Optimized Synthesis of Hydrogen-Bond Surrogate Helices: Surprising Effects of Microwave Heating on the Activity of Grubbs Catalysts

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Supporting Information

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General. Commercial-grade reagents and solvents were used without further purification except as indicated. CH_2Cl_2 and DMF were dried prior to use by percolation through anhydrous Al_2O_3 as described by Grubbs and coworkers.[1] All reactions were stirred magnetically; moisture-sensitive reactions were performed under nitrogen in flame-dried glassware. Thin-layer chromatography (TLC), usually using either ethyl acetate/hexane or methanol/CH_2Cl_2 as the solvent system, was used to monitor reactions. Visualization was accomplished by either ultraviolet light or by immersing the plate in a 1% aqueous solution of potassium permanganate and heating. Flash chromatography with silica gel was performed following the conditions described by Still and coworkers.[2] Solvents were removed by rotary evaporation under reduced pressure; where appropriate, the residue was further dried using a vacuum pump. Reverse-phase HPLC experiments were conducted with 4.6 x 150 mm (analytical scale) or 21.4 x 150 mm (preparative scale) Waters C_{18} reverse phase columns using a Beckman Coulter HPLC equipped with a System Gold 168 Diode array detector. The typical flow rates for analytical and preparative HPLC were 1 mL/min and 8 mL/min, respectively. In all cases, 0.1% aqueous TFA and acetonitrile buffers were used. Proton NMR spectra were obtained on a Bruker AV-400 (400 MHz), Bruker AV-500 (500 MHz), or Varian-200 (200 MHz) spectrometer. Carbon NMR spectra were obtained on a Bruker (100.5 MHz) spectrometer. Proton chemical shifts are reported as d values relative to tetramethylsilane (0.00 ppm) or to the particular solvent used in the experiment ($CDCl_3$: 7.26 ppm or D_2O: 4.80 ppm). Carbon chemical shifts are reported as d values relative to the particular solvent used in the experiment ($CDCl_3$: 77.0 ppm). Data is reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, br = broad), coupling constant, and integration. Infrared

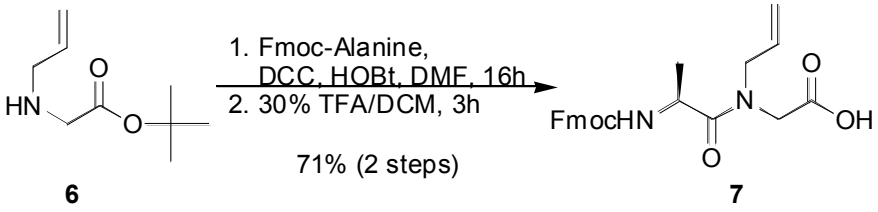
[1] Pangborn, A. B.; Giardello, M. A.; Grubbs, R. A.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518-1520.
[2] Still,W.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925.

(IR) spectra were obtained with a Thermo Nicolet Avatar 360 FTIR. High-resolution mass spectra (HRMS) were obtained by fast atom bombardment (FAB) of samples in *m*-nitrobenzyl alcohol with Cs^+ ions. FAB experiments were performed by MSU-NIH Mass Spectrometry Facility, East Lansing, MI. LCMS data was obtained on an Agilent 1100 series LC/MSD (XCT) electrospray trap.

Synthesis of *N*-allyl dipeptides

The Synthesis of Fmoc-Ala-*N*-allyl-Ala-OH was synthesized as previously reported.[3]

Scheme S1



Fmoc-Ala-(*N*-allyl)-Gly-OH (7). A solution of *N, N*-dicyclohexylcarbodiimide (DCC, 0.66 g, 3.21mmol), 1-hydroxybenzotriazole (HOBt, 0.43 g, 3.21 mmol) and Fmoc-Ala-OH (0.99 g, 3.21 mmol) was stirred in DMF (10 mL) for 10 minutes. *N*-allyl-Gly-*t*-butyl ester **6** (0.50 g, 2.92 mmol) was then added to the flask and the resulting mixture was stirred at room temperature overnight. The reaction mixture was then poured into 30 mL water and extracted with ether (3 x 25 mL). The combined organic layers were combined and dried with anhydrous magnesium sulfate, filtered and concentrated by rotary evaporation. The resulting yellow oil was then

3. Chapman, R. N.; DiMartino, G.; Arora, P. S.; *J. Am. Chem. Soc.* **2004**, *126,* 12252-12253.

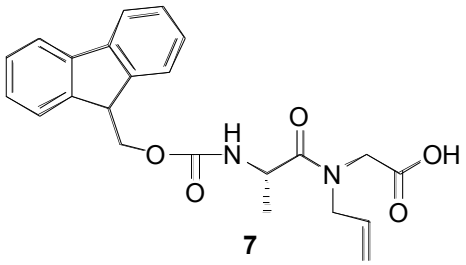
purified by flash chromatography (gradient of 10-30% ethyl acetate in hexanes) to afford the dipeptide Fmoc-Ala-*N*-allyl-Gly-O*t*Bu as a colorless oil (1.06 g, 2.28 mmol, 78%)

^{1}H NMR (400 MHz, $CDCl_3$, 248 K, mixture of rotamers 1:3) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.52-7.58 (m, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.27-7.32 (m, 2H), 6.14 (d, *J* = 7.8 Hz, 0.25H), 6.06 (d, *J* = 7.7 Hz, 0.75 Hz), 5.70-5.80 (m, 0.75H), 5.61-5.69 (m, 0.25H), 5.10-5.25 (m, 2H), 4.62 (quintet, *J* = 7.1 Hz, 0.75H), 4.43-4.49 (m, 0.25H), 4.14-4.30 (m, 4H), 3.80-4.07 (m, 2H), 3.68 (d, *J* = 17.3 Hz, 1H), 1.40 (s, 9H), 1.37 (d, *J* = 6.7 Hz, 2.75H), 1.31 (d, *J* = 6.7 Hz, 0.25H); ^{13}C NMR (100 MHz) δ 167.76, 155.62, 143.93, 143.81, 141.29, 132.01, 127.07, 125.19, 119.96, 118.52, 82.09, 67.08, 51.18, 48.07, 47.13, 33.14, 28.04, 19.32; IR (film) 3303, 1741, 1656 cm^{-1}; HRMS *m/z* for $C_{27}H_{32}N_2O_5$ $[M + H]^+$ calcd 465.2390, found 465.2387

A solution of Fmoc-Ala-*N*-allyl-Gly-O*t*Bu (1.06 g, 2.28 mmol) in 35 mL dichloromethane, 15 mL trifluoroacetic acid was stirred for 2 h. The solution was then poured into 75 mL water and extracted with DCM (3 x 50 mL). The organic layers were combined and dried with anhydrous magnesium sulfate, filtered and concentrated to afford 0.84 g of **7** as a white powder (91%).

^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers 1:3) δ 7.67 (d, *J* = 7.5 Hz, 2H), 7.48-7.53 (m, 2H), 7.31 (t, *J* = 7.4 Hz, 2H), 7.13 (m, 2H), 5.99 (d, *J* = 8.2 Hz, 0.25H), 5.90 (d, *J* = 8.1 Hz, 0.75H), 5.51-5.69 (m, 1H), 4.97-5.11 (m, 2H), 4.66 (quintet, *J* = 7.1 Hz, 0.75H) 4.40-4.48 (m, 0.25H), 4.25 (t, *J* = 6.8 Hz, 1.50H), 4.19 (d, *J* = 17.4 Hz, 1H), 4.11 (t, *J* = 17.4 Hz, 1H), 3.84 (d, *J* = 17.4 Hz, 1H), 1.31 (d, *J* = 6.8 Hz, 2.25H), 1.27 (d, *J* = 6.7 Hz, 0.75H); ^{13}C NMR (100 MHz) δ 173.92, 172.39, 156.07, 143.92, 141.29, 131.95, 127.74, 127.07, 125.2, 119.96, 118.88, 77.27, 67.36, 51.29, 47.13, 19.08; IR (film) 3305, 1717, 1653 cm^{-1}; HRMS *m/z* for $C_{27}H_{32}N_2O_5$ $[M + H]^+$ calcd 409.1734, found 409.1763

NMR spectra of *N*-allyl dipeptide 7 (mixture of rotamers).



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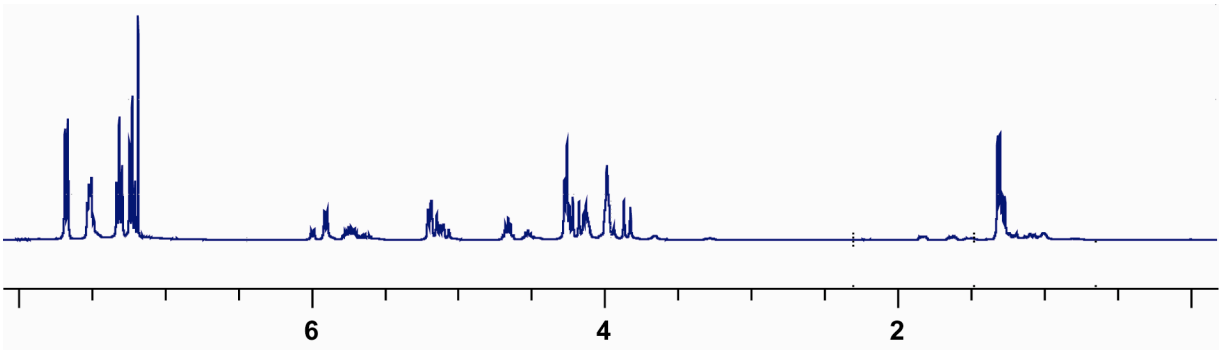
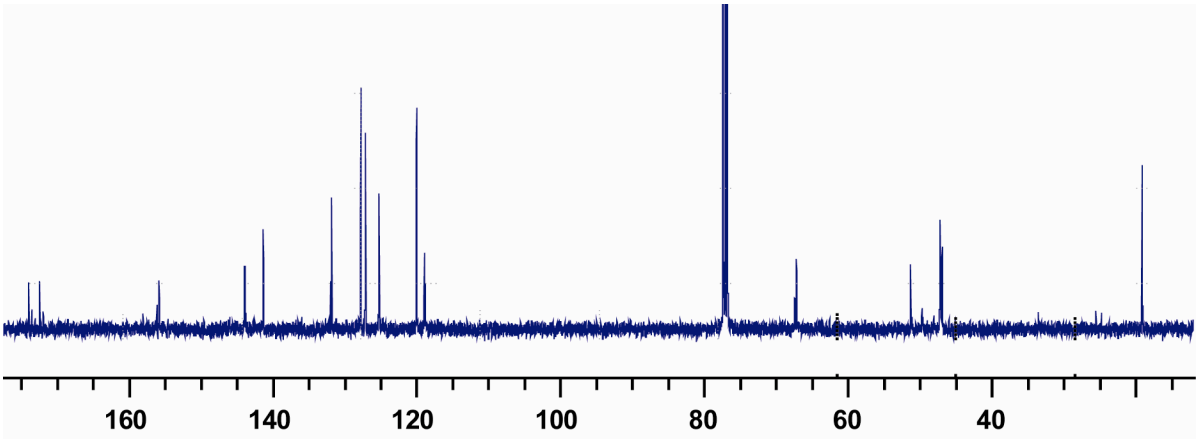


^{1}H NMR for **7** in $CDCl_3$



^{13}C NMR for **7** in $CDCl_3$

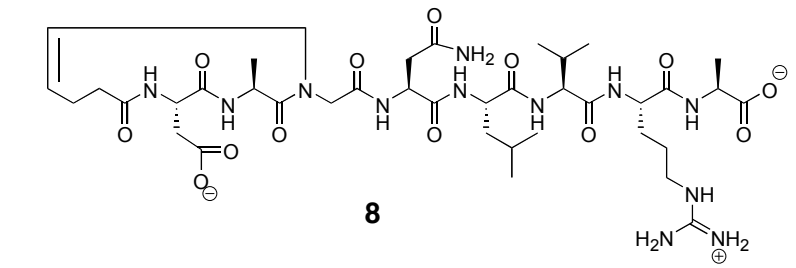
Synthesis of resin bound bis-olefin peptides. Resin bound bis-olefin peptides were synthesized by conventional Fmoc solid-phase chemistry on Rink amide resin or pre-loaded Wang resin with appropriate substitutions of N-allylated dipeptide and 4-pentenoic acid. In each coupling step, the appropriate Fmoc amino acid (5 equiv) was activated with HBTU (4.9 equiv) in 10% DIPEA/NMP solution for 10 min and added to the resin bound free amine. The resulting mixture was shaken for 30–45 min. The coupling efficiency was monitored by ninhydrin test. After each coupling step, the Fmoc group was removed by treatment with 20% piperidine in NMP (2 x 20 min). The bis-olefin containing resin was thoroughly washed with DMF and dichloromethane respectively, and dried under vacuum overnight. Peptides were stored at 4°C until needed.

General method for RCM in the microwave. The resin bound bis-olefin (20 mg) was placed in a thick wall glass tube (CEM) and sealed with a cap. An appropriate amount of catalyst in 200µl of freshly distilled solvent was added, the tube was purged with argon and the resin allowed to swell for 20 min. All reactions were carried out in the CEM discover microwave reactor with a run time of no more than 3 minutes, while the hold time and temperature varied as indicated in Table S1. All microwave reactions were conducted in a sealed glass tube; the pressure was monitored and did not exceed 40 psi, while the temperature was monitored by an IR sensor at the base of the reaction vessel. After the indicated time had elapsed, the solution was cooled rapidly by compressed air, and the resin washed with DCM (3x), 10% 1,3-bis(diphenylphosphino)propane in DCM (6x) and DCM (3x), then dried for 3 h under vacuum. The peptide was cleaved from the resin with 95% TFA: 2.5% triisopropylsilane : 2.5% water for

90 min, filtered, placed on a rotary evaporator for 5 minutes at 40 ^{o}C, then dissolved in 0.2 mL 0.1% TFA in water. The resulting solution was then monitored by LC-MS and analytical HPLC.

RCM under conventional heating conditions. Reactions with HGII were carried out at 60 °C in DCE with 15 mol% catalyst for 24-72 h, and those with GII at 35 °C in DCE with 15 mol% catalyst for 48 h, as previously reported.[4] The reaction was carried out in a temperature-controlled oil-bath.

Figure S1. **HBS Peptide 8 (from bis-olefin 1): HPLC trace (216 nm)**



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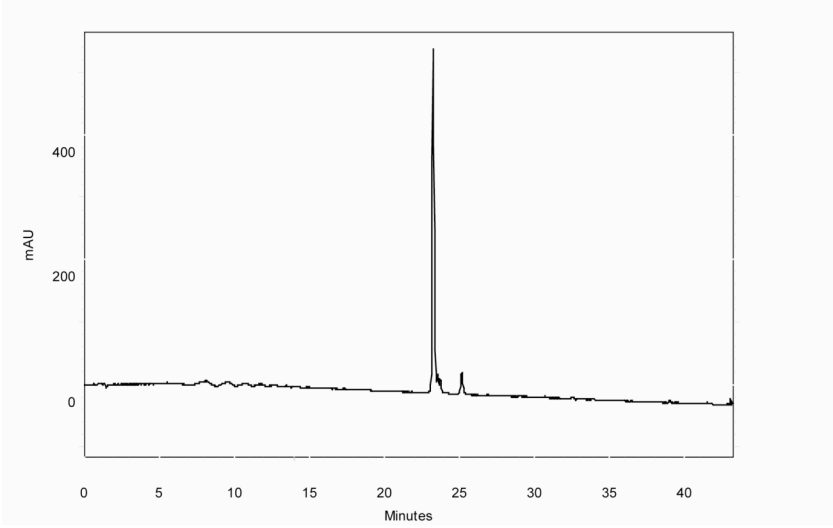
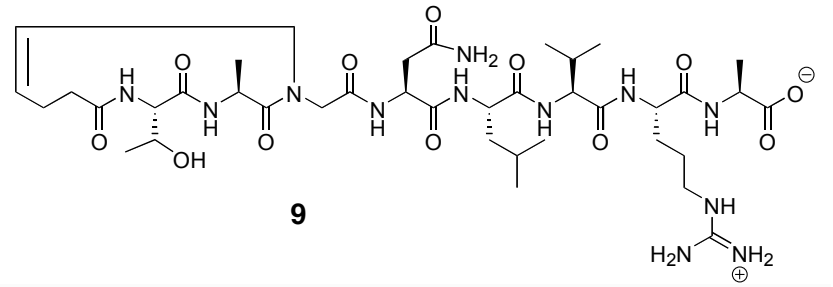


ESIMS *m/z* $C_{39}H_{63}N_{12}O_{13}$ $[M + H]^+$ calcd 909.0, found 909.7

4. Dimartino, G.; Wang, D.; Chapman, R. N.; Arora, P. S.; *Org. Lett.* **2005**, *7*, 2389-2392.

Figure S2. HBS Peptide 9 (from bis-olefin 2): HPLC trace (216 nm)



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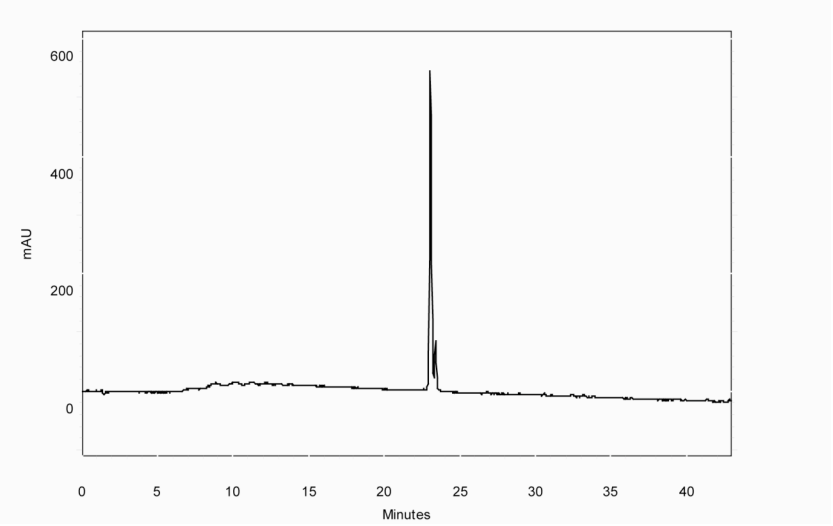
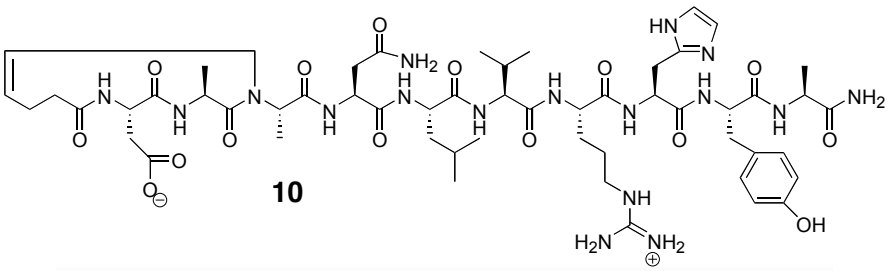
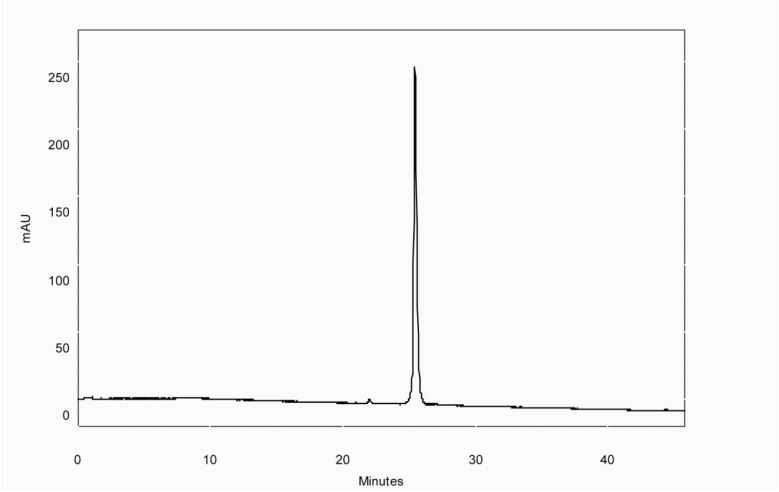


ESIMS *m/z* $C_{39}H_{65}N_{12}O_{12}$ $[M + H]^+$ calcd 895.0, found 895.5

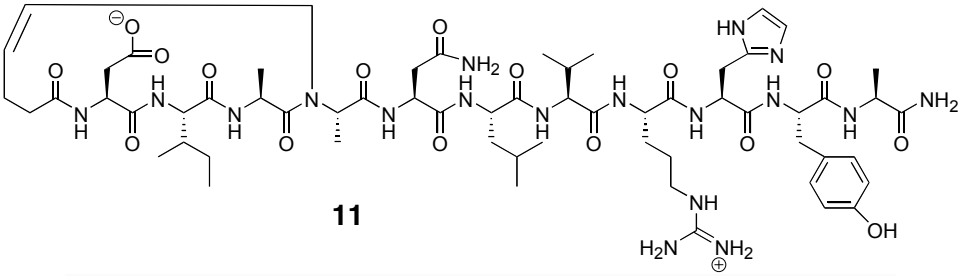
Figure S3. HBS **Peptide 10 (from bis-olefin 3): HPLC trace (216 nm)**





ESIMS *m/z* $C_{55}H_{81}N_{16}O_{16}$ $[M + H]^+$ calcd 1222.6, found 1222.6

Figure S4. HBS Peptide 11 (from bis-olefin 4) : HPLC trace (216 nm)



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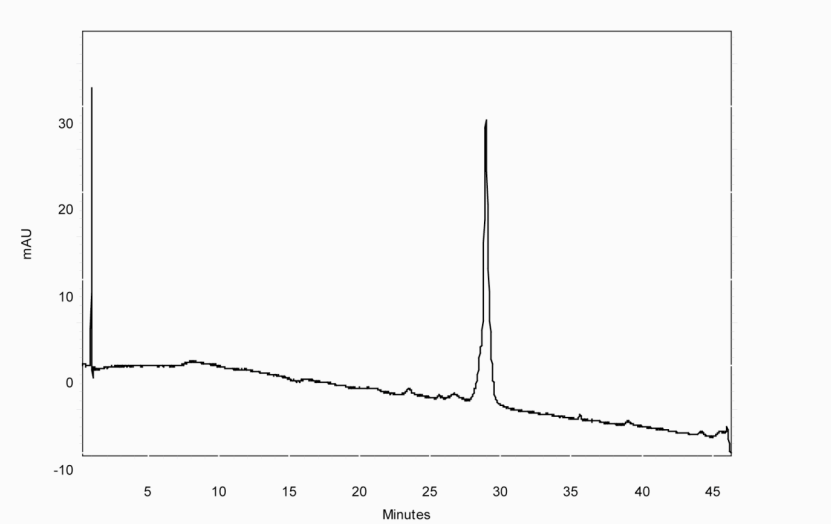


ESIMS *m/z* $C_{61}H_{92}N_{17}O_{17}$ $[M + H]^+$ calcd 1335.7, found 1335.7

Table S1. Summary of microwave-assisted RCM reactions performed on **1-5**

Peptide	Catalyst	Temp (°C)	Time (minutes)	Solvent	RCM (%)	CM (%)
1	HGII	120	1	DCE	Trace	0
1	HGII	120	5	DCE	18	0
1	HGII	120	10	DCE	24	0
1	HGII	200	0.5	Bmim	0	0
1	HGII (30 mol %)	200	0.5	Bmim	0	0
1	HGII	120	5	DCE	24	0
1	GII	100	2	DCE	70	0
1	HGII	160	10	Toluene	0	89
1	GII	160	10	Toluene	56	0
1	HGII	200	5	DCB	81	0
1	GII	120	2	DCB	80	0
2	HGII	100	2	DCE	34	0
2	HGII	100	5	DCE	46	0
2	HGII	120	5	DCE	52	0
2	HGII	150	5	DCE	66	0
2	HGII	120^	5	DCE	23	0
2	HGII	120^^	5	DCE	46	0
2	HGII (7 mol %)	120	5	DCE	26	0
2	HGII (7 mol %)	120	10	DCE	26	0
2	HGII (7 mol %)	120	20	DCE	23	0
2	HGII (7 mol %)	120	30	DCE	25	0
2	HGII	60	5	DCE	Trace	0
2	HGII	100	5	DCE	9	0
2	HGII	120	5	DCE	22	0
2	HGII	140	5	DCE	46	0
2	HGII	160	5	DCE	69	0
2	GI	120	5	DCE	17	0
2	CR	120	5	DCE	0	0
2	GII	120	1	DCE	47	0
2	GII	60	2	DCE	21	0
2	GII	100	2	DCE	55	0
2	GII	120	2	DCE	71	0
2	GII	140	2	DCE	61	0
2	GII	160	5	Toluene	44	0
2	HGII	160	10	Toluene	0	92
2	GII (1 mol %)	100	2	DCE	15	0
2	GII (5 mol %)	100	2	DCE	29	0
2	GII (10 mol %)	100	2	DCE	41	0
2	GII (20 mol %)	100	2	DCE	63	0
2	HGII (1 mol %)	160	5	DCE	Trace	0
2	HGII (7 mol %)	160	5	DCE	36	0
2	HGII (25 mol %)	160	5	DCE	56	0
2	HGII	200	5	10 % Bmim/DCE	Trace	0
2	HGII	200	5	10 % DMF/DCE	Trace	0
2	HGII	160	10	Toluene	4	75
2	GII	160	10	Toluene	19	0
2	GII	120	2	DCB	84	0
2	HGII	200	5	DCB	83	0
3	HGII	160	10	Toluene	12	43
3	GII	160	5	Toluene	34	0
3	GII	120	2	DCE	48	0
3	GII	160	2	DCE	41	0
3	HGII	200	10	DCB	67	0
3	GII	200	10	DCB	11	0
3	HGII	160	5	DCE	42	0
3	HGII	120	2	DCE	11	0
3	HGII	60	5	DCB	4	0
3	HGII	80	5	DCB	9	0
3	HGII	100	5	DCB	11	0
3	HGII	120	5	DCB	20	0
3	HGII	140	5	DCB	34	0
3	HGII	160	5	DCB	58	0
3	HGII	180	5	DCB	63	0
3	HGII	200	5	DCB	69	0
3	HGII	220	5	DCB	62	0

3	GII	60	2	DCB	10	0
3	GII	80	2	DCB	22	0
3	GII	100	2	DCB	38	0
3	GII	120	2	DCB	76	0
3	GII	140	2	DCB	61	0
3	GII	160	2	DCB	62	0
3	GII	180	2	DCB	46	0
3	GII	200	2	DCB	41	0
3	GII	220	2	DCB	36	0
4	HGII	200	5	DCB	76	0
4	GII	120	2	DCB	58	0
5	HGII	200	5	DCB	Trace	0
5	GII	120	2	DCB	0	0
5	HGII	160	10	Toluene	0	71
5	GII	160	10	Toluene	0	0
5	HGII (100 mol %)	200	5	DCB	Trace	0

All reactions contained 15 mol % unless otherwise stated. All yields are reported as % conversion of bis-olefin to either the RCM or CM product as determined by the rpHPLC peak areas at 216 nm. Abbreviations: GII = Grubbs II, HGII = Hoveyda-GrubbsII, GI = Grubbs I, CR = ciba-ruthenium, RCM = Ring Closing Metathesis, CM = Cross Metathesis, SM = Starting material (bis-olefin), DCE = Dichloroethane, DCB = dichlorobenzene, bmim = 1-butyl-3-methylimidazolium tetra fluoroborate, DMF = Dimethyformide, ^ = 100 W microwave power, ^^ = 200 W microwave power.

Description of microwave conditions and variables

All microwave reactions were performed at the desired temperatures as stated. Typical microwave power, pressure and rate of temperature acceleration are as follows:

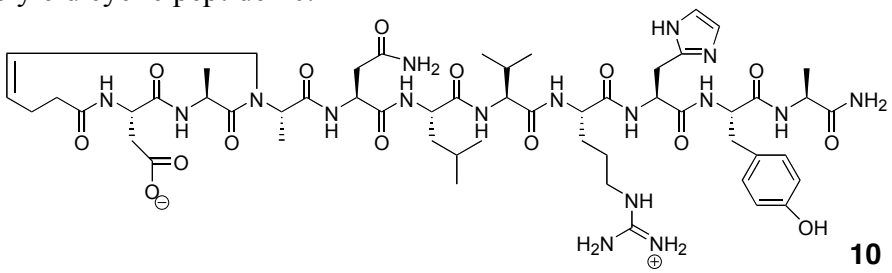
Hoveyda - Grubbs II:
RT - 200°C in 110 seconds (Ramp = 120 seconds)
Pressure reaches 30 psi (maximum)
Hold time varies according to data in table
Microwave Power reaches 275W after 110 seconds then decreases to 180W for remainder of reaction
200°C - 50°C in 60 seconds

Grubbs II:
RT - 120°C in 45 seconds (Ramp = 60 seconds)
Pressure reaches 10 psi (maximum)
Hold time varies according to data in table
Microwave Power reaches 75W after 30 seconds then decreases to 25W for the remainder of the reaction
120°C - 50°C in 30 seconds

Power control:

For the reactions where power is stated, the amount of power to reach the desired temperature was achieved by allowing different pressures of compressed air to hit the sealed microwave vessel. This works by keeping the internal temperature down, so an increase in microwaves is seen by the reaction in progress. At 300W, more compressed air is cooling the reaction than at 200W. *However, to our knowledge it has not been clearly proven that this procedure does not alter the temperature of the reaction at the side of the vessel compared to the internal solution, or indeed, if the whole temperature of the reaction is altered significantly. As a result, we run the reactions as a function of the target temperatures, as monitored by the IR probe, rather than by controlling power.*

Figure S5. Selected HPLC traces (216 nm) of CRUDE reaction mixtures. Bis-olefin **3** was treated with either Hoveyda-Grubbs II or Grubbs II catalyst for the indicated duration and temperature to yield cyclic peptide **10**.



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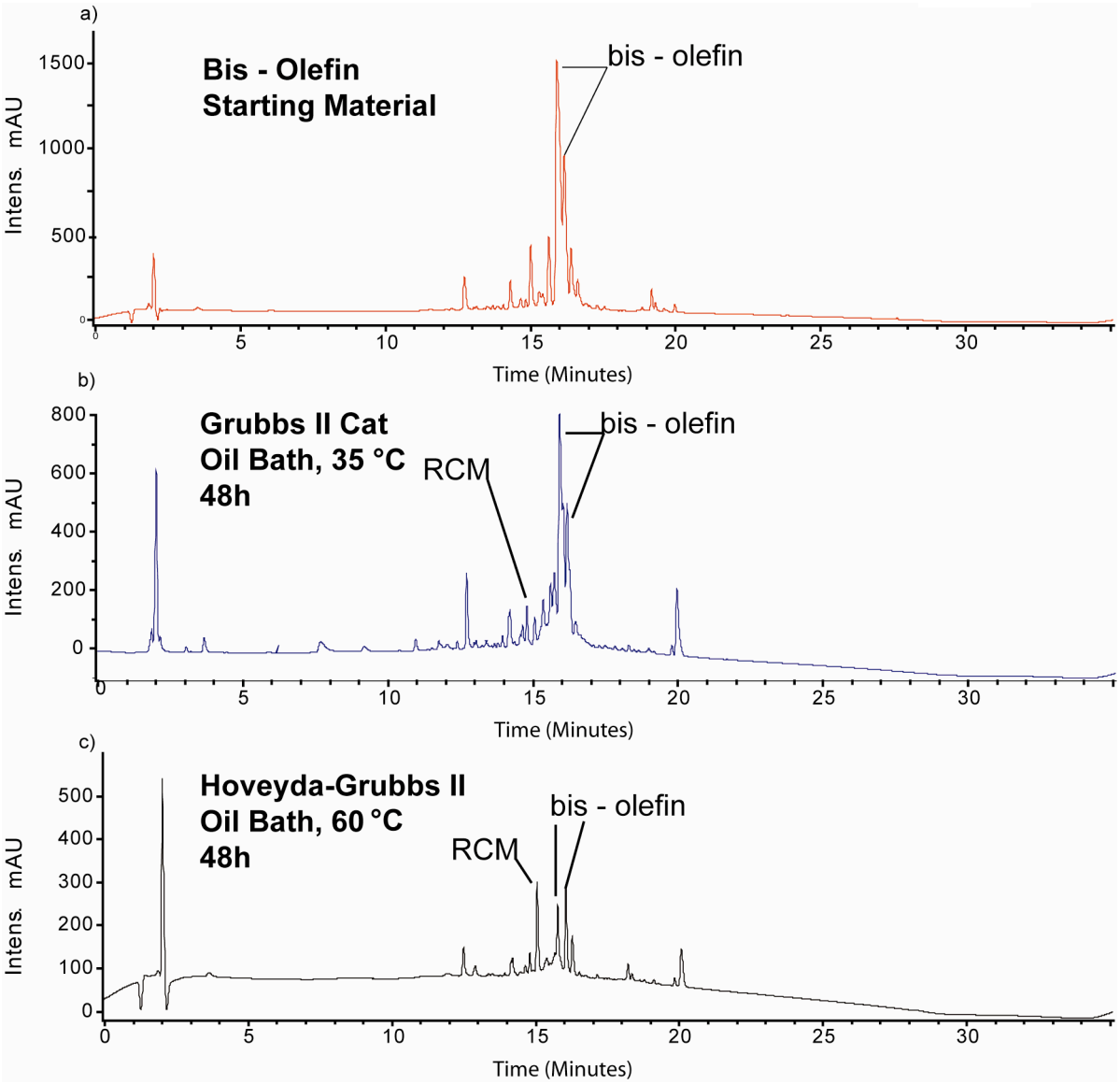

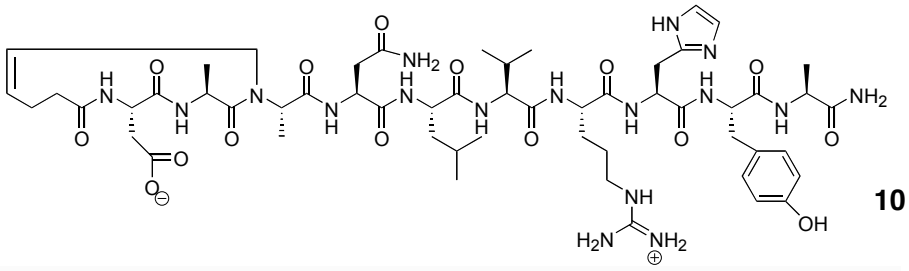

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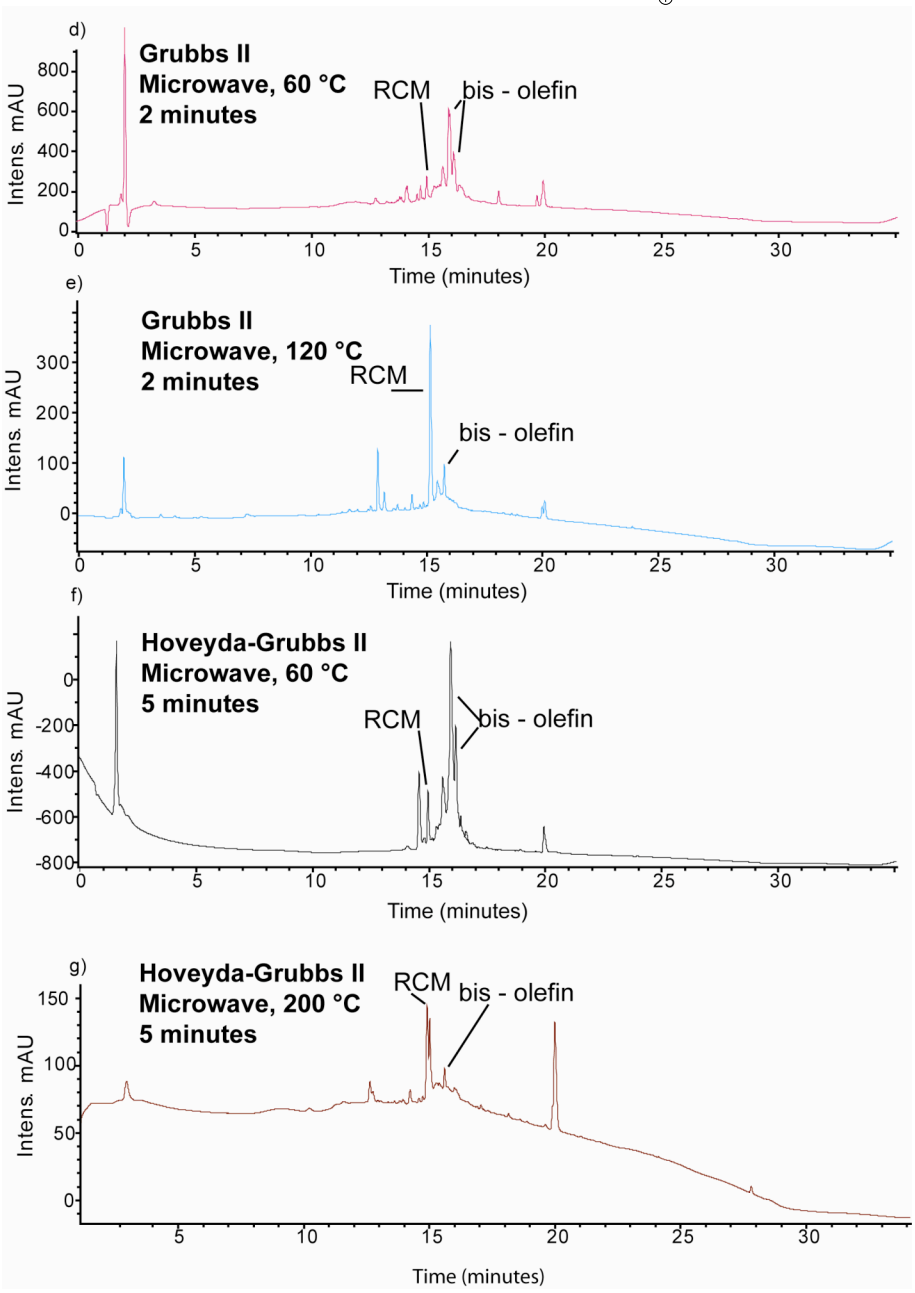

d)
Grubbs II
Microwave, 60 °C
2 minutes
RCM
bis - olefin
Intens. mAU
Time (minutes)
e)
Grubbs II
Microwave, 120 °C
2 minutes
RCM
bis - olefin
Intens. mAU
Time (minutes)
f)
Hoveyda-Grubbs II
Microwave, 60 °C
5 minutes
RCM
bis - olefin
Intens. mAU
Time (minutes)
g)
Hoveyda-Grubbs II
Microwave, 200 °C
5 minutes
RCM
bis - olefin
Intens. mAU
Time (minutes)

Effect of bis-olefin sequence on the RCM reaction under microwave conditions

The sequences listed in Table S2 are different than those shown in the main manuscript, and are included to assess the effect of sequence (beta-branched amino acids, etc) on the ring-closing metathesis reaction under the microwave conditions. We find that the microwave procedure affords high yields of the respective macrocycles from a diverse set of bis-olefins. Bis-olefins **14** and **15** represent the *same macrocycle sequences as previously described*[4] *for the optimization of the oil-bath conditions.* Our best yields for RCM **14** and **15** with oil-bath method were in the 65-80% range and are a bit higher under the microwave conditions.[4, 5] The data shows that microwave conditions perform as well or better than the oil-bath conditions regardless of the sequence.

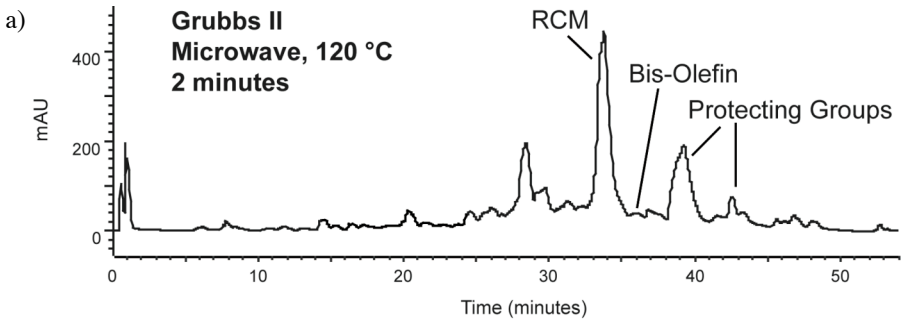
Table S2. Effect of bis-olefin sequence on the metathesis yields under the *optimized* microwave or oil-bath conditions.

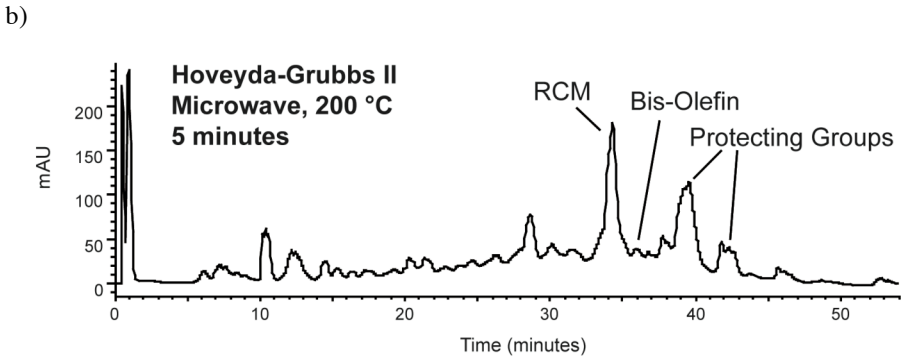
bis-olefin	sequence	% conversion with HGII in microwave	% conversion with GII in microwave	% conversion with HGII in oil-bath
12	XEVA*QRLAIIAY	84%	NP	NP
13	XQQL*EEDLKAYLDWITQ	81%	NP	NP
14	XQVG*RQLAAIYR[§]	92%	95%	74%[§]
15	XRIA*RLEEKYK[§]	80%[†]	NP	65%[§]
16	XTAA*DCEYNAR	85%	NP	NP
17	XDAA*NYRHK	88%	NP	NP

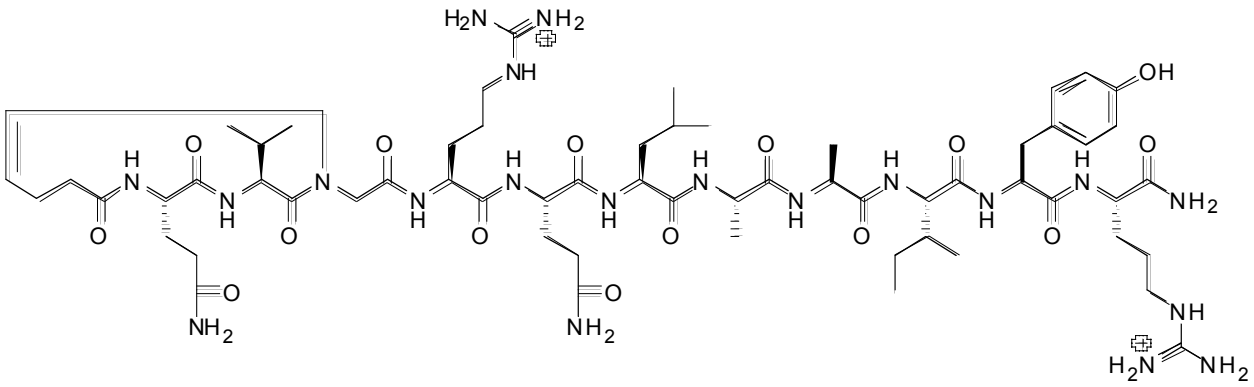
X= pentenoic acid; * denotes the *N*-allyl amino acid; NP: not performed; [†]complete consumption of the bis-olefin but a small amount of the cross-metathesis product observed. [§] data from references 4 and 5.

5. Wang, D.; Chen. K.; Kulp, J. K. III, Arora, P. S. *J. Am. Chem. Soc.* **2006**, *128*, 9248-9256.

Figure S6. HPLC traces (216 nm) of crude reaction mixtures illustrating metathesis of bis-olefin **14** to yield cyclic **18** with (a) GII and (b) HGII. (RCM refers to the metathesis product.)







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