conditions developed in our earlier study to maintain active catalyst[2] (i.e., 3 separate additions of Grubbs second generation catalyst and 18 h reaction, conditions A in Table 1). This gave the required macrocycles **1**−**6** as mixtures of geometric isomers in modest yields of 49, 22, 51, 34, 22, and 41%, respectively, after purification by column chromatography. Microwave-assisted RCM of **7**−**12** under Conditions B, again using 3 separate additions of Grubbs second generation catalyst, resulted in slightly improved yields of **1**−**6** isolated as the same mixtures of alkene isomers in 58, 37, 58, 38, 24, and 42% respectively, Table 1.

Importantly, the addition of chlorodicyclohexylborane to the thermal RCM of dienes **8** and **9** (Conditions C, Table 1) resulted in a significantly improved yield of **2** and **3**. These were isolated in 82 and 74%, respectively, as the same mixtures of isomers after purification by column chromatography. The addition of chlorodicyclohexylborane under microwave-assisted conditions (D) gave still further improvement, with 91% and quantitative yield of **2** and **3** respectively (Table 1). Here chlorodicyclohexylborane is considered to disrupt the stable and nonproductive six-membered ruthenium carbene chelate that would form on reaction of the catalyst with the alkene proximal to the ester of the respective dienes (see Figure 1).

Some comparative studies were carried out using titanium(IV) isoproxide as an alternative Lewis acid, given reports[8] of its use in disrupting such chelates. In particular, RCM of **8** in the presence of titanium(IV) isoproxide under thermal conditions (analogous to conditions C) gave a much reduced yield of **2** (34%). In addition, RCM of **8** with a single addition of catalyst (10 mol %) with either titanium(IV) isoproxide or chlorodicyclohexylborane as additive, gave alkene along with significant

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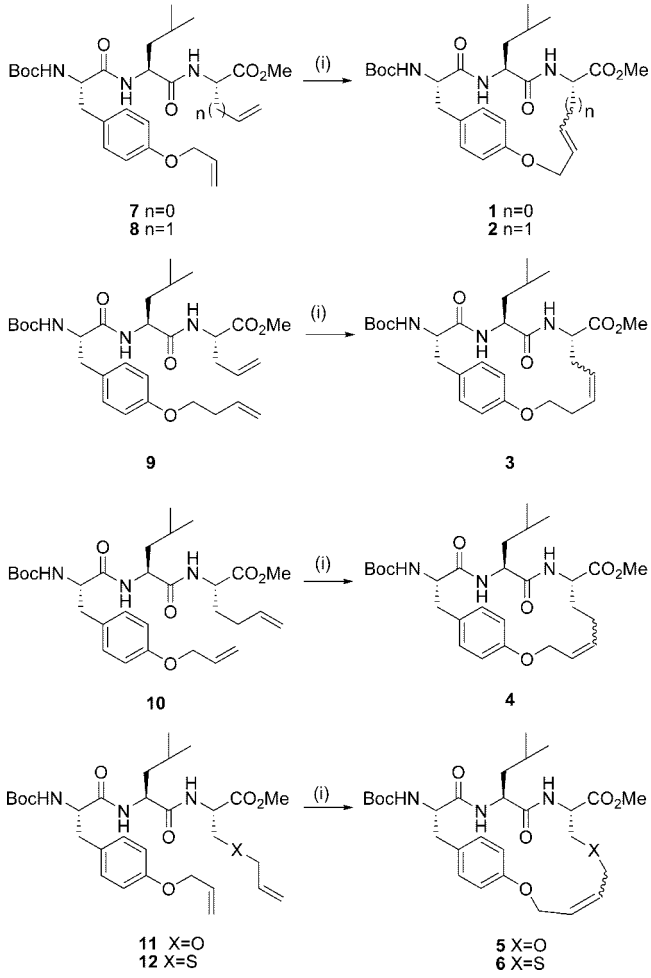
SCHEME 1. RCM of Dienes 7−12[a]



[a] Reagents and conditions: (i) Grubbs 2nd generation catalyst, see Table 1.

returned starting material. Thus the optimum conditions for RCM would appear to be the use of chlorodicyclohexylborane as Lewis acid and 3 sequential additions of Grubbs second generation catalyst, with microwave irradiation showing some advantage.

Next we investigated the influence of chlorodicyclohexylborane on the RCM of dienes **7** and **10**−**12**. Here the addition of chlorodicyclohexylborane under both thermal and microwave conditions did not give a significant improvement in the efficiency of macrocyclisation, Table 1. The less stable seven- and eight-membered chelates that might form in reactions of **10**−**12** (Figure 1) would unlikely interfere with cyclization. Thus we expect these reactions to be unaffected by the addition of a Lewis acid, as was observed. Reaction of the vinyl group of diene **7** with Grubbs catalyst would give a particularly stable five-membered ring chelate that would likely be unaffected by the addition of chlorodicyclohexylborane, again as was the case. The near quantitative formation of **2** and **3** using chlorodicyclohexylborane and microwave RCM conditions thus provides the optimum route for scale up synthesis of these important macrocycles.[2]

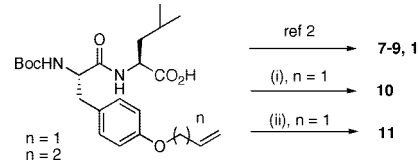
The ratio of isomers of alkenes was similar in samples of **1**−**6** formed under each of the four reaction conditions, with Table 1 showing results for Conditions D. RCM of **10** resulted in some ring contraction to give a mixture of 16-, 17- and 18-membered macrocycles as revealed by mass spectrometry and

TABLE 1. RCM of Dienes 7−12

diene	RCM condition[a]	product[b]	ring size	ratio[c]	conversion (%)[d]
7	A	**1**	16	19(*E*):1(*Z*)	49
	B			20(*E*):1(*Z*)	58
	C			19(*E*):1(*Z*)	50
	D			20(*E*):1(*Z*)	48
8	A	**2**	17	10(*E*):1(*Z*)	22
	B			9(*E*):1(*Z*)	37
	C			9(*E*):1(*Z*)	82
	D			9(*E*):1(*Z*)	91
9	A	**3**	18	1.3(*E*):1(*Z*)	51
	B			1.7(*E*):1(*Z*)	58
	C			1.9(*E*):1(*Z*)	74
	D			1.8(*E*):1(*Z*)	quant
10	A	**4**[e]	18	−	34
	B			−	38
	C			−	41
	D			−	39
11	A	**5**	19	8(*E*):1(*Z*)	22
	B			8(*E*):1(*Z*)	24
	C			9(*E*):1(*Z*)	30
	D			10(*E*):1(*Z*)	33
12	A	**6**[f]	19	10:1	41
	B			9:1	42
	C			9:1	45
	D			11:1	40

[a] All reactions performed in 1,1,2 TCE with 3 separate additions of 10 mol % Grubbs 2nd generation catalyst. Condition A: Thermal reflux 18 h. Condition B: Microwave reflux 1 h. Condition C: Thermal reflux with Lewis acid 18 h. Conditions D: Microwave reflux with Lewis acid 1 h. [b] RCM metathesis result in mixtures of geometric isomers. [c] Ratios determined by ^{1}H NMR before purification (conditions D). [d] Isolated yields following column chromatography. [e] Multiple macrocyclic products were formed due to alkene migration. [f] Configuration of alkene unable to be assigned.

SCHEME 2. Preparation of Dienes 7−12 from *O*-Allyl or *O*-Homoallyl *N*-Boc-Tyr-LeuOH[a]



[a] Reagents and conditions: (i) (S)-homoallyl-Gly-OMe, DMF, DIPEA, EDC, HOAT (76%); (ii) (*S*)-O-allyl-Ser-OMe.HCl, DMF, DIPEA, HATU (77%).

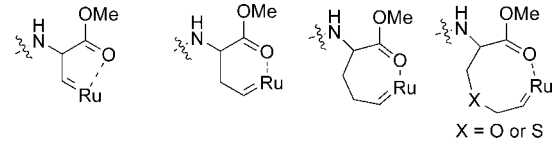


FIGURE 1. Structure of ruthenium chelates.

as such isomer ratios are not reported for this case. Macrocycle **2** was recrystallized from methanol to give the major alkene as fine-plate like needles. Single X-ray analysis of this material revealed the (*E*)-configuration (Figure 2).[10] This is consistent[11] with the coupling constant observed for the alkenic protons (J = 15.5 Hz) in the major alkene of the mixture. The crystal structure also revealed a β-strand geometry for the peptide backbone as defined by P2(Leu) Φ and Ψ torsion angles within

(10) Structures were solved by standard methods and atomic coordinates have been deposited with the Cambridge Structural Database.

(11) Coupling constants of J = 13.0 to 15.0 Hz are associated with *E*-alkenes (Kessler, H.; Seip, S. NMR of Peptide. In *Two-dimensional NMR Spectroscopy: Application for Chemists and Biochemists*, 2nd ed.; Croasmun, W. R., Carlson, R. M. K., Eds.; VCH Publishers: New York, 1994; pp 619−650).

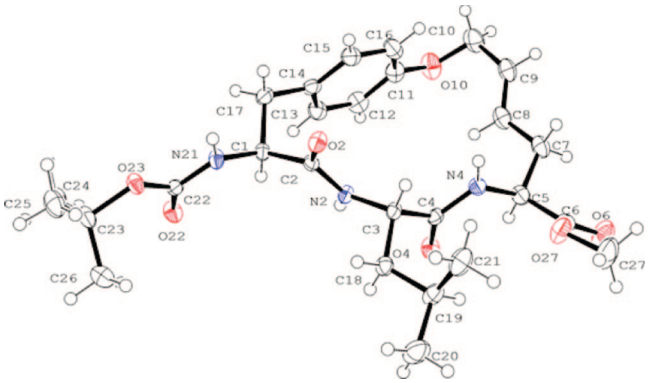


FIGURE 2. ORTEP diagram of the X-ray crystal structure of (*E*)-**2** showing a β-strand peptide backbone with $\Phi = -147.3°$ and $\Psi = 119.7°$ with respect to the P2(Leu) Φ and Ψ angles.

the ranges $-160° < \Phi < -100°$ and $90° < \Psi < 160°$ (Figure 2). This conformation is central to the ability of these derivatives to bind to proteases and is thus a key finding.[1]

The configuration of the alkenes in macrocycles **1**, **3**, and **5** were established by ^{1}H NMR analysis of the major isomer evident in each of the crude reaction mixtures. Alkene coupling constants (J = 15.5, 15.0, and 16.0 Hz, respectively) were observed in each case which is consistent with an (*E*) configuration.[11] The ^{1}H NMR spectrum of the metathesis product mixture from reaction of **10** was complicated due to the before mentioned formation of multiple macrocyclic products resulting from alkene migration. The alkene vicinal coupling constant of **6** was not measurable due to the chemical shift of the vinyl protons of the major isomer having identical chemical shifts. The major geometric alkene isomer from the RCM of **10** and **12** could, therefore, not be definitively assigned.

In conclusion, addition of chlorodicyclohexylborane to thermal and microwave promoted RCM of dienes **8** and **9** affords near quantitative formation of macrocycles **2** and **3**. This is of particular significance in that the macrocycle **2** is a key intermediate in the synthesis of a potent inhibitor of calpain 2, which shows much promise in the treatment of cataract.[2] These results may also be applicable to other studies where long reactions times for RCM have been reported.[12] The yield of macrocycle reflects inversely on the stability of a reuthenium carbene chelate and directly on the ability of chlorodicyclohexyl borane to disrupt chelation. The crystal structure of (*E*)-**2** defined its absolute configuration and revealed a β-strand geometry for its peptide backbone as is required for binding to a protease.

Experimental Section

Ring Closing Metathesis: Condition A. Thermal reflux. To a solution of diene in anhydrous 1,1,2-trichloroethane (0.01M) under an inert atmosphere was added Grubb's second generation catalyst (10 mol %) and the mixture heated under reflux. After 1 h a second portion of catalyst (10 mol %) was added, and the mixture washeated for 1 h before the final portion (10 mol %) was added. The reaction mixture was heated under reflux for a further 16 h, cooled, stirred overnight with activated charcoal, filtered, and the solvent was removed *in vacuo*. **Condition B. Microwave reflux.** To a solution of diene in anhydrous 1,1,2-trichloroethane (0.01 M) under an inert atmosphere was added Grubb's second generation catalyst (10 mol %), and the reaction mixture was heated for 20 min in a microwave (1200 W, 110−115 °C). Two further portions of catalyst (2 × 10 mol %) were added with 20 min heating between each addition. The reaction mixture was cooled, stirred overnight with activated charcoal, filtered, and the solvent removed *in vacuo*. **Condition C. Thermal reflux with Lewis acid.** To a solution of diene in anhydrous 1,1,2-trichloroethane (0.01 M) under an inert atmosphere was added Grubb's second generation catalyst (10 mol %) and chlorodicyclohexyl borane (1 M solution in hexane, 10 mol %). The reaction mixture was heated under reflux for 1 h and then treated as for **A** with two further equivalents of catalyst. **Condition D. Microwave reflux with Lewis acid.** To a solution of diene in anhydrous 1,1,2-trichloroethane (0.01 M) under an inert atmosphere was added Grubb's second generation catalyst (10 mol %) and chlorodicyclohexyl borane (1 M solution in hexane, 10 mol %). The reaction mixture was then heated for 20 min in a microwave (1200 W, 110−115 °C) and then treated as for **B** with two further equivalents of catalyst.

(*E*)/(*Z*)-(7*S*,10*S*,13*S*)-13-*tert*-Butoxycarbonylamino-10-isobutyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),4,15(19),16-tetraene-7-carboxylic acid Methyl Ester (2). Diene **8** (2.00 g, 3.67 mmol) was subjected to RCM under optimum conditions D. The crude material was purified by flash chromatography on silica using a gradient of ethyl acetate and (50/70) petrol ether to give **2**, a white solid (1.73 g, 91%) as a 9:1 mixture of isomers. mp 241−243 °C; $[\alpha]_D^{20}$ +32.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR for major isomer from mixture (500 MHz in $CDCl_3$): δ 7.01 (2H, app d, J = 5.5 Hz, *H*ArO), 6.78 (2H, app d, J = 5.5 Hz, *H*ArO), 5.87 (1H, d, J = 8.5 Hz, N*H*), 5.81 (1H, d, J = 7.0 Hz, N*H*), 5.54 (1H, app dt, J = 16.0, 3.8, and 3.8 Hz, $OCH_2CHCHCH_2$), 5.44 (1H, ddd, J = 16.0, 6.5, and 1.5 Hz, $OCH_2CHCHCH_2$), 5.34 (1H, d, J = 8.5 Hz, N*H*), 4.73 (1H, ddd, J = 9.0, 8.5, and 3.0 Hz, $CHCO_2CH_3$), 4.64 (1H, J = 15.5 Hz $OCHH$-$CHCHCH_2$) 4.59 (1H, J = 15.5 and J = 4.2 Hz, $OCHHCHCHCH_2$), 4.09−4.20 (2H, m, $CHCH_2ArO$ and $CHCH_2CH(CH_3)_2$), 3.74 (3H, s, OCH_3), 3.08 (1H, dd, J = 12.7 and 5.0 Hz, CHCHHAr), 2.73 (1H, J = 12.7 and 12.1 Hz, CHCHHAr), 2.67 (1H, J = 14.6 Hz, $CHHCHCO_2CH_3$), 2.34 (1H, m, $CHHCHCO_2CH_3$), 1.52−1.58 (3H, m, $CH(CH_3)_2$ and $CHCH_2CH(CH_3)_2$), 1.44 (9H, s, $(CH_3)_3$), 0.84−0.88 (6H, m, $CHCH_2CH(CH_3)_2$); ^{13}C NMR for major isomer from mixture (75 MHz, $CDCl_3$): δ 171.9, 171.0, 170.7, 156.1, 155.0, 129.7, 128.4, 128.1, 127.5, 115.8, 79.8, 66.4, 57.0, 52.5, 51.8, 51.6, 42.8, 38.8, 34.7, 28.3, 24.5, 24.3, 22.7, 22.5; HRMS (ES) 518.2869 (MH^+). $C_{27}H_{40}N_3O_7$ requires 518.2866.

Diene **8** (50 mg, 0.1 mmol) was also subjected to RCM under conditions C using titanium(IV) isoproxide (10 mol %) in place of chlorodicyclohexyl borane to give (*E*)-**2** as a white solid after purification by chromatography as described above (16 mg, 34%). Data as recorded above.

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Supporting Information Available: ^{1}H NMR spectra for all compounds, X-ray structural analysis of (*E*)-**2**, and the preparation of diene **11** and compounds **1** and **3**−**6** under Conditions D for RCM. This material is available free of charge via the Internet at http://pubs.acs.org.

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